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FABRIZIO MARCONDES MACHADO

DESVENDANDO A DIVERSIDADE DOS ANOMALODESMATA (MOLLUSCA: BIVALVIA): UMA ABORDAGEM MORFOLÓGICA E FILOGENÉTICA

UNRAVELLING THE DIVERSITY OF ANOMALODESMATA (MOLLUSCA: BIVALVIA): A MORPHOLOGICAL AND PHYLOGENETIC APPROACH

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Tese apresentada ao Instituto de Biologia da Universidade Estadual de Campinas como parte dos requisitos exigidos para a obtenção do título de Doutor em Biologia Animal na área de Biodiversidade Animal.

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Orientador: Prof. Dr. Flávio Dias Passos

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“Everybody is a genius. But if you judge a fish by its ability to climb a tree, it will live its whole life believing that it is stupid".

(Albert Einstein)

“-Lembre-se meu filho, somos dois contra o mundo”

(Minha mãe)

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RESUMO

Considerado um grupo monofilético, os Anomalodesmata Dall, 1889 possuem atualmente mais de 800 espécies, abrigando alguns dos bivalves marinhos mais raros, bizarros e especializados. Essa raridade aliada a uma carência de informações anatômicas detalhadas sobre suas espécies sempre foram obstáculos para a compreensão de suas relações internas, consequentemente, resultando também em uma baixa representatividade de táxons em reconstruções filogenéticas. Com o intuito de preencher algumas das principais lacunas no conhecimento dos Anomalodesmata, a presente Tese propôs três objetivos específicos, buscando, através de diferentes ferramentas, ampliar, atualizar e apresentar novas idéias ao conhecimento evolutivo, morfológico e taxonômico desse importante grupo de bivalves. O primeiro objetivo se deu por meio de coletas realizadas na Baía do Araçá, litoral Norte do Estado de São Paulo, onde foi possível observar e descrever em detalhes a morfologia funcional e o comportamento de *Cardiomya cleryana*, trazendo novos *insights* sobre os bivalves carnívoros como um todo. O segundo objetivo foi realizado por meio do acesso à coleção malacológica do Museum of Comparative Zoology - Harvard University, onde grande parte dos lotes depositados nessa instituição foram revisados, gerando novos dados conquiliológicos e anatômicos para a elaboração de uma análise morfológica, gerando uma nova filogenia para os Anomalodesmata. Por fim, o terceiro objetivo versou sobre o uso de microtomógrafos de raios-x para ampliar o conhecimento anatômico dos anomalodesmados, culminando nos primeiros protocolos de contraste para bivalves marinhos, comparações entre diferentes técnicas invasivas, além das primeiras descrições anatômicas de bivalves baseadas exclusivamente em imagens tomográficas e reconstruções 3D.

ABSTRACT

Considered a monophyletic group, the Anomalodesmata Dall, 1889 currently have more than 800 species, bearing some of the rarest, bizarre and specialized marine bivalves. This rarity combined with the scarcity of detailed anatomical information about their species has always been obstacles to the understanding of their internal relationships, consequently, also resulting in a low representativeness of taxa in phylogenetic reconstructions. In order to fill some of the main gaps in the knowledge of Anomalodesmata, the present thesis proposed three specific objectives, by using different tools, to expand, update and present new insights about the evolutionary, morphological and taxonomic knowledge of this important group of bivalves. The first objective took place through field collections in the Araçá Bay, north coast of the State of São Paulo, where it was possible to observe and to describe in detail the functional morphology and behaviour of *Cardiomya cleryana*, bringing new ideas about the carnivorous bivalves as a whole. The second goal was through the access to the malacological collection of the Museum of Comparative Zoology - Harvard University, where most of the lots deposited in this institution were reviewed, generating new conchological and anatomical data for the elaboration of a morphological analysis, generating a new phylogeny for the Anomalodesmata. Finally, the third objective was to increase the anatomical knowledge of anomalodesmatans by using X-ray microtomography, culminating in the first contrast protocols for marine bivalves, comparisons between different invasive techniques, and the first anatomical descriptions of bivalves based exclusively on tomographic images and 3D reconstructions.

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INTRODUÇÃO GERAL

Considerado o segundo maior grupo de metazoários, o Filo Mollusca inclui cerca de 70.000 espécies fósseis e mais de 80.000 pertencentes a fauna Recente (Margulis & Schwartz 1998, Ponder & Lindberg 2008, Rosenberg 2014, Moretzsohn 2009). Contudo, apesar de ser considerado um grupo de invertebrados bem conhecido, abrigando formas típicas como lesmas, caramujos, bivalves, polvos e lulas, estima-se que ainda existam milhares de novas espécies de moluscos a serem descritas (Bouchet *et al*. 2002, Groombridge & Jenkins 2002). Atualmente, estudos filogenéticos sugerem que os Mollusca estão representados por oito clados, distribuídos dentre dois importantes agrupamentos monofiléticos: (i) Aculífera, formado pelos clados Polyplacophora + Caudofoveata + Solenogastres e (ii) Conchífera, reunindo Monoplacophora + Gastropoda + Bivalvia + Cephalopoda + Scaphopoda (Kocot *et al*. 2011, Smith *et al*. 2011, Sherhorlz *et al*. 2017). De maneira geral, a presença de escleritos e de uma concha primariamente univalve, correspondem a algumas das sinapomorfias que caracterizam os Aculífera e Conchífera, respectivamente (Sigwart & Lindberg 2015).

Dentre os clados que compõem Mollusca, Bivalvia (= Pelecypoda, = Lamellibranchiata) se destaca por sua incrível diversidade morfológica, importância econômica, ecológica e até mesmo biomédica (Faust *et al*. 2009, Pawiro 2010, Elshawari *et al*. 2013), sendo o segundo maior grupo de moluscos em número de espécies (~8.000) (Boss 1982, Coan *et al*. 2000, Huber 2010, Sharma *et al*. 2012). Caracterizados por apresentarem uma compressão lateral do corpo, que por sua vez se encerra em uma concha bivalve dorsalmente articulada *via* charneira e ligamento (Coan & Valentich-Scott 2000), os bivalves são extremamente abundantes dentre a fauna marinha, distribuindo-se desde o entremarés até regiões abissais, constituindo um importante elemento na composição da biomassa bêntica (Giribet 2008).

Atualmente seis grupos principais compõem Bivalvia, *Protobranchia* (nuculídeos, nuculanídeos e solemyiídeos); *Pteriomorpha* (mexilhões, ostras, vieiras e arcídeos); *Palaeoheterodonta* (mexilhões de água doce e trigonídeos); *Archiheterodonta* (bivalves dotados de hemoglobina); Anomalodesmata (bivalves tubículas e carnívoros) e Imparidentia (o maior e mais amplamente distribuído grupo de bivalves) (Bieler *et al*. 2014, González *et al*. 2016).

Conhecida por abrigar as mais raras e bizarras espécies de bivalves marinhos, a subclasse Anomalodesmata Dall, 1889 se destaca por apresentar a maior diversidade de hábitos de vida dentre todos os Bivalvia (Machado *et al*. 2016). De difícil acesso, as espécies desse grupo geralmente ocorrem em águas profundas (> 500 metros) e quase sempre estão associadas a nichos ecológicos altamente especializados (Harper *et al*. 2000; Allen 2008; Morton *et al*. 2016a). Como consequência, os Anomalodesmata possuem também uma elevada diversidade morfológica. Escavadores de sedimento, endolíticos, cimentantes, bissados, construtores de tubo, predadores e até mesmo microcarnívoros incubadores são alguns exemplos dessa importante diversidade (Harper *et al*. 2000; Morton *et al*. 2016a, b).

Considerado um grupo monofilético, os Anomalodesmata possuem atualmente mais de 800 espécies distribuídas entre oito superfamílias, Clavagelloidea d’Orbigny, 1844, Myochamoidea P.P. Carpenter, 1861, Pandoroidea Rafinesque, 1815, Pholadomyoidea King, 1844, Thracioidea Stoliczka, 1870, Cuspidarioidea Dall, 1970; Poromyoidea Dall, 1886 e Verticordioidea Stoliczka, 1870 (Runnegar 1974, Bieler *et al*. 2010, Morton 1981, Bieler & Gofas 2016). Comparado a outros grupos de Bivalvia os Anomalodesmata possuem um considerável número de espécies, no entanto, o conhecimento sobre sua morfologia (anatomia) e relações filogenéticas ainda está aquém do necessário para entender as relações internas de seus grupos (Harper *et al*. 2006). Isto porque, na maioria dos casos a maior parte desse conhecimento está baseado apenas em detalhes da concha como, por exemplo, formato, cor, escultura externa, ligamento e detalhes da charneira (Pimenta & Oliveira 2003, Oliveira & Absalão 2009, Oliveira & Absalão 2010, Absalão & Oliveira 2011, Coan *et al*. 2000; Coan & Valentich-Scott 2012). Apesar de ser um importante elemento taxonômico, a morfologia da concha por si só impossibilita a robustez das reconstruções filogenéticas, tendo, portanto, que estar aliada à anatomia (Harper *et al*. 2000, 2006, Bieler *et al*. 2014). Contudo, o conhecimento anatômico sobre os Anomalodesmata ainda é muito escasso, principalmente quando comparado à totalidade de suas espécies (~811 spp); apenas 7,5% (~61 espécies) tiveram seus tecidos internos estudados em detalhes (órgãos da cavidade palial e da massa visceral) e destas apenas 27% (~17 espécies) foram observadas vivas (Machado *et al*. 2018- submitted, Worms 2018, NCBI 2018). Consequentemente, a maior parte dos resultados sobre a anatomia do grupo são quase que exclusivamente baseados em espécimes fixados, o geralmente dificulta a interpretação de possíveis homologias, gera incertezas durante a codificação de caracteres (órgãos contraídos, quebrados e/ou deformados) e principalmente cria ruídos nas reconstruções filogenéticas.

Com o advento de técnicas moleculares, o início dos anos 2000 foram marcados por importantes trabalhos sobre a evolução dos Bivalvia e em especial dos Anomalodesmata, como por exemplo, as primeiras análises cladísticas morfológicas e combinadas entre dados morfológicos e moleculares (Harper *et al*. 2000, 2006; Dreyer *et al*. 2003). Contudo, não apenas o conhecimento morfológico anatômico como também o molecular se desenvolveu muito nos últimos 17 anos, trazendo uma significativa quantidade de novos dados e ferramentas que poderão auxiliar na ampliação e reinterpretação do conhecimento evolutivo dos Anomalodesmata (Bieler *et al*. 2014). Vale destacar ainda que nesse período, dezenas de anomalodesmados tiveram seus tecidos internos estudados em detalhes, estudos sobre a ultraestrutura dos espermatozoides ganharam destaque, novas ideias sobre o hábito de vida dos bivalves carnívoros foram apresentadas e até mesmo uma nova família, Clistoconchidae Morton, 2012, foi descrita (Krylova 2001; Morton 2003, 2015; Healy *et al*. 2008; Leal 2008; Simone & Cunha 2008; Morton 2012; Temkin & Strong 2013; Machado *et al*. 2016; Morton *et al*. 2016a, b). Além disso, importantes ferramentas como micro tomógrafos (técnica não-destrutiva) e o *Next-generation sequencing* (nova tecnologia de sequenciamento) também começaram a ser utilizadas (Goulding *et al*. 2009, Handschuh *et al*. 2013, González *et al*. 2015). E é nesse novo cenário que a presente Tese de Doutorado está inserida, não apenas revisando a taxonomia e descrevendo em detalhes a anatomia de algumas espécies, como também propondo pela primeira vez a utilização de ferramentas não-invasivas para o estudo dos Anomalodesmata, assim como, elaborando testes cladísticos morfológicos que irão incorporar e reinterpretar a relações internas desse importante grupo de bivalves.

OBJETIVOS

A presente Tese tem como objetivo principal ampliar, atualizar e apresentar novas idéias ao conhecimento evolutivo, morfológico e taxonômico dos Anomalodesmata; gerando três diferentes objetivos específicos:

- *Taxonomia e morfologia funcional* - (Capítulo 1)

1- Descrever em detalhes a anatomia e o comportamento de *Cardiomya cleryana* (d'Orbigny, 1842) ampliando o conhecimento sobre a família Cuspidariidae Dall, 1886 e fornecendo novas informações sobre os bivalves carnívoros como um todo; além de elaborar uma breve revisão taxonômica sobre o gênero *Cardiomya* A. Adams, 1864 no Oceano Atlântico.

- *Sistemática filogenética* - (Capítulo 2)

2- Elaborar uma análise filogenética morfológica mais abrangente e atual para os Anomalodesmata, consequentemente, promovendo uma rediscussão sobre as relações internas de suas famílias e apresentando uma nova filogenia para esse importante grupo de bivalves marinhos.

- *Morfologia não-invasiva* - (Capítulo 3)

3- Expandir o conhecimento sobre a morfologia funcional dos Anomalodesmata por meio de técnicas não invasivas (microtomografia de raios-x), elaborando protocolos de contraste e propondo pela primeira vez a descrição anatômica de algumas espécies de Anomalodesmata, baseado exclusivamente em imagens tomográficas e reconstruções 3D.

METODOLOGIA

- Área de estudo, coletas e preparação dos espécimes estudados

A baía do Araçá, localizada no canal de São Sebastião, no Litoral Norte do Estado de São Paulo, se constitui no último testemunho existente preservado das áreas de manguezal no trecho entre Bertioga e Ubatuba. O Araçá é uma área de grande complexidade constituída por uma pequena baía limitada por flancos rochosos que abrange quatro praias (Deodato, Pernambuco, Germano e Topo), duas ilhotas (Pernambuco e Pedroso), três núcleos principais de bosques de mangue, e uma extensa planície de fundo mole, inteiramente descoberta em períodos de maré baixa de sizígia. A fisionomia resultante é de declividade suave (planície de maré), com largura da zona entremarés de 50-300 m, constituídas por sedimentos areno-lamosos, relativamente compactos, e cascalhos que também constituem o sublitoral. Assim, o Araçá pode ser classificado como uma baía muito protegida, com hidrodinâmica dominada pela maré, mas que também sofre a ação de ondas de baixa amplitude (Amaral *et al*. 2010).

Todas as estações foram efetuadas de modo a amostrar, da melhor maneira possível, a região de estudo, até a isóbata de 20 m de profundidade (Fig. 1). As coletas foram efetuadas com auxílio de embarcação do Instituto Oceanográfico da USP. Foram escolhidos 18 pontos mediante o posicionamento das isóbatas. As posições exatas das estações foram determinadas com um GPS Garmin Map 185 Sounder, com acoplamento de carta náutica. As coletas do sedimento para estudo da fauna dos Mollusca foram efetuadas com pegador do tipo van Veen, com área de amostragem de 0,25 m^2. Além disso, uma draga de arrasto retangular, medindo aproximadamente 70 cm de abertura (rede cônica de 2 cm de abertura entrenós) também foi usada em alguns pontos selecionados para a ampliação do material coletado.

Todo o sedimento referente à coleta do sublitoral foi inicialmente levado para o CEBIMar (Centro de Biologia Marinha da USP), peneirado em malha de 0,3 mm. Os espécimes encontrados foram triados e identificados até o nível de espécie utilizando-se de literatura especializada. Foram selecionados e acondicionados em aquários os indivíduos mais ativos e bem preservados de *C. cleryana*, para as análises do comportamento de escavação e atividade sifonal.

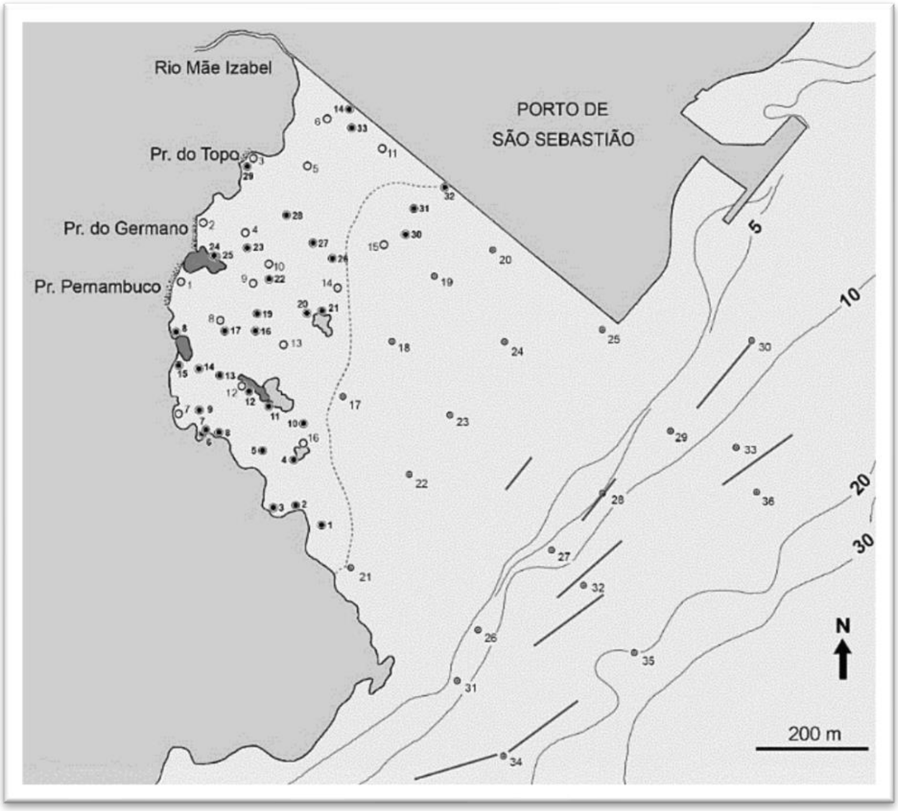

Figura 1: Baía do Araçá. Desenho amostral contendo todas as estações de coleta, com destaque para os pontos 21-36, ambos localizados no sublitoral

Alguns indivíduos foram então fotografados, filmados, dissecados e desenhados com o auxílio de um microscópio estereoscópico com câmara clara.

Alguns exemplares foram anestesiados em mentol, e trazidos para o laboratório de Malacologia, no Departamento de Biologia Animal da Universidade Estadual de Campinas (UNICAMP), onde foram submetidos à microscopia eletrônica de varredura (MEV) e histologia.

Para a descrição da morfologia funcional de *C. cleryana*, a presente Tese seguiu uma sequência metodológica de observações baseado nos trabalhos de Domaneschi (1995), Passos & Domaneschi (2004) e Piffer *et al.* (2011). Essa sequência se aplica apenas a espécimes vivos e inicia-se pela descrição detalhada da morfologia da concha (dimensões, formato, escultura externa, dentes da charneira e cicatrizes musculares) com subsequente análise dos tecidos internos como: sifões (quando presentes), bordas do manto, musculatura (adutores, retratores do pé, protratores do pé, entre outros), cavidade do manto (disposição dos principais órgãos na cavidade palial), caracterização dos septos musculares, correntes ciliares na superfície da massa visceral, caracterização dos palpos labiais e definição da estrutura e do funcionamento do estômago. Para mais detalhes, consultar o Capitulo 1.

- Análise filogenética

Com o intuito de elaborar a análise morfológica foi criada uma matriz contendo 61 táxons + 61 caracteres, ambos obtidos por meio de uma extensa revisão bibliográfica e também pela reanálise de táxons depositados em instituições de pesquisa ao redor do mundo. O teste cladístico foi elaborado a partir de uma análise de *implied weigth parcimony*, com diferentes valores de K, baseado em metodologia previamente usada por Mirande (2009) e Guadanucci (2009) utilizando-se do programa TNT (Goloboff *et al*. 2008). Os índices de consistência (CI) e de retenção (RI) foram efetuados no programa WINCLADA

1.00.08, assim como a otimização dos caracteres e edição das árvores (Nixon 2002). Os índices de suporte de ramos *(Bremmer support)* também foram devidamente fornecidos (Goloboff & Farris 2001). Para mais detalhes, consultar o Capítulo 2.

- Microtomografia de raios-x: especificações e contraste

Imagens tomográficas de algumas espécies de Anomalodesmata foram adquiridas por meio de dois diferentes modelos de microtomógrafos (µCT) - SkyScan 1272 e 1172 (Bruker MicroCT, Kontich, Bélgica) - através de duas importantes parcerias: LNNano (Brazilian Nanotechnology National Laboratory) e Harvard University. Durante a aquisição de imagens, com o intuito de estabelecer uma comparação entre os protocolos de contraste e tempo de aquisição, foram selecionados espécimes recém coletados e espécimes depositados em Museus. Para os recém coletados, os espécimes foram previamente anestesiados (Mentol) e fixados em glutaraldeído; posteriormente imersos em solução de contraste (ácido fosfotungstico + DMSO) durante 3 dias e então levados ao Micro-CT; já para os espécimes depositados em museus, geralmente preservados em álcool 70%, o tempo em solução de contraste variou entre 10 e 35 dias. De maneira geral os parâmetros utilizados durante a aquisição das imagens foram: voltagem= 30 Kv; corrente= 140µA; tempo de exposição= 1,699ms; média de frames= 3-10; filtro de alumínio= sim 0,5mm; rotação 360º= não; escala da imagem em pixel= 1,5 a 6 µm; *flat field correction* = ativado; câmera binning= 2x2; tempo de aquisição= 2 a 6 horas. Reconstruções tridimensionais dessas espécies foram elaboradas por meio dos programas NRecon 1.6.8.0 e CTvox 2.4.0 Bruker, Bélgica. Imagens tomográficas em cortes transversais e sagitais também foram analisadas por meio do programa DataViewer 1.4.4.0, Bruker, Bélgica. Para mais detalhes, consultar o Capítulo 3.

- Visitas às coleções malacológicas

Embora muitos lotes de Anomalodesmata depositados em Instituições nacionais tenham sido analisados e fotografados, a maior parte das análises e dados morfológicos foram obtidos por meio do acesso a espécimes depositados em Instituições estrangeiras, como por exemplo, o Museum of Comparative Zoology - Harvard University (MCZ), e o Smithsoniam Institution (USNM), devido a maior representatividade do grupo nesses locais. Com o intuito de revisar e codificar novos caracteres morfológicos, assim como elaborar a revisão taxonômica de alguns gêneros, foi priorizada inicialmente a análise de lotes das séries tipo (holótipos e parátipos), além daqueles contendo tecidos internos em um bom estado de preservação. Utilizando-se de microscopia de luz e microtomografia de raios-x novos registros da concha e partes moles foram elaborados, complementando o conhecimento do grupo e consequentemente revisando a identificação de algumas espécies. Detalhes sobre as instituições visitadas, quantidade de lotes e *voucher numbers*, consultar o Capítulo 2.

RESULTADOS

A presente Tese optou por apresentar todos os resultados e discussões em formato de manuscritos, previamente redigidos em língua inglesa. Portanto, cada capítulo corresponde a um manuscrito, sendo os dois primeiros já publicados e/ou submetidos.

CAPÍTULO 1

Functional morphology of *Cardiomya cleryana* (d'Orbigny, 1842) (Bivalvia: Anomalodesmata: Cuspidariidae) from Brazilian waters: new insights into the lifestyle of carnivorous bivalves

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Abstract

Of the more than 800 Recent species of Anomalodesmata only 16 have been studied alive. The Septibranchia, comprising a number of carnivorous bivalve superfamilies, are no exception to this generalisation and, until recently, no living member of this group has been studied since the 1980's. Collected from the shallow, shelf, seabed off Brazil, Cardiomya cleryana *is one of only a few species to have its morphology described in recent years and the first ever anomalodesmatan to be filmed alive. Important anatomical features such as a greatly extensible foot to secure itself in the sediment with a single byssal thread, exhalant siphon inter-tentacular projections and micro-papillae on the surface of the siphonal tentacles are described. Observations on the species' behaviour have revealed a lifestyle hitherto not recorded for any septibranch, indeed any anomalodesmatan. The anchoring mechanism of* C. cleryana *using an extraordinarily long byssal thread is described for the first time. The life position of* C. cleryana *is at 45° to the sediment-water interface whereas other cuspidariids have been considered to orient themselves vertically. An anatomical comparison between* Cardiomya *and* Bathyneaera *has revealed close affinities. This study thus provides new insights into the morphology and behaviour of the Cuspidariidae.*

INTRODUCTION

The Anomalodesmata Dall, 1889 is the richest subclass of marine bivalves in terms of the variety of life habits expressed and the most specialized and often bizarre species. Their living representatives are typically rare, since many occur in deeper waters while others are restricted to highly specialized niches (Morton 1985a; Morton *et al*. 2015a; Harper *et al*. 2000; Allen 2008). Long considered a monophyletic group, the anomalodesmatans are currently placed within the basal Imparidentia (Bieler *et al*. 2014) and encompass ten superfamilies, with eight occurring in the Recent fauna, that is, the Clavagelloidea d'Orbigny, 1844, Myochamoidea P.P. Carpenter, 1861, Pandoroidea Rafinesque, 1815, Pholadomyoidea King, 1844, Thracioidea Stoliczka, 1870, Cuspidarioidea Dall, 1970, Poromyoidea Dall, 1886, Verticordioidea Stoliczka, 1870, and two extinct groups - the Ceratomyoidea Arkell, 1844 and Edmondioidea King, 1850 (Runnegar 1974; Bieler *et al*. 2010; Morton 1981a, 2012).

Among the estimated more than 800 Recent species of Anomalodesmata (Gofas 2015), few have been studied alive and, as a consequence, most of the available information pertaining to their functional morphology and behaviour (lifestyle) is mainly based on preserved specimens highlighted mostly in the works of Morton (1973, 1980, 1982, 1984a, b, 2002a, 2003, 2005, 2006, 2012, 2015) and Morton *et al*. (2015a, b). Living individuals of only eight families (16 species) of Anomalodesmata have been studied, that is, the Cuspidariidae - *Cuspidaria cuspidata* (Olivi, 1792), *Cuspidaria rostrata* (Spengler, 1793), *Cuspidaria obesa* (Lóven, 1846) and *Cardiomya planetica* (Dall, 1908) (Yonge 1980; Allen & Morgan 1981; Reid & Reid 1974; Reid & Crosby 1980); Penicillidae – *Brechites* (=*Verpa*) *penis* (Linnaeus, 1758), *Brechites vaginiferus* (Lamarck, 1818) and *Foegia novaezelandiae* (Bruguière, 1789) (Purchon 1955, 1960; Morton 2002b, 2004); Lyonsiidae - *Lyonsia californica* Conrad, 1837, *Entodesma navicula* (A. Adams & Reeve, 1850) new comb. of *E. saxicola* and *Mytilimeria nuttalli* Conrad, 1837 (Narchi 1968; Yonge 1952); Myochamidae - *Myadora striata* (Quoy & Gaimard, 1835) (Morton 1977); Periplomatidae- *Offadesma angasi* (Crosse & P. Fischer, 1864) (Morton 1981a); Poromyidae - *Poromya granulata* (Nyst & Westendorp, 1839) (Morton 1981b); Pandoridae - *Pandora filosa* (Carpenter, 1864) (Thomas 1994) and *Frenamya ceylanica* (G.B. Sowerby I, 1835) (Morton 1984c); and Thraciidae - *Trigonothracia jinxingae* Xu, 1980 and *Thracia meridionalis* E. A. Smith, 1885 (Morton 1995; Sartori & Domaneschi 2005). Among these, only the Cuspidariidae and Poromyidae have carnivorous representatives.

According to Bieler *et al*. (2010), the carnivorous bivalves (clade Septibranchia) are currently represented by three superfamilies: the Cuspidarioidea, Verticordioidea and

Poromyoidea. Typically, but not wholly, these carnivorous bivalves are characterized by the presence of a muscular septum which functions in prey capture. Of these superfamilies, the Cuspidarioidea has the most representatives with about 300 species distributed in four families: the Cuspidariidae Dall, 1886, Halonymphidae Scarlato & Starobogatov, 1983, Protocuspidariidae Scarlato & Starobogatov, 1983 and Spheniopsidae J. Gardner, 1928 newly allocated by Morton *et al*. (2015a).

The Cuspidariidae stands out among the Anomalodesmata in comprising about 32% (~260 spp) of all the species described (Gofas & Bouchet 2015). Commonly occurring in deep and abyssal waters, the family is composed exclusively of carnivorous bivalves, which have wide geographic distributions and can generally be identified by the presence of a posteriorly rostrate shell (Allen 2008, 2011; Mikkelsen & Bieler 2008; Coan & Valentich-Scott 2012). Despite showing great diversity in terms of anatomical characters, the cuspidariids generally have a muscular septum pierced by pores, sensory siphonal tentacles and a stomach of Type II (Yonge 1928; Purchon 1956; Allen & Morgan 1981; Krylova 1993; Poutiers & Bernard 1995). According to Gofas & Bouchet (2015), the Cuspidariidae is represented today by 18 living genera. Of these, eleven genera and 100 species have been recorded from the Atlantic Ocean while from Brazilian waters representatives of only five genera are known, that is, *Cuspidaria* Nardo, 1840, *Cardiomya* A. Adams, 1864, *Plectodon* Carpenter, 1865, *Myonera* Dall & Smith, 1886, and *Octoporia* Scarlato & Starogobatov, 1983 (Allen & Morgan 1981; Rios 1994, 2001; Absalão *et al*. 2003; Absalão & Pimenta 2005; Absalão & Oliveira 2011; Allen 2011). Oliveira & Absalão (2009) also reported three species of *Protocuspidaria* Allen & Morgan, 1981, and included them as cuspidariids, but now this genus is considered in a separated family, Protocuspidariidae (Scarlato & Starogobatov, 1983; Krylova, 1995).

The genus *Cardiomya* is represented in Brazilian waters by only five species that are identified by rostrate shells with radial ribbing. The anatomy of *Cardiomya* was first described by Allen & Morgan (1981) who examined the Atlantic species *C. perrostrata* (Dall, 1881), *C. costellata* (Deshayes, 1835), *C. knudseni* Allen & Morgan, 1981 and *C. curta* (Jeffreys, 1876) (= *C. cadiziana* M. Hubber, 2010) and, more recently, by Morton (2015) who described the anatomy of *C. costellata*. More than three decades after the last species of Cuspidariidae were observed alive, individuals of *C. cleryana* (d'Orbigny, 1842) have been collected from relatively shallow waters off southeastern Brazil. This material has provided not only the first anatomical description of a Brazilian cuspidariid but also allowed for a detailed examination of the functional morphology and behaviour of living individuals of this species, providing new insights into the lifestyle of this carnivorous family of bivalves.

MATERIALS AND METHODS

Living individuals of *C. cleryana* were obtained from bottom samples collected with a rectangular dredge (40 x 80 cm and 90 cm bag length) in waters off southeastern Brazil, specifically from the São Sebastião Channel, Araçá Bay (23º 49' 20.1" S; 45º 24' 10.3" W), off the northern coast of São Paulo State by the BIOTA-FAPESP Program, between October 2012 and December 2014. Hundreds of intertidal and subtidal samples were obtained from between 3 to 20 metres depths and sieved using a 0.5 mm mesh. From ten of these, samples collected at depths of between 10 to 20 metres, five empty shells and 33 living individuals of *C. cleryana* were obtained. Of these, the most active individuals were selected for behavioural observations. To do this, aquaria were filled to a depth of 15 cm with the ambient sediment (fine sand and gravel) from the collection station and then filled with seawater. Ten individuals were photographed and observed for about two hours. Some were also videotaped. Behaviours, such as the digging process, digging depth and siphonal movements were recorded. Other individuals were anaesthetized with menthol and magnesium chloride and dissected to remove the mantle, foot and adductor muscles (for future molecular analysis) and male gonads (for future TEM analysis). Other individuals were selected for SEM examination of the shells and internal tissues. For histological purposes, the most relaxed individuals were decalcified in a solution of 100 ml distilled water containing 0.89 g of NaCl and 1.02 g of ascorbic acid and embedded in Historesin® in order to obtain serial transverse and sagittal sections of between 3-5 µm thick. All specimens, SEM stubs and histological slides are deposited in the Museum of Zoology "Prof. Adão José Cardoso" of the University of Campinas (ZUEC), with the following accession numbers: ZUEC-BIV 5119 to 5141.

RESULTS

SYSTEMATICS

Order ANOMALODESMATA Dall, 1889
Superfamily CUSPIDARIOIDEA Dall, 1886
Family CUSPIDARIIDAE Dall, 1886
Genus *Cardiomya* A. Adams, 1864
Cardiomya cleryana (d'Orbigny, 1846)

(Figures 1-10)

Cardiomya A. Adams, 1864

Cardiomya has an inflated globular to ovate shell, with an elongated rostrum. The sculpture is of strong radial ribs posteriorly and commarginal striae. Right valve with a posterior lateral tooth, which may be obsolete; left valve edentate. Resilifer shallow to deep, sub-vertical. Muscular septum with four pairs of pores and small lateral septal muscles attached close to the anterior end of the posterior siphonal retractor muscles (Poutiers & Bernard 1995; Coan *et al*. 2000; Coan & Valentich-Scott 2012).

Original description

Sphène de Cléry (Homage to Mr. Cléry) or *Sphena cleryana* d'Orbigny, 1845: 572, pl. LXXXIII, figs 16-18 or in d'Orbigny 1853: 285.

Synonymy

Sphena cleryana d'Orbigny, 1846; *Cuspidaria simillima* E.A. Smith, 1915; *Cuspidaria* (*Cardiomya*) *simillima* E.A. Smith, 1915.

Type locality

Cape St. Thomé Peninsula, Campos dos Goytakazes - off Rio de Janeiro State, Brazil from 80 metres depth.

Conchological remarks

According to Gofas & Bouchet (2015), 53 recent species constitute the genus *Cardiomya*. Of these, the Pacific *C. lanieri* (Strong & Hertlein, 1937), *C. gouldiana* (Hinds, 1843) and *C. pectinata* (P.P. Carpenter, 1864) most resemble *C. cleryana* particularly in terms of the outline and arrangement of the radial ribs. Although similar, however, some significant conchological differences can be identified, for example, the presence of two sharp and widely-spaced radial ribs in the posterior portion of the disk of *C. lanieri*; the presence of regular commarginal lirae between all the ribs and a reduced rostrum in *C. gouldiana* and the undifferentiated shape of the ribs in *C. pectinata* (Poutiers & Bernard 1995, figures 59, 60; Coan & Valentich- Scott 2012, plate 318; Coan *et al*. 2000, plate 120).

Only seven species of *Cardiomya* are known to occur in the Western Atlantic Ocean. Of these, according to Rios (1994, 2009) and Absalão & Pimenta (2005), but five species occur in Brazilian waters. These are: *C. cleryana*, *C. ornatissima* (d'Orbigny, 1853), *C. perrostrata* (Dall, 1881), *C. striata* (Jeffreys, 1876) and *C. surinamensis* van Regteren Altena, 1871. Among the possible Brazilian species, *C. ornatissima* is different from the others in having prominent radial ribs with broad interspaces, resembling *C. costata* (G.B. Sowerby I, 1834), which occurs from Baja California to Ecuador, and *C. glypta* Bush, 1898, which occurs off French Guiana. Both of these taxa are illustrated by Coan & Valentich-Scott (2012, plate 317) and Massemin *et al*. (2009, p. 349), respectively. No other Brazilian records have been identified with regard to *C. striata* and *C. surinamensis* other than Rios (1994, figure 1486; 2009, figure 1681) and Absalão & Pimenta (2005, figure 135), respectively; and though similar to *C. cleryana* in the number of ribs, *C. surinamensis* has an oval shape and a much reduced rostrum (Altena 1971, figure 3).

Cardiomya cleryana and *C. perrostrata* are the most well known taxa from Brazilian waters and are similar to each other and easily confused. After analyzing photographs of museum lots (NMR- Natuurhistorisch Museum, Rotterdam, Holland; ZUEC- Museum of Zoology "Prof. Adão José Cardoso" of the University of Campinas, Brazil; USNM - United States National Museum, Smithsonian Institution, USA) containing shells of different sizes of these two species this paper describes and illustrates some characteristics that help in their differentiation. It is also worth noting that there are no significant differences between their hinge plates. We, therefore, highlight four distinctive shell characters that separate them: the contours of the posterior-anterior dorsal margin, the shape of the rostrum, the presence or absence of a rostral rib and the position of the umbones. Individuals of *C. perrostrata* are on average smaller than congenerics (2.4 to 7.3 mm in shell length) and have a straight and continuous postero-dorsal shell margin (pdm), confluent with the rostrum (r); a straight antero-dorsal margin (adm) forming a small shoulder more obvious in larger individuals; a straight, thick and slightly upwardly pointing rostrum with one diagonal rostral ridge (rr) on the surface and umbones (u) small, low and positioned more medially (Figure 1A, B). *Cardiomya perrostrata* is, therefore, different from *C. cleryana* (4.5 to 11.8 mm in shell length), which has rounded anterior and slightly concave posterior dorsal shell margins, a thin, slightly recurved, rostrum and more prominent umbones slightly displaced towards the posterior (Figure 1C, D).

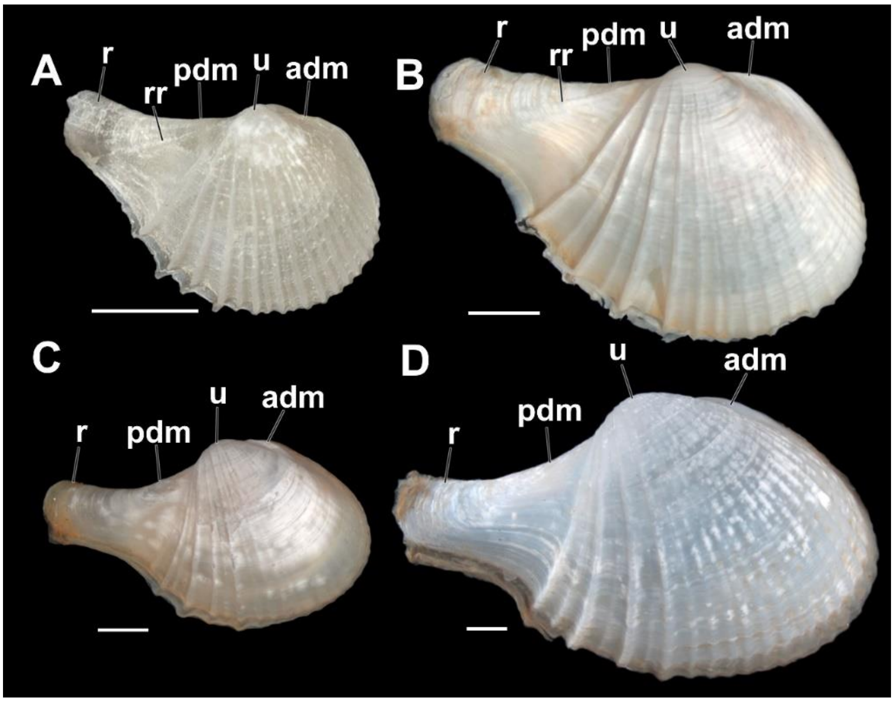

Fig. 1. Comparison between the shells of *Cardiomya perrostrata* and *Cardiomya cleryana.* (A, B) outer view of right valve of two specimens of *C. perrostrata,* with straight dorsal margins, umbones smaller and low with a straighter, thicker and slightly pointed up rostrum; (A) a specimen with ~2.4 mm in length - USNM 832408; (B) a specimen with ~7.3 mm in length – ZUEC-BIV 5130. (C, D) outer view of right valve of two specimens of *C. cleryana,* showing the contour of dorsal margins, umbones more prominent with rostrum thinner and slightly recurved. (C) a specimen with ~6.3 mm in length – ZUEC-BIV 5133; (D) a specimen with ~11.7 mm in length – ZUEC-BIV 2218. (See the list of abbreviations). Scale bars: A-D, 1mm.

BIOLOGY AND BEHAVIOUR

Distribution

Rio de Janeiro to Tierra del Fuego and the Falklands Islands (Carcelles &Williamson 1950; Rios 1994, 2009; Scarabino 2003). Specifically with regard to Brazil: Bahia de Paranaguá (25°30’S; 48°30’ W), Paraná State (Boehs *et al*. 2004); Ilha Grande Bay (44°45’W; 23° 20’S), Angra dos Reis, Rio de Janeiro State (Grillo *et al*. 1998); Channel of São Sebastião, Ponta do Guaecá (23°50’S, 45°27’W) to Baía do Araçá (23°48’S, 45°24’W), 5-10 m, São Paulo State (Migotto *et al*. 1993). This species was also originally reported upon from Jamaica, Cuba, St. Thomas, Guadalupe (d’Orbigny, 1846).

The living animal

Siphonal movements

Ten living individuals of *C. cleryana* were observed in relation to their siphonal movements and five of these also had these filmed with high-resolution cameras providing the first such record of a carnivorous bivalve. This tool was important for the interpretation of the complex movements exhibited by the siphons. The siphons of *C. cleryana* are sensitive to vibrations in the water column and these and other mechanical disturbances caused their rapid retraction within the rostrum. When they re-emerged, the seven sensory tentacles and the two sets of exhalant inter-tentacular projections were also extended. In living individuals also it was possible to see the colour of the siphonal sheath, which was also extended from the posterior margins of the rostrum. Even though buried at 45° to the sediment-water interface, the siphons of *C. cleryana* are always extended in a near-vertical position.

Three sequential siphonal movements undertaken by *C. cleryana* were observed: (i) water circulation, (ii) cleaning and (iii) feeding.

(i) The circulation of water into and out of the mantle cavity of *C. cleryana* was characterized by the simultaneous extrusion of the inhalant and exhalant siphons, creating currents that circulate through the infra- and supra-septal chambers *via* the septal pores. This movement was not constant, occurring at intervals of between 5-10 seconds and was synchronized with movements of the muscular septum that creates these currents. When the exhalant siphon was extended its length was greater than that of the inhalant whereas the latter had a much wider aperture. It is noteworthy that relaxation of the septum resulted in the retraction of the exhalant siphon, the two sets of inter-tentacular projections thereby closing the exhalant opening. The projections resemble two small opercula that open and close according to the status of the exhalant siphon. This movement possibly assists in the oxygenation of tissues and removes faeces and other nitrogenous wastes.

(ii) A cleaning action was observed during the digging process of *C. cleryana* and appears to be associated mainly with removing sediment and pseudofaeces accumulated in the infra-septal chamber. This movement was performed exclusively by the inhalant siphon and lasted for but 1-2 seconds. It resulted in the extension of the inhalant siphon while simultaneously reducing its diameter, creating a more elongate but narrower tube. The simultaneous depression of the septum increases the pressure in the infra-septal chamber creating this action and which in conjunction with valve closure results in sediment and/or pseudofaeces being ejected from the inhalant siphon thereby cleaning the infraseptal chamber. Throughout a digging cycle this movement was repeated two to three times.

(iii) Feeding is uniquely associated with prey capture in *C. cleryana* and was more difficult to observe, because it was typically confused with water circulation movements. Feeding was only observed in completely buried individuals and it was thus not possible to observe the associated movements of the muscular septum. During feeding, however, the aperture of the inhalant siphon was narrowed and pointed towards potential prey, capturing it by means of the dramatic extension and opening of the siphonal aperture, as described and illustrated for *C. planetica* by Reid and Crosby (1980, figure 1).

The digging and re-burial process

Cardiomya cleryana naturally occurs in a sediment characterized by a mix of fine sand and gravel. A change in the type of sediment appears to affect the ability to dig in this species. After being collected, ten individuals of *C. cleryana* were kept in aquaria without sediment but with running seawater for 30 minutes. The most active individuals of these were then placed in Petri dishes with a thick bed of sediment. Some individuals were filmed. During these observations, three combinations of sediment were offered, that is, fine sand + gravel (original combination), gravel only and fine sand only. When exposed to the original sediment (fine sand + gravel) most individuals buried themselves with in 6-7 minutes. In gravel, no individuals buried themselves whereas in fine sand most re-buried within 2-4 minutes. No significant differences were observed either between adults and young individuals or between males and females. All the observed individuals behaved in the same way during the re-burial process. The digging sequence can be divided into three parts: (i) recognition of sediment type, (ii) pedal digging movements, and (iii) final positioning in the sediment as follows (*see video in https://www.researchgate.net/publication/301697915_Behaviour_movie_Cardiomya_cleryana_Bivalvia_Septibranchia_Anomalodesmata*).

(i) When placed on the sediment, the foot of each individual was extended from the pedal gape to touch the sediment and was then retracted immediately into the infra-septal chamber bringing with it some of the particles. Possibly, this helps the bivalve to evaluate grain size and thus its resistance to re-burial. The same movement could be repeated several (2-20) times and may or may not result in re-burial. When exposed to fine sand, the individual’s foot made similar movements but digging movements commenced immediately.

(ii) When digging commenced, the foot penetrated the sediment and initially turned an individual onto its side. As the foot began the re-burial process, contraction of the pedal retractor muscles straightened the shell so that the ventral margin was in contact with the sediment surface. From this point onwards, the foot began to burrow deeper and deeper and the pedal retractor muscles interracted with movements of the septum to complete the re-burial process in a series of downward oriented jerky movements. These intermittent movements were not continuous but occurred at intervals of about 2-3 seconds. The direction of the excavation was always the same for all individuals, that is, the anterior region was buried first and, then, in a series of antero-posterior rocking movements, the shell was eventually positioned at 45° to the sediment surface. *Cardiomya cleryana* continued the re-bural process until only about 1/3 of the rostrum was exposed above the sediment surface. This region of the shell and siphonal sheath is camouflaged within the sediment by the adhesion of sand grains to it using arenophilic gland secretions.

(iii) After removing each of the individuals carefully from the sediment, it was clear that they were all buried at an angle of approximately 45° (Figure 2), different to the vertical position (90°) observed by Yonge (1928) for *Cuspidaria obesa* and *Cuspidaria cuspidata* and for *Cuspidaria rostrata* by Reid & Reid (1974) and as generally postulated by Morton (1987, figure 13) for species of *Cardiomya* and *Cuspidaria*. In addition to observing position in the sediment, burial depth was measured and this was shown to approximate individual shell length.

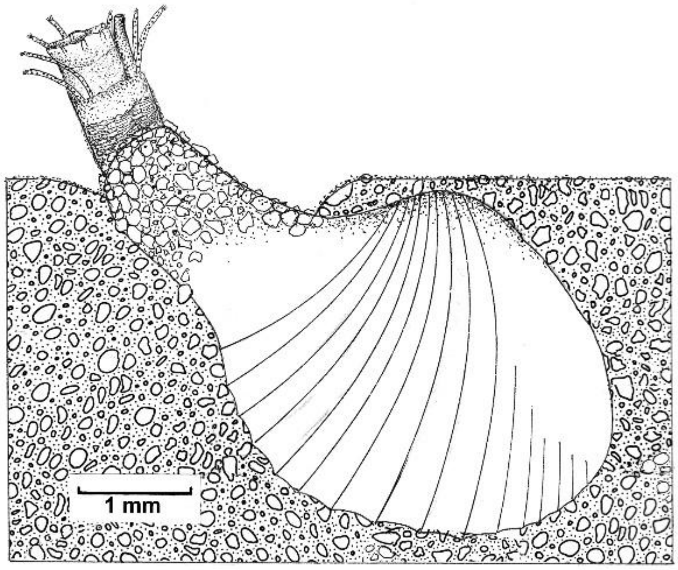

Fig. 2. *Cardiomya cleryana*. An adult individual in its life position in the sediment.

ANATOMY

The shell

Shell small (length: 4.5–11.7 mm; height: 3-6 mm; width: 2.3–5.1 mm), elongate, pear-shaped, inequilateral, slightly inequivalve, rostrate, inflated (Figure 3A-C); shell valves thin, whitish to somewhat transparent; left valve (lv) larger than the right (rv) and overlapping it ventrally (Figure 3A). Rostrum (r) moderately narrow (~800-1,100 µm), long (~1/2 the length of the shell), slightly recurved, subtruncate (Figure 3A-C); with sinuous radial lines (rl) on the external surface (Figure 3B, D). Umbones prominent and slightly displaced to the posterior. Prodissoconch I (pr I) small (~130 µm in length), circular and smooth; limits of prodissoconch II not visible (Figure 3H). Antero-dorsal shell margin small, rounded, merging with the umbones and confluent with the anterior margin; posterodorsal shell margin larger than the anterior, straight to slightly concave, confluent with the posterior margin and merging with the rostrum (Figure 3F, G). In some individuals the anterior dorsal margin may be slightly prominent and convex (Figure 3B, F, G). Lunule and escutcheon absent. Periostracum thin, adherent and translucent white to light brown (Figure 3A). External surface of the valves with fine commarginal striae, stronger ventrally; main body of the shell or disk (dk) characterised by 13-20 complete radial ribs which extend from close to the umbones of each valve to the ventral margin; anterior slope with 10-15 fine and narrow radial ribs (fr); posterior portion with 3-5 heavy radial ribs (hr) with short interspaces, sometimes with fine radial ribs (Figure 3A-C). Micropits (mp) present but restricted to around prodissoconch I, only visible with SEM (Figure 3E). Inner surface whitish and smooth, anterior slope and ventral margin with crenulations corresponding in position with the external ribs. Hinge plate very narrow. Right valve with only one strong, moderately short, posterior lateral tooth (lt) (Figure 3F, H); left valve edentulous (Figure 3G). Amphidetic ligament (li) fibrous (fp) and with a calcified lithodesma (lit), deeply located on a subtriangular resilifer (re) placed directly below the umbones (Figure 3I, J). Anterior adductor muscle scar (aam) elongate, barely visible; posterior adductor muscle scar (ppm) more rounded than the anterior, deep and well marked (Figure 3F-H). Pallial line entire and not visible, even with SEM.

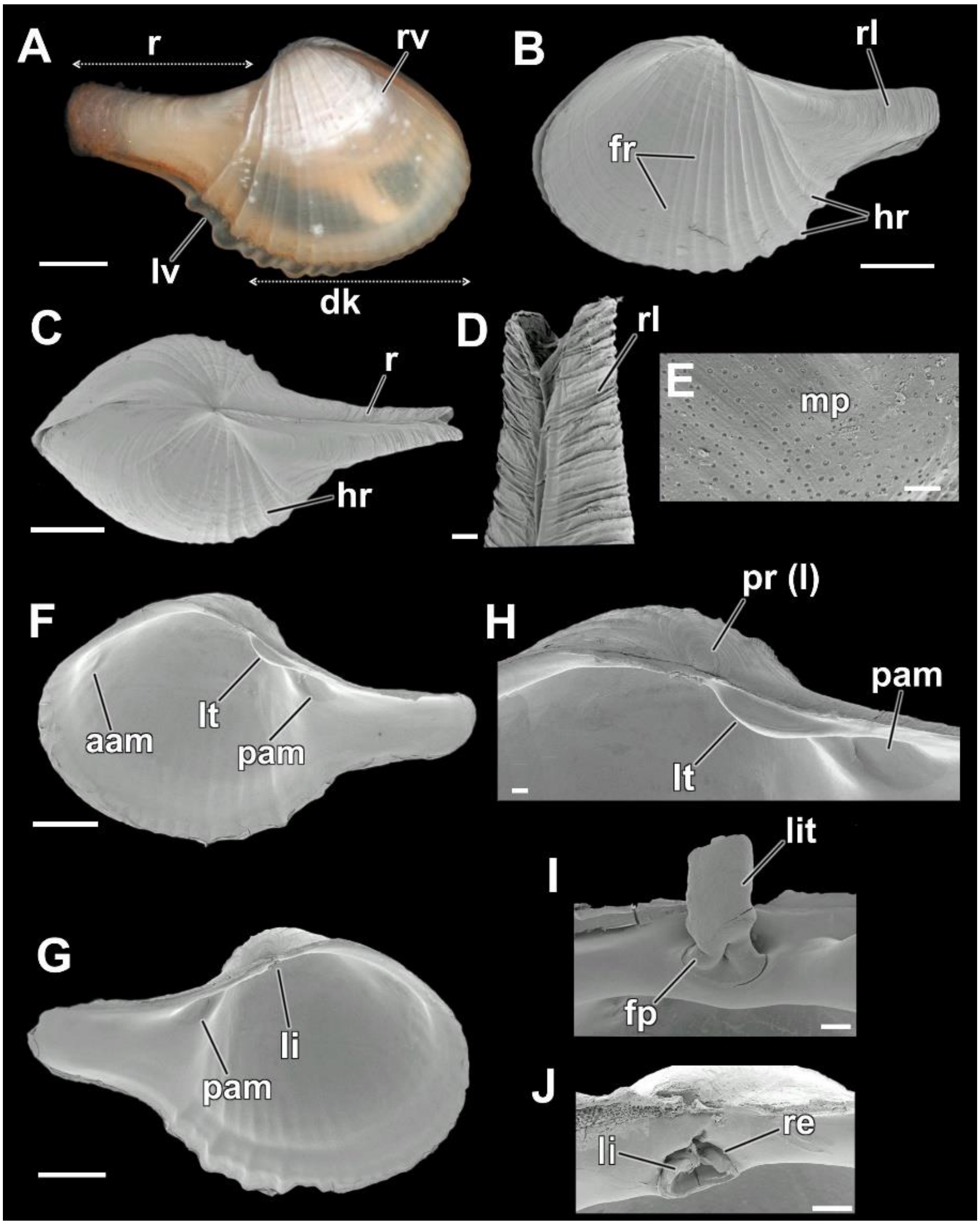

Fig. 3. *Cardiomya cleryana*, details of shell. (A) photomicrograph of a living specimen (right view) with the limits of disk and rostrum, showing the overlap between the valves – ZUEC-BIV 5132; (B-J) SEM views – ZUEC-BIV 5119, 5120; (B) external view of the left valve, showing the disk decorated with fine and heavy radial ribs and the rostrum with fine radial lines; (C) dorsal view; (D) higher magnification of rostrum with radial lines; (E) magnification of dissoconch, near its limits with the prodissoconch, showing micropits; (F) inner view of right valve, showing the

posterior lateral tooth and the adductor muscle scars; (G) inner view of left valve, with ligament bellow the umbo; (H) magnification of the right hinge plate, showing the prodissoconch I, posterior lateral tooth and adductor muscle scar; (I) ligament, with its calcified (lithodesma) and fibrous parts; (J) resilifer with a part of the ligament. (See the list of abbreviations). Scale bars: A-C, F, G, 1mm; D, H, I, J, 100 µm; E, 10 µm.

The siphons

Posteriorly, the siphonal apparatus (sa) comprises two siphons, inhalant (is) and exhalant (es), surrounded by small sensory tentacles and encased in a thick and muscular siphonal sheath (ss) (Figure 4A, B & D). The siphons are formed by fusion of the inner folds and inner surfaces of the middle mantle folds (type B of Yonge 1957) and are separate. This separation occurs by means of a specific structure, the inter-siphonal septum, a delicate posterior extension of the muscular septum. This same extension also forms the sphincter at the base of the inhalant siphon, which is dorsally attached to the posterior septal retractor muscle. The inhalant sphincter is important in regulating the water flow and input of prey into the infra-septal chamber. In anesthetized individuals, the sphincter measures ~570 µm in length and 340 µm in diameter.

The siphons are supported by a set of muscle bundles, peripherally traversing the entire length of the siphonal apparatus and terminating near the posterior septal muscle as the siphonal retractor muscles. The inhalant siphon is large and possesses four finger-shaped sensory tentacles (it) arising from its ventral and lateral base. The exhalant siphon is about one third smaller in diameter than the inhalant, with three tentacles arising from its latero-dorsal base. Also identified are two projections attached to the free edge between the exhalant siphonal tentacles (et). These inter-tentacular projections (ip) are formed by three small papillae resembling siphonal tentacles still in the process of formation (Figure 4B, C, D). Similar structures have been observed previously in some species of *Bathyneaera* (Cuspidariidae) (Krylova 1993).

Using SEM, the opening at the tip (to) of each siphonal tentacles comprises a pit containg a bundle of cilia (ci) (Figure 4F). The seven siphonal tentacles are similar in shape and size and measure about 100 µm in length. In living individuals, the tentacles are pigmented with small white spots. Using SEM, it is possible to observe the presence of micro-papillae (tp) on the external surfaces of the siphonal tentacles these being larger and more prominent on the lateral and ventral faces (Figure 4E). Such micro-papillae were not observed on the surfaces of the inter-tentacular projections.

In cross section, the siphons are formed by five tissue layers: (i) an outer epithelium characterized by the presence of cylindrical cells; (ii) an arenophilic layer with numerous arenophilic glands; (iii) a layer of circular muscles; (iv) an innermost layer of longitudinal muscles overlying; and (v) the internal squamous epithelium. The arenohilic glands (ag) characterized by a core (cg) and a duct (gd) that in living individuals extends outwards forming a small U-shaped papilla (Figure 4G, H). In fixed individuals these glandular papillae and their ducts are inverted (Oliveira & Sartori 2013, figure 3). Using SEM, the glandular papillae can be observed easily on the surface of the siphons and are cylindrical in shape sometimes with a constriction apically (Figure 4E).

The siphonal sheath that surrounds the siphons is a tubular extension of the outer fold of left and right mantle lobes that extend from the posterior end of the septum into the rostrum. In living individuals, the most posterior portion of the sheath is pigmented with small white spots as in the inhalant siphon and its tentacles. Anteriorly, the sheath is light reddish pigmented.

The mantle

The mantle of *Cardiomya cleryana* is thin, transparent and formed by three marginal folds (inner, middle and outer), which are antero-ventrally unfused forming a large pedal gape occupying approximately one-third the length of the ventral margin. A single mantle fusion occurs in the middle of the ventral margin and is formed by union of the inner folds and the inner surfaces of the middle folds, that is, type B fusion of Yonge (1948, 1982). Ciliated rejection tracts were not observed here. Except for the pedal gape, therefore, there are no unfused mantle areas, that is, there is no 4th pallial aperture, as in some anomalodesmatans (Morton 1981c).

A more detailed cross-section of the ventral mantle margin of *Cardiomya cleryana* is illustrated in Figure 5. Here, each lobe comprises an elongated inner fold (imf), a reduced middle fold (mmf) and a large outer fold (omf) – the latter containing a large haemocoelic space (ha). From the periostracal groove (pg) between the latter two folds arises an exceedingly thin periostracum (p). A well developed pallial retractor muscle (prm) is present and connects the mantle margin with the shell. Two different glandular regions are present in the ventral mantle margin along the entire length of the pedal gape. The first, mantle margin gland I (mmg I), is located dorsally and parallels the fibres of the pallial retractor musculature. Well developed, this gland stains dark blue and is thus basophilic. The second region, mantle margin gland II (mmg II) stains light blue and is located internally between inner and middle mantle folds. Both glands may be mucus secreting. In life, a large amount of mucus was observed around the region of the pedal gape during the re-burrowing process and may prevent sediment entering the infra-septal chamber *via* this large aperture.

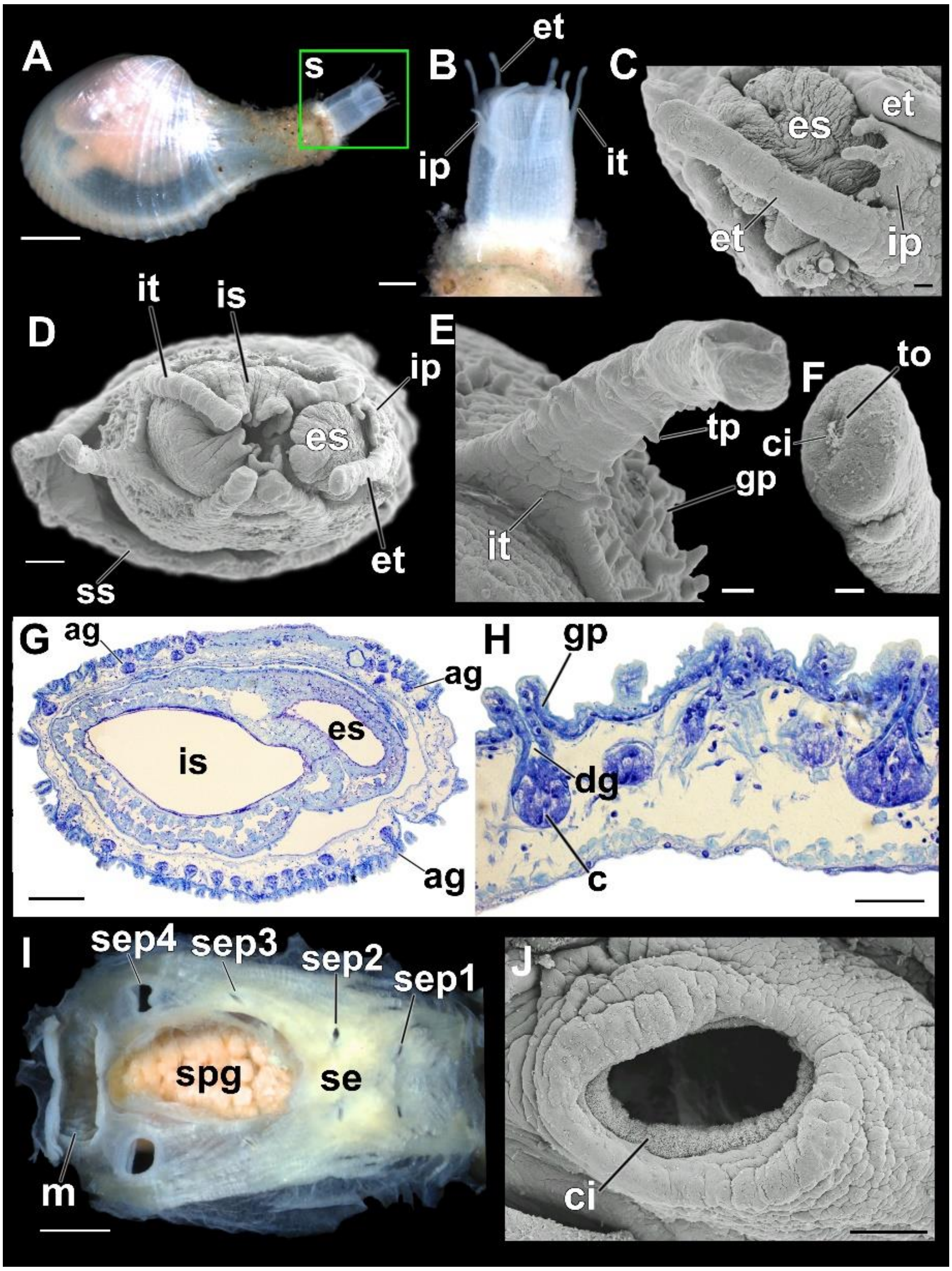

Fig. 4. *Cardiomya cleryana*, details of anatomy. (A) photomicrograph of a living specimen with the siphons protruded; (B) magnification of siphons, showing the seven siphonal tentacles (4 in the inhalant and 3 in the exhalant) and one set of inter-tentacular projections between the exhalant siphonal tentacles. (C-F) SEM views of siphons – ZUEC-BIV 5123, 5124. (C) an exhalant inter-

tentacular projection, formed by three small papillae; (D) frontal view of the siphonal apparatus with inhalant and exhalant siphons, showing the siphonal sheath; (E) siphonal tentacle, with micro-papillae in the surface and glandular papillae in the surface of siphons; (F) apical ciliated tip, showing cilia inside. (G-H) histological sections – ZUEC-BIV 5139. (G) transverse section of siphons, showing the arenophilic glands; (H) sagittal section of arenophilic layer, showing the glandular papillae, duct and core; (I) dissected specimen – ZUEC-BIV 5127, showing the septum with four pairs of pores, mouth and septal pedal opening; (J) higher magnification of one septal pore, with internal ring of cilia. (See the list of abbreviations). Scale bars: A, 1 mm; B, 200 µm; C, 30 µm; D, J, 100 µm; E, F, 10 µm; G, 200 µm; H, 50 µm; I, 500 µm.

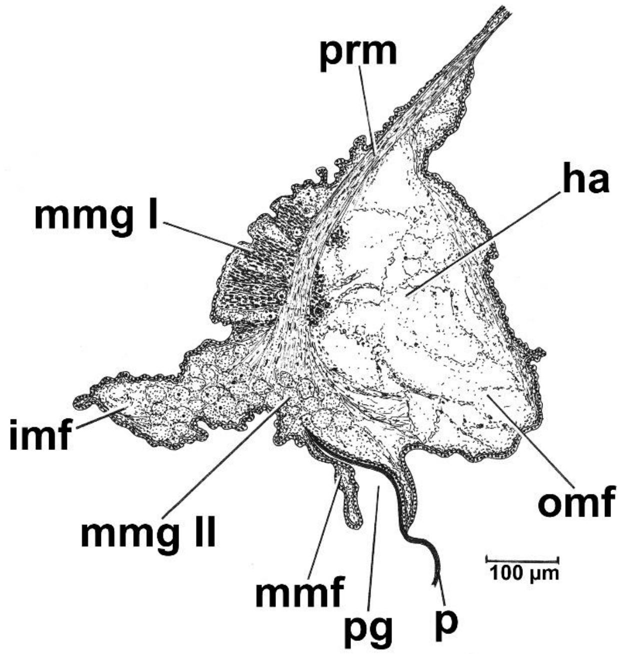

Fig. 5. *Cardiomya cleryana.* Transverse section through right mantle margin. (See the list of abbreviations).

The septum

A horizontal septum (se) is present in *Cardiomya cleryana* dividing the mantle cavity into infra and supraseptal chambers. The infraseptal chamber is capacious (Figure 6). The septum is long and wide, ~2.5 to 3.5 mm in length and 400-750 µm in width, and comprises longitudinal muscle bands. It is suspended in the mantle cavity by robust posterior and anterior septal retractor muscles (psm/asm) that are attached to the shell valves just above their respective adductor muscles and lie close to the minute pedal retractor muscles (pprm/arpm) (Figure 6). A pair of narrow inner longitudinal septal muscles are also present lateral to the pores and septal pedal gape (spg), and attach close to the anterior septal retractor muscle (Figure 6). Lateral septal retractor muscles are also present being more concentrated posteriorly. This lateral septal retractor musculature is formed by finely separated bundles of delicate muscles that extend tangentially from the posterior septal retractor muscles and to the left and right shell valves dorsal to the visceral mass.

In *Cardiomya cleryana* there is also an extra lateral septal retractor muscle attached close to the anterior end of the siphonal retractor muscles, as reported for some species of *Cardiomya*, *Cuspidaria* and *Myonera* by Allen & Morgan (1981) and Morton 2015). Left and right halves of the septum are united by the septal membrane (sm), which separates anteriorly to create the siphonal gape (~1.5 mm in length) through which the foot can protrude. Unlike in *Grippina coronata* Machado & Passos, 2015 (Spheniopsidae) (Morton *et al*. 2015) no cilia were observed along the margin of this membrane. The septum is perforated ventrally by four pairs of pores (sep 1-4) more or less equally spaced and measuring about 250 µm in diameter (Figure 4I). Inside these pores are small internal lips (100-150 µm) similar to those observed in *Cuspidaria parva* Verril & Bush, 1898 by Allen & Morgan (1981, figure 17). These lightly muscular lips are ciliated dorsally forming a ring. A ring of such cilia (ci) is illustrated in Figure 4J.

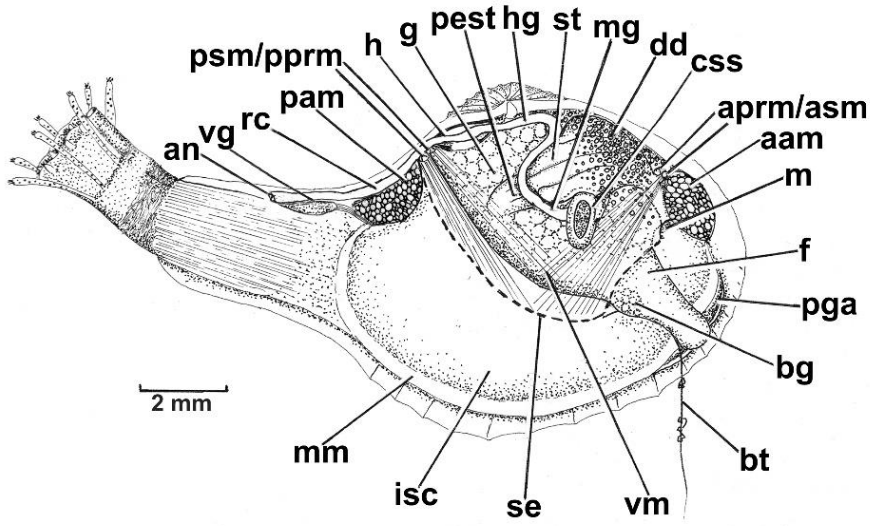

Fig. 6. *Cardiomya cleryana*. An adult individual as seen from the right side, with the right shell valve and mantle lobe removed to illustrate the organs of the mantle cavity and visceral mass. (See the list of abbreviations).

The foot and byssus

The visceral mass is situated above the septum and is continuous with an anterior foot that extends through a septal gape in the septum anteriorly and can also be extended outwards from the similarly anterior pedal gape (pga) in the mantle margin (mm) to effect burrowing. The foot (f) is large and during the digging process can reach a length that almost equals the length of the shell. On average, living individuals each had a foot about

4-5 mm in length (Figures 7E). In fixed individuals, the foot is contracted (0.5–1.5 mm), anteriorly pointed with a narrow sole and a defined heel. In SEM, cilia can be seen to cover the ventral surface of the foot. Ventrally too, the sole has a deep pedal groove (peg) extending from the end of the heel to almost the tip of the foot (Figure 7F, G). No cilia occur on the dorsal surface of the foot.

The foot of *Cardiomya cleryana* is invaded by two pairs of pedal retractor muscles. These are a shorter anterior pair attached to the shell valves above the anterior adductor muscle. The posterior pedal retractor muscles are long, bifurcated at two points, and attach to the shell close to the posterior adductor muscle. The first bifurcation occurs in the middle region of the muscles fusing with the visceral mass. The second bifurcation occurs before the attachment to the shell valves, creating two small insertion points. A pair of short, fine, muscle bundles from the visceral mass usually attach to the gonads and merge with the posterior pedal retractor muscles just above the first bifurcation.

Internally, through sagittal and parasagittal sections it is possible to see the byssal gland (bg) that is formed by two glandular regions. The first of these is larger (bg I), wide and well developed throughout the length of the foot. The second is smaller (bg II) and restricted to the anterior region, close to the tip of the foot (Figure 7H). Posteriorly, the developed glandular region (bg I) opens *via* about seventeen small ducts which converge and secrete a single long byssal thread (bt) (Figure 7H). In almost all individuals such a transparent thread was apparent and which in some could measure up to 20 mm in length, that is, three to four times the length of shell (Figures 6, 8 & 9C). This long byssal thread demonstrates that the foot must extend to an equal length so that it can plant it within the sediment (Figure 8) thereby securely anchoring each individual. During planting of the thread, the foot also twists to facilitate the necessary sediment probing action (Figure 8, f (tw)).

The alimentary system

The alimentary system of *C. cleryana,* as seen from the right side, is illustrated in Figure 6. Anteriorly, the septum ends at a large mouth (m) (~800 µm in diameter), which, in turn, has lateral muscular extensions forming the walls of the buccal funnel (Figures 9A & B). Small labial palps are present. The anterior palps (ap) are narrow, horn-shaped and measure ~400 µm in length. The posterior palps (pp) are reduced, flattened (~300 µm in length) and are located along the postero-lateral margins of the mouth (Figure 9A, B). The palps are ciliated and similar in shape to type I described by Allen & Morgan (1981, figure 7). From the mouth a short, thick and muscular oesophagus (o) (~340 µm in diameter) opens into the anterior-dorsal part of the elongated-oval stomach (st) (Figure 9A, C). The stomach (type II of Purchon, 1956) is large (1.5 to 2.1 mm in length), thick, with a well defined posterior extension (pest) and a reduced crystalline style sac (css) (Figure 6). Laterally, the stomach is lined by an epithelium that secretes an internal thick layer of cuticle, the gastric shield (gs) (Figure 9A). Ventral and antero-ventral portions of the stomach are laterally covered by the digestive diverticulae (dd) while the posteroventral region is laterally covered by the gonads (g/te) (Figure 9A). The dorsal area of the stomach is not covered. In immature individuals, the stomach is surrounded by the digestive diverticulae.

In all individuals of *C. cleryana* examined, the stomach always contained ingested prey (ipi), either whole or partially digested (Figure 9A). In section, the sizes of these prey items ranged from 150 to 250 µm in length and most resembled small copepods (Crustacea) (Figure 9A). Sorting areas were also observed on the stomach floor. From the right side of the stomach floor arises the small crystalline style sac (css) (~350 µm in length) and the mid gut (mg), close to each other but separated other than at their origins with the stomach (Figures 6 & 9C), similar to *C. knudseni* (Allen & Morgan 1981, figure 32). The style-sac of *C. cleryana* is a short oval-circular cavity (~400 µm in diameter) inside which is a cylindrical style (~150-200 µm in diameter) (Figure 6 & 9C). Close to the style-sac opening into the stomach are two typhlosoles, one smaller the other larger.

The mid gut (mg) begins on the right side of the stomach floor, extends ventrally from the base of the style-sac and travels dorsally along the border of the right lateral wall of the stomach. Here it curves posteriorly and then its lumen expands and eventually gives rise to the hind gut (hg) (Figures 6 & 9C). In sagittal section, the mid gut is large and contains many skeletal remains of digested prey (ipi) (Figure 9A). Within the visceral mass, the digestive diverticulae (dd) are located anterodorsally. In living individuals, the rectum (rc), anus (an) and visceral ganglia (vg) are displaced posterior to the posterior adductor muscle when the siphons are extended (Figure 6).

The hind gut (hg) is straight, long and narrow eventually giving rise to the rectum (rc). Smaller fragments of prey skeletons occur inside the hind gut. The hind gut penetrates the pericardial cavity/heart (pc/h) traversing the ventricle of the heart from front to back (Figures 6 & 9A). In living individuals, with siphons extended, the rectum and the anus (an) are displaced by about 1 mm from the posterior end of the posterior adductor muscle (Figures 6 & 9D). The anus opens between the visceral ganglia (vg), releasing the faeces into the exhalant chamber and siphon.

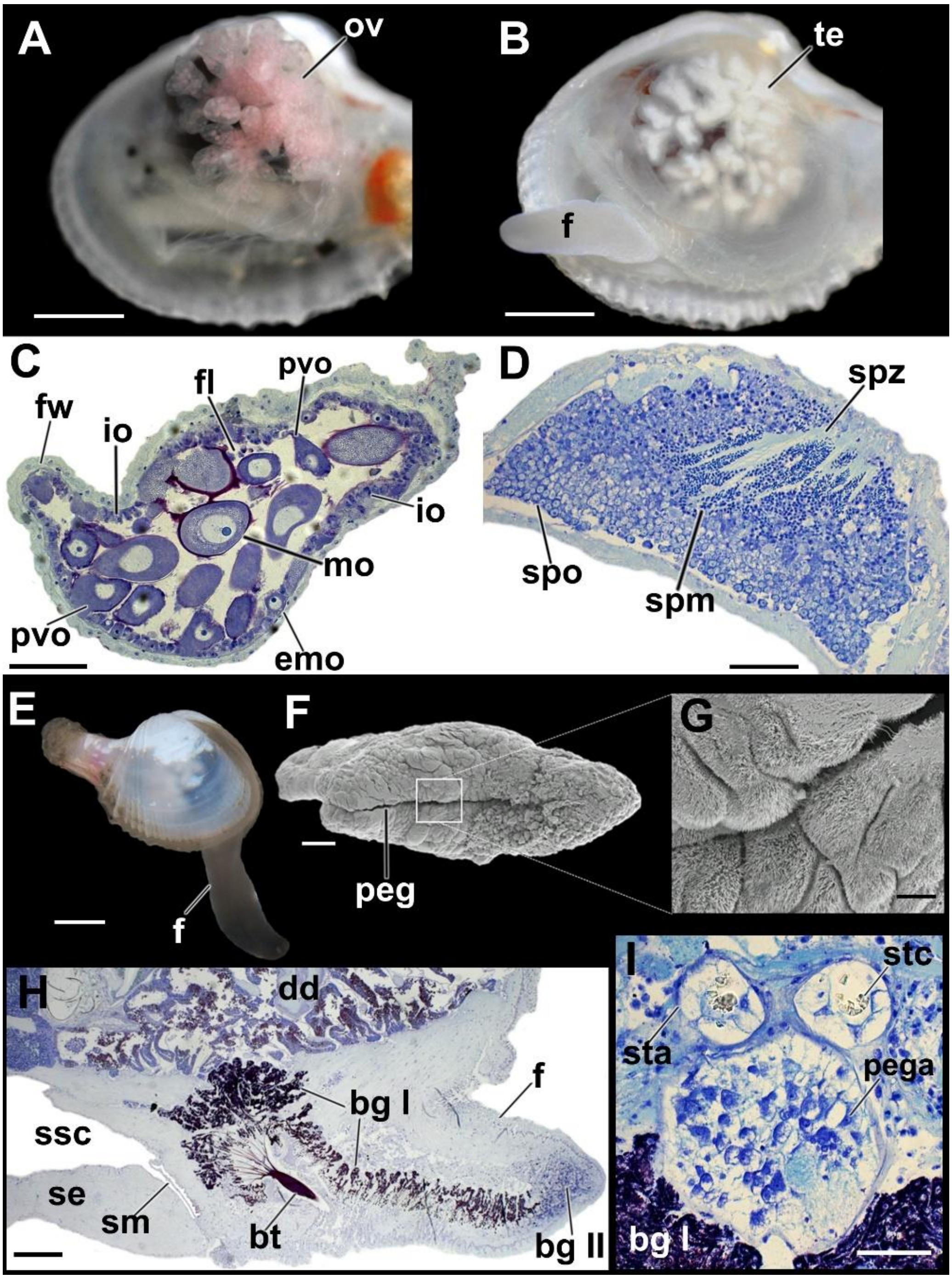

Fig. 7. *Cardiomya cleryana* details of anatomy. (A-B) photomicrographs of two living specimens. (A) a female, showing pink ovaries; (B) a male with whitish testes; (C) sagittal section in a single ovarian follicle, showing the follicular wall, lumen and oocytes in different stages of maturity; (D) transverse section of testes, showing spermatogonia, spermatocyte and spermatozoa; (E) living specimen with the protruded extensible foot. (F-G) SEM images – ZUEC-BIV 5127. (F) ventral view of foot showing the pedal groove; (G) higher magnification of the pedal groove,

showing the cilia. (H-I) histological sections of the foot – ZUEC-BIV 5139. (H) sagittal section showing dorsally the digestive diverticulae, septum, supraseptal chamber, septum membrane, byssal gland with the two glandular regions and the formation of a single byssal thread; (I) transverse section, showing the byssal gland region I, pedal ganglia and statocysts with small, irregular and cristal-like statoconia inside. (See the list of abbreviations). Scale bars: A, B, E, 1 mm; C, 100 µm; D, 50 µm; F, H, 200 µm; G, 20 µm; I, 50 µm.

The reproductive system

All sectioned specimens were either males or females, none was obviously hermaphroditic, and *C. cleryana* is, thus, possibly dioecious. However, only three individuals were sectioned and the smallest of these (5.2 mm shell length) was a male. The other two larger individuals (5.5 and 6.3 mm shell length) were both females, raising the possibility of protandric hermaphroditism as in *C. costellata* (see Morton 2015). Moreover, the sex ratio was biased in favour of males (2:1) and since no individuals of a shell length <5 mm was collected, the expression of sexuality in *C. cleryana* remains speculative.

The gonads, testes (t) and ovaries (ov) of *C. cleryana* are located near the posterolateral and dorsal walls of the stomach and occupy a large part of the visceral mass (vm) (Figure 7A & B). In some cases, the gonads extend around almost all the organs of the visceral mass, and are evaginated as lateral pouches into the supraseptal chamber. The gonads of *C. cleryana* are formed by several follicular lobes. These lobes each has a small duct that connects to a wider one, the gonoduct. This opens into the posterodorsal region of the supraseptal chamber. The follicular lobes vary in size and shape depending on the individual. In living individuals, males and females can be identified by the shape and colour of their follicular lobes. The testes are usually thinner (0.1–0.4 mm) and whitish whereas the ovarian lobes are thicker (0.7–1.0 mm) with a transparent outer wall and containing small pink spheres - the oocytes (Figure 7A & B).

Although less common than males, it was possible to observe female gametogenesis, which in *C. cleryana* comprises oocytes in four stages of maturity: (i) immature oocytes (io) located in the wall of the ovarian follicle are the smallest (5-10 µm in diameter) with an oval to flattened shape. Each follicle may contain 70 to 100 immature oocytes; (ii) early maturing oocytes (emo) are also located in the walls of follicles (fw) close to the immature cells but are larger (20-30 µm in diameter) with a rounded shape and a large central nucleus; (iii) pre-vitellogenic, teardrop shaped, oocytes (pvo) (50-110 µm in diameter) are the most common cells in mature individuals and are always attached to the follicular wall *via* a stalk. Within the follicles are many; (iv) mature oocytes (mo) (80-150 µm in diameter), which are characterized by a large amount of yolk displacing the nucleus to the cell periphery. This cell type was not attached to the follicle wall and was free in the lumen (Figure 7C). The testes could be differentiated into spermatogonia (spo), spermatocytes (spm) and spermatozoa (spz) (Figure 7D).

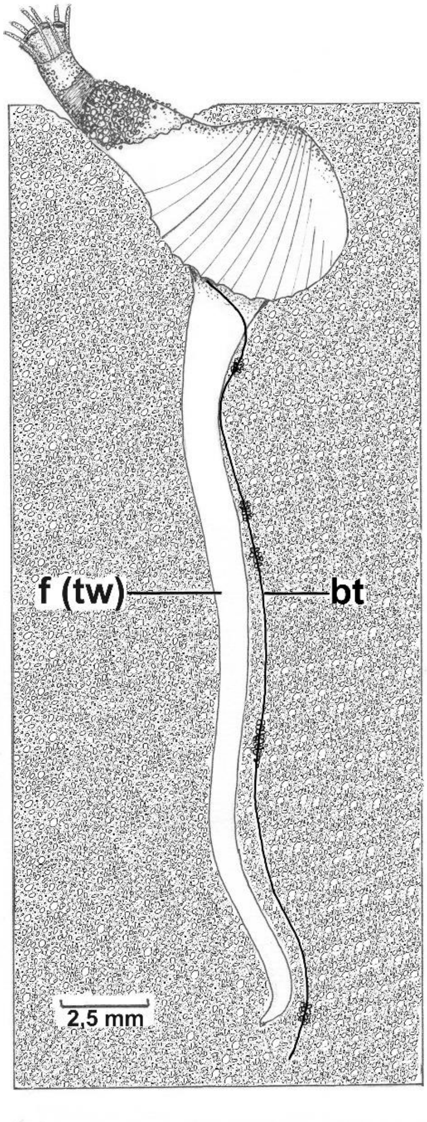

Fig. 8. *Cardiomya cleryana*. An adult individual in its natural position in the sediment, with the elongated foot and the anchoring byssal thread. (See the list of abbreviations).

The nervous system

The nervous system of *C. cleryana* is similar to that described for most species of Cuspidariidae, with three pairs of ganglia: the cerebropleural, pedal and visceral. The cerebropleural (cg) pair is the largest with each ganglion measuring approximately ~80 µm in diameter. These are located close to the anterior adductor muscle and dorso-laterally to the mouth. This pair is formed by elaborated nerve connections and each ganglion is formed by six branches: (i) a large commissure nerve (cn) (~800 µm in length) linking the pair; (ii) two divergent nerves (~350 µm in length), a labial nerve (ln) and a buccal nerve (bn) innervating the anterior labial palps and mouth; (iii) the dorsal adductor muscle nerve (an), inserted ventrally in the anterior adductor muscle. In *C. cleryana* the anterior adductor muscle is divided somewhat and has a thin groove in the middle; (iv) a muscular nerve (mmn), innervating the anterior septal and pedal retractor muscles with some of these nerve bundles also extending towards the mantle margin, and a visceral nerve (vn) extending into the visceral mass (Figure 9E).

The pedal ganglia (pega) (diameter ~150 µm) are located in the dorsal region of the foot near the ventral margin of the visceral mass and anterior to the byssal glands. Closely applied to the dorsal surface of the pedal ganglia is a pair of separate statocysts (sta) (~60 µm in diameter) (Figures 7I & 10). The statocysts comprise a small capsule of but six or eight cells in transverse section and inside each capsule are numerous crystal-like statoconia (stc). The number (20-30) and size (10 to 15 µm in diameter) of these statoconia differ in each capsule. There is no statolith. The statocysts of *C. cleryana* resemble statocyst types B and C described by Morton (1985b, figures 3a, b). A similar situation was also observed for *Spheniopsis brasiliensis* Machado & Passos, 2016 (Spheniopsidae) (Morton *et al*. 2015b).

When the siphons are contracted the visceral ganglia (vg) (~100 µm in diameter) of *C. cleryana* are situated beneath the posterior adductor muscle. In individuals with relaxed siphons, the ganglia are displaced by about 1 mm behind the posterior adductor muscle (Figures 6 & 9D). In both positions, the visceral ganglia are situated close to the anus and the base of the exhalant siphon. The same situation also applies to the rectum and the anus of *C. cleryana*, linked as they are to the visceral ganglia (Figures 6 & 9D). Between five to seven nerve bundles (cnb) pass from the ganglia into the visceral mass and foot and the ventral margin of the posterior adductor muscle and rectum (Figure 9D). Small nerve fibres also innervate the siphons.

DISCUSSION

Anatomical remarks

According to Poutiers & Bernard (1995), Coan *et al*. (2000) and Coan & Valentich-Scott (2012) the genus *Cardiomya* possesses only two diagnostic anatomical features, that is: a septum perforated by four pairs of pores, and a small lateral septal retractor muscle attached close to the anterior end of the siphonal retractor muscles. The second feature is not, however, unique to this genus and has been described for species of *Bathyneaera* such as *B. hadalis* (Knudsen, 1970) by Allen & Morgan (1981, figure 34). Among the 30 individuals of *C. cleryana* herein examined, all possessed the two diagnostic features identified above, although as described other important behavioural and morphological characters have also been identified and will be discussed below.

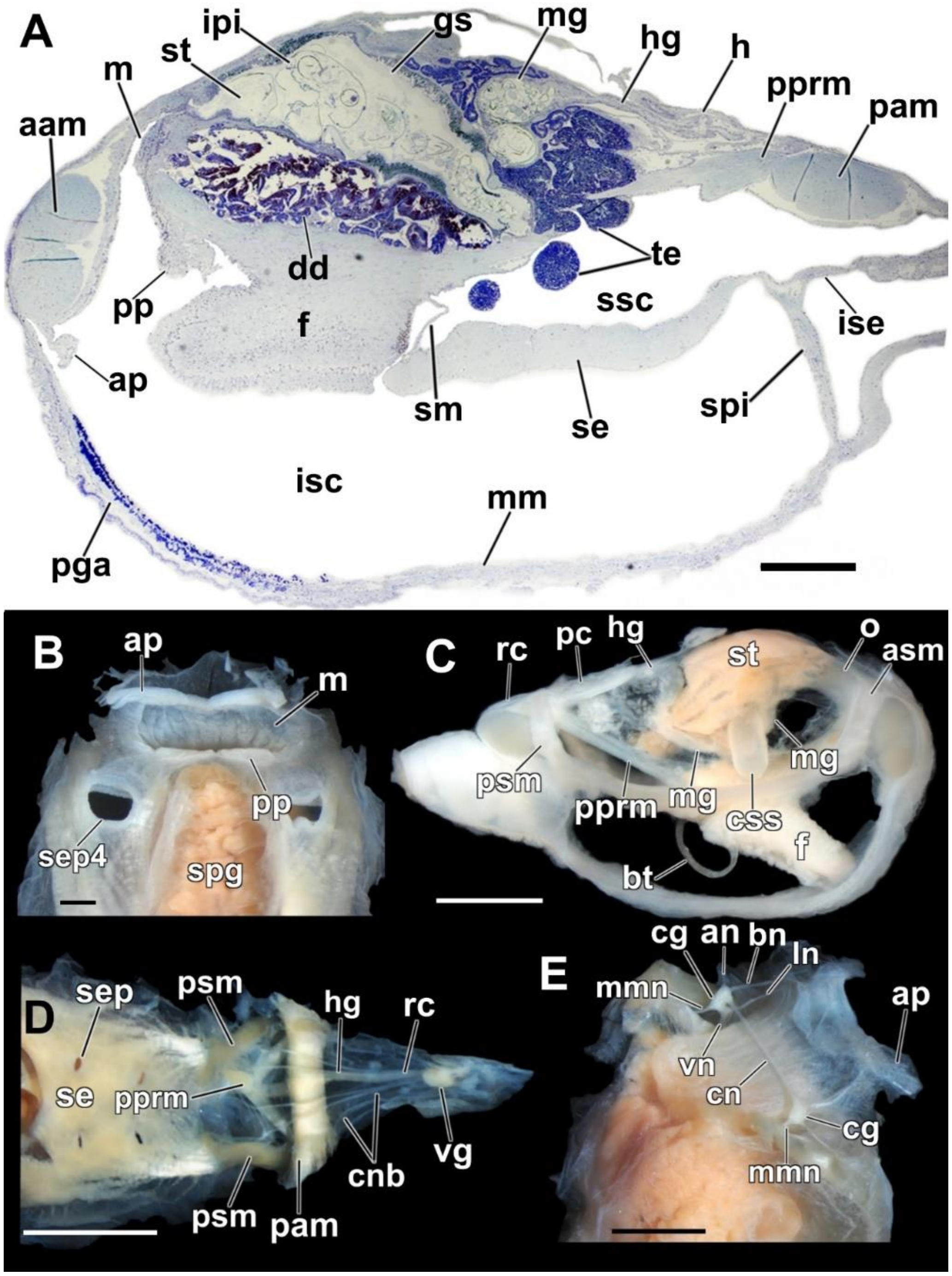

Fig. 9. *Cardiomya cleryana* details of anatomy. (A) sagittal section from the left side without siphons, showing details of organs of pallial cavity and visceral mass – ZUEC-BIV 5139. (B-E) photomicrographs made from dissected specimens – ZUEC-BIV 5127, 5129. (B) anteroventral view showing the mouth with labial palps, septal pores and septal pedal opening; (C) right view showing some organs of pallial cavity and visceral mass; (D) posteroventral view showing the posterior septal muscle, retractor pedal muscle bifurcated before attachment in the shell, posterior adductor muscle, connectives nerve bundles, hind gut, rectum and visceral ganglia; (E) dorsal view of the buccal funnel after removal of the anterior adductor muscle, showing the

cerebropleural ganglia and its branches. (See the list of abbreviations). Scale bars: A, E, 500 µm B, 200 µm; C, D, 1000 µm.

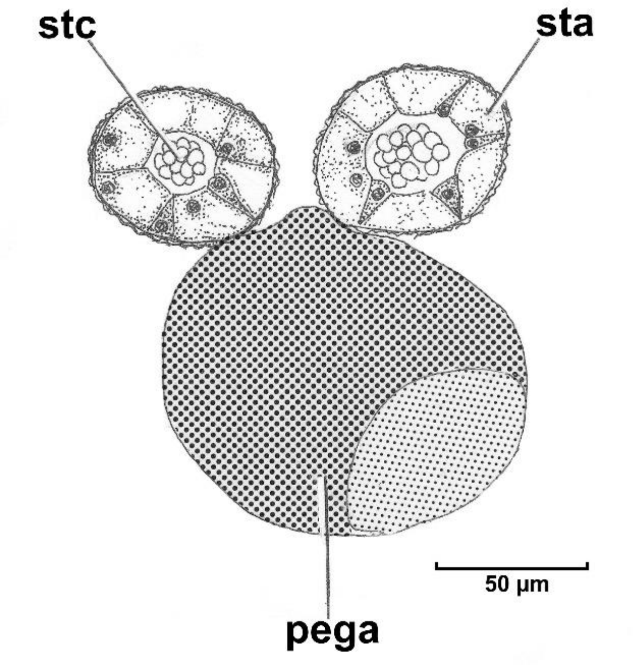

Fig. 10. *Cardiomya cleryana*. A transverse section through the pedal ganglia and paired statocysts at the base of the foot. (See the list of abbreviations)

Life orientation

Few species of the Cuspidariidae, have ever been examined alive with information about the behaviour of this carnivorous family restricted to the work of Yonge (1928) on *Cuspidaria rostrata* and *Cuspidaria cuspidata*, Reid & Reid (1974) on *C. rostrata* and *Cuspidaria obesa*, Reid & Crosby (1980) on *Cardiomya planetica* and Allen & Morgan (1981) on *C. cuspidata*.

This study examined ten living individuals of *Cardiomya cleryana.* The main difference found between *C. cleryana* and other Cuspidariidae species was the life orientation adopted by this bivalve in the sediment. Morton (1987, figure 13) postulated, based on the illustrations of *Cardiomya planetica* by Reid and Reid (1974), that members of the Cuspidariidae adopt a position approximately perpendicular to the sediment surface. The orientation adopted by *C. cleryana* in the sediment, however, is ~45° degrees to the sediment surface (Figures 2 & 8).

The siphons

The siphons of *Cardiomya cleryana* are similar to those described by Yonge (1928), Reid & Reid (1974), Allen & Morgan (1981) and Morton (2015) for *Cuspidaria rostrata, Cuspidaria cuspidata, Cuspidaria obesa* and *Cardiomya costellata,* respectively. The presence of finger-shaped siphonal tentacles with micro papillae on the surface, each tipped with a pit occupied by sensory cilia, as well as two sets of inter-tentacular projections between the exhalant siphonal tentacles, differentiates *C. cleryana* from the above species. The siphonal tentacles of *Cuspidata cuspidata* and *Cuspidaria obesa* are club-shaped at their tips, whereas in *Cuspidaria rostrata* they are more elaborately expanded at the tips into foliate structures each of which has two notches (Reid & Reid, 1974, figure 2), and are thus different from the rounded structures seen in *C. cleryana* (Figure 4E & F).

The presence of siphonal tentacles (=siphonal papillae) with a ciliated apical pit have also been observed in *Cardiomya planetica* (Cuspidariidae) by Reid & Crosby (1980, figure 5); *Multitentacula venusta* Krylova, 1995 (Protocuspidariidae) Krylova (1995, figure 3D); *Grippina coronata* and *Spheniopsis brasiliensis* (Spheniopsidae) Morton *et al*. (2015a, b) and more recently in *C. costellata* (Morton 2015, figures 6-7). According to Reid & Crosby (1980) and Morton *et al.* (2015a) these apical cilia are used as mechanoreceptors of the vicinal movements of potential prey. The same function may also be attributed to those of *C. cleryana*.

The colour pattern of the siphons of *Cardiomya cleryana* (orange red siphonal sheath and small white spots on the surface of the inhalant siphon and siphonal tentacles) has also been observed in other anomalodesmatans including *Lyonsia californica* Conrad, 1837 (black spots on both siphons and tentacles), *Pandora filosa* (brownish pigment spots on both siphons and tentacles), *Cuspidaria cuspidata* (scarlet pigments on the inhalant siphon and its tentacles) and both *C. obesa* and *Cardiomya costellata* (siphons coloured red in life) by Narchi (1968, figure 2), Thomas (1994, figure 3), Allen & Morgan (1981, p. 529), Yonge (1928) and Martins *et al.* (2009), respectively. The red end of the spectrum is lost quickly with depth so that it appears black, and is thus characteristic of many deeper water species. It is thus not unusual to see it in deeper water cuspidariids and their relatives.

The relationship with *Bathyneaera*

Similar structures to the exhalant inter-tentacular projections observed in *Cardiomya cleryana* have been reported previously in the literature by Knudsen (1970) and Reid & Crosby (1980) for *Myonera mexicana* Knudsen, 1970 and *Cardiomya planetica* (Dall, 1908), respectively, and thereafter by Allen & Morgan (1981, figure 34b) and Krylova (1993, figures 1, 2) for species of *Myonera* and *Bathyaneaera*, again respectively. According to Knudsen (1970) and Allen & Morgan (1981), *M. mexicana*, *Myonera demistriata* (Allen & Morgan, 1981) (=*Bathyaneaera hadalis* Knudsen, 1970) and *Myonera garretti* Dall, 1908 have an '*inter-tentacular tuberculous web*' along the free edge of the exhalant tentacles. Reid & Crosby (1980, figure 3b) also observed '*minor tentacles arising between the three exhalant siphonal tentacles and exhalant siphon*' in

C. planetica. Later, Krylova (1993) observed this same feature, now called '*projections between the dorsal exhalant tentacles*' in *Bathyaneaera hadalis, B. tillamookensis* (Dall, 1916), *B. paleifera* Krylova, 1993, *B. disa* (Bernard, 1989) and *B. bernardi* Krylova, 1993. Subsequently, this feature has been included in the diagnosis for species of *Bathyaneaera*. The presence of these inter-tentacular projections in *C. cleryana* further adds to the arguments for a close relationship between *Cardiomya* and *Bathyaneaera* corroborating the initial observations made by Scarlato & Storobogatov (1983) and Krylova (1993).

The function of these inter-tentacular projections remains uncertain however. Histological examination of *Cardiomya cleryana* suggests that the origin of the small papillae that form these projections is the same as that of the siphonal tentacles, that is, they originate on the middle (typically sensory) mantle fold. In addition, observations on living individuals also showed that these projections assist in closing the exhalant opening when the siphons are retracted. It is therefore suggested that the inter-tentacular projections function as: extra sensory structures assisting the siphonal tentacles in detecting potential prey; replacing damaged siphonal tentacles; and assist in the closing of the exhalant opening preventing unwanted particles from entering the supraseptal chamber.

In addition, the shape of the *Cardiomya cleryana* stomach is similar to the description given by Temkin & Strong (2013) of that of *Bathyaneaera demistriata*. In both species, an expansion of the stomach wall is evident. This expansion is only to the posterior region of the stomach in *C. cleryana* that is fused to the posterior pedal retractor muscle whereas in *B. demistriata* the stomach is anteroposteriorly expanded.

According to Krylova (1993), the main differences between *Cardiomya* and *Bathyaneaera* include: (i) occurrence depth - *Cardiomya* 0-4,000 m, *Bathyanearea* 439-8,430 m; (ii) the absence of a lateral tooth in the hinge plate and (iii) a reduced rostrum in *Bathyanearea* species. In addition, (iv) the tips of the siphonal tentacles of *C. cleryana* but not *Bathyaneaera* spp have an apical ciliated pit and micro-papillae on their surface (this study).

The foot and byssal thread

Other new morphological features concerning *Cardiomya cleryana* and reported upon in this study include the presence of a long byssal thread and a greatly extensible foot (Figures 7E & 8). Although byssal threads have been reported upon for the representatives of a number of anomalodesmatan families such as the Lyonsiidae (*Entodesma saxicola* (Baird, 1863) =*E. navicula* (A. Adams & Reeve, 1850)), Lyonsiellidae (*Lyonsiella horrida* Allen & Turner, 1974, *Lyonsiella frielei* Allen & Turner, 1974 and *Policordia jeffreysi* (Friele, 1879)), Verticordiidae (*Verticordia triangularis* Locard, 1898, *Verticordia quadrata* Smith, 1885 and *Spinosipella deshayesiana* (P. Fisher, 1862)) and, more recently, the Poromyidae (*Dillema frumarkernorum* Leal, 2008 and *Dillema spectralis* Leal, 2008) (Yonge 1952; Allen & Turner 1974; Morgan & Allen 1976; Leal 2008; Simone & Cunha 2008; Simone 2009) such a long thread seen in *C. cleryana* has never been reported upon before for any anomalodesmatan.

More specifically, with regard to the Cuspidariidae, Allen & Morgan (1981, p. 440) reported that '*Threads have been seen in only two species*' but did not identify which taxa nor illustrate them. Krylova (1993) reported upon the presence of a byssal thread in a single individual of *Bathyaneaera tillamokensis* (Cuspidariidae) but also did not either describe or illustrate this feature. The byssal thread identified in *Cardiomya cleryana* seems to be an important adaptation to survival in an area of the sea bed where there are strong sea bed currents (Dottori *et al*. 2015). In small Araçá Bay, strong currents (South Atlantic Central Water), constantly cause the sediment to be displaced from the sea bed and they thereby modify the benthic environment and displace unsecurely anchored species. The long byssal thread thus favours survival in such a disturbed habitat.

A similarly long foot has, however, been described for species of the Mytilidae such as *Modiolarca subpicta* (Cantraine, 1835) (=*Musculus subpictus* Cantraine, 1835) and is similarly responsible for the planting of byssal threads in the juvenile (Morton & Dinesen, 2011). Another example is provided by *Crenella decussata* (Montagu, 1808) which has a long foot mainly used to construct an adventitious tube within which an individual resides (Morton *et al*. 2015c). Some representatives of the Lucinoidea such as *Diplodonta punctata* (Say, 1822) (Domaneschi 1979), *Lucina pectinata* (Gmelin, 1791) [=*Phacoides pectinatus* (Gmelin, 1791)] (Narchi & Assis 1980), *Anodontia philippiana* (Reeve, 1850) [=*Pegophysema philippiana* (Reeve, 1850)] (Taylor & Glover 2006, fig. 4) also have a long, highly extensible, foot. This is also the case for some thyasirids such as those reported by Payne and Allen (1991), Dufour & Felbeck (2003) and Passos *et al*. (2007).

The statocysts

The nervous system of *Cardiomya cleryana* is similar to that described by Allen & Morgan (1981) for other species of the Cuspidariidae and is formed by three pairs of ganglia: cerebropleural, pedal and visceral. The position of the visceral ganglia in *C. cleryana* is similar to that observed in *Cuspidaria rostrata* by Reid & Reid (1974: figure 3) and *Cardiomya planetica* by Reid & Crosby (1980, figure 4) where the ganglia are situated distant from the posterior adductor muscle (Figures 6 & 9D). They are, however, different from the situation observed in *Cardiomya costellata* by Morton (2015, figure 21A), where the ganglia are located beneath the posterior adductor muscle (but with the siphons retracted).

The only feature of the nervous system observed in *Cardiomya cleryana* that differs from other Cuspidariidae is the morphology of the statocysts. According to Morton (1985b), three types of statocyst occur in representatives of the Anomalodesmata: A, B and C. Type B can, however, be sub-divided into three groups on the basis of differences in statolith structure. In *C. cleryana* the statocyst are closely applied to the dorsal surface of the pedal ganglia with each capsule formed by between 5-6 cells (in transverse section) and with a large number of statoconia in the capsule's cavity (Figures 7I & 10). This is similar in part to Morton's Type B_2 seen in *Parilimya maoria* Dell (1963) (Parilimyiidae) (statocysts closely applied to the pedal ganglia with both a statolith and statoconia inside) and to Type C, exclusive to the Cuspidariidae. This latter type is formed by 4-5 cells, each with little cytoplasm. *Spheniopsis brasiliensis* (Spheniopsidae) also has statocsyts which resemble both Type B_1 and the Type C; although Morton *et al*. (2015b) defined them as Type C. The presence of many crystalline statoconia in *Cardiomya cleryana* suggests that though a sedentary lie-in-wait predator, it is capable of fine orientation within the sediment as demonstrated in this study.

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List of abbreviations used in figures

aam	Anterior adductor muscle
adm	Anterior dorsal margin
ag	Arenophlic gland
an	Anus
ap	Anterior labial palp
aprm	Pedal retractor muscle
asm	Anterior septal muscle
bg	Byssus gland
bg I	Byssal gland type I
bg II	Byssal gland type II
bn	Buccal nerve
bt	Byssal thread
c	Core of the arenophilic gl
cg	Cerebropleural ganglia
ci	Cilia
cn	Comissure nerve
cnb	Connectives nerve bundle
css	Crystalline style sac
dd	Digestive diverticulae
dg	Duct of arenophilic gland
dk	Disk (shell)
es	Exhalant siphon
et	Exhalant tentacle
emo	Early maturing oocyte
f	Foot
fl	Follicular lumen
fp	Fibrous part of ligament
fr	Fine radial ribs
f (tw)	Foot twisted
fw	Follicle wall
g	Gonad
gp	Glandular papillae
gs	Gastric shield
h	Heart
ha	Haemocoel
hg	Hindgut
hr	Heavy radial ribs
imf	Inner mantle fold
ipi	Ingested prey item
io	Immature oocyte
is	Inhalant siphon
ise	Intersiphonal septum
isc	Infra-septal chamber
it	Inhalant tentacle
ip	Intertentacular projection
li	Ligament
lit	Lithodesma

ln	Labial nerve	mm	Mantle margin
lt	Lateral tooth	mmf	Middle mantle fold
lv	Left valve	mmg I	Mantle margin gland I
m	Mouth	mmg II	Mantle margin gland II
mg	Midgut	mmn	Muscular nerve
mo	Mature oocyte	rr	Rostral ridge
mp	Micropits	rv	Right valve
o	Oesophagus	s	Siphons
ov	Ovaries	se	Septum
omf	Outer mantle fold	sm	Septal membrane
p	Periostracum	sep	Septal pore
pc	Pericardial cavity	sep (1)-(4)	Septal pores (1-4)
pg	Periostracal groove	spg	Septal pedal gape
pp	Posterior labial palp	spm	Spermatocyte
pam	Posterior adductor muscle	spo	Spermatogonia
pdm	Posterior dorsal margin	spz	Spermatozoan
peg	Pedal groove	spi	Sphincter inhalant siphon
pega	Pedal ganglia	ss	Siphonal sheath
pest	Extension of stomach	ssc	Supra-septal chamber
pga	Pedal gape	st	Stomach
psm	Posterior septal muscle	sta	Statocyst
pr I	Prodissoconch I	stc	Statoconia
prm	Pallial retractor muscle	te	Testes
pprm	Pedal retractor muscle	tp	Tentacle papillae
pvo	Pre-vitellogenic oocyte	to	Tip of siphonal tentacle
r	Rostrum	u	Umbones
rc	Rectum	vg	Visceral ganglia
re	Resilifer	vn	Visceral nerve
rl	Radial lines	vm	Visceral mass

CAPÍTULO 2

A morphological analysis of the Anomalodesmata (Mollusca, Bivalvia) and a new phylogeny for this eccentric, sometimes bizarre, group

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Abstract

The Anomalodesmata Dall, 1889 comprises a diverse sub-class of the Bivalvia, composed by some of the rarest and most specialized species. This rarity has consequently constituted the greatest obstacle for a comprehensive understanding of the internal relationships of this group due to the low representativeness of species present in any phylogenetic reconstructions. Herein, therefore, with the primary purpose of creating the first comprehensive morphological analysis of the sub-class, data concerning all the, as currently recognized, anomalodesmatans families were gathered into the same cladistic analysis including, for the first-time, information about members of the Clistoconchidae, Cetoconchidae, Protocuspidariidae and Spheniopsidae. Plus, the identification and description of a new family - the Bentholyonsiidae. Information about shell morphology, anatomy and behaviour of anomalodesmatans were compiled through a review of the literature (from 1895 to 2017) and by a re-analysis of shells and internal tissues of some species deposited in museum collections around the world. More than 190 museum lots have been analysed, 60 papers reviewed and 61 taxa selected. Our sensitivity analysis (equal and implied weighting) suggests a deep division of the Anomalodesmata into four main clades: the first three composed generally by shallow marine water species bringing together 12 families and treated here as non-carnivorous lineages and a carnivorous lineage mainly comprising deep water species (10 families). As a consequence, a new anomalodesmatan phylogenetic tree is presented bringing new insights into the internal relationships of this, sometimes bizarre, sub-class.

Introduction

Different from all other groups, the Anomalodesmata Dall, 1889 stands out in comprising the most rarely encountered and specialized species of the Bivalvia, occurring in virtually all marine habitats and, as a consequence, demonstrating a wide range of morphological adaptations to them (Morton 1981, 1982, 1985a). These morphological features variously include, for example, a dorsal crack in both valves of the shell, a ligamental lithodesma, pallial arenophilic glands, a fourth pallial aperture, a raptorial inhalant siphon, ectopic pallial eyes, taenioid muscles, a ctenidium modified into a muscular septum, an opisthopodium, a post-larval byssus, and an ability to build either calcareous adventitious crypts or tubes. Such features, and many others, have allowed representatives of the Anomalodesmata to explore a great diversity of highly specific ecological niches and providing them with the epithet of 'bizarre' (Morton 1985a; Harper *et al.* 2000).

Knowledge of the above diversity of eccentric morphological features, contained in the descriptions of the functional morphology of many anomalodesmatan taxa, commenced in the 19th century (Smith 1885; Dall 1895; Pelseneer 1891) and continued into the 1900s, driven mainly by important marine expeditions such as the British Antarctic *Terra Nova* Expedition, the Woods Hole Oceanographic Institution's deep-sea benthic programme, the John Murray Expedition and the French expeditions of 1978-1989 among others (Pelseneer 1906, 1911; Burne 1920; Yonge 1928; Knudsen 1967, 1970; Allen & Turner 1974; Allen & Morgan 1981; Krylova 1991, 1993, 1994a, b, 1995; Poutiers & Bernard 1995). Contemporaneously, other important studies on the biology, anatomy, ecology and evolution of anomalodesmatans have been conducted, many highlighted in the researches of Brian Morton (second author of this paper) (Adal & Morton 1973; Morton 1973, 1974, 1976, 1977, 1980, 1981a, 1981b, 1982, 1984a, b, c, d, 1985a, b, 1987, 1995; Yonge & Morton 1980).

The first cladistical analysis of the sub-class was undertaken by Harper *et al.* (2000), who gathered together much of the anomalodesmatan morphological knowledge elucidated over the last 100 years. The same century was also marked by an expansion of knowledge about the biology, anatomy and life style habits of a highly specialized group of anomalodesmatans colloquially referred to as the watering pot shells (Morton & Harper 2001; Morton 2002a, b, 2003a, b, 2004a, b, c, 2005a, b, 2006a, b, c, 2007, 2009, 2011; Harper & Morton 2004). This century too saw the first researches using techniques that increased the number of anomalodesmatan molecular sequences (Dreyer *et al.* 2003; Harper *et al.* 2006; Williams *et al.* 2017) with the aim of resolving the overall phylogenetic complexity of the Bivalvia (Giribet & Wheeler 2002; Taylor *et al.* 2007; Sharma *et al.* 2012; Bieler *et al.* 2014; González *et al.* 2016; Combosh *et al.* 2017). In another way, the 2000s were marked by the first study of spermatozoan ultrastructure in some members of the Anomalodesmata (Healy *et al*. 2008); the description of a new anomalodesmatan family, the Clistoconchidae by Morton (2012) and of important studies on the anatomy and behaviour of carnivorous bivalves (Krylova 2001; Morton 2003a, 2015; Leal 2008; Simone & Cunha 2008; Bieler *et al*. 2014; Temkin & Strong 2013; Morton *et al*. 2016a, b; Safonova & Barwick 2016) generating the first high definition moving images of a living species of this group (Machado *et al*. 2017).

Although, therefore, there has been a significant increase in morphological and molecular data concerning the Anomalodesmata in recent years, the sub-class is still poorly studied in comparison with other groups of the Bivalvia, for example the Imparidentia Bieler, Mikkelsen & Giribet, 2014 (= Euheterodonta, excluding the Anomalodesmata). In addition, due to the rarity of virtually all anomalodesmatan taxa, their low representativeness in the most recent phylogenetic analyses remains the greatest challenge to a comprehensive understanding of their internal relationships, although its monophyly is well demonstrated by many authors such as Giribet & Wheeler (2002), Giribet & Distel (2003), Dreyer *et al.* (2003), Healy *et al.* (2008), Harper *et al*. (2006), Sharma *et al.* (2012), Bieler *et al.* (2014), González *et al.* (2016), Combosh *et al.* (2017) and Williams *et al.* (2017).

With the primary purpose of creating the first comprehensive morphological analysis of the Anomalodesmata, therefore, this work has reviewed and analyzed all the available information on the sub-class contained within the literature. The principal aim of our study was to propose a new phylogeny for Anomalodesmata using a cladistical analysis that has gathered together, for the first time, all that is known about the morphological attributes of the 21 Recent and, as currently recognized, families of this sub-class.

Following completion of the resulting, based on the consensus tree, we have also identified and herein describe a new anomalodesmatan family - the Bentholyonsiidae. Finally, we engage in a discussion on the evolution of the sub-class to try and put, as described herein, its extraordinary phylogeny showing a broader picture of evolution and adaptive radiation that Morton (1985a) has attempted previously, but which, now, in the light of this study, revealed by the increase in the taxa representativeness new internal relationships for this important group of marine.

Materials and Methods

Literature review

This study commenced with a review of the extensive, but disparate, literature containing anatomical information about the diverse species of extant Anomalodesmata. More than 60 papers have been analyzed, 180 species reviewed and 61 taxa selected to create the present cladistical analysis (Table 1). Species were selected taking into account those taxa studied previously in detail (functional morphology, including histology) and with the shell and internal details of each species' anatomy illustrated through drawings and/or photographs, for example, *Grippina coronata* Machado & Passos, 2016 (Morton *et al*. 2016a), *Lyonsiella abyssicola* (G. O. Sars, 1872) (Allen & Turner 1974), *Cardiomya cleryana* (d'Orbigny, 1842) (Machado *et al*. 2017) and *Thracia meridionalis* Smith, 1885 (Sartori & Domaneschi 2005). In addition, there was also a preference for species that besides having information available in the literature, could also be re-accessed through lots deposited in Museums.

For the 61 chosen taxa, 61 morphological characters were coded and categorized as follows: 12 characters on the *shell microstructure and shell morphology*; 3 on the *general features of the mantle*; 16 on the *siphonal features*; 6 on the *musculature*; 16 on the *visceral mass organs* (*alimentary tract* + *nervous system*); 3 on the *reproductive biology*; and 5 on the *life style*. Ultrastructural characters, such as sperm morphology, have not been included in this analysis.

Table 1. A list of the extant families of Recent Anomalodesmata and all the species for which anatomical information is available as well as the main reference sources in chronological order. The sixty-one taxa from the 180 species reviewed that were selected to compose the present cladistical analysis are identified in bold. Basic statistical information on each of the families is also provided, as well as an estimation of the representativeness (%) of each family within the framework of the present cladistical analysis. Abbreviations: Σ= total of species recorded for the family (based in WoRMS); R= number of species that were reviewed by the present study; U= number of species used in this cladistic analysis and the percentage in relation to the total of registered species.

Families	Species	Main references
Pholadomyidae King, 1844 Σ= 1spp R= 1spp U= 1spp (100%)	***Pholadomya candida*** Sowerby, 1863	Morton (1980)
Parilimyidae Morton, 1981 Σ= 17 spp R= 1spp U= 1spp (5,8%)	***Parilimya fragilis*** (Grieg, 1920)	Morton (1982)
Clavagellidae d'Orbigny, 1844 Σ= 12 spp R= 8 spp U= 5 spp (41,6%)	*Bryropa melitensis* (Broderip, 1824)	Pelseneer (1911)
	Dacosta australis (Sowerby, 1829)	Morton (1984a)
	Bryropa lata (Broderip, 1824)	Savazzi (2000), Pallazzi & Villari (2000)
	Humphreyia strangei (Adams, 1854)	Morton (2002b)
	Dianadema multangularis (Tate, 1887)	Morton (2003a)
	Bryropa aligamenta Morton, 2005	Morton (2005)
	Stirpulina ramosa (Dunker, 1882)	Morton (2006c), Morton (2013)
	Dianadema minima G.B Sowerby III, 1889	Morton (2009)
Penicillidae Gray, 1858 Σ= 9 spp R= 6 spp U= 5 spp (55,5%)	*Verpa penis* (Linnaeus, 1758)	Purchon (1956a), Purchon (1960)
	Brechites attrahens (Lightfoot, 1786)	Morton (1984b), Morton (2002a)
	Kendrickiana veitchi (Smith, 1971)	Morton (2004b)
	Nipponoclava gigantea (Sowerby, 1888)	Morton (2004c)
	Foegia novaezelandiae (Bruguière, 1789)	Morton (2004a)
	Verpa philippinensis (Chenu, 1843)	Morton (2006b), Liu (2008)
Lyonsiidae P. Fisher, 1887 Σ= 34 spp R= 7 spp U= 4 spp (11,7%)	*Lyonsia norwegica* (Gmelin, 1791)	Pelseneer (1911)
	Entodesma navicula (Adams & Reeve, 1850)	Yonge (1952), Morgan & Allen (1976), Morton (1987b), Huber (2010)
	Mytilimeria nuttalli Conrad, 1839	Yonge (1952)
	Lyonsia californica Conrad, 1837	Narchi (1968)
	Lyonsia hyalina (Conrad, 1831)	Prezant (1979), Thomas (1993)
	Entodesma beana (Gould, 1850)	Mikkelsen & Bieler (2008), Simone *et al.* (2015)
	Lyonsia floridana Conrad, 1849	Bieler *et al.* (2014)
Pandoridae Rafinesque, 1815 Σ= 46 spp R= 5 spp U= 2 spp (4,3%)	*Frenamya elongata* Carpenter, 1846	Pelseneer (1911), Bieler *et al.* (2014)
	Pandora inaequivalvis (Linnaeus, 1758)	Allen (1954)
	Frenamya ceylanica (Sowerby, 1835)	Morton (1984d)
	Pandora filosa (Carpenter, 1864)	Thomas (1994)
	Pandora brevirostris Güller & Zelaya, 2016	Güller & Zelaya, 2016
	Pandora braziliensis G. B. Sowerby II, 1874	Güller & Zelaya, 2016

Myochamidae Carpenter, 1861 Σ= 35 spp R= 3 spp U= 2 spp (5,7%)	*Myadora complexa* Iredale, 1924	Pelseneer (1911)
	Myadora striata (Quoy & Gaimard, 1835)	Morton (1977)
	Myochama anomioides Strutchbury, 1830	Yonge & Morton (1980), Harper & Morton (2000), Bieler *et al.* (2014)
Cleidothaeridae Hedley, 1918 Σ= 2 spp R= 1 spp U= 1 spp (50%)	***Cleidothaerus albidus*** (Lamarck, 1819)	Morton (1974)
Thraciidae Stoliczka, 1870 Σ= 81 spp R= 6 spp U= 3 spp (3,7%)	*Thracia conradi* Couthouy, 1839	Thomas (1967)
	Trigonothracia jinxingae Xu, 1980	Morton (1995)
	Parvithracia lukini Kamenev, 2002	Kamenev (2002)
	Parvithracia sirenkoi Kamenev, 2002	Kamenev (2002)
	Thracia meridionalis Smith, 1885	Sartori & Domaneschi (2005)
	Thracia phaseolina (Lamarck, 1818)	Sartori & Ball (2009), Bieler *et al.* (2014)
Periplomatidae Dall, 1895 Σ= 44 spp R= 4 spp U= 2 spp (4,5%)	*Pendaloma otohimeae* (Habe, 1952)	Pelseneer (1911)
	Cochlodesma praetenue (Pulteney, 1799)	Allen (1958, 1960), Bieler *et al.* (2014)
	Offadesma angasi (Crosse & P. Fischer, 1864)	Morton (1981b)
	Periploma margaritaceum (Lamarck, 1801)	Rosewater (1984)
Laternulidae Hedley 1918 Σ= 20 spp R= 3 spp U= 2 spp (10%)	***Laternula elliptica*** (King, 1832)	Burne (1920), Morton (1976), Sartori *et al.* (2006), Bieler *et al.* (2014)
	Laternula truncata (Lamarck, 1818)	Adal & Morton (1973), Morton (1973), Morton (1976)
	Laternula spengleri (Gmelin, 1791)	Morton (1976), Savazzi (1990)
Clistoconchidae Morton, 2012 Σ= 1 spp R= 1 spp U= 1 spp (100%)	***Clistoconcha insignis*** E. A. Smith, 1980	Morton (2012)
Bentholyonsiidae *fam. nov* Σ= 1 spp R= 1 spp U= 1 spp (100%)	***Bentholyonsia teramachii*** Habe, 1952	Morton (2003b)

Cetoconchidae Ridewood, 1903 Σ= 23 spp R= 7 spp U= 1 spp (4,34%)	*Cetoconcha pelseneeri* Pelseneer, 1911	Pelsenner (1911)
	Cetoconcha ceylonensis Knudsen, 1970	Knudsen (1970)
	Cetoconcha galatheae Knudsen, 1970	Knudsen (1970)
	Cetoconcha braziliensis Allen & Morgan, 1981	Allen & Morgan (1981)
	Cetoconcha angolensis Allen & Morgan, 1981	Allen & Morgan (1981)
	Cetoconcha alephtinae (Krylova, 1991)	Krylova (1991)
	Cetoconcha elegans (Krylova, 1991)	Krylova (1991)
Poromyidae Dall, 1886 Σ= 52 spp R= 17 spp U= 4 spp (7,7%)	***Cetomya tornata*** (Jeffreys, 1876)	Pelseneer (1906), Knudsen (1970), Allen & Morgan (1981)
	Poromya australis Smith, 1885	Pelseneer (1911), Krylova (2001)
	Cetomya eximia (Pelseneer, 1911)	Pelseneer (1911), Knudsen (1967), Krylova (2001)
	Poromya granulata (Nyst & Westendorp, 1839)	Yonge (1928), Yonge & Morton (1980), Morton (1981c)
	Dermatomya tenuiconcha (Dall, 1913)	Knudsen (1970)
	Cetomya perla (Dall, 1908)	Knudsen (1970)
	Lissomya rotundula Krylova, 1997	Krylova (1997)
	Cetomya butoni (Prashad, 1932)	Krylova (2001)
	Cetomya poutiersi Krylova, 2001	Krylova (2001)
	Cetomya nataliae Krylova, 2001	Krylova (2001)
	Cetomya voskresenskii Krylova, 2001	Krylova (2001)
	Cetomya bacata Krylova, 2001	Krylova (2001)
	Cetomya celsa Krylova, 2001	Krylova (2001)
	Poromya undosa Hadley & Petterd, 1906	Krylova (2001)
	Dillema frumarkernorum Leal, 2008	Leal (2008)
	Dillema spectralis Leal, 2008	Leal (2008)
	Poromya illevis Hedley, 1913	Bieler *et al.* (2014)
Lyonsiellidae Dall, 1895 Σ= 49 spp R= 22 spp U= 4 spp (8,16%)	*Policordia pilula* (Pelseneer, 1911)	Pelseneer (1911)
	Lyonsiella abscissa Pelseneer, 1911	Pelseneer (1911)
	Policordia murrayi (Knudsen, 1967)	Knudsen (1967)
	Lyonsiella galatheae (Knudsen, 1970)	Knudsen (1970)
	Dallicordia alaskana (Dall, 1895)	Dall (1895), Knudsen (1970)
	Policordia lisbetae Knudsen, 1970	Knudsen (1970)
	Policordia densicostata (Locard, 1898)	Allen & Turner (1974), Morton (1987c)
	Policordia olivacea Poutiers & Bernard, 1995	Poutiers & Bernard (1995)
	Policordia atlantica Allen & Turner, 1974	Allen & Turner (1974)
	Policordia gemma (A. E. Verril, 1880)	Allen & Turner (1974)
	Policordia papyracea (E. A. Smith, 1885)	Allen & Turner (1974)
	Policordia jeffreysi (Friele, 1879)	Allen & Turner (1974)
	Policordia laevis Allen & Turner, 1974	Allen & Turner (1974)
	Allograma formosa (Jeffreys, 1881)	Allen & Turner (1974), Morton (1984c)
	Lyonsiella subquadrata (Jeffreys, 1882)	Allen & Turner (1974)
	Lyonsiella perplexa Allen & Turner, 1974	Allen & Turner (1974)
	Lyonsiella frielei Allen & Turner, 1974	Allen & Turner (1974)
	Lyonsiella smidti Friele, 1886	Allen & Turner (1974)
	Lyonsiella abyssicola (G. O. Sars, 1872)	Allen & Turner (1974), Morton (1987c)
	Lyonsiella fragilis Allen & Turner, 1974	Allen & Turner (1974)
	Lyonsiella horrida (Allen & Turner, 1974)	Allen & Turner (1974)
	Policordia hispida Safonova & Barwick, 2016	Safonova & Barwick, (2016)

Verticordiidae Stoliczka, 1870 Σ= 58 spp R= 10 spp U= 5 spp (8,6%)	*Spinosipella acuticostata* (Philippi, 1894)	Dall (1895)
	Halicardia nipponensis Okutani, 1967	Nakazima (1967)
	Verticordia triangularis Locard, 1898	Allen & Turner (1974), Morton (1987c)
	Verticordia quadrata Smith, 1885	Allen & Turner (1974)
	Halicardia flexuosa (Verril & S. Smith, 1881)	Allen & Turner (1974)
	Halicardia philippinensis Poutiers, 1981	Morton (1987c)
	Spinosipella deshayesiana (P. Fischer, 1862)	Simone & Cunha (2008)
	Spinosipella costeminens (Poutiers, 1981)	Simone & Cunha (2008)
	Haliris tenerrima (Jaeckel & Thiele, 1931)	Bieler *et al.* (2014)
	Trigonulina ornata d'Orbigny, 1853	Analysed by the authors
Euciroidae Dall, 1895 Σ= 13 spp R= 3 U= 2 (15,3%)	***Euciroa pacifica*** Dall, 1895	Dall (1895)
	Euciroa rostrata Jaeckel & Thiele, 1931	Knudsen (1967), Poutiers & Bernard (1995)
	Euciroa eburnea (Wood-Mason & Alcock, 1891)	Knudsen (1967), Poutiers & Bernard (1995)
Spheniopsidae J. Gardner, 1928 Σ= 14 spp R= 2 spp U= 2 spp (14,3%)	***Grippina coronata*** Machado & Passos (2016)	Machado & Passos (2015), Morton *et al.* (2016a)
	Spheniopsis brasiliensis Machado & Passos (2016)	Machado & Passos (2015), Morton *et al.* (2016b)
Protocuspidariidae Scarlato & Starogobatov, 1983 Σ= 19 spp R= 11 spp U= 2 spp (10,5%)	***Protocuspidaria atlantica*** Allen & Morgan, 1981	Allen & Morgan (1981), Krylova (1995)
	Protocuspidaria pusilla Krylova, 1995	Krylova (1995)
	Protocuspidaria speciosa Krylova, 1995	Krylova (1995)
	Protocuspidaria fragilis Krylova, 1995	Krylova (1995)
	Multitentacula composita Krylova, 1995	Krylova (1995)
	Multitentacula venusta Krylova, 1995	Krylova (1995)
	Multitentacula admirabilis Krylova, 1995	Krylova (1995)
	Multitentacula parvula Krylova, 1995	Krylova (1995)
	Multitentacula paulula Krylova, 1995	Krylova (1995)
	Multitentacula parilis Krylova, 1995	Krylova (1995)
	Multitentacula amoena Krylova, 1995	Krylova (1995)
Halonymphidae Scarlato & Starogobatov, 1983 Σ= 17 spp R= 7 spp U= 2 spp (11,7%)	***Halonympha depressa*** (Jeffreys, 1882)	Allen & Morgan (1981)
	Halonympha atlanta Allen & Morgan, 1981	Allen & Morgan (1981)
	Octoporia octoporosa (Allen & Morgan, 1981)	Allen & Morgan (1981), Krylova (1994b)
	Octoporia podobeda Krylova, 1994	Krylova (1994b)
	Octoporia rugosa Krylova, 1994	Krylova (1994b)
	Octoporia sinuosa Krylova, 1994	Krylova (1994b)
	Octoporia poutiera Allen, 2011	Allen (2011)
Cuspidariidae Dall, 1886 Σ= 254 spp R= 54 spp U= 9 spp (3,54%)	***Cuspidaria cuspidata*** (Olivi, 1792)	Pelseneer (1906); Yonge & Morton (1980), Allen & Morgan (1981), Morton (1987b)
	Cuspidaria convexa Pelseneer, 1911	Pelseneer (1911)
	Rhinoclama dubia (Pelseneer, 1911)	Pelseneer (1911), Knudsen (1967), Krylova (1994a)
	Pseudoneaera thaumasia Sturany, 1901	Pelseneer (1911)

Cuspidaria rostrata (Spengler, 1793)	Yonge (1928), Reid & Reid (1974)
Cuspidaria approximata E. A. Smith, 1896	Knudsen (1967)
Cuspidaria gigantea Prashad, 1932	Knudsen (1967)
Rengea caduca (E. A. Smith, 1894)	Knudsen (1967)
Cuspidaria barnardi Knudsen, 1970	Knudsen (1970), Allen & Morgan (1981)
Cuspidaria undata (Verril, 1884)	Knudsen (1970), Allen & Morgan (1981)
Cuspidaria delli Knudsen, 1970	Knudsen (1970)
Cuspidaria guineensis Knudsen, 1970	Knudsen (1970)
Cuspidaria haasi Knudsen, 1970	Knudsen (1970)
Cuspidaria natalensis Knudsen, 1970	Knudsen (1970)
Cuspidaria parkeri Knudsen, 1970	Knudsen (1970)
Myonera garretti Dall, 1908	Knudsen (1970)
Bathyneaera hadalis (Knudsen, 1970)	Knudsen (1970), Allen & Morgan (1981), Krylova (1993), Bieler *et al.* (2014)
Myonera tasmanica (Knudsen, 1970)	Knudsen (1970)
Cuspidaria obesa (Lóven, 1846)	Reid & Reid (1974), Allen & Morgan (1981)
Cuspidaria parva Verril & Bush, 1898	Allen & Morgan (1981)
Cuspidaria jeffreysi (Dall, 1881)	Allen & Morgan (1981)
Cuspidaria atlantica Allen & Morgan, 1881	Allen & Morgan (1981)
Cuspidaria circinata (Jeffreys, 1876)	Allen & Morgan (1981)
Cuspidaria ventricosa Verril & Bush, 1898	Allen & Morgan (1981)
Cardiomya planetica (Dall, 1908)	Reid & Crosby (1980)
Cardiomya perrostrata (Dall, 1881)	Allen & Morgan (1981)
Cardiomya knudseni (Allen & Morgan, 1881)	Allen & Morgan (1981)
Cardiomya cadiziana Huber, 2010	Allen & Morgan (1981)
Rhinoclama abrupta (Allen & Morgan, 1981)	Allen & Morgan (1981)
Rhinoclama halimera (Dall, 1886)	Allen & Morgan (1981), Krylova (1994a)
Cardiomya costellata (Deshayes, 1835)	Allen & Morgan (1981), Morton (2015)
Myonera alleni Poutiers in Poutiers & Bernard, 1995	Allen & Morgan (1981)
Myonera pauscistriata Dall, 1886	Allen & Morgan (1981)
Luzonia simplex (Allen & Morgan, 1981)	Allen & Morgan (1981)
Tropidomya abbreviata (Forbes, 1843)	Allen & Morgan (1981)
Rhinoclama notabilis (Jeffreys, 1876)	Allen & Morgan (1981)
Tropidomya diagonalis (Allen & Morgan, 1981)	Allen & Morgan (1981)
Bathyneaera tillamookensis (Dall, 1916)	Allen & Morgan (1981), Krylova (1993)
Bathyneaera quadrostrata (Poutiers, 1984)	Krylova (1993)
Bathyneaera globulosa Krylova, 1993	Krylova (1993)
Bathyneaera laticella (Dall, 1886)	Krylova (1993)
Bathyneaera paleifera Krylova, 1993	Krylova (1993)
Bathyneaera disa (Bernard, 1989)	Krylova (1993)
Bathyneaera bernardi Krylova, 1993	Krylova (1993)
Rhinoclama similis Krylova, 1994	Krylova (1994a)
Rhinoclama semistrigosa (Jeffreys, 1881)	Krylova (1994a)
Rhinoclama teres (Jeffreys, 1881)	Krylova (1994a)
Rhinoclama raoulensis (Powel, 1958)	Krylova (1994a)
Rhinoclama dorsirecta (Verco, 1908)	Krylova (1994a)
Cuspidaria concentrica Thiele (1912)	Zelaya & Ituarte (2006)
Cuspidaria minima (Egorova, 1993)	Zelaya & Ituarte (2006)
Cuspidaria krylovae Allen, 2011	Allen (2011)
Luzonia morganae Allen, 2011	Allen (2011)
Cardiomya cleryana (d'Orbigny, 1842)	Machado *et al.* (2016)

Material examined

Of the 61 species selected for this cladistical analysis, 35 had previously been studied by their respective authors and voucher specimens are deposited in different institutions around the word (Appendix 1), the anatomical data about another 26 species were obtained exclusively from literature. In addition, the private collection of Dr Flávio Dias Passos (Universidade Estadual de Campinas, Brazil) has been consulted and from it *Trigonulina ornata* d'Orbigny, 1853, *Laternula elliptica* (King, 1832) and *Thracia meridionalis* Smith, 1885 have been examined, plus the MCZ collection where more than 40 species of Anomalodesmata had their shells and internal tissues re-analyzed. These are all illustrated in Figures 1-5.

Figure 1. Shells of some species of Anomalodesmata analysed in this paper. A, *Pholadomya candida* (MCZ 387668), outer and inner views of the right shell valve; B, *Dacosta australis* (MCZ 387672), adventitious

tube and a frontal view of the anterior pedal disc; C, *Brechites attrahens* (MCZ 32406), shell, adventitious tube and a frontal view of the anterior pedal disc; D, *Verpa phillipinensis* (MCZ 85837), shell and adventitious tube; E, *Foegia novaezelandiae* (MCZ 387671), adventitious tube; F, *Nipponoclava gigantea* (MCZ 44735), adventitious tube and a magnification of the shell valves; G, *Entodesma navicula* (MCZ 239531), adult and a juvenile (below) specimens; H, *Pandora inaequivalvis* (MCZ 374289), outer and inner view of the valve surfaces of four individuals; I, *Cleidothaerus albidus* (MCZ 182201) outer and inner views of the valves surfaces plus an isolated lithodesma. Scale bar = 2 cm. Abbreviations: il, internal ligament; lit, lithodesma; lv, left valve; lt, lateral tubules; pep, perforate plate; ps, pedal slit; rv, right valve; v, valves.

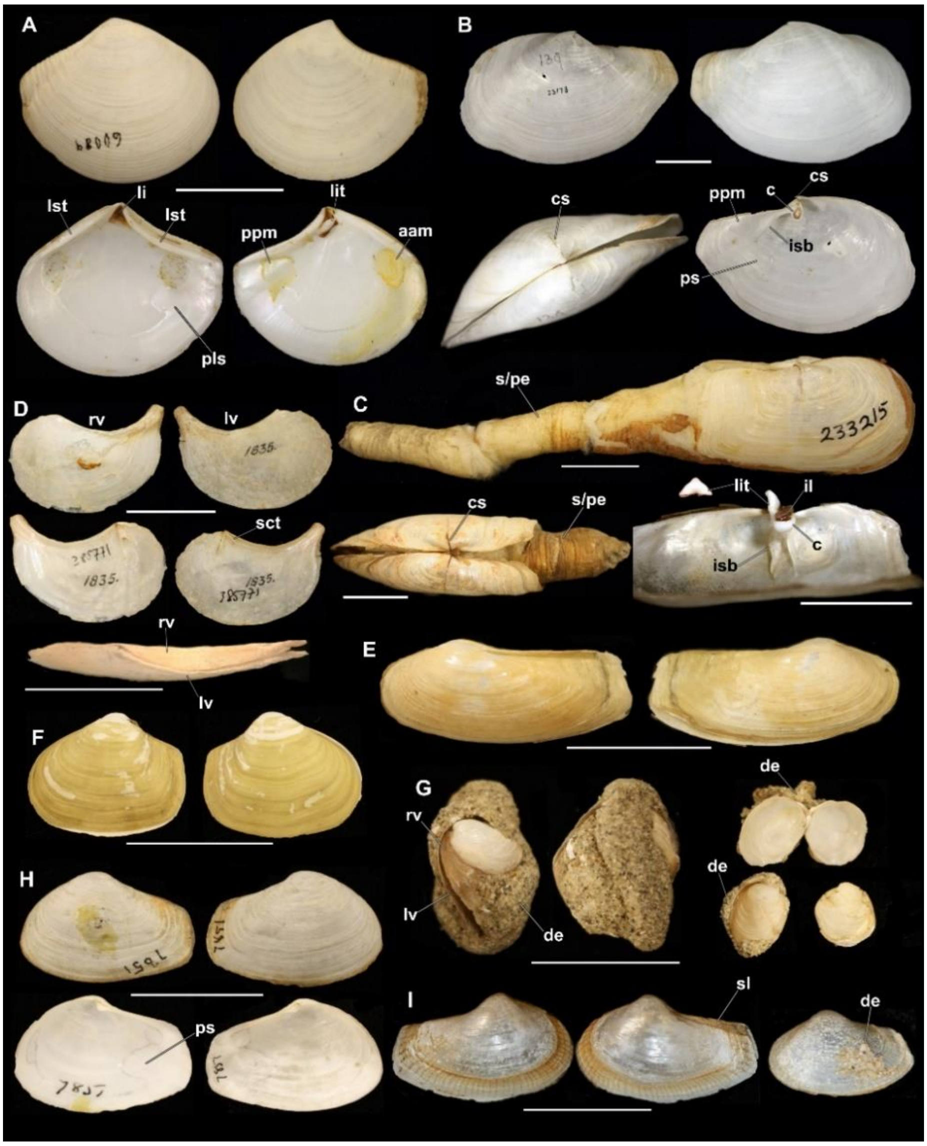


Figure 2. Shells of some species of Anomalodesmata analysed in this paper. A, *Myadora striata* (MCZ 60089), outer and inner views of the shell valve surfaces highlighting some internal details; B, *Offadesma angasi* (MCZ 23173) outer, inner and dorsal views highlighting shell details; C, *Laternula truncata* (MCZ 233215) outer, inner and dorsal views of three individuals showing some details of the shells, siphons and an isolated lithodesma; D, *Frenamya ceylanica* (MCZ 385771) outer, inner and dorsal views of the right

and left valves; E, *Lyonsia californica* (MCZ 63945) an outer view of the valve surface; F, *Thracia meridionalis* (MCZ 27/880), outer view of the valve surface; G, *Mytilimeria nuttalli* (MCZ 61821) outer and inner views of the surfaces of four individuals showing a large amount of debris attached to the shell surface; H, *Thracia similis* (MCZ 387667), outer and inner views of the right and left shell valves; I, *Lyonsia hyalina* (MCZ 182003), outer and inner views of the right and left valves plus debris attached to the shell surface of the juvenile specimen (right). Scale bar: A-H = 2cm, I = 1cm. Abbreviations: aam, anterior adductor muscle scar; c, chondrophore; cs, crack in the shell; de, debris attached in the valve surface; il, internal ligament; isb, internal shell buttress; li, ligament; lit, lithodesma; lst, lateral secondary tooth; lv, left valve; pls, pallial sinus; ppm, posterior adductor muscle scar; rv, right valve; s, shell; sct, secondary cardinal tooth; sl, secondary ligament; s/pe, siphons/periostracal sheath.

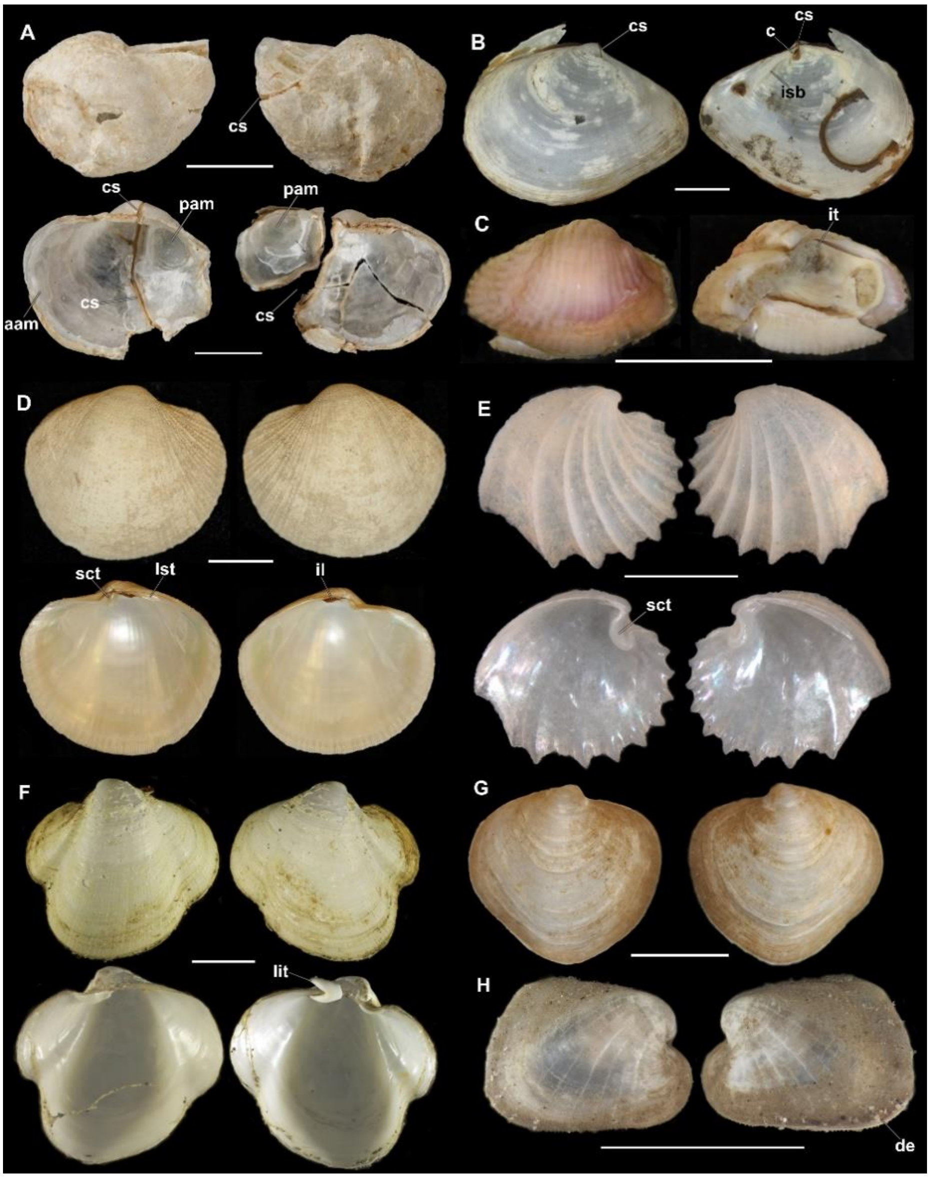


Figure 3. Shells of some species of Anomalodesmata analysed in this paper. A, *Clistoconcha insignis* (NHMUK 1911.30.19-20 paralectotypes, courtesy of A. Salvador/photos by Kevin Webb), outer (above) and inner views (below) of the shell valves; B, *Cochlodesma praetenue* (MCZ 375545), outer and inner

views of the left shell valve; C, *Myochama anomioides* (MCZ 379067), outer and inner views of the unattached left shell valve showing the internal tissues; D, *Euciroa eburnea* (MCZ 387669), outer and inner views of the shell valves; E, *Trigonulina ornata* (personal collection of Dr. Flávio D. Passos), outer and inner views of the shell valves; F, *Halicardia nipponensis* (personal collection, courtesy of Dr. Takuma Haga), views of the outer surfaces of the right and left shell valves; G, *Vertambitus triangularis* (MCZ 348045), views of the outer surfaces of the right and left shell valves; H, *Lyonsiella abyssicola* (MCZ 348034), views of the outer surfaces of the right and left shell valves. Scale bars: A, C, E = 2mm; D, F, G = 1cm; B, H = 5mm. Abbreviations: aam, anterior adductor muscle scar; c, chondrophore; cs, crack in the shell; de, debris attached in the valve surface; il, internal ligament; it, Internal tissue; isb, internal shell buttress; lit, lithodesma; lst, lateral secondary tooth; ppm, posterior adductor muscle scar; r, rostrum; sct, secondary cardinal tooth; sl, secondary ligament.

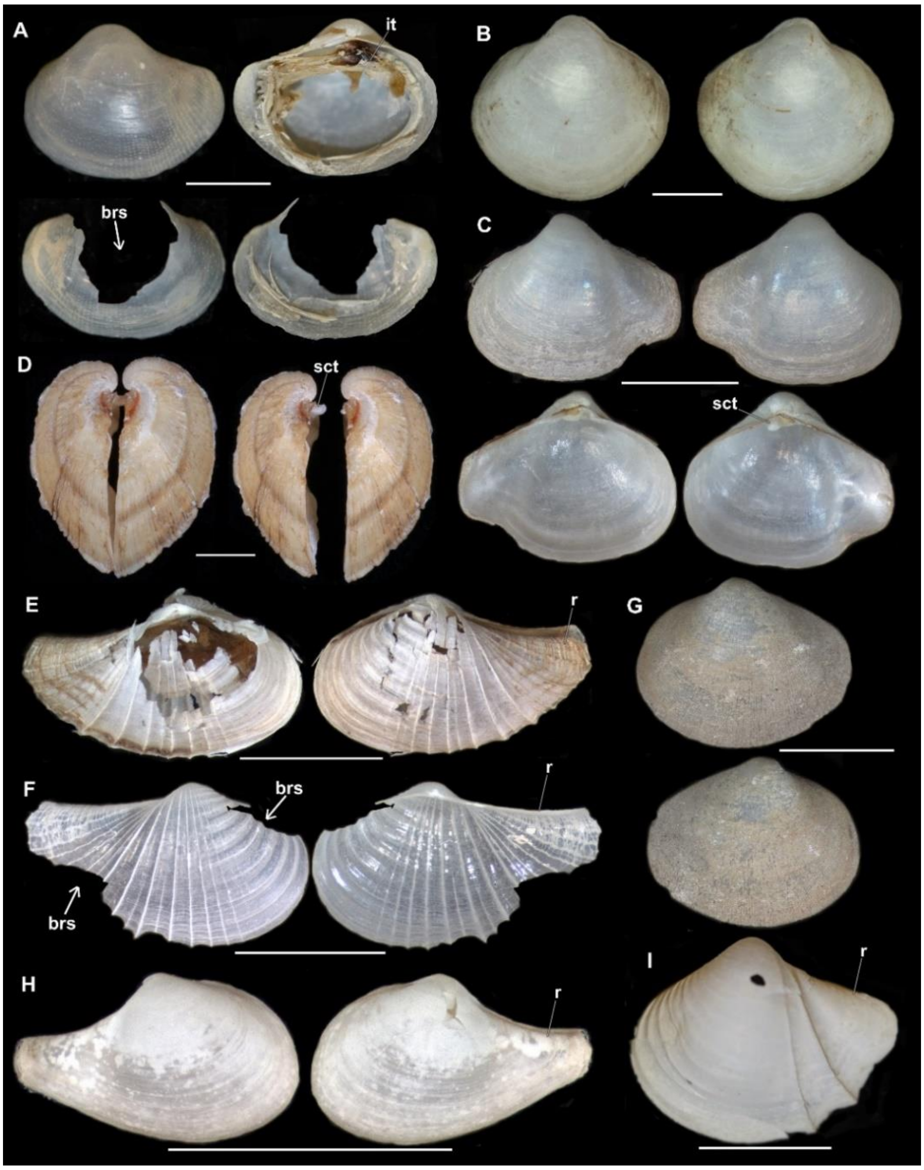


Figure 4. Shells of some species of Anomalodesmata analysed in this paper. A, *Cetoconcha braziliensis* (MCZ 281315- holotype), views of the outer and inner surfaces of the right (whole) and left (broken) shell

valves; B, *Cetomya tornata* (MCZ 353657), views of the outer surfaces of the right and left shell valves; C, *Poromya rostrata* (ZUEC 2240), views of the outer and inner surfaces of the right and left shell valves; D, *Dilemma frumarkenorum* (BSMN 15029 – holotype, courtesy of Prof José H. Leal), views of the outer surfaces of the right and left shell valves articulated and non-articulated; E, *Bathyneaera hadalis* (MCZ 377976), views of the outer surfaces of the right and left shell valves; F, *Bathyneaera hadalis* (MCZ 281308- holotype) views of the outer and inner surfaces of the right shell valve; G, *Poromya granulata* (MCZ 163/77), views of the outer surfaces of the right and left shell valves; H, *Halonympha depressa* (MCZ 348009), views of the outer surfaces of the right and left shell valves; I, *Myonera paucistriata* (MCZ 349123), an outer view of the left valve. Scale bars: A-I = 5mm. Abbreviations: brs, broken shell region; it, internal tissue; r, rostrum; sct, secondary cardinal tooth.

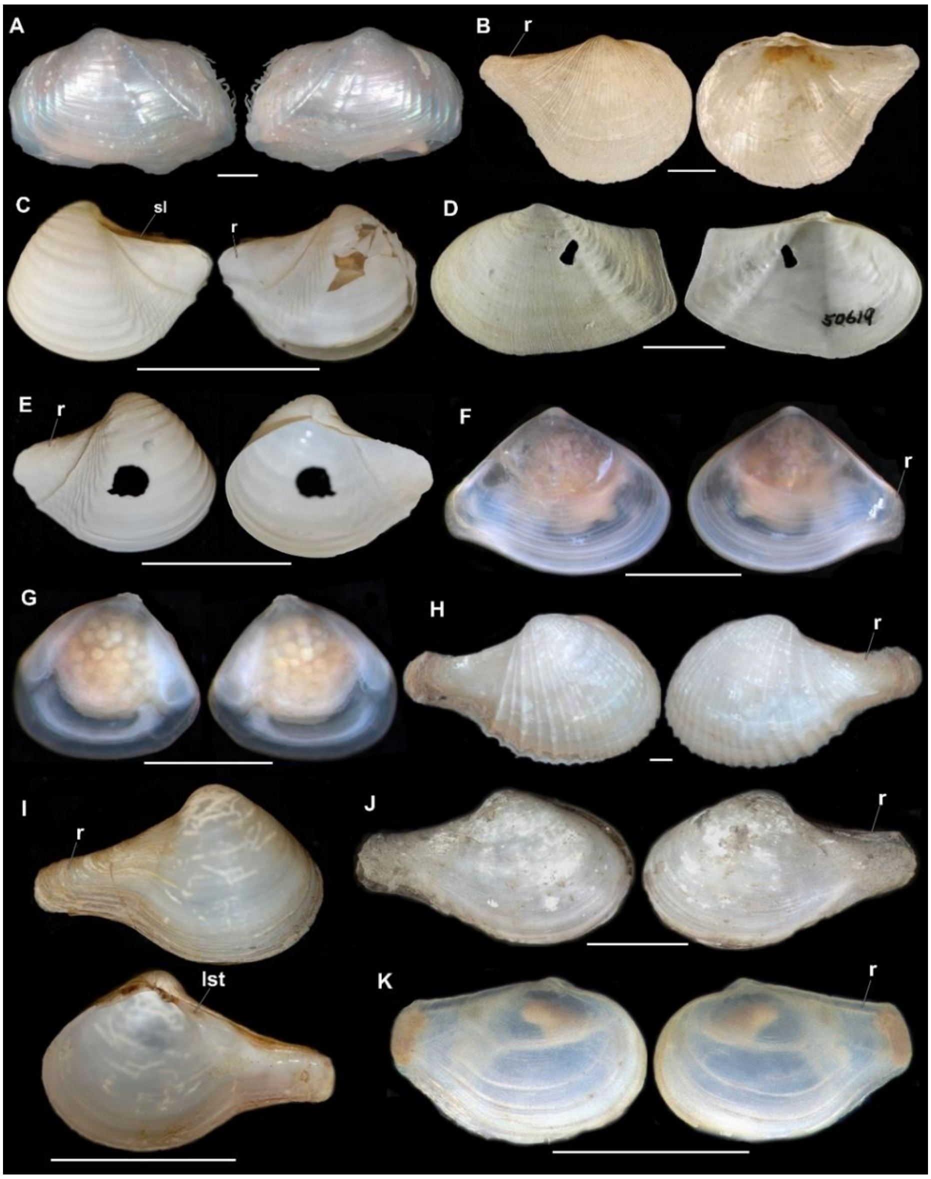


Figure 5. Shells of some species of Anomalodesmata analysed in this paper. A, *Allograma formosa* (Museo Argentino de Ciencias Naturales “Bernardino Rivadavia”), outer and inner views of the shell valves; B, *Acreuciroa rostrata* (MCZ 387671), outer and inner views of the right valve; C, *Tropidomya diagonalis*

(MCZ 348004), views of the outer surfaces of the right and left shell valves; D, *Bentholyonsia teramachii* (Mo50619- courtesy of Dr Takuma Haga and Dr Hiroshi Saito), views of the outer and inner surfaces of the left shell valve; E, *Tropidomya diagonalis* (MCZ 281311- holotype), views of the outer and inner surfaces of the right shell valve; F, *Spheniopsis brasiliensis* (ZUEC 6483), views of the outer surfaces of the right and left shell valves, showing part of internal tissues due to the transparency of the shells; G, *Grippina coronata* (ZUEC 6181), views of the outer surfaces of the right and left shell valves, showing part of internal tissues due to the transparency of the shells; H, *Cardiomya cleryana* (ZUEC 2218), views of the outer surfaces of the right and left shell valves; I. *Cuspidaria cuspidata* (MCZ 226442), views of the outer and inner surfaces of the right shell valve; J, *Cuspidaria obesa* (MCZ 387673), views of the outer surfaces of the right and left shell valves; K, *Cardiomya costellata* (MCZ 243626), views of the outer surfaces of the right and left shell valves. Scale bars: A, J, K = 2mm; B-D, E = 1cm, F-H = 1000µm. Abbreviations: sl, secondary ligament; r, rostrum.

Phylogenetic analysis

The character matrix derived from analyses of the shell and internal tissue morphology of the studied taxa relates to representatives of all 22 families of the Anomalodesmata including the new, herein described, Bentholyonsiidae. The data matrix obtained included 61 ingroup taxa plus three outgroup taxa, with shell features and anatomical data available from the literature (Morton 1987a; Simone *et al.* 2015; Paine & Allen 1991), including: (i), the Palaeoheterodonta - *Neotrigonia margaritacea* (Lamarck, 1804); (ii), the Archiheterodonta - *Cardites floridanus* (Conrad, 1835), new comb. to *Carditamera floridana*; and (iii), Imparidentia - *Thyasira trisinuata* (d'Orbigny, 1853), all representing distinctive phylogenetic groups that are broadly considered distinct from the Anomalodesmata (Combosh *et al*. 2017).

The matrix was scored for 61 morphological characters (12 from the shell, 41 from internal tissues and eight associated with reproductive biology/life style characters) (Appendix 2). Of the 61 characters, 26 (43,3%) were coded as multistate and treated as non-additive while 35 (57,3%) other characters were coded as binary. The data matrix was assembled and edited using the computer software NDE 0.5.0 (Page 2001) and analysed by a heuristic search under different parameters of character weighting with the computer program TNT (Goloboff *et al*. 2008). Parsimony analysis was conducted using equally-weighted characters and implied weighting (with differing parameters), in order to perform a sensitivity analysis of the dataset under distinct situations (Wheeler 1995). To establish the K-values that would be used in our implied weighting analyses, we followed Mirande (2009) and Guadanucci (2014) dividing the values of fit/distortion into regular intervals, obtained under different K-values. The values of K used were those that can be assigned to the '*not perfectly hierarchical'* character fits of 70%, 72.7%, 75.5%, 78.3%, 81.1%, 83.8%, 86.6%, 89.4%, 92.2% and 95% (script commands aaa 3 10 70 95 7) of the fit of a 'perfectly hierarchical' one (regular intervals of 2.7%). According to Goloboff (1993), due to the concave/convex shape of the curve of K-values *versus* fit/distortion, higher values of K tend to produce more similar results. Mirande (2009) and Guadanucci (2014), therefore, drew attention to the importance of setting K-values that permit appropriate comparisons, and this was accordingly also undertaken herein.

Most parsimonious trees were searched by heuristic methods, with 100,000 replications, holding five trees per search and collapsing trees after searching (the number of trees were identical when used collapsing rules either 1 or 3). In order to eliminate '*zero length branches'*, the nodes without support were collapsed ('*hard collapse unsupported nodes in all trees*') and only the best trees kept ('*keep best tree only - delete suboptimal*'). Strict consensus was calculated for all searches. Branch support was evaluated through relative Bremer support (Goloboff & Farris 2001). All character optimizations and tree editing were performed with the software WINCLADA 1.00.08 (Nixon 2002).

Results

Morphological characters and states

Of the 61 characters constituting this analysis, about 35 (57%) had previously been coded in earlier publications (Harper *et al.* 2000, 2006; Giribet & Wheeler 2002; Bieler *et al.* 2014) and others were described and discussed by Morton (1985a). Of these 35 previously coded characters, some have been re-interpreted and new character states either included and/or modified. The other 26 characters (~43%) have been coded for the first time. Our final matrix consisted of 3,904 cells with ~3.3% missing data most of which were related to details of the visceral mass and alimentary tract, reproductive features and the nervous system (characters 46, 47, 48, 49, 53, 54 and 55). The descriptions of all 61 characters including the number of steps (L), consistency (CI) and retention (RI) indexes of each character are described below. The autapomorphies (characters 28, 34, 50, 51) were retained in our analyses for three reasons: (i), there is no reduction of resolution in our analyses if they remain; (ii), there is no increase in the values of CI and RI indexes in the consensus trees; and (iii), future researches will be able to assess the evidential support for the monophyly of ingroup terminals. In addition, important anatomical features of the main groupings recovered in our analyses are illustrated in Figures 6 and 7.

Shell microstructure and shell morphology

1- **Shell microstructure**: (0) prismatic + nacreous microstructures; (1) fine homogenous + nacreous microstructures; (2) homogenous + homogeneous microstructure; (3) coarse (>µm) homogenous + nacreous microstructure; (4) Prismatic + lenticular and sheet nacre microstructures; (5) Prismatic + complex crossed lamellar (aragonite); (6) Prismatic + crossed lamellar + complex crossed (aragonite) (L= 6, CI= 66, RI= 88). This character was coded by Harper *et al*. (2000, character 1). Although the authors know that many efforts were made to improve this character (see Harper et al. 2006: p. 412, Bieler et al. 2014: p. 49), the impossibility of a more detailed shell analysis for some species led us to coded multiple layers in a single data.

2- **Valve equality**: (0) equivalve; (1) slightly inequivalve; (2) strongly inequivalve (L= 12, CI= 16, RI= 62). All species that have one of the valves either cemented or associated intimately with the substratum (rocks, tubes) were also coded as strongly inequivalve, state '2', for example, *Dianadema multangularis* (Tate, 1887) (Morton 2003a). This character was coded by Harper *et al*. (2000, character 3).

3- **Dorsal, left to right crack in the shell**: (0) absent; (1) present, partially; (2) present, entire (L= 2, CI= 100, RI= 100). According to Morton (2002: figs. 3-11), unlike the dorsal cracks observed in other anomalodesmatans (Laternulidae and Periplomatidae), those of *Clistoconcha insignis* E. A. Smith, 1980 extend from the shell's dorsal apex to the ventral margins of both shell valves so that, effectively, each valve is split into two pieces, anterior and posterior, with a sharp distinction between morphologies on either side of the crack. According to Savazzi (1990), the cracks ameliorate mechanical stresses to the posterior regions of the shell during valve adduction. Herein, therefore, the crack in the shell of *C. insignis* was coded as being present and entire, whereas representatives of the Periplomatidae and Laternulidae were coded as being partially present. This character was coded by Harper *et al*. (2000, character 4) and Bieler *et al*. (2014, character 19)

4- **Internal shell buttress**: (0) absent; (1) present (L= 2, I= 50, RI= 80) (Fig. 2C). According to Morton (1976), extending from the resilifer of each valve in a posteroventral direction are the clavicles, or buttresses, which follow the umbonal ridge and act as strengthening struts to the shell. These are usually seen in representatives of the Laternulidae and Periplomatidae and have, more recently, been described for *C. insignis* (Morton 2002). New character.

5- **Posterior rostrum**: (0) absent; (1) present (L= 2, CI= 50, RI= 92) (Figs 3F, H, J, 4E, F, H, I, 5A-D, F-I). Unique among the Bivalvia, the rostrum is a posterior extension of the shell valves secreted by the mantle that usually house the siphons when they contract. New character.

6- **Hinge teeth**: (0) edentulous; (1) secondary teeth; (2) schizodont type (L= 14, CI= 14, RI= 58). The presence of secondary teeth is not an exclusive to some representatives of the Anomalodesmata but, herein, this character is most common, for example, the crura/cru structure in species of *Pandora* (Pandoridae) or the cardinal-like tooth in species of *Dilemma* (Poromyidae) (Figs 1H, 4D). Here, as with Bieler *et al*. (2014, character 24), anomalodesmatan secondary teeth are interpreted as being non-homologous structures to the cardinal and lateral teeth present throughout the other taxa of the Bivalvia. The state ‘2’ was also considered here as a secondary dentition type, but exclusive of the species *Neotrigonia margaritacea* (Lamarck, 1804) (Morton 1987: fig. 14). *Carditamera floridana* Conrad, 1838 that has heterodont (cardinal and lateral) hinge teeth was coded as ‘not applicable’. This character was coded by Giribet & Wheeler (2002, character 41).

7- **Denticulate structure(s) located below the umbones**: (0) absent; (1) present (L= 5, CI= 20, RI= 42) (Figs 3D-E, 4C-D). This denticulate structure is non-homologous with cardinal teeth but are similar in position. Species either without teeth (edentulous) or with heterodont hinge teeth were coded as ‘not applicable’. New character.

8- **Denticulate structure(s) located lateral to the umbones**: (0) absent; (1) present (L= 6, CI= 16, RI= 50) (Figs 2A, 3E). This denticulate structure is non-homologous with lateral teeth but are similar in position. Species either without teeth (edentulous) or with heterodont hinge teeth were coded as ‘not applicable’. New character.

9- **Primary ligament**: (0) external; (1) internal; (2) absent at least in the adult (L= 5, CI= 40, RI= 50). This character was initially coded by Harper *et al*. (2000, character 9), but here a state ‘2’ was included in the analyses due to the absence of a primary ligament in adult individuals of *Bryopa aligamenta* (Morton 2005).

10- **Ligament and resilifer**: (0) not sunken; (1) simple sunken; (2) sunken between chondrophore; (3) sunken between coiled chondrophore (L= 8, CI= 37, RI= 82) (Fig. 2B-C). Some observations: not sunken = species without resilifer; simple sunken = ligament inserted in a shallow resilifer; sunken between chondrophores = ligament inserted into a usually deep and spoon-shaped resilifer; sunken between coiled chondrophores = ligament inserted in a deep and coiled spoon-shaped resilifer (exclusively coded for the coiled shell of *Cleidothaerus albidus*). This character was coded by Harper *et al*. (2000, character 10).

11- **Secondary ligament**: (0) thin; (1) thick (L= 7, CI= 28, RI= 73) (Fig. 2I). All anomalodesmatans reviewed in this study have a secondary ligament or a periostracal ‘ligament’. According to Yonge (1982), a secondary ligament of fused periostracum assists in the alignment of the edentulous shell valves. The term ‘fused periostracum’ was established by Owen (1958) although, since periostracum must cover the entire shell, there is no fusion. The term is nevertheless retained herein simply to emphasise that it is present and, thus, even those species with secondary teeth have a ‘secondary ligament’ although such structures may not function as such. This character was coded by Harper *et al*. (2000, character 11).

12- **Lithodesma**: (0) absent; (1) present in adult individuals; (2) present in only juvenile individuals (L= 5, CI= 40, RI= 81) (Figs 1I, 2A, C). The lithodesma is a solid structure, like an ossicle, formed by calcification of the medial portion of the internal ligament layer (Yonge 1976). This character was also coded by Harper *et al*. (2000, 2006, characters 12 and 4, respectively). Herein, it has also been included as state ‘2’ based on observations made on *Thracia meridionalis* and *Thracia phaseolina* (Lamarck, 1818) by Sartori & Domaneschi (2005) and Sartori & Ball (2009), respectively.

General features of the mantle

13- **Ventral mantle fusion (Yonge 1982)**: (1) Type A; (1) Type B; (2) Type C (L= 7, CI= 28, RI= 66). This character was coded by Harper *et al*. (2000, character 13).

14- **Arenophilic glands and their secretions**: (0) absent; (1) present only on the posterior valve surfaces; (2) present over entire valve surfaces; (3) present only over the siphons; (4) present both on the posterior surface of the shell valves and the siphons (L= 13, CI= 30, RI= 67) (Figs. 2I, 4G). This character was coded by Harper *et al*. (2000, character 14) and more recently by Bieler *et al*. (2014, character 46). Herein, a state '4' has been added to accommodate some species that had arenophilic gland secretions on the surface of the posterior shell rostrum and on the outer surface of the siphonal sheath, for example, *Cardiomya cleryana* (d' Orbigny, 1842) (Machado *et al*. 2016). *Pholadomya candida* G. B. Sowerby I, 1823 was codified here as '1' based in the observations made by Sartori & Harper (2009: fig. 1a-c) and more recently by the photographs of a specimen collected alive from the shallow waters of the National Natural Park Tayrona on the Colombian Caribbean coast by Juan M. Díaz, CoML Caribbean's Colombian country co-ordinator.

15- **Fourth pallial aperture**: (0) absent; (1) present in adults; (2) present only in juvenile individuals (L= 8, CI= 25, RI= 60) (Figs 6, 7). Located close to the base of the siphons, the fourth pallial aperture probably has the function of facilitating the elimination of pseudofaeces from the interior of the pallial cavity or functions as a pressure release valve through which some of the mantle water is ejected following rapid adduction in fast-burrowing bivalves (Atkins 1937, Yonge 1952b). This character was coded by Harper *et al*. (2000, 2006, characters 14 and 5). Herein a state '2' has been added to accommodate two species, that is, *Brechites attrahens* (Lightfoot, 1786) – new combination for *Brechites vaginiferus* (Lamarck, 1818), and *Humphreyia strangei* (A. Adams, 1854) (Morton 1984b, 2002b: fig. 8). The fourth pallial aperture is also seen in representatives of a few non-anomalodesmatan taxa, for example, *Spisula*, *Lutraria* (Imparidentia, Mactridae), *Ensis* (Pharidae), *Tagelus* (Solecurtidae) and *Siliqua* (Solenidae), but the homology between these structures has never been tested (Yonge 1948, 1952b; Morton 2010).

Siphonal characters

16- **Siphons**: (0) absent; (1) present (L= 1, CI= 100, RI= 100). The state '0' was used to code the out-group species that do not have siphons or that have only an inhalant aperture and exhalant siphon. According to Morton (1987), for example, *Neotrigonia margaritacea* (out-group) has two functional 'siphons', which are not, however, morphologically delineated by pallial fusions. This character was coded by Giribet & Wheeler (2002, character 55) and Bieler *et al*. (2014, character, 63).

17- **Siphon length**: (0) pallial sinus absent; (1) pallial sinus present but does not extend beyond the mid dorsal-ventral axis of the shell; (2) pallial sinus present and extends beyond the mid dorso-ventral axis of the shell (L= 6, CI= 33, RI= 82) (Fig. 2A, B, H). This character was coded by Harper *et al*. (2000, character 16), Giribet & Wheeler (2002, character 25) and Bieler *et al*. (2014, character, 8).

18- **Siphonal separation**: (0) separated; (1) fused (L= 3, CI= 33, RI= 85). Here the inhalant and exhalant siphons were coded as ‘separated’ when completely unfused or partially fused (about 1/3 of it extension free). Siphons were considered ‘fused’ when they are fused completely (90 to 100% fusion when fully extended). This character was coded by Harper *et al*. (2000, character 17)

19- **Symmetrical relationship between inhalant and exhalant siphons**: (0) similar in shape and size; (1) different in shape and size (L= 2, CI= 50, RI= 96) (Figs 6, 7). This character was coded by Harper *et al*. (2000, character 18) but has been re-interpreted herein.

20- **Siphons encased in a periostracal sheath**: (0) absent; (1) present (L= 2, CI= 50, RI= 90) (Figs 2C, 6). New character.

21- **Siphons encased in a tissue sheath**: (0) absent; (1) present. (L= 1, CI= 100, RI= 100) (Fig. 7). This tissue or siphonal sheath is a tubular muscular extension of the outer fold of the left and right mantle lobes that extend from the posterior end of the septum into the rostrum, consequently surrounding, protecting and camouflaging the siphons. This structure probably promotes support and help in guiding the siphons during protraction (Machado *et al*. 2016: see supplementary material). Usually associated with the presence of modified siphons for prey capture, this muscular sheath is only seen in species of the Cuspidarioidea, for example, *Cardiomya cleryana* (d’Orbigny, 1842). New character.

22- **Siphonal fusion type (Yonge 1982)**: (0) Type A; (1) Type B; (2) Type C (L= 6, CI= 33, RI= 84). This character was coded by Harper *et al*. (2000, character 19).

23- **Ciliary sense organs**: (0) absent; (1) present (L= 5, CI= 20, RI= 80) (Fig. 7). Associated to the siphonal tentacles of some bivalves, these sensory organs are more common found in carnivorous bivalves probably receptive to the vibrations made by their potential prey. These organs have, however, also been identified in non-carnivorous species probably helping them to control the input of water into the mantle cavity. This character was coded by Harper *et al*. (2000, character 20).

24- **Siphonal tentacles**: (0) absent from both siphons; (1) present on the exhalant siphon only; (2) present on the inhalant siphon only; (3) present on both (L= 5, CI= 40, RI= 40). This character was coded by Harper *et al*. (2000, character 21).

25- **Siphonal tentacles (position)**: (0) on siphonal apertures; (1) on siphonal base; (2) present on both (L= 2, CI= 100, RI= 100). Species without siphonal tentacles were coded as 'not applicable', for example, *Parilimya fragilis* (Grieg, 1920). New character.

26- **Siphonal tentacles (4I + 3E arrangement)**: (0) present; (1) absent (L= 2, CI= 50, RI= 92). This arrangement is typical of the Cuspidariidae and is characterised by four ventral tentacles around the inhalant siphon and three dorsal ones around the exhalant siphon (Machado *et al*. 2016: fig. 4B). Species without siphonal tentacles were coded as 'not applicable'. New character.

27- **Branched siphonal tentacles**: (0) absent; (1) present (L= 1, CI= 100, RI= 100). Characteristic of species of the Lyonsiellidae and Verticordiidae, these siphonal tentacles have either a fringing or arborescent appearance usually associated with the presence of a large number of gland cells and haemocoelomic spaces (Allen & Turner 1974: figs 57 & 75). Species without siphonal tentacles were coded as 'not applicable'. New character.

28- **Ectopic pallial eyes**: (0) absent; (1) present (L= 1 UNINF) (Fig. 6). Arranged in a circle around the siphons, ectopic eyes comprise a cornea, sclerotic coat and a double retina. Hitherto only recorded for *Laternula truncata* (Lamarck, 1818) (Morton 1973: fig. 4; Adal & Morton 1973: fig. 1). Species without siphons were coded as 'not applicable'. New character. (UNINF= uninformative character, this character is an autapomorphy).

29- **Papillate tentacles covering the surface of the siphons**: (0) absent; (1) present (L= 1, CI= 100, RI= 100) (Fig. 6). These are typical of some species of the Lyonsiidae, for example, *Entodesma navicula* (Adams & Reeve, 1950) (Yonge 1952a: figs 5 & 6; Morton 1985: fig. 8). New character.

30- **Exhalant inter-tentacular margin**: (0) free; (1) with projections (L= 4, CI= 25, RI= 0). Also called 'inter-tentacular projections web' or 'projections between the dorsal exhalant tentacles' by Knudsen (1970: fig. 92C) and Krylova (1993: fig. 1D, F-H), respectively, this structure is formed on the middle mantle folds. The function of these exhalant inter-tentacular projections is little understood although observations of living individuals of *C. cleryana*, Machado *et al*. (2016: fig. 4C) suggested three possible hypotheses to explain their function: (i), extra sensory structures assisting the siphonal tentacles in detecting potential prey; (ii), replacing damaged siphonal tentacles and/or (iii), assisting in the closure of the exhalant opening preventing unwanted particles from entering the supraseptal chamber. Species either without siphonal tentacles or with tentacles only on the inhalant siphon were coded as 'not applicable'. New character.

31- **Inhalant siphon**: (0) long, simple tube shaped; (1) short to long, cone shaped; (2) *Parilimya* type; (3) extensible type; (4) raptorial type (L= 5, CI= 80, RI= 96). Siphons coded as long and tube shaped represent the typical bivalve form, for example, the inhalant siphon of *Thracia meridionalis* Smith, 1885. Siphons coded as cone shaped represent a type of short inhalant siphon observed in some species of carnivorous bivalves, for example, *Lyonsiella abyssicola* (G. O. Sars, 1872). The *Parilimya* type corresponds to the extensible inhalant siphon with an internal posterior membranous tongue. This siphon type has only been identified in *P. fragilis*. The state '3', extensible type, is proposed here to represent a typical form of inhalant siphon observed in the majority of Cuspidariidae species, for example, *Cardiomya costellata* (Deshayes, 1835). Although it is also considered an extensible inhalant siphon, the raptorial, or hood-like, form represents a modification only seen in active predatory species, for example, the raptorial inhalant siphon of *Allograma formosa* (Jeffreys, 1881). This type of raptorial siphon encompasses those species that possesses a 'siphonal cowl' or 'inner valve', formerly interpreted by Allen & Turner (1974) as a tongue of tissue attached peripherally to the inside of the lateral and ventral walls of the inhalant siphon extending into the mantle cavity forming a broad channel that assists in the transport of food from the inhalant aperture to the mouth, that is, a structure associated with the inhalant siphon. Herein, and according to Morton (1981b), the raptorial siphon type was interpreted as being the inhalant siphon itself that, in most cases, in fixed specimens, is inverted into the mantle cavity. This character was coded, in part, by Harper *et al*. (2000, character 22).

Musculature

32- **Adductor musculature**: (0) present; (1) absent (L= 3, CI= 33, RI= 0). Herein, state '1' refers to the species where the adults are amyarian, for example, *Brechites attrahens* (Lightfoot, 1786) (Morton 1984). New character.

33- **Relationship between the adductor muscles**: (0) isomyarian; (1) anterior larger; (2) posterior larger (L= 8, CI= 25, RI= 40) (Fig. 6). This character was used by Harper *et al*. (2000, character 24). Species that do not have adductor muscles as adults were coded as 'not applicable'.

34- **Pedal gape muscles**: (0) normal; (1) *Pholadomya* type (Morton 1980) (L= 1 UNINF). According to Morton (1980), the pedal gape of *Pholadomya candida* possesses a unique pedal gape musculature resembling the cruciform muscles of the Tellinoidea. According to this author, these muscles provide the species with an exclusive (to the Anomalodesmata) type of feeding, that is, feeding *via* the pedal gape in the nutrient poor waters of the Caribbean. This character was used by Harper *et al*. (2000, character 25). (UNINF= uninformative character, autapomorphy).

35- **Pedal musculature**: (0) normal; (1) reduced; (2) absent (L= 11, CI= 18, RI= 40). This character was coded by Harper *et al*. (2000, character 26).

36- **Pedal disc**: (0) absent; (1) present (L= 1, CI= 100, RI= 100) (Fig. 6). This structure is a muscular thickening of tissues surrounding the pedal gape with the prime function of pumping interstitial water into and out of the mantle cavity *via* the pedal gape to generate the hydrodynamic pressures necessary in the pallial haemocoels to extend the siphons following retraction (Morton 1984b, 2002b) and to facilitate reburial (Purchon 1956). New character.

37- **Taenioid muscles**: (0) absent; (1) present; (2) present, very reduced (L= 4, CI= 50, RI= 0) (Figs 6, 7). This pair of muscles are elongate siphonal retractors that have separate insertions on the shell valves. In *P. fragilis,* for example, they serve to pull the inhalant siphon into the mantle cavity (Morton 1982). Herein, *Bentholyonsia teramachii* (Habe, 1952) was coded as ‘1’, based exclusively in the presence of shell scars located on each valve, internal to the anterior adductor and pedal retractor muscle scars (Morton 2003: fig. 2F). This character was coded, in part, by Harper *et al*. (2000, character 27).

Visceral mass

38- **Mouth**: (0) tube-shaped, usually reduced; (1) funnel-shaped, usually large (L= 2, CI= 50, RI= 96). Among the Bivalvia, the most common mouth form is tube-shaped. With a small buccal aperture, this mouth form is usually associated with a thin and ciliated oesophagus. This mouth shape is also associated with suspension and deposit-feeding species, for example, *Lyonsia californica* Conrad, 1837 (Narchi 1968: fig. 6). The large funnel-shaped mouth facilitates the ingestion of large prey and is usually associated with a short, muscular and non-ciliated, oesophagus; this mouth form is commonly observed in carnivorous bivalves, for example, *Cardiomya knudseni* (Allen & Morgan, 1981) (Allen & Morgan 1981: figs 5 & 32). New character.

39- **Labial palps**: (0) absent; (1) present; (2) present, but reduced (L= 1, CI= 100, RI= 100) (Figs 6, 7). The state ‘0’ was coded to include members of the Spheniopsidae. This character was coded by Bieler *et al*. (2014, character 136).

40- **Labial palps (type)**: (0) lamellate (with sorting ridges); (1) non-lamellate (either reduced or non-sorting ridges) (L= 1, CI= 100, RI= 100) (Figs 6, 7). *Parilimya fragilis* was coded as ambiguous ‘0/1’ in presenting a condition intermediate between the lamellate palps of *Pholadomya* and the non-lamellate palps of some families of carnivorous bivalves such as, for example, representatives of the Cuspidariidae and Poromyidae (Morton 19821: figs 26 & 27). New character.

41- **Anterior and posterior labial palps**: (0) symmetrical (typical bivalve plan); (1) slightly asymmetrical; (2) anterior pair reduced and posterior pair well developed; (3) anterior pair well developed and posterior pair reduced; (4) medially fused into a pouch/buccal sac (L= 6, CI= 66, RI= 91). New character.

42- **Ctenidia**: (0) plicate, usually large, vertically aligned, eulamellibranch condition (Ridewood 1903); (1) non-plicate, usually reduced, horizontal aligned (typical Lyonsiellidae condition); (2) modified into a thick septum (typical septibranch condition); (3) reduced and associated with a thin septum (intermediate condition); (4) modified into a membranous septum (pseudoseptal condition, Krylova 1995: figs 1D, 3A, 5C); (5) *Neotrigonia* type (intermediate fillibranch condition). (L= 7, CI= 85, RI= 96) (Figs 6, 7). This character was also coded, in part, by Harper *et al*. (2000, characters 29 and 30), Giribet & Wheeler (2002, characters. 64, 65 and 68) and Bieler *et al*. (2014, character 149), but herein these are gathered into the same character representing the five possible morphological types of ctenidia characteristic of the Anomalodesmata.

43- **Septum perforated by isolated pores**: (0) absent; (1) present (L= 1, CI= 100, RI= 100) (Fig. 7). Species without a septum were coded as ‘not applicable’. New character. (Allen & Morgan 1981: figs. 3, 22 & 24; Morton *et al*. 2016: figs. 2B, E & 11A).

44- **Septum with branchial sieves (with inter-filamentar connections)**: (0) absent; (1) present (L= 1, CI= 100, RI= 100) (Fig. 7). Species without a septum were coded as ‘not applicable’. New character. (Allen & Morgan 1981: figs 71 & 74).

45- **Septum perforated by grouped pores (without inter-filamentar connections)**: (0) absent; (1) present (L= 2, CI= 50, RI= 50) (Fig. 7). Species without a septum were coded as ‘not applicable’. New character. (Allen & Morgan 1981: fig. 86; Leal 2008: figs. 52, 58).

Alimentary tract

46- **Stomach type (Purchon 1956)**: (0) stomach Type II; (1) stomach Type IV (L= 1, CI= 100, RI= 100). Herein, stomach Type II was characterized by the presence of thick muscular folds, an oval to elongate-oval chamber (with a posterior extension), extensive protective chitinous lining, reduced crystalline style sac, sorting areas either reduced or absent, few openings (two or three) to the digestive diverticulae and usually associated with a large, muscular and non-ciliated, oesophagus; and of Purchon’s type IV, with an oval dorsal chamber elongated ventrally, an absence of muscular folds, well development crystalline style sac, the presence of sorting areas, numerous ducts to the digestive diverticulae and usually associated with a narrow, ciliated, oesophagus. This character was used, in part, by Harper *et al*. (2000, 2006: characters 33 and 8, respectively). *Parilimya fragilis* (Grieg, 1920) has been coded in our matrix as ambiguous '0/1', since it represents a stomach considered morphologically intermediate between types II and IV (Purchon 1987, 1990; Temkin & Strong 2013).

47- **Style sac**: (0) free; (1) conjoined with mid gut (L= 3, CI= 33, RI= 81). This character was coded in a different way by Bieler *et al*. (2014, character 123).

48- **Rectum**: (0) located above heart; (1) located below heart; (2) penetrates heart (L= 4, CI= 50, RI= 0). This character was coded by Harper *et al*. (2000, character 35).

49- **Rectum**: (0) passes over kidneys; (1) penetrates kidneys (L= 10, CI= 10, RI= 35). This character was coded by Harper *et al*. (2000, character 36).

50- **Accessory excretory organ**: (0) absent; (1) present (L= 1 UNINF). According to Morton (1980: fig. 19), this organ is a branched appendage flanked ventrally and posteriorly by two small papillae and located on the posterior border of the visceral mass. The presence of densely packed amoebocytes in the interior of this structure suggested a possible excretory function. New character. (UNINF= uninformative character, this feature is an autapomorphy for the Pholadomyidae).

51- **Waste storage pouch**: (0) absent; (1) present (L= 1 UNINF). Located in the left posterior end of the stomach, this pouch has the function of storing exoskeletal remains of ingested prey (Morton *et al*. 2016: figs. 2H, 18-20). New character. (UNINF= un-informative character, this character is an autapomorphy).

Nervous system

52- **Opisthopodium**: (0) absent; (1) present, bifurcated; (2) present, non-bifurcated (L= 2, CI= 100, RI= 100) (Fig. 6). This organ is a posterior slender process ('elongated appendage') arising from the postero-ventral edge of the visceral mass and is formed mainly by nerve bundles, indicating a possible sensory function (Morton 1980). Two different morphological shapes of this organ have been identified: (i) either enlarged at the base and bifurcated at the tip as shown, for example, in *Pholadomya candida* Sowerby, 1863 or (ii) enlarged at the base and non-bifurcated at the tip as shown, for example, in *Halicardia nipponensis* Okutani, 1967 (Nakazima 1967: plates VI-3, VII-3, VIII-1,2). According to Dall (1895), the ophisthopodium observed in *Halicardia flexuosa* (Verrill & S. Smith [in Verrill], 1881) is analogous to the structure described initially by Owen (M.S) for *Pholadomya* (Morton 1985: pp. 86-95). Herein, they are considered to be homologous structures. New character.

53- **Statocyst (Morton 1985)**: (0) absent; (1) type A; (2) type B1; (3) type B2; (4) type B3; (5) type C (L= 10, CI= 50, RI= 72). This character was used by Harper *et al*. (2000, character 37), but herein the state 'absent' is used to include certain species of tube builders, for example, *Stirpulina ramosa* (Dunker, 1882) (Morton 2013).

Reproductive biology

54- **Expression of sexuality**: (0) dioecious; (1) hermaphrodite (L= 5, CI= 20, RI= 69). Herein, character state '1' is considered to represent both hermaphrodite types, simultaneous and consecutive. This character was coded by Harper *et al*. (2000, character 39).

55- **Gonadial apertures**: (0) united; (1) separated; (2) united with the urinary ducts (L= 7, CI= 28, RI= 68). This character was coded by Harper *et al*. (2000, character 40).

56- **Fertilized eggs incubated in the visceral mass**: (0) absent; (1) present (L= 1, CI= 100, RI= 100) (Fig. 7). This feature has been identified only for *Grippina coronata* Machado & Passos, 2015 (Spheniopsidae) by Morton *et al*. (2016a: fig. 2G, H). New character.

Life style characters

57- **Cementation to the substratum by glandular secretions**: (0) absent; (1) present, *via* mantle margin glands; (2) present, *via* siphonal glands; (3) present, *via* sticky outer periostracal layer (L= 4, CI= 75, RI= 0). Knowledge of the production site for the cementing substance is unresolved in such anomalodesmatans, although some hypotheses have been discussed by Harper & Morton (2000), Morton & Harper (2001) and Morton (2007) and herein these have been considered to code this character. This character was coded by Giribet & Wheeler (2002, character 53) and Bieler *et al*. (2014, character 21) but is re-interpreted herein.

58- **Calcareous adventitious tube/crypt**: (0) absent; (1) present (L= 1, CI= 100, RI= 100). According to Morton (2004c), calcareous adventitious tube formation probably results from the mantle epithelium that secretes it. This character was coded for the first time by Harper *et al*. (2000, character 42) and later by Giribet & Wheeler (2002, character 136) and Bieler *et al*. (2014, character 20).

59- **Post-larval byssus**: (0) absent; (1) anchorage *via* a single byssal thread; (2) anchorage *via* more than one byssal thread (L= 11, CI= 18, RI= 25) (Fig. 7). This character was coded, in part, by Harper *et al*. (2000, character 43).

60- **Water input (feeding mode):** (0) *via* siphons; (1) *via* pedal gape (L= 1, CI= 100, RI= 100). Possible path of water from the external environment to the interior of the mantle cavity (Morton, 1980: fig. 57). New character.

61- **Digging**: (0) active burrower; (1) passive burrower (sedentary life); (2) non-borrower (tube-builder/cemented/endolithic) (L= 7, CI= 28, RI= 85). New character.

Phylogenetic analyses

The results of all analyses (equal and implied weighting) are summarized in Table 2. The final cladogram obtained from the results of these analyses (Figs. 8, 9) shows the strict consensus tree obtained under implied weighting analysis with K-values of 7.319, 9.137, 11.911, 16.667 and 26.708. Preference for this topology, which has been used to discuss phylogenetic relationships, is due to it being the most consistent cladogram resulting from sensitivity analysis implemented with TNT (script aaa.run).

Herein, the description of results is subdivided into two main elements: the non-carnivorous lineages, composed by three different clades, and a single carnivorous lineage (Figs. 8, 9). The analysis described herein recovered the Anomalodesmata as monophyletic, supported by the following synapomorphies: the presence of siphons (character 16, non-ambiguous), hermaphroditism as the main expression of sexuality (54, ambiguous), united gonadial apertures (55, ambiguous) and passive burrowing sedentary life-style (61, ambiguous).

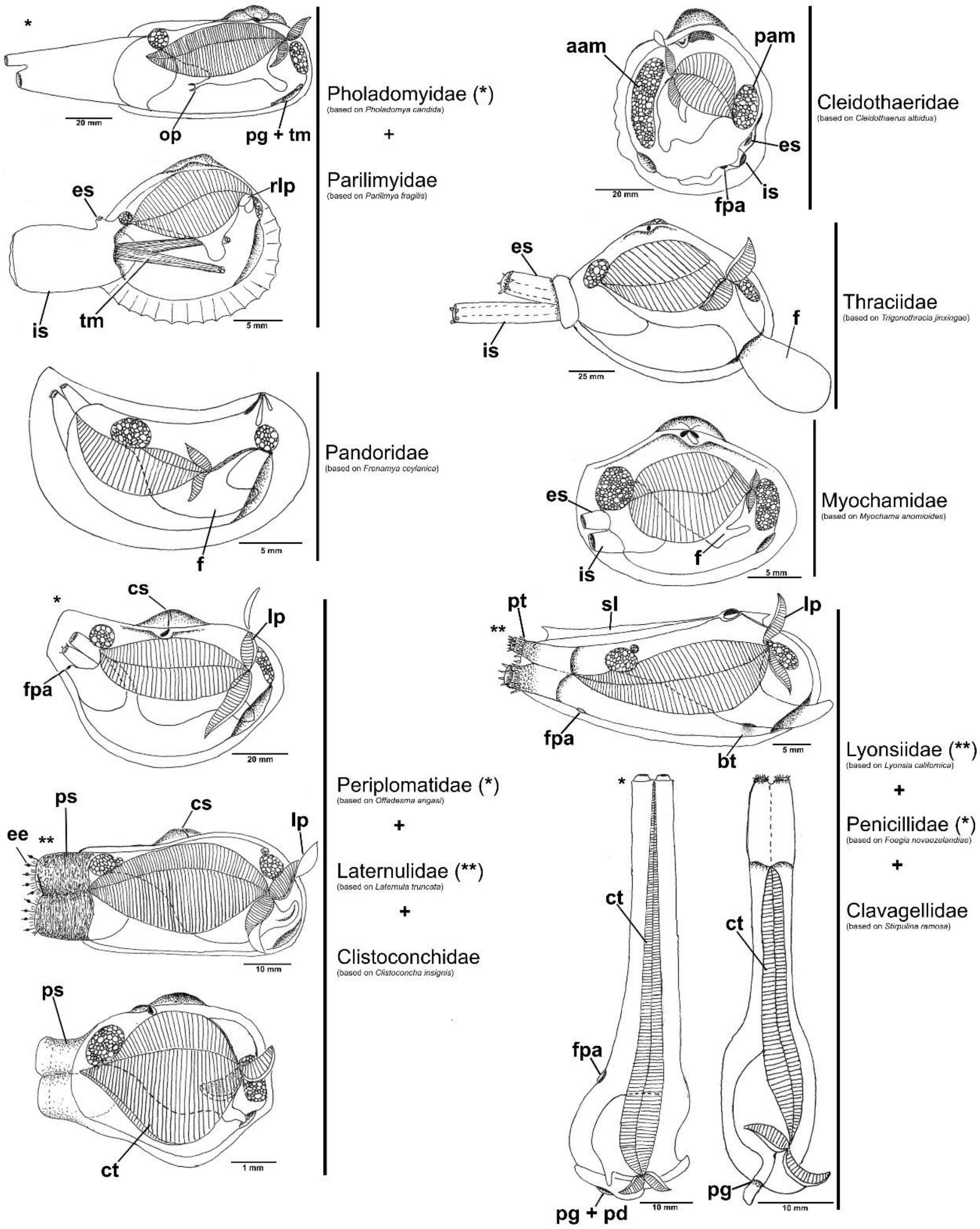


Figure 6. Illustrations of some anatomical features of the 'non-carnivorous' lineages, showing all families and sister groups analysed in this paper. In clades formed by either two or more families, the presence of the symbol (*) identifies the illustration and its respective family. All drawings were based on species already illustrated previously by Brian Morton that in some cases were re-designed either to show new features or to highlight a specific composition of characters of the same sister group. Abbreviations: aam, anterior adductor muscle; ct, ctenidia; cs, crack in the shell; ee, ectopic eyes; es, exhalant siphon; f, foot;

fpa, fourth pallial aperture; is, inhalant siphon; lp, lamellate labial palps; op, ophistopodium; pam, posterior adductor muscle; pg + pd, pedal gape + pedal disc; pg + tm, pedal gape + taenioid muscle; ps, periostracal sheath; pt, papillate tentacles; rlp, reduced labial palps; sl, secondary ligament; tm, taenioid muscle.

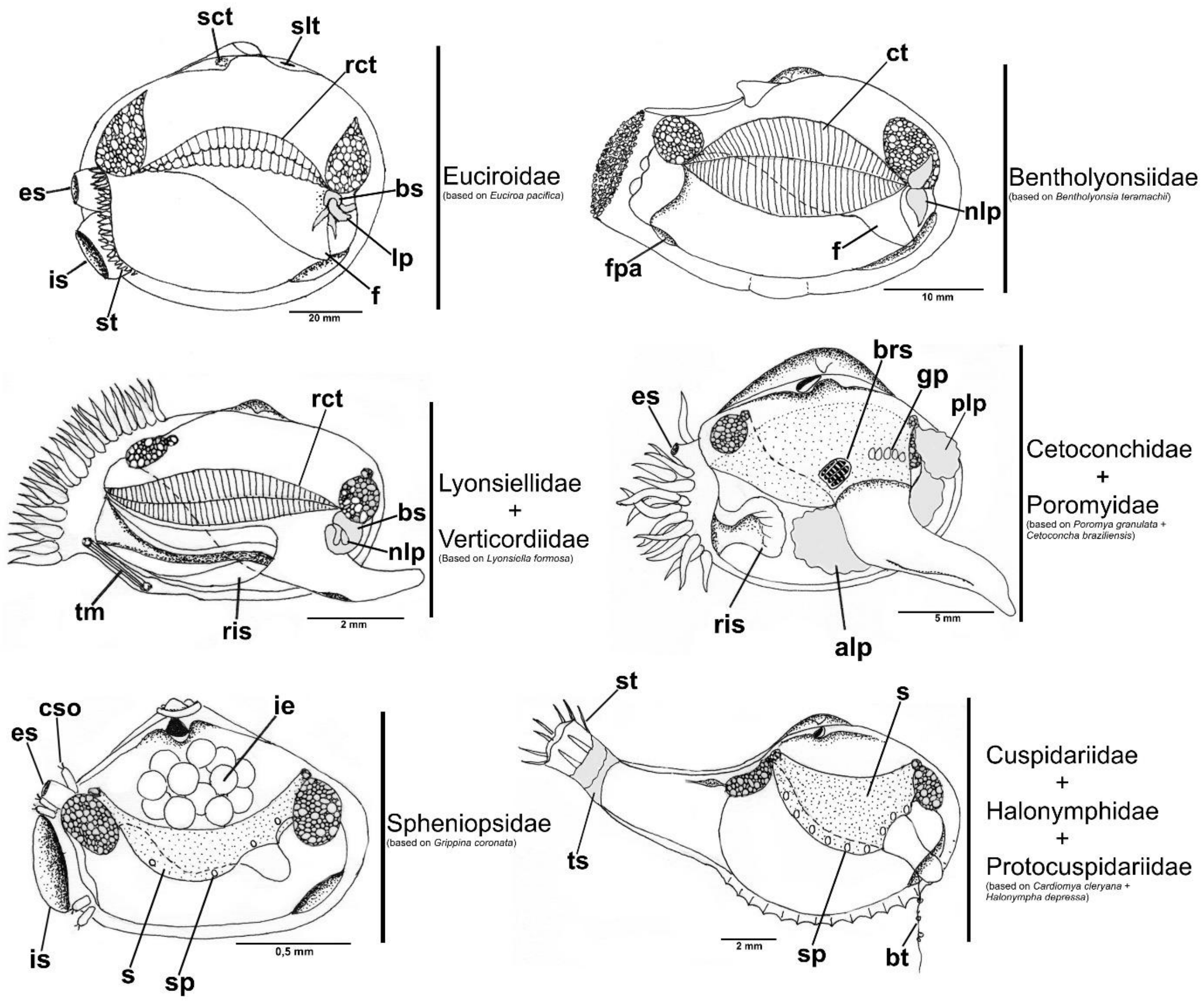


Figure 7. Illustrations of some anatomical features of the ‘carnivorous’ lineage, showing all families and sister groups analysed in this paper. All drawings were based on species already illustrated previously by Brian Morton that in some cases were re-designed either to show new features or to highlight a specific composition of characters of a same sister group (ex. Poromyidae + Cetoconchidae), except for the Euciroidae illustration that was based on the drawings of Dall (1895, plate. XXIII, figs 2 & 4 and XXIV figs 4, 5, 7 & 8) for *Euciroa pacifica* and the description provided by Poutiers & Bernard (1995, pp 116-118) for *Euciroa eburnea*. Abbreviations: alp, anterior labial palp; bt, byssal thread; bs, branchial sieve (with inter-filamentar connections); bcs, buccal-sac; cso, ciliary sense organs; es, exhalant siphon; fpa, fourth pallial aperture; gp, grouped pores (without inter-filamentar connection); ie, incubated eggs in the visceral mass; is, inhalant siphon; lp, labial pals; nlp, non-lamellate labial palp; plp, posterior labial palp; rct, reduced ctenidia; ris, retracted inhalant siphon; s, septum; sct, secondary cardinal tooth; slt, secondary lateral tooth; sp, septal pore; st, siphonal tentacle; tm, taenioid muscle; ts, tissue sheath.

Table 2. Results obtained from the trees identified from the herein undertaken analysis of the anomalodesmatan characters identified for all studied taxa and described and discussed in this research paper. **EW**= Equal weighted; **IW**= Implied weighting (k=values); **MPTs**= Most parsimonious trees; **Ci/Ri**= Consistency index/Retention index in the consensus trees.

Analyses	MPTs	Length (MPTs/consensus)	Ci/Ri (MPTs)	Total fit	Percentage weight of characters
EW	3978	255/311	0.41/0.80	-	-
IW (k0= 3.280)	60	-	0.40/0.79	18.596	70
IW (k1= 3.758)	16	-	0.40/0.80	17.491	72.778
IW (k2= 4.345)	16	-	0.40/0.80	16.277	75.556
IW (k3= 5.082)	10	-	0.40/0.80	14.985	78.333
IW (k4= 6.036)	10	-	0.40/0.80	13.600	81.111
IW (k5= 7.319)	12	-	0.41/0.80	12.102	83.889
IW (k6= 9.137)	12	-	0.41/0.80	10.486	86.667
IW (k7= 11.911)	12	-	0.41/0.80	8.731	89.444
IW (k8= 16.667)	12	-	0.41/0.80	6.802	92.222
IW (k9= 26.708)	12	-	0.41/0.80	4.655	95

The non-carnivorous anomalodesmatans

Herein, the sister group of all other anomalodesmatans, the nested Pholadomyidae + Parilimyidae, was recovered in 100% of all implied weighting analyses and is supported by the following synapomorphies: the presence of an external primary ligament (9, ambiguous); arenophilic gland secretions on the posterior valve surface (14, ambiguous); a fourth pallial aperture (15, ambiguous) and a pallial sinus that does not extend beyond the mid dorso-ventral axis of the shell (17, ambiguous).

The Pholadomyidae, represented here only by *Pholadomya candida*, is supported by five apomorphies, that are: a pedal gape musculature of the *Pholadomya* type (34); an accessory excretory organ (50); a rectum passing below the heart (48, ambiguous); a bifurcated opisthopodium (52, non-ambiguous) and statocysts of Type A (53, ambiguous). In turn, the also monotypic Parilimyidae, represented by *P. fragilis*, is supported by six apomorphies: the absence of hinge teeth (6, ambiguous), non-symmetrical siphons (19, ambiguous), a *Parilimya* type inhalant siphon (31, ambiguous), the absence of a pedal musculature (35, ambiguous), a funnel-shaped mouth (38, non-ambiguous) and slightly asymmetrical labial palps (41, ambiguous). The grouping between these two families, recovering the taxonomic superfamily Pholadomyoidea and its consequent topology as sister-group to all other anomalodesmatans, as previously argued for, on the basis of its fossil history, by Pojeta (1971), Runnegar (1974), Morton (1985a) and Harper *et al*. (2000, 2006).

In addition, the new topology observed for *P. fragilis* did not recover it as a carnivorous member, as initially proposed by Morton (1982). The sharing of some morphological features with the Pholadomyidae, however, such as: the presence of taenioid muscles (37, ambiguous), a fourth pallial aperture (15), arenophilic secretions on the posterior valve surfaces (14) and the absence of siphonal tentacles (24, ambiguous), suggests the current topology for the Parilimyidae. However, it should be noted that the presence of two characters exclusively associated with the carnivorous anomalodesmatans, such as non-lamellate labial palps (40, ambiguous) and a stomach of Type II (46, ambiguous), were coded in our matrix as ambiguous '0/1' for *P. fragilis* and, thereby, probably, reinforcing a closer proximity to the Pholadomyidae. There is no direct evidence that *P. fragilis* is carnivorous such as, for example, the presence of prey items inside the stomach although the species' funnel shaped labial palps would suggest this strongly (Morton 1982: figs. 26, 27).

The monophyletic Pandoridae was recovered in 100% of all sensitivity analyses and this node is supported by the one synapomorphy: a rectum that penetrates the kidney (49, ambiguous). Usually recovered as a sister group of the Lyonsiidae in the most recent molecular phylogenetic researches (Harper *et al.* 2006; Bieler *et al*. 2014; Combosh *et al*. 2017), this family appears herein as an independent clade among the non-carnivorous lineages of Anomalodesmata.

The cladogram brought together the other nine families of the non-carnivorous lineages, and are supported mainly by the combination of two ambiguous synapomorphies: a pallial sinus that does not extend beyond the mid dorso-ventral axis of the shell (17) and siphonal fusions of type A (22, ambiguous).

One branch of the large non-carnivorous clade is formed by three families, the paraphyletic Periplomatidae with the Laternulidae + Clistoconchidae. The node that united these families is supported by two non-ambiguous synapomorphies: the presence of a dorsal crack in the shell (3) and an internal shell buttress (4). For the Periplomatidae, specifically, the non-monophily is explained mainly by the difference in the valve equality (2) between *Offadesma angasii* (Crosse & P. Fischer, 1864) and *Cochlodesma* *praetenue* (zero branch length), with a slightly inequivalve shell in the latter. In addition, *O. angasii* demonstrates other differences in relation to *C. praetenue* such as: the presence of a strongly inequivalve shell (2), a ventral mantle fusion of Type A (13, ambiguous), arenophilic secretions on the posterior valve surfaces (14), presence of a fourth pallial aperture (15), siphonal tentacles only on the inhalant siphon (24) and a rectum that penetrates the kidneys (49).

The well supported grouping between the paraphyletic Laternulidae and the monotypic Clistoconchidae is supported by the following synapomorphies: the presence of fused siphons (18, ambiguous), siphons encased in a periostracal sheath (20, ambiguous), fused siphons of Type C (22) and gonadial apertures united with the urinary ducts (55). Herein, for the first time, we provide a phylogenetic analysis based on morphological data obtained by Morton (2012) for the Clistoconchidae. Our analysis shows a strong affinity between the Clistoconchidae and Laternulidae (a topology recovered in all implied and equal weight analysis), a somewhat different relationship to that hypothesised by Morton (2012) who suggested an affinity between the Clistoconchidae and Thraciidae. In addition, a clear sub-division between two species of Laternulidae, that is, *Laternula elliptica* (King, 1832) and *Laternula truncata* (Lamarck, 1818) (Fig. 8*), is caused mainly by the absence of a lithodesma in the former and by its presence in the latter.

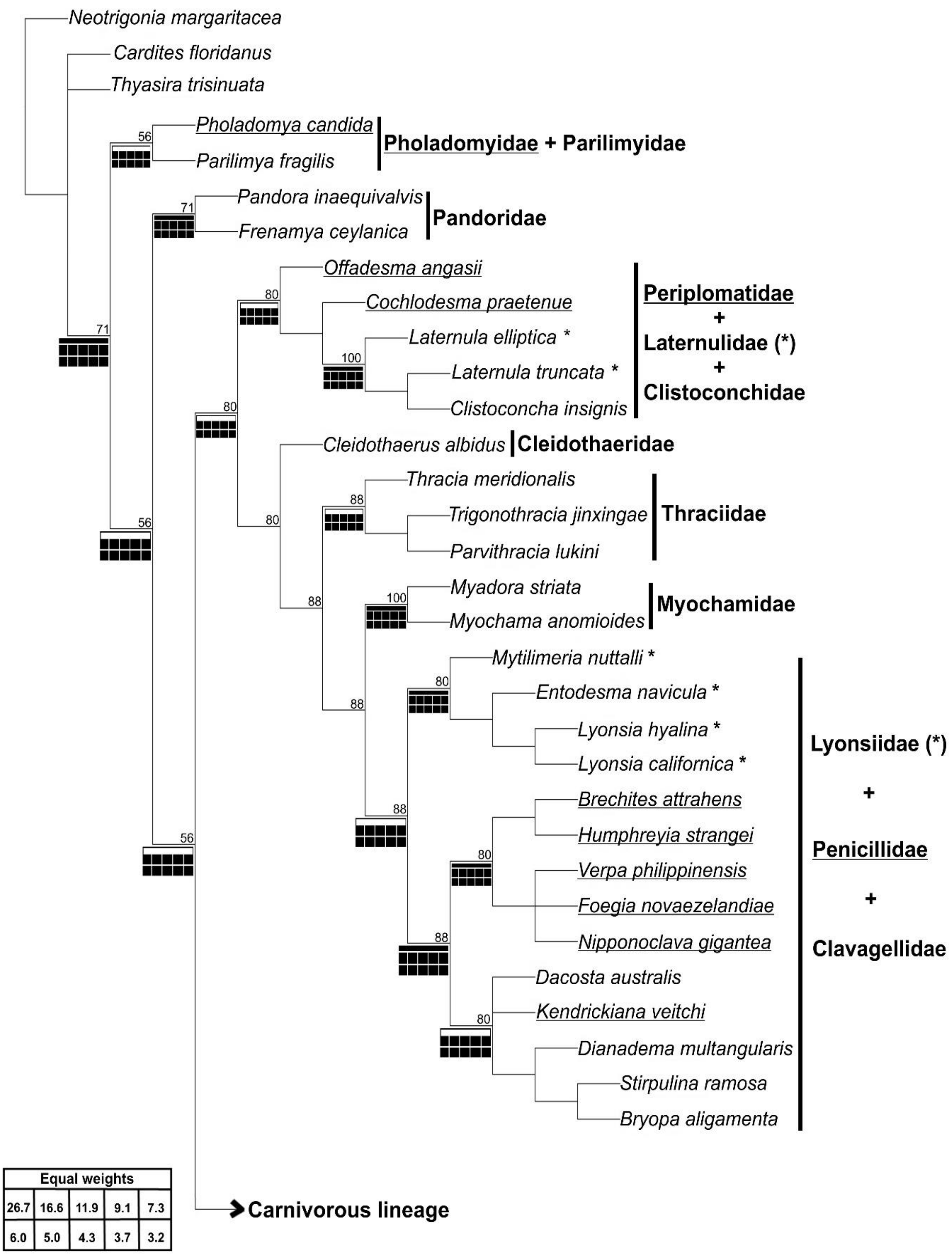
The monotypic Cleidothaeridae, represented by *Cleidothaerus albidus,* also arises here as an independent clade, supported by six apomorphies: the presence of a ligament between a sunken coiled chondrophore (10, ambiguous), siphonal tentacles only on the inhalant siphon (24), an anterior adductor muscle larger than the posterior (33, ambiguous), a rectum passing below the heart (48), cementation to the substratum *via* a sticky layer of outer periostracum associated with mantle glands (57, non-ambiguous) and the absence of a pedal musculature (35),

The monophyletic clade Thraciidae comprising species of genera *Trigonothracia*, *Thracia* and *Parvithracia,* being the latter used for the first time in a cladistical analysis. This clade is supported by three synapomorphies: the presence of a shell microstructure characterized for two homogenous layers (1, ambiguous), slightly inequivalve shells (2) and ciliary sense organs (23, ambiguous). In addition, *Thracia meridionalis* E. A. Smith, 1885 does not group closely with other thraciids due to the presence of a lithodesma only in juvenile individuals (12). Other taxa, such as *Trigonothracia jinxingae* Xu, 1980 and *Parvithracia lukini* Kamenev, 2002, are grouped by the presence of a pallial sinus that extends beyond the mid dorso-ventral axis of the shell (17).

The monophyletic Myochamidae was recovered in all the implied and equal weighting analyses, but this clade is supported herein only by one ambiguous synapomorphy, that is, the presence of a thin secondary ligament (11). Usually recovered as a group closer to the Cleidothaeridae and/or Thraciidae in most publications on anomalodesmatan phylogeny (Harper *et al.* 2000, 2006; Healy *et al*. 2008; Bieler *et al*. 2014; Combosh *et al*. 2017), here Myochamidae was recovered as an independent clade, forming a sister-group with Lyonsiidae + Clavagelloidea (Clavagellidae + Penicillidae).

The Lyonsiidae clade was specifically recovered in all our analyses and their monophyly is supported by the following synapomorphies: the presence of papillate tentacles covering the surface of the siphons (29, non-ambiguous) and by a posterior adductor muscle larger than the anterior (33). Herein, *Mytilimeria nuttalli* Conrad, 1837 is separated from other lyonsiid taxa in being considered endolithic, living in association with ascidian colonies and consequently coded as a non-burrower (61, ambiguous). Recently, however, Combosh *et al.* (2017) have suggested, following a molecular analysis of representatives of the same three genera examined in the present study (*Mytilimeria*, *Entodesma*, *Lyonsia*), that the Lyonsiidae is not monophyletic and should be nested within the Pandoridae, an opinion thus differing from the morphological analyses described and discussed herein.

Another important cluster among the non-carnivorous lineages is formed by the monophyletic Lyonsiidae and by its the sister group the Penicillidae + Clavagellidae. The grouping between these three families has been recovered in 100% of all implied weighting analyses and is supported by only one ambiguous synapomorphy, that is, the presence of tentacles at the base and on the external apertures to the siphons (25).


Neotrigonia margaritacea
Cardites floridanus
Thyasira trisinuata
Pholadomya candida
Parilimya fragilis
Pholadomyidae + Parilimyidae
Pandora inaequivalvis
Frenamya ceylanica
Pandoridae
Offadesma angasii
Cochlodesma praetenue
Laternula elliptica *
Laternula truncata *
Clistoconcha insignis
Periplomatidae
+
Laternulidae (*)
+
Clistoconchidae
Cleidothaerus albidus
Cleidothaeridae
Thracia meridionalis
Trigonothracia jinxingae
Parvithracia lukini
Thraciidae
Myadora striata
Myochama anomioides
Myochamidae
Mytilimeria nuttalli *
Entodesma navicula *
Lyonsia hyalina *
Lyonsia californica *
Brechites attrahens
Humphreyia strangei
Verpa philippinensis
Foegia novaezelandiae
Nipponoclava gigantea
Dacosta australis
Kendrickiana veitchi
Dianadema multangularis
Stirpulina ramosa
Bryopa aligamenta
Lyonsiidae (*)
+
Penicillidae
+
Clavagellidae
Carnivorous lineage
56
71
71
80
100
80
80
56
88
80
88
100
80
88
88
88
80
80
56
Equal weights
26.7
16.6
11.9
9.1
7.3
6.0
5.0
4.3
3.7
3.2

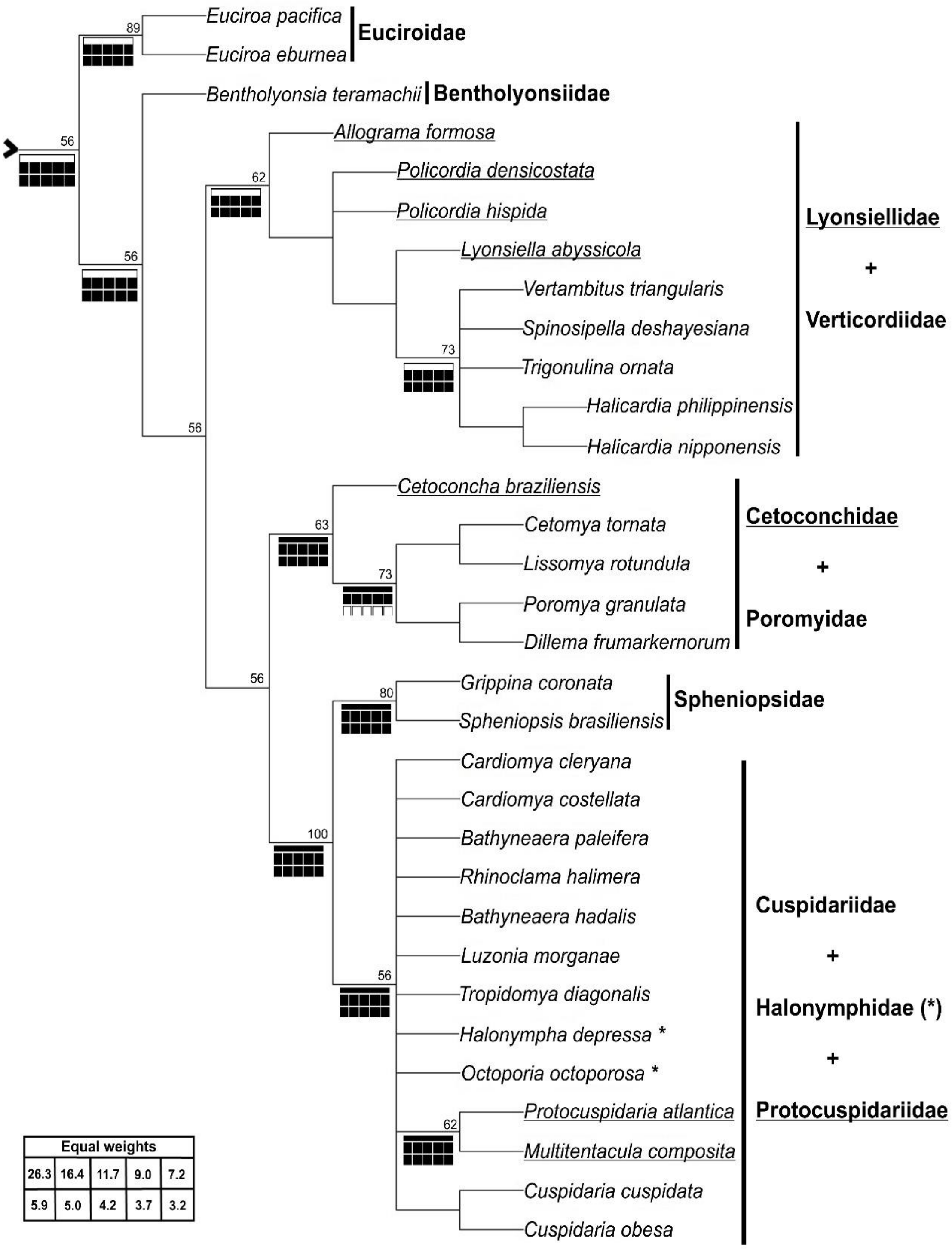


Figure 8. Strict consensus of 12 trees obtained under implied weighting analysis with K-values of 7.319 (topology identical to K-values of 9.137, 11.911, 16.667 and 26.708). Numbers above nodes are Relative Bremer Support. In clades formed by either two or more families, their underlining or the presence of the symbol (*) identifies the species and its respective family. Sensitive plots on key nodes: black squares are recovered topologies; white squares are non-recovered topologies.

In our analyses, the Clavageloidea (adventitious tube-building group), represented here by the Clavagellidae and Penicillidae, has been consistently recovered as monophyletic. The main synapomorphies that support the monophyletic status of the Clavagelloidea are: a ventral mantle fusion of Type C (13), the presence of fused siphons (18), siphons encased in a periostracal sheath (20), siphonal fusions of type A (22), an ability to build a calcareous adventitious tube or crypt (58, non-ambiguous) and a water input (feeding) preferably *via* pedal gape (60). The topology of the Clavagelloidea was also recovered in all our implied and equal weights analyses. In addition, our analyses show a clear division within the Clavagelloidea into two well supported paraphyletic assemblages represented by (i), five species (terminal taxa underlined) of the Penicillidae, excluding *Kendrickiana veitchi* (B. J. Smith, 1971) (but see discussion), and supported by two non-ambiguous synapomorphies, that is, the presence of an equivalve shell (2) and a pedal disc (36, non-ambiguous); and (ii), four species plus *K. veitchi* of the Clavagellidae and supported by two synapomorphies, that is, the absence of a lithodesma in adult individuals (12) and of a fourth pallial aperture (15). The monophyly of these two families requires the inclusion of *K. veitchi* within the Penicillidae [as originally placed by Morton (2004b)] although the absence of a fourth pallial aperture and of a pedal disc corroborates the current positioning of this species within the Clavagellidae clade. In addition, it is interesting to note that the Penicillidae identifies an internal subdivision separating *Brechites attrahens* (Lightfoot, 1786) and *Humphreyia strangei* (A. Adams, 1854) from other taxa. These species (juvenile only for *H. strangei*) are linked by two features, that is, the presence of a fourth pallial aperture only in juvenile individuals (15) and the absence of a pallial sinus (17, ambiguous); while *Verpa philippinensis* (Chenu, 1843), *Foegia novaezeladiae* (Bruguière, 1789) and *Nipponoclava gigantea* (Sowerby, 1888) are supported by two ambiguous synapomorphies, that is, an anterior adductor muscle larger than the posterior (33) and the absence of a pedal musculature (35).

The carnivorous anomalodesmatans

The carnivorous lineage, is supported by 100% of implied weighting analyses, gathering together 32 taxa supported by the following synapomorphies: the presence of a slightly inequivalve shell (2), ciliary sense organs (23), siphonal tentacles located on the siphonal base (25) and by a stomach of Type II (46).

The monophyletic Euciroidae is recovered in 100% of all implied weighting analyses and supported by the following synapomorphies: the presence of denticulate structure located laterally to the umbones (8, ambiguous), a ligament sunken between chondrophores (11), a short and cone-shaped inhalant siphon (31), a normal pedal musculature (35) and labial palps medially fused into a pouch/buccal sac (41). Herein only two species of Euciroidae, that is, *Euciroa eburnea* (Wood-Mason & Alcock, 1891) and *Euciroa pacifica* Dall, 1895, were tested revealing the monophyly of this family and arising as an independent clade among the Anomalodesmata.

Here a new family, the Bentholyonsiidae also is described based upon the morphological data obtained for *Bentholyonsia teramachii* by Morton (2003a), which provides sufficient information to justify this decision. Below is the description for this new family of the Anomalodesmata.

Bentholyonsiidae *fam. nov.* Morton & Machado, 2017 (this study)

Type genus: *Bentholyonsia* Habe, 1952

Type species by original designation: *Bentholyonsia teramachii* Habe, 1952 (Genera of Japanese shells, 3: 257) (Figs. 5D, 7)

<u>Composition</u>. This new family includes only one species: *Bentholyonsia teramachii* Habe, 1952. This taxon is known only from the Pacific Ocean, specifically the central coast of Japan and Western Australia, occurring at depths of between 100 to 150 metres.

<u>Diagnosis</u>. *Bentholyonsiidae fam. nov.,* can be identified by the combination of eleven important features: the presence of (i), ventral mantle fusions of Type C (13); (ii), arenophilic gland secretions only on the posterior external margin of the valves (14); (iii), a fourth pallial aperture (15); (iv), a pallial sinus that does not extend beyond the mid dorso-ventral axis of the shell (17); (v), siphonal tentacles located at the base of the siphons (25); (vi), taenioid muscles (37); (vii), a funnel-shaped mouth (38); (viii) non-lamellate labial palps (40); (ix), a stomach of Type II (46); (x), statocysts of the Type B3 (53) and (xi), a dioecious sexuality (54).

<u>Description of the shell</u>. The shells of *B. teramachii* were measured by Morton (2003a: figs. 1, 2) and ranged in length from 22.5 to 39.0 mm and are slightly inequivalve, equilateral, rounded anteriorly, squarely truncate posteriorly, slightly pointed postero-

dorsally with valve gapes, especially posteriorly. Right valve slightly larger than the left overlapping it ventrally giving a sinuous form to the posterior and anterior shell areas. Umbones central, slightly inflated, opisthogyrate. Outer sculpture composed of fine radial striae with a bulbous, medial, dorso-ventrally aligned, ridge. A yellow periostracum covered ventrally and posteriorly with attached sand grains and other debris. Hinge plate weak, without teeth. Ligament internal, opisthodetic, sunken between chondrophores. Calcified central portion of the ligament formed into a large lithodesma. Secondary ligament of fused periostracum connecting the two valves anterior and posterior to the ligament. The pallial line scar thick, concave posteriorly and convex anteriorly. Pallial sinus short. Taenioid muscle scars visible and located on each valve just internal to the anterior adductor and pedal retractor muscle scars on each valve. Suspensory muscle scar present, dorsally located just anterior to the umbones. Scars of adductor muscles visible and of a similar shape and size.

Internal morphology. Short, sensory, siphons. Ctenidia of Type E, non-plicate, an absence of food grooves and the outer demibranch aligned horizontally. Extendable and non-lamellate labial palps with a simple lips and mouth. Non-ciliated oesophagus. Stomach Type II, muscular, with a thick gastric shield. Midgut-rectum ciliated. Midgut and style sac conjoined, the latter without a crystalline style (in the intact specimen examined); hindgut passing under the ventricle of the heart and over the kidneys. Kidneys paired with baggy distal limbs and opening into the suprabranchial chamber at the reno-pericardial apertures. Arenophilic glands occurring along all the mantle margins. Foot well development with a ventral and vertically-aligned byssal groove, but no byssal threads reported upon. Statocysts of type B_3. A pair of anterior suspensory muscles attach to the shell valves antero-ventral to the umbones. Probably dioecious, but only a female individual examined.

Remarks. An important discussion regarding the taxonomy of the type species of this new family was made by Morton (2003a) when preserved specimens collected from Japan and Australia were compared, and both previously deposited in the National Science Museum, Tokyo, Japan and in the Western Australian Museum, Perth, Western Australia, respectively. Recently, in the WoRMS web site Marshall & Bouchet (2015) proposed that the genus *Bentholyonsia* Habe, 1952 should be a synonym of *Lyonsia* Turton, 1822, citing Coan & Valentich-Scott (2012) as the source for this synonymization. Detailed information on the genus *Bentholyonsia* was not, however, found in this work. In addition, Bouchet (2013) in the same web site, cite Huber (2010) as the ‘basis of record’ for re-allocating *Bentholyonsia teramachii* Habe, 1952 into *Lyonsia teramachii* (Habe, 1952). This work provided basic data about *Bentholyonsia*, based on Morton (2003a), but does not present, in our opinion, sufficiently detailed information to justify this change (Huber, 2010 pp 520 and 796).

We, thus, consider *Bentholyonsia* Habe, 1952 to be a valid genus. In addition, the Bentholyonsiidae *fam. nov* has been recovered, distinctively, as a member of the carnivorous lineage, from all consensus trees with the same topology identified in all our analyses. In general, therefore, *B. teramachii* can be considered to represent a lyonsiid-like carnivore, sharing some morphological features with the Lyonsiidae (non-carnivorous clade), such as: a possible potential for suspension feeding, the presence of a thick secondary ligament uniting the valves (11), the presence of a fourth pallial aperture in adult individuals (15); a long, tube-shaped, inhalant siphon (31) and symmetrical labial palps (41) but, as with some other carnivorous species, siphonal tentacles located on a siphonal base (25), a funnel-shaped mouth (38), non-lamellate labial palps (40), non-plicate and reduced ctenidia aligned horizontally (42, ambiguous) and a stomach of Type II (46).

The Lyonsiellidae and Verticordiidae form a large, sister-group, cluster recovered from 100% of implied weighting analyses. This clade is supported by the following synapomorphies: branched siphonal tentacles (27, ambiguous), a rectum that penetrates the kidneys (49) and a post-larval byssus (59, ambiguous). The Lyonsiellidae is represented by an unresolving internal relationship between the species *Allograma formosa*, *Lyonsiella abyssicola* and the polytomy of *Policordia densicostata* and *Policordia hispida*. In our consensus tree, the last three species share with the Verticordiidae the presence of arenophilic gland secretions over the entire valve surfaces (14) and a short and cone-shaped inhalant siphon (31) - features absent in another lyonsiellid - *Allograma formosa*.

The monophyletic Verticordiidae, also recovered in 100% of implied weighting analyses, is supported by a single synapomorphy, that is, the presence of secondary hinge teeth (6). The family Verticordiidae also represents an unresolving internal relationship (polytomy) between some of their species, that is, *Vertambitus triangularis* (Locard, 1898), *Spinosipella desahyesiana* (P. Fischer, 1862) and *Trigonulina ornata* d'Orbigny,

1853. Herein, *V. triangularis* is supported by the presence of gonadial apertures separated from the urinary duct (55), while *T. ornata* is supported by: the presence of a slightly inequivalve shell (2), Type B2 statocysts (53) and the absence of a post-larval byssus (59). *Spinosipella deshayesiana*, in turn, is only supported by one ambiguous synapomorphy, that is, the presence of reduced ctenidia associated with a thin septum (42). In addition, the grouping formed by the two species of *Halicardia* is supported by three synapomorphies: the presence of siphonal fusion Type A (22), siphonal tentacles only on the inhalant siphon (24) and by a non-bifurcated opisthopodium (52).

The clade, formed by the Cetoconchidae + Poromyidae (=Poromyoidea Dall, 1886), also has a topology recovered in all our analyses and is supported by the following synapomorphies: the presence of an external primary ligament (9) and a thin secondary ligament (11) and asymmetrical labial palps, that is, well developed anterior palps and reduced posterior ones (41), and by the absence of a lithodesma (12). The Cetoconchidae, herein analysed for the first time in a morphological cladistical analysis and represented by *Cetoconcha braziliensis* Allen & Morgan, 1981, is supported by but one apomorphy, that is, the presence of a normal pedal musculature (35). This analysis recovers a morphological proximity between the Cetoconchidae and Poromyidae as has been suggested previously by Knudsen (1970), Allen & Morgan (1981) and Krylova (1991), although such a monophyly has not been tested hitherto.

The monophyletic Poromyidae, in turn, was recovered in 50% of implied and equal weighting analyses and is supported by two ambiguous synapomorphies: siphonal fusions of type A (22) and a free style sac (47, ambiguous), remembering that this latter character was coded as missing data ‘?’ for *Lissomya rotundula* Krylova, 1997 and *Dillema frumarkernorum* Leal, 2008 due to the absence of such information in the literature. Internally, this clade has two well defined clusters, the first formed by *Cetomya tornata* + *L. rotundula* supported by the presence of a muscular septum with branchial sieves (44, ambiguous) and the second represented by *Poromya granulata* (Nyst & Westendorp, 1839) plus *D. frumarkernorum* supported by ventral mantle fusions of Type A (13). Despite presenting a low Bremmer support, the relationship between the Cetoconchidae and Poromyidae shows a topology similar to that reported upon previously (Harper *et al*. 2000, 2006; Bieler *et al*. 2014), that is, forming a clade with the Cuspidarioidea Dall, 1886. This large clade composed of the Poromyoidea and

Cuspidarioidea is, in turn, supported by the presence of ctenidia modified into a variously thin to thick muscular septum (42) and by an active burrowing life-style (61, ambiguous).

With a topology recovered in all our analyses, the well supported Cuspidarioidea clade is the largest amongst the other, carnivorous, groups of Anomalodesmata analyzed and comprises the Spheniopsidae (2 spp), Cuspidariidae (9 spp), Halonymphidae (2 spp) and Protocuspidariidae (2 spp). This clade is supported mainly by the following synapomorphies: a pallial sinus that does not extend beyond the mid dorso-ventral axis of the shell (17), siphons encased in a tissue sheath (21, non-ambiguous), siphonal tentacles in a 4I + 3E arrangement (26, ambiguous), an extensible inhalant siphon (31, ambiguous), a septum perforated by isolated pores (43, ambiguous) and Type C statocysts (53), noting that the latter was coded as missing data '?' for nine of the fifteen species that form this clade due the absence of such information in the literature.

Inside this clade there are two main groupings: the first comprising the Spheniopsidae and the second comprising the Cuspidariidae, Halonymphidae and Protocuspidariidae, forming a sister-group. This analysis recovered the Spheniopsidae as being monophyletic supported by the following synapomorphies: the presence of fertilized eggs incubated in the visceral mass (56, non-ambiguous) and by the absence of labial palps (39, non-ambiguous). Members of this family have been used for the first time in a cladistical analysis and it demonstrates their relationship with other Cuspidarioidea. This new topology for the Spheniopsidae corroborates the previously observation made by Marshall (2002) and most recently by Machado *et al.* (2015) and Morton *et al.* (2016a, b) upon shell morphology plus anatomy, about a possible relationship between the Spheniopsidae and the Cuspidarioidea.

The large sister-group formed by the Cuspidariidae, Halonymphidae and Protocuspidariidae is supported by four ambiguous synapomorphies: the presence of denticulate structures located lateral to the umbones (8), arenophilic gland secretions present on the posterior surfaces of the shell and siphons (14), a free style sac (47) and a dioecious sexuality (54), the latter three coded as missing data '?' for some of the species analysed. This clade appears in our consensus tree with a large polytomy, that is, with an unresolved relationship between taxa analysed resulting from zero length branches. This topology is probably associated with the poor anatomical knowledge about the members of these families, and reflected in a low representativeness of them in our cladistic framework, mainly for Cuspidariidae (Table 1). As a consequence, internally, only the monophyly of the Protocuspidariidae is recovered, where *Protocuspidaria atlantica* Allen & Morgan, 1981 and *Multitentacula composita* Krylova, 1995 are nested and supported by the following synapomorphies: an equivalve shell (2), a normal pedal musculature (35), reduced anterior and well developed posterior labial palps (41) and by ctenidia modified into a membranous septum (42).

The polytomy between the Cuspidariidae and Halonymphidae is seen in all the most parcimonious trees, while the Protocuspidariidae has always been recovered as monophyletic. Figure 10, however, brings an alternative topology for Cuspidarioidea showing a grouping between *Bathyneaera paleifera* Krylova, 1993, *Bathyneaera hadalis* (Knudsen, 1970), *Cardiomya cleryana* (d'Orbigny, 1842), *Cardiomya costellata* (Deshayes, 1835), except *Tropidomya diagonalis* (Allen & Morgan, 1981), supported by the presence of projections on the exhalant inter-tentacular margin (30, ambiguous). This grouping is interesting because may suggest the possibility of a subdivision into Cuspidariidae to include the Cardiomyinae as previously proposed by Scarlato & Storagobatov (1983) and accepted by Krylova (1993: pp 52), but never before recovered by a phylogenetic analysis. A description and discussion about the possible functions and significance of these inter-tentacular projections was recently provided by Machado *et al.* (2017: pp 458).

The consensus tree obtained from a total of 12 most parsimonious trees, resulting from our sensitivity analyses, are identified in Figure 8 showing the sensitivity plots (navajo rugs) on nodes of interest and in Figure 9 providing the set of synapomorphies cited above. No different overall resolutions were obtained for most of the analysed clades, indicating the strong stability of the phylogenetic relationships presented.

Discussion

For the purposes of this study, the shell features and internal anatomies of 61 species of Anomalodesmata were selected to make up the present cladistical analyses. For the first time, representatives of all 22 families of Anomalodesmata, included the newly, herein, described, Bentholyonsiidae and members of Clistoconchidae, Cetoconchidae, Protocuspidariidae and Spheniopsidae, were gathered together in the same phylogenetic analysis. In comparison with the previous morphological cladistic work undertaken by Harper *et al*. (2000), this study contains about 258% more species (61 versus 17), 117% more genera (37 versus 17), 57% more families (22 versus 14), and 41% more characters (61 versus 43) thereby considerably increasing the representativeness of these groups and, as a consequence, allowing for a greater number of phylogenetic relationships to be identified for the Anomalodesmata.

These results identify a different path towards our greater understanding of the internal relationships within the Anomalodesmata, where the most accepted sub-division into three clades, Septibranchia (Verticordiidae + Euciroidae + Lyonsiellidae + Poromyidae + Halonymphidae + Cuspidariidae), 'thraciid' (Thraciidae + Cleidothaeridae + Myochamidae + Periplomatidae) plus 'lyonsiid' (polyphyletic Lyonsiidae + Clavagellidae + Pandoridae + Laternulidae) lineages supported by Harper *et al* (2006), Healy *et al* (2008), Bieler *et al* (2014) and in part by Combosh *et al* (2017), have not been wholly recovered here.

The analysis herein suggests a deep division within the Anomalodesmata into four clades: (i), the Pholadomyoidea (Pholadomyidae + Parilimyidae) as sister group of all other Anomalodesmata; (ii), the Pandoridae as an monophiletic group, (iii) a large clade that includes nine families (Periplomatidae, Laternulidae, Clistoconchidae, Cleidothaeridae, Thraciidae, Myochamidae, Lyonsiidae, Clavagellidae, Penicillidae), and (iv), a generally deep water marine carnivorous lineage formed by 10 families (Euciroidae, Bentholyonsiidae *fam. nov.*, Lyonsiellidae, Verticordiidae, Cetoconchidae, Poromyidae, Spheniopsidae, Cuspidariidae, Halonymphidae, Protocuspidariidae). Herein, the first three lineages referred in this paper as non-carnivorous, are usually composed of shallow-water marine anomalodesmatans.

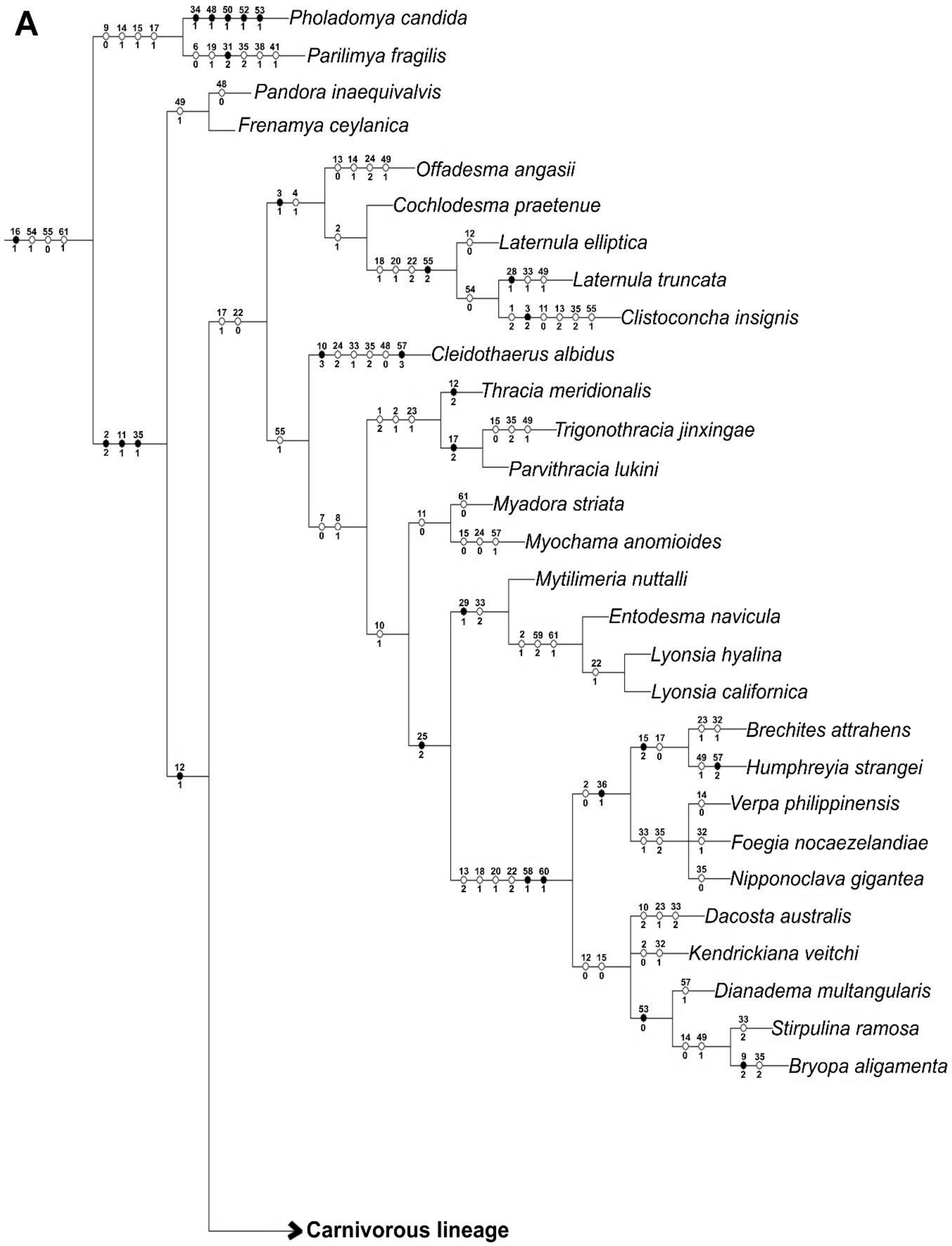

A
16 54 55 61
1 1 0 1
9 14 15 17
0 1 1 1
34 48 50 52 53
1 1 1 1 1
Pholadomya candida
6 19 31 35 38 41
0 1 2 2 1 1
Parilimya fragilis
2 11 35
2 1 1
49
1
48
0
Pandora inaequivalvis
Frenamya ceylanica
12
1
17 22
1 0
3 4
1 1
13 14 24 49
0 1 2 1
Offadesma angasii
2
1
Cochlodesma praetenue
18 20 22 55
1 1 2 2
12
0
Laternula elliptica
54
0
28 33 49
1 1 1
Laternula truncata
1 3 11 13 35 55
2 2 0 2 2 1
Clistoconcha insignis
55
1
10 24 33 35 48 57
3 2 1 2 0 3
Cleidothaerus albidus
7 8
0 1
1 2 23
2 1 1
12
2
Thracia meridionalis
17
2
15 35 49
0 2 1
Trigonothracia jinxingae
Parvithracia lukini
10
1
11
0
61
0
Myadora striata
15 24 57
0 0 1
Myochama anomioides
25
2
29 33
1 2
Mytilimeria nuttalli
2 59 61
1 2 1
Entodesma navicula
22
1
Lyonsia hyalina
Lyonsia californica
13 18 20 22 58 60
2 1 1 2 1 1
2 36
0 1
15 17
2 0
23 32
1 1
Brechites attrahens
49 57
1 2
Humphreyia strangei
33 35
1 2
14
0
Verpa philippinensis
32
1
Foegia nocaezelandiae
35
0
Nipponoclava gigantea
12 15
0 0
10 23 33
2 1 2
Dacosta australis
2 32
0 1
Kendrickiana veitchi
53
0
57
1
Dianadema multangularis
14 49
0 1
33
2
Stirpulina ramosa
9 35
2 2
Bryopa aligamenta
Carnivorous lineage

Figure 9. Strict consensus of 12 trees obtained under implied weighting analysis with K-values of 7.319 (topology identical to K-values of 9.137, 11.911, 16.667 and 26.708). A. non-carnivorous lineages; B. carnivorous lineage. Black circles = synapomophies; white circles = homoplasies. Numbers above branches are characters; numbers below branches are character states.

The non-carnivorous lineages

Although a greater genus-level sampling is necessary, more specifically for the Periplomatidae, Clavagellidae and Penicillidae, our morphological analyses point to new taxonomic relationships within the Anomalodesmata when compared with some previous taxonomic groupings. According to the classification proposed by Bieler *et al.* (2010), for example, the superfamilies Pholadomyoidea (Pholadomyidae + Parilimyidae) and Clavagelloidea (Clavagellidae + Penicillidae) should be maintained, that is, have been recovered in our analysis, while the Pandoroidea (Pandoridae + Lyonsiidae), Myochamoidea (Myochamidae + Cleidothaeridae) and Thracioidea (Thraciidae + Periplomatidae + Laternulidae) need to be re-interpreted as will be discussed below. In addition, the monophyly of the Lyonsiidae and Thraciidae has been identified for the first time, while the Periplomatidae, Clavagellidae and Penicillidae were recovered as paraphyletic groups. For the monophyly of these two crypt/tube-building families, that is, the Clavagellidae and Penicillidae, specifically, our analysis suggests that *Kendrickiana veitchi* (B. J. Smith, 1971) may be a clavagellid due the absence of a fourth pallial aperture (15) and of a pedal disc (36), therefore, their re-allocation to the Clavagellidae would be necessary. The presence of both valves fused into the fabric of the adventitious tube of *K. veitchi*, however, does not support this re-allocation (Morton 2005, 2007) and which, therefore, the second author of this paper declines to support.

Herein, the Pholadomyidae and Parilimyidae are nested forming a monophyletic group, the Pholadomyoidea, this consisting the sister-group to all other anomalodesmatans. As a consequence, some of character states shared by these families also represent the plesiomorphic states of the Anomalodesmata as a whole, for example: an equivalve shell (2), absence of a dorsal crack (3), absence of an internal shell buttress (4), absence of a posterior rostrum (5), absence of a lithodesma (12), absence of a pedal disc (36), labial palps (39), stomach Type IV (46) and a conjoined mid gut and style sac (47).

The phylogenetic position observed to the deep-water species, *Parilimya fragilis* (Parilimyidae), has been recovered as a non-carnivorous member of the Anomalodesmata. In 10% of the implied weighting analyses (K value 3.280), however, this family was recovered from the Cuspidarioidea, specifically grouped with the families Cetoconchidae and Poromyidae (Poromyoidea). For this K value, not considered here due to low support, the Parilimyidae shared three ambiguous synapomorphies with these carnivorous families, that is: the presence of an external primary ligament (9), a thin secondary ligament (11) and the absence of a ligamental lithodesma (12). Although, this topology has not been considered here, this specific situation deserves further attention because of an early suggestion made by Morton (1985a) in which it was hypothesised that species of *Parilimya* could reveal more about the origin of the Poromyidae and here, for the first time, a possible relationship between *P. fragilis* and a septibranch clade has been identified.

In addition, although the shell morphology and details of the internal tissues of *P. candida* and *P. fragilis* have been described by Morton (1980, 1982), some limitations experienced during the dissections of these long-term preserved specimens plus the absence of any new fresh individuals, have prevented access to some important anatomical details, such as that of the stomach, labial palps and structures associated with the siphons. Some of these limitations have directly influenced the coding of some characters of *P. fragilis,* such as character 40 (labial palps type) and character 46 (stomach type), both coded as an ambiguous character '0/1'. In addition, a tentative re-analysis of the shells of *P. fragilis* is not possible since the type-specimen deposited in the Zoological Museum of the University of Bergen, Norway (ZMUB 17192), is missing. The topologies observed to these families (Pholadomyidae + Parilimyidae), however, in the present cladistical analysis, that is, as a sister-group to all other anomalodesmatans, re-inforces the evolutionary concept hypothesized by Morton (1985a) for the extant superfamilies of the Anomalodesmata as evolving from a pholadomyoidean stock is herein upheld.

The Pandoridae topology was recovered in most of the analyses is different from those reported upon in studies where the Pandoridae generally appears to be associated with either the Lyonsiidae (Harper *et al.* 2006; Combosh *et al.* 2017), Lyonsiidae + Laternulidae (Bieler *et al.* 2014) or with the Cleidothaeridae (Harper *et al*. 2000). In addition, it also shows a conflicting result in relation to fossil records that usually indicate a more recent origin for the Pandoridae, that is, the Cenozoic (Boss 1978). Herein, therefore, two hypotheses were considered in an attempt to explain the topology of the Pandoridae: (i), the absence of a lithodesma in the species analysed and (ii), the low representativeness of members of this family in our matrix dataset (Table 1). Our results suggest that the absence of a lithodesma (12) is a plesiomorphic state for the Anomalodesmata, showing that this calcified ligament arose only once during the radiation of this sub-class and that was variably lost in some clades such as, for example, Poromyidae and in some representatives of the Laternulidae and Pandoridae. In *Thracia meridionalis* E. A. Smith, 1885 (Thraciidae) too, the presence of a lithodesma is seen only in juvenile individuals, suggesting a different interpretation for the function of this structure in the Anomalodesmata and questioning, if observed in other taxa, their real phylogenetic signal (Sartori & Domaneschi 2005). In addition, although neither *Pandora inaequivalvis* (Linnaeus, 1758) and *Frenamya ceylanica* (G. B. Sowerby I, 1835) have a lithodesma, other pandoriids such as, for example, *Pandora braziliensis* G. B. Sowerby II, 1874 and *Pandora brevirostris* Güller & Zelaya, 2016 have a well-development one (Güller & Zelaya 2016). Perhaps the inclusion of pandoriids with a lithodesma in our analyses could change the topology of this family within the Anomalodesmata although a more complete study of the anatomy of the above species would be first necessary.

Traditional morphology-based classifications have consistently grouped the Thraciidae, Laternulidae and Periplomatidae within the Thracioidea (Boss 1978; Yonge & Morton 1980; Morton 1981, 1985a; Harper *et al*. 2000). Most recently, however, molecular approaches have suggested that these families could be, for example, separated into two different lineages, 'thraciid' (Thraciidae, Periplomatidae) and 'lyonsiid' (Periplomatidae) (Harper *et al*. 2006; Bieler *et al*. 2014). Now, with the inclusion of morphological data available for *Clistoconcha insignis* E. A Smith, 1910 (Clistoconchidae), our results suggest a remodeling of the grouping of these families with the Periplomatidae + Laternulidae + Clistoconchidae forming a clade not directly related to the Thraciidae. The group Periplomatidae + Laternulidae + Clistoconchidae is supported by the presence of a dorsal crack in the shell (3) plus the presence of an internal shell buttress (4), both considered exclusive to those families and consequently absent in thraciids.

Although, the Thraciidae share other important characters with the Periplomatidae + Laternulidae + Clistoconchidae, the fact that could perhaps justify a greater proximity between them, would be the inclusion of *Parvithracia lukini* Kamenev, 2002 into our analyses. If so, this would apparently displace the clade Thraciidae due to the presence of hinge teeth (secondary teeth) – a feature usually absent among confamilials. It is, however, true that the Thraciidae is known to be morphologically variable and, therefore, a greater representativeness should be considered within future researches to include anatomical data about *Asthenothaerus*, *Bushia*, *Cyathondonta* and *Skoglundia* – all hitherto unstudied in any sufficient detail.

The cementing bivalves belonging to the Cleidothaeridae (*Cleidothaerus*) and Myochamidae (*Myochama* and the non-cementing *Myadora*), specifically, herein considered to arise as independent clades different from previous analyses that have generally recovered these families either as sister-groups (Combosh *et al*. 2017) or nested with other anomalodesmatan clades (Dreyer *et al*. 2003; Harper *et al*. 2006; Bieler *et al*. 2014). Our results suggest that the Cleidothaeridae and Myochamidae are valid families, although the monophyly of the former has not been tested, and neither has the probability that the cemented habit has arisen independently in these two families. Cementation in representatives of these families is from glandular secretions, the mechanisms appearing to be different between *Myochama* and *Cleidothaerus*, being *via* mantle margin glands in the former and *via* a sticky layer of outer periostracum in the latter (Harper & Morton 2000; Morton & Harper 2001; Morton 2007). In addition, the ability to cement is not exclusive to the species belonging to the genera identified above, but can also be observed in the tube-builders *Humphreyia strangei* (Penicillidae) and *Dianadema multangularis* (Clavagellidae) (Morton 2003b, 2002b), although there is no ecological equivalence. These observations suggest that maybe this ability has arisen at least three times during the adaptive radiation of the Anomalodesmata, possibly at different times in the Myochamidae, Cleidothaeridae and Clavagelloidea.

Considered by Yonge & Morton (1980) as the most primitive family among the Pandoroidea (Lyonsiidae, Myochamidae, Pandoridae, Cleidothaeridae), especially in terms of ligament structure, the Lyonsiidae presents their monophyly supported by two synapomorphies, that is, the presence of papillate tentacles covering the surface of the siphons (29) and a posterior adductor muscle larger than the anterior (33). The papillate tentacles covering the surface of the inhalant and exhalant siphons, seems to be an exclusive feature of lyonsiids, being observed for most the species reviewed in this study. Observed and illustrated for the first time by Yonge (1952a), the function of these small structures is unknown. They probably act as sensory organs, however, helping the short siphons to regulate the input/output of water into and out of the mantle cavity. The reduction of the anterior adductor muscle is also a common feature shared among the Lyonsiidae due to the effects of byssal attachment; although it is more evident in *Entodesma navicula* and *Mytilimeria nuttali* than in species of *Lyonsia*. Herein, a new topology for the monophyletic Lyonsiidae is presented, nesting this family close the Clavagelloidea (Clavagellidae + Penicillidae) and sharing with them a single synapomorphy, that is, the presence of siphonal tentacles on the apertures and at the bases of the siphons (25). The close relationship between the Lyonsiidae and Clavagelloidea has been reported upon in part by Dreyer *et al*. (2003), Harper *et al*. (2006) and more recently by Combosh *et al.* (2017) and Williams *et al*. (2017), the latter using mitochondrial molecular data.

Our analysis has also brought together representatives of all extant genera of the Clavagelloidea, showing for the first time a deep division of this superfamily into two main clades, the Penicillidae and Clavagellidae. This division is similar to that proposed by Morton (2007) where the Clavagelloidea would be divided into two functional clades, but now including information on *Dianadema minima* and *Stirpulina ramosa* (Morton 2009, 2013).

In summary, some factors such as the analysis of members of the Pholadomyidae and Parilimyidae as an anomalodesmatan ingroup, the inclusion of information on *Clistoconcha insignis* (Clistoconchidae) plus the considerable increase in the representativeness of the Clavagelloidea (10 species) in our morphological dataset, have identified new internal topological configurations relating to the phylogeny of the Anomalodesmata, specifically to the set of 12 families (Pholadomyidae, Parilimyidae, Pandoridae, Periplomatidae, Laternulidae, Clistoconchidae, Cleidothaeridae, Thraciidae, Myochamidae, Lyonsiidae, Clavagellidae, Penicillidae) now representing the non-carnivorous lineages.

The carnivorous lineage

Our analysis recovered the Euciroidae, Verticordiidae, Poromyidae and Spheniopsidae as monophyletic clades, the Lyonsiellidae as paraphyletic and a part of the clade Cuspidarioidea (that is, Cuspidariidae + Halonymphidae + Protocuspidariidae + Spheniopsidae) as unresolved (polytomy), except for the Protocuspidariidae and Spheniopsidae. The monotypic Bentholyonsiidae *fam. nov* and Cetoconchidae were recovered in all our analyses and are supported by a set of seven and one synapomorphy, respectively. Our results suggest a possible re-interpretation of the Verticordioidea (that is, it is formed by the non-monophyletic families Lyonsiellidae and Verticordiidae plus the Euciroidae) and confirm for the first time the Cetoconchidae as a member of the

Poromyoidea (that is, Cetoconchidae + Poromyidae) and the Spheniopsidae as member of the Cuspidarioidea.

Usually associated with the Verticordioidea Stoliczka, 1870, the Euciroidae arises here as an independent clade among the carnivorous lineage. Although sharing some characters with some Verticordioidea such as, for example: the presence of secondary hinge teeth, a thin secondary ligament, arenophilic secretions over the entire shell, a short and cone-shaped inhalant siphon and labial palps medially fused into a pouch/buccal sac, our analyses suggest that the Euciroidae should be removed from the Verticordioidea, although the present work recognizes some limitations during the encoding of some characters for the Euciroidae, based exclusively on data contained in the literature. In fact, there is a gap in current knowledge of the Euciroidae, in that all anatomical knowledge of this family is restricted to the works of Dall (1895), Poutiers & Bernard (1995) and Knudsen (1967: pp 302-304) who recorded for the first time prey inside of stomach of *Euciroa eburnea* (foraminifera + copepods) and *Euciroa rostrata* (20 foraminifera). An interesting discussion concerning *Euciroa eburnea* and other members of the Verticordiidae and Poromyidae was also provided by Poutiers & Bernard (1995) and, although short, the authors suggested that predation in *Euciroa* could be similar to the strategy used by representatives of the Verticordiidae, which, due to the absence of a raptorial inhalant siphon, probably capture their prey *via* sticky siphonal tentacles (Allen & Turner 1974; Morton 1987b).

According to Allen & Morgan (1981), in general, members of *Cetoconcha* (Cetoconchidae) are basically ‘poromyids’, possessing similar labial palps, siphons, foot and stomach structure. Conversely, the same authors also called attention towards possible anatomical differences between *Cetoconcha* and *Poromya* such as the presence of an intermediate muscular septum between the condition observed in *P. granulata* and species of *Cuspidaria* plus three rows of grouped pores perforating it (without interfilamentar connections). Krylova (1991), analysing Pacific species, described a similar pattern of septal pores for *Cetoconcha alephinae* (Krylova, 1991) and *Cetoconcha elegans* (Krylova, 1991) with three rows of pores, but with the presence of interfilamentar connections. In addition, Krylova (1991) also highlighted the number of siphonal tentacles and the number of pores of each row to differentiate the species analysed. Herein, therefore, the morphological information concerning only one species of Cetoconchidae, *Cetoconcha braziliensis*, has been analysed, due to the availability of anatomical information in the literature and the presence of some specimens deposited in the Museum of Comparative Zoology- Harvard University (MCZ 281315- holotype, 357597), allowing a re-analysis of this species. In our re-analyses, no differences were found between the muscular septum of *C. braziliensis* and *Poromya* species; the presence of three rows of septal pores were confirmed; and a difference between the musculature associated with the foot of these species has been identified. Despite the contracted state of the museum specimens, *C. braziliensis* has a large foot with a normal pedal musculature similar to other carnivorous taxa, such as, for example, *Protocuspidaria atlantica* (MCZ 352417), while some species of Poromyidae, such as *Poromya granulata* (ZUEC 2238) and *Cetomya tornata* (MCZ 348406), have a reduced foot associated with a reduced pedal musculature. Our analysis has, therefore, recovered for the first time the Cetoconchidae as a member of the Poromyoidea corroborating some previous classifications as, for example Bieler *et al*. (2010) and Carter *et al*. (2011).

The Cuspidarioidea has been recovered in all our analyses and is supported by a set of six synapomorphies and including now the monophyletic Spheniopsidae. The inclusion of spheniopsids in our analyses was only possible due to recent researches on the taxonomy and functional morphology of *Grippina coronata* and *Spheniopsis brasiliensis* from Brazilian waters (Machado & Passos 2015; Morton *et al.* 2016a, b). These studies provided, for the first time, details about the muscular septum, siphons, stomach and reproductive features of this family. Although the Spheniopsidae share some characteristics with other representatives of the Cuspidarioidea such as, for example, the presence of siphons encased in a tissue sheath (21), a 4I + 3E siphonal tentacle arrangement (31), a septum perforated by isolated pores (43) and statocysts of Type C (53); other characters such as the presence of fertilized eggs in the visceral mass (56) and the absence of labial palps (39) support our conclusion of the Spheniopsidae being monophyletic and seem to suggest that this clade may be a valid family. In addition, it is worth mentioning the absence of an arenophilic system (14) in members of the Spheniopsidae, at least in the species analysed here, showing for the first time the loss of this character within the Cuspidarioidea. Commonly associated as a synapomorphy of the Anomalodesmata (Sartori *et al*. 2006; Sartori 2008; Oliveira & Sartori 2013), the presence of arenophilic glands/secretions is not always recorded for representatives of this sub-class, being absent in the Myochamidae, Thraciidae, Cleidothaeridae, Clistoconchidae and in some members of the Pandoridae, Clavagellidae, Penicillidae, Periplomatidae, Poromyidae and Lyonsiellidae. So, we argue that the arenophilic glands and their secretions has been probably lost on multiple occasions during the adaptive radiation of the Anomalodesmata.

The Cuspidariidae, in turn, is the most diverse family among the Anomalodesmata with more than 250 species recorded (Gofas & Bouchet 2015). Herein, this family was recovered as an unresolved clade nested within the Halonymphidae and Protocuspidariidae, probably due to its low representativeness in our matrix dataset, 3.54% (see Table 1). Even with such low representativeness, however, previous researches have been successful in obtaining a monophyly for this family, although none of them have used representatives of the Halonymphidae and Protocuspidariidae in the same analyses (Dreyer *et al*. 2003; Harper *et al*. 2000, 2006; Combosh *et al*. 2017). Another unresolved topology for Cuspidarioidea was highlighted from the other 12 equally parsimonious trees suggesting a grouping between species of *Bathyneaera* and *Cardiomya* (Cuspidariidae) - a relationship previously pointed to by Scarlato & Storagobatov (1983), Krylova (1993) and, most recently, by Machado *et al*. (2017) (see Fig. 10).

Initially proposed by Scarlato & Storagobatov (1983) in a work with little morphological detailing, the Halonymphidae and Protocuspidariidae have been, until now, the target of many doubts in relation to their taxonomic status, being accepted as a valid-families by Krylova (1994b, 1995), Bieler *et al*. (2010), Carter *et al*. (2011) and rejected by Poutiers & Bernard (1995), Morton (2003a), Oliveira & Absalão (2009), and Allen (2011). This taxonomic rejection by some authors is based mainly in the inconsistency of morphological characters, generating insufficient data that, as a consequence, could help differentiate them from the Cuspidariidae. Conversely, the presence of more than five pairs of septal pores in species of *Halonympha* and *Octoporia* (Halonymphidae) and *Protocuspidaria* and *Multitentacula* (Protocuspidariidae) (Allen & Morgan 1981; Krylova 1994b, 1995) has been identified as an important morphological character to justify this taxonomic status. In addition, hinge dentition, the muscularised septum, the labial palps and the number of siphonal tentacles have also been recognised by some authors to differentiate these families from the Cuspidariidae (Poutiers 1984; Allen & Morgan 1981; Krylova 1994b, 1995; Allen 2011).

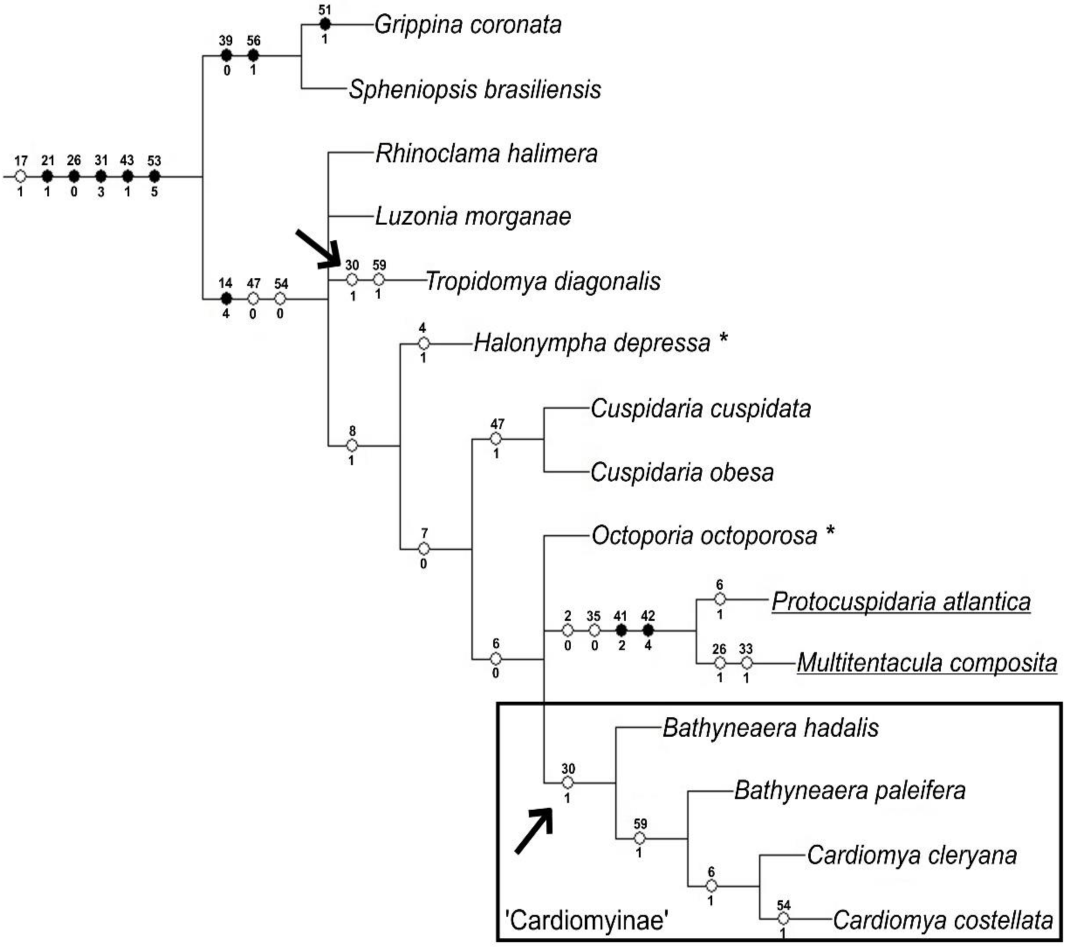


Figure 10. One distinct topology for the clade Cuspidarioidea, showing a grouping between species that have projections in the exhalant intertentacular margin (character 30), except *Tropidomya diagonalis*, suggesting a subfamilial division in the Cuspidariidae to include the 'Cardiomyinae'. The Protocuspidariidae (underlined species) and Halonymphidae (*****) are highlighted. Black circles = homologies; white circles = homoplasies. Numbers above branches are characters; numbers below branches are character states.

Herein, therefore, we consider the Halonymphidae and Protocuspidariidae to be valid families following Bieler *et al*. (2010). Some problematic characters, such as the number of septal pores and siphonal tentacles, however, were not encoded in this study in an attempt to avoid misinterpretation of internal relationships among these families constituting the carnivorous lineage. The number of septal pores is highly variable among members of the Halonymphidae (8-20) and Protocuspidariidae (5-30) (Allen & Morgan 1981; Krylova 1994b, 1995) suggesting, at least to us, that this variation could be associated with intra-specific variations in individual size and shape perhaps related to age. The same phenomenon has been identified for the number of siphonal tentacles, that may vary from 7 to 34 in species of *Octoporia* and 9 to 33 in *Multitentacula* (Krylova

1994b, 1995). In addition, according to Oliveira & Sartori (2013: pp. 7), the siphonal tentacles described by Krylova (1995) may be arenophilic papillae, which could generate a mis-interpretation of this structure and mis-represent the real number of siphonal tentacles in species of *Multitentacula*. Our analyses, however, show that even without the use of these characters, the clade formed by *Protocuspidaria atlantica* + *Multitentacula composita* (that is, the Protocuspidariidae) is always recovered, while the Halonymphidae (*Halonympha depressa* and *Octoporia octoporosa*) was not recovered in any of our analyses. This absence of support for the Halonymphidae, specifically, may suggest that: (i), the use of characters such as the number of septal pores and siphonal tentacles, though problematic, should be considered or (ii), the Halonymphidae do not have sufficient unique morphological features to justify their familial status and, therefore, the genera *Halonympha* and *Octoporia* should be considered for relocation within the Cuspidariidae.

In addition, it is worth noting that although the vast majority of carnivorous anomalodesmatans live in deep waters, recent works also have shown the presence of living carnivorous in shallow waters as, for example, *Cardiomya cleryana* (10 to 20m) and *Grippina coronata* (21 to 53m) (Machado *et al*. 2017; Machado & Passos 2015; Morton *et al*. 2016a, b, respectively), suggesting that there may have been an evolutive divergence within the Cuspidarioidea specifically, shaping the evolution of this clade in two different types of carnivorous, that is, shallow and deep waters predators. Up until now, no morphological pattern has been observed that could differentiate these two possible sub-lineages of predators, however, more and more studies about the natural history of these organisms has brought new clues for understanding of the evolution of the Cuspidarioidea and consequently about the carnivorous lineage as a whole.

Conclusions

Probably evolving in the Palaeozoic, representatives of the Pholadomyidae and its sister group the Parilimyidae have subsequently and respectively radiated into shallower subtidal waters as suspension feeders and typically as predators of, generally, small benthic invertebrates. From such beginnings, this study has shown through a greater increase in genus-level representativeness and of characters choice and scoring, new internal relationships for this important marine bivalve sub-class. Morphological data on representatives of the Clistoconchidae, Cetoconchidae, Spheniopsidae and Protocuspidariidae have been gathered together for the first time and analysed in a cladistical analysis and a new family has been described - the Bentholyonsiidae. Into the non-carnivorous lineages, the Lyonsiidae has been specifically recovered in all our analyses and the monophyly of this clade and its clustering with its the sister group the Penicillidae + Clavagellidae is an important step towards our understanding of the bizarre Clavagelloidea. We have also identified a well-supported grouping between the paraphyletic Laternulidae and the monotypic Clistoconchidae, forming a deeply nested sister group. For the monophyletic carnivorous clade, this study presents for the first time an imagined but never before analysed phylogenetic diversity of carnivorous anomalodesmatans. Herein, we have identified, for the first time, the Euciroidae as an independent clade from the Verticordioidea. The topology of the Cetoconchidae and Spheniopsidae within the Poromyoidea and Cuspidarioidea, respectively, has been confirmed for the first time in a cladistical analysis. In summary, therefore, our analyses have identified new internal relationships for the Anomalodesmata as well as, new elements for the reconstruction of the evolutionary history of this important group of marine bivalves, using a morphological perspective.

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Appendix 1. List of museum lots containing voucher specimens that were previously analysed and/or re-analysed by the authors. Museum abbreviations: BMNH/NHM, Natural History Museum, London, United Kingdom; MCZ, Museum of Comparative Zoology- Harvard University, Cambridge, USA; ZMUB, Zoological Museum of the University of Bergen, Norway; WAM, Western Australian Museum, Perth, Australia; SAM, South Australian Museum, Adelaide, Australia; NSMT-MoR, Imperial Household to the National Museum of Nature and Science, Tokyo, Japan; NMV, National Museum of Victoria, Melbourne, Australia; SAM, South Australian Museum, Adelaide, Australia; SBMNH, Santa Barbara Museum of Natural History, California, USA; MA, Auckland War Memorial Museum, New Zealand; AM, Australian Museum, Sydney, Australia; MZUSP, Museu de Zoologia da Universidade de São Paulo, Brazil; SAM, South African Museum, Cape Town, Africa; WAM, Western Australian Museum; ZUEC, Museu de Zoologia da Universidade Estadual de Campinas- São Paulo, Brazil; PBBM, Bernice Pauahi Bishop Museum, Hawai, USA; MNRJ, Museu Nacional do Rio de Janeiro, Brazil; DBUA, Department of Biology of the University of the Azores, Portugal; BMSM, Bailey-Matthews National Shell Museum- Florida, USA; AM, Australian Museum- Australia.

Species used as ‘outgroup’:

Family Trigoniidae (Bivalvia: Palaeoheterodonta)
Neotrigonia margaritacea: The original specimens used by Morton (1987) were not found, probably were not deposited in a Museum; MCZ 83283.

Family Carditidae (Bivalvia: Archiheterodonta)
Cardites floridanus (new comb. to *Carditamera floridana*): MCZ 383274, 295688, 289937, 289953.

Family Thyasiridae (Bivalvia: Imparidentia)
Thyasira trisinuata: MCZ 347671, 355327, 355166, 375810.

Species of Anomalodesmata:

Family Pholadomyidae
Pholadomya candida: NHM 1969266, 19525108, 19677688; MCZ 387668.

Family Parilimyidae
Parilimya fragilis: ZMUB 17192, this lot is missing.

Family Pandoridae
Frenamya ceylanica: BMNH 1983102; MCZ 385771.
Pandora inaequivalvis: MCZ 374289.

Family Periplomatidae

***Offadesma angasi**: NHM 1894; MCZ 23173.*
Cochlodesma praetenue: MCZ 375545.
Family Laternulidae
Laternula truncata: The original specimen(s) used by Morton (1973) were not found, probably were not deposited in a Museum; MCZ 233215.
Laternula elliptica: 10 preserved specimens in the private collection of Dr. Flávio Dias Passos, Laboratory of Malacology, Universidade Estadual de Campinas, São Paulo, Brazil.

Family Clistoconchidae
Clistoconcha insignis: NHM 1911.8.30.18, 1911.8.30.19, 1911.8.30.20, 19911.8.30.21, 19911.8.30.23; SAM A37285.

Family Cleidothaeridae
Cleidothaerus albidus (new comb. to *Cleidothaerus maorianus*): The original specimen(s) used by Morton (1974) to described the anatomy were not found, probably were not deposited in a Museum; MCZ 182201.

Family Thraciidae
Trigonothracia jinxingae: BMNH 1994049;
Thracia meridionalis: MZUSP 40934, 40935, 40936, 27/880; 5 preserved specimens in the private collection of Dr. Flávio Dias Passos, Malacology laboratory, Universidade Estadual de Campinas, São Paulo, Brazil.

Family Myochamidae
Myadora striata: MA104092, MA80208; MCZ 60089.
Myochama anomioides: NHM 1887.2.9.2452, 1887.2.9.2453, 1857.11.18.62, 1887.2.9.2454, 1857.11.18.8, 1887.2.9.2455; MCZ 379067.

Family Lyonsiidae
Entodesma navicula (new comb. to *Entodesma saxicola*): SBMNH 3119, 3120; MCZ 239531.
Lyonsia californica: MCZ 63945.
Lyonsia hyalina: MCZ 182003.
Mytilimeria nutalli: MCZ 61821.

Family Penicillidae
Humphreyia strangei: BMNH 1910, 12.31.1; WAM S12727, S12726, S12725.
Brechites attrahens (new comb. to *Brechites vaginiferus*): NHM 1984026; MCZ 31, 32406.
Kendrickiana veitchi: NMV F27419; SAM D14890, D14992, D14993.
Nipponoclava gigantea: NSMT 73509; MCZ 44735.
Foegia novaezelandiae: WAM S10960; MCZ 387671.
Verpa philippinensis (new comb. to *Penicillus philippinensis*): MCZ 342343.

Family Clavagellidae

Dacosta australis (new comb. to *Clavagella australis*): BMNH 198249; MCZ 387672.

Stirpulina ramosa*:* NSMT 20709.

Dianadema multangularis: SAM TD16110.

Bryopa aligamenta: NSMT 73507.

Family Euciroidae

Euciroa eburnea: MCZ 387669.

Family Bentholyonsiidae *fam. nov*

Bentholyonsia teramachii: WAM S14105; NSMT Mo50619; Mo73490 (missing), Mo56343.

Family Lyonsiellidae

Policordia densicostata: BMNH 1985078, 1985079, 1985080; MCZ 272769, 348053, 357595.

Allograma formosa: NHM 1885.11.5.1210; PBBM 204791.

Lyonsiella abyssicola: BMNH 198575, 198576, 198577; MCZ 272772, 348034.

Family Verticordiidae

Vertambitus triangularis (new comb. to *Verticordia triangularis*): BMNH 198574; MCZ 353653, 348045.

Halicardia philippinensis*:* The original specimen(s) used by Morton (1973) were not found, probably were not deposited in a Museum; MCZ 85837.

Halicardia nipponensis: Personal collection of Prof. Dr. Takuma Haga.

Trigonulina ornata: NMNH 856388, 1237124, 1237122; SMNH 856388, 1237124, 843174, 800037, 891378; ZUEC 2227, 2228 plus four preserved specimens in process of description (Museu de Zoologia da Universidade Estadual de Campinas- Unicamp, Brazil.

Family Cetoconchidae

Cetoconcha braziliensis: MCZ 281315.

Family Poromyidae

Poromya granulata: The original specimen(s) used by Morton (1973) were not found, probably were not deposited in a Museum; ZUEC 2238; MCZ 163/77.

Poromya rostrata: MCZ 2240; ZUEC 2240, 2242.

Cetomya tornata: MCZ 353657, 348406.

Dillema frumarkernorum: BMSM 15029.

Family Spheniopsidae

Grippina coronata: ZUEC BIV 6167, 6181, 6183, 6203, 6204; MNRJ 26279, 26280; MZUSP 117138, 117139.

Spheniopsis brasiliensis: ZUEC BIV 6173, 6486, 6192; MNRJ 26281, 26282, 26283, 26284; MZSP 117134, 117135, 117136, 117137.

Family Cuspidariidae
Cuspidaria cuspidata: The original specimen(s) used by Yonge & Morton (1980) were not found, probably were not deposited in a Museum; MCZ 378947, 226442.
Cuspidaria obesa: MCZ 387673.
Cardiomya costellata: DBUA 158, 162, 606, 614; MCZ 352385
Cardiomya cleryana: ZUEC-BIV 5119 to 5141.
Bathyneaera hadalis: MCZ 281308, 377976.
Myonera paucistriata: MCZ 349123.
Tropidomya diagonalis: MCZ 281311, 348004.
Luzonia morganae: MCZ 375553.

Family Halonymphidae
Halonympha depressa: MCZ 348009.
Octoporia octaporosa: MCZ 281307.

Family Protocuspidariidae
Protocuspidaria atlantica: MCZ 281314

Appendix 2. Data matrix scored by 61 taxa plus 61 morphological characters. Abbreviations: **n**= not applicable, **?**= miss data, **x/y**= ambiguous.

	000000000	**1111111111**	**2222222222**	**3333333333**	**4444444444**	**5555555555**	**66**
	123456789	**0123456789**	**0123456789**	**0123456789**	**0123456789**	**0123456789**	**01**
Neotrigonia margaritacea	400002100	n20n0000nn	nnnnnnnnnn	nn00000001	005nnn112?	000?010000	n0
Carditamera floridana	50000nnn0	n00?0000nn	nnnnnnnnnn	nn00000001	000nnn1101	000?010002	n0
Thyasira trisinuata	600001101	n0000000nn	nnnnnnnnnn	nn02000001	??6nnn1120	000?0?0000	n0
Pholadomya candida	000001100	0001111110	00100nnn00	n000100201	000nnn1110	1011100000	0/11
Parilimya fragilis	000000nn0	0000111111	00100nnn00	n200020111	0/110nnn0/1120	0002100000	01
Dacosta australis	020000nn1	2102301110	1021321000	0002010001	000nnn1120	0004110010	12
Stirpulina ramosa	020000nn1	1102001110	1020321000	0002010001	000nnn?121	00001?0010	12
Dianadema multangularis	020000nn1	1102301110	1020321000	0000010001	000nnn??20	0000??0110	12
Bryopa aligamenta	020000nn2	n102001110	1020321000	0000020001	000nnn??21	000??10010	12
Brechites attrahens	000000nn1	1112121010	1021321000	001n011001	000nnn1?20	0004110010	12
Humphreyia strangei	000000nn1	1112121010	1020321000	0000011001	000nnn?121	0004110210	12
Verpa philippinensis	000000nn1	11?2011?10	1020321000	0001021001	000nnn??20	0004110010	12
Foegia novaezelandiae	000000nn1	11?2111110	1020321000	001n021001	000nnn??20	000???0010	12
Kendrickiana veitchi	000000nn1	11?2301110	1020321000	001n010001	000nnn??20	0004??0010	12
Nipponoclava gigantea	000000nn1	11?2111110	1020321000	0001001001	000nnn??20	0004??0010	12
Cleidothaerus albidus	020001101	3111011100	0000201000	n001020001	000nnn1100	0003110300	02
Cuspidaria cuspidata	210011011	2111401101	0111310000	0300010011	1121000120	0005010000	00
Cuspidaria obesa	210011011	2111401101	0111310000	0300010011	1121000120	0005010000	00
Cardiomya cleryana	210011011	2111401101	0111310000	1300010011	1121000020	0005010001	00
Cardiomya costellata	210011011	2111401101	0111310000	?300010011	1121000020	00051100?	00
Bathyneaera paleifera	210010nn1	21?1401101	0111310000	1300010011	11210000??	000???0001	00
Rhinoclama halimera	210011011	21?1401101	0111310000	03000?0011	11210000??	000???0000	0?
Bathyneaera hadalis	210010nn1	21?1?01101	0111310000	1300010011	11210000??	000?0?0000	00
Luzonia morganae	210011101	2111?01101	011?310000	0300010011	112100002?	000?010000	00
Tropidomya diagonalis	210011101	2111?01101	0111310000	1300010011	1121000???	000?0?0001	00
Halonympha depressa	210111111	2111?01101	0111310000	0300010011	11/221000??	000?0?0000	00

Appendix 2. Data matrix (continue)

	000000000	**1111111111**	**2222222222**	**3333333333**	**4444444444**	**5555555555**	**66**
	123456789	**0123456789**	**0123456789**	**0123456789**	**0123456789**	**0123456789**	**01**
Octoporia octoporosa	210010nn1	2111401101	0111310000	0300010011	11/221000020	000???0000	00
Protocuspidaria atlantica	200011011	2111?01101	0111310000	0300000011	1241000020	000?010000	00
Multitentacula composita	200010nn1	2111401101	0111311000	0301000011	12410000??	000???0000	00
Grippina coronata	210001111	2111001101	0111310000	0300010010	nn2100012?	01051?1000	00
Spheniopsis brasiliensis	210011111	2111001101	0111310000	0300010010	nn2100012?	00051?1000	00
Poromya granulata	1/200001100	1000001001	0001311000	0400010011	1320010020	0002100000	00
Cetomya tornata	1/210001100	1001001001	0001311000	0400010011	1320100020	000?100000	00
Lissomya rotundula	1/200001101	1001?01001	0001311000	04000?0011	1320100???	000?1?0000	00
Dillema frumarkernorum	1/200001100	1000001001	0001311000	0400010011	1320010???	000?1?0002	02
Cetoconcha braziliensis	1/210000nn0	0001?01001	0011311000	0400000011	1320010120	000?100000	00
Allograma formosa	000000nn1	1111001001	0011311100	0400010111	141nnn0121	0002100002	01
Lyonsiella abyssicola	010000nn1	1011201001	0011311100	0100010011	111nnn0121	0002100001	01
Policordia densicostata	010000nn1	1111201001	0011311100	0100010011	111nnn012?	000?100002	01
Policordia hispida	010000nn1	111?201001	00?1311100	0100010011	111nnn012?	000?100001	0?
Bentholyonsia teramakii	010000nn1	2112111100	0011311000	0000010111	101nnn0120	00040?0000	01
Vertambitus triangularis	010001101	1011201001	0011311100	0100010011	111nnn0121	0002110002	01
Halicardia philippinensis	010001101	1011?01001	0001211100	n100010011	111nnn012?	002???000?	01
Halicardia nipponensis	010001101	1011?01001	0001211100	n100010011	111nnn0120	002???0001	01
Spinosipella deshayesiana	010001101	1011201001	0011311100	0100010011	1130000121	000?100001	01
Trigonulina ornata	000001101	1011201001	0011311100	0100010011	111nnn0121	0003100000	01
Euciroa pacifica	0/210001111	201??01000	00?1311000	0100000001	0430000121	000?1?0000	01
Euciroa eburnea	0/210001111	201?201000	00?1311000	0100000001	043000012?	000?1?0000	01
Entodesma navicula	0/310000nn1	1111211100	0000321001	0002010001	000nnn1120	0002110002	01
Lyonsia hyalina	0/310000nn1	1111211100	0010321001	0002010001	000nnn1120	00021?0002	01
Lyonsia californica	0/310000nn1	1111211100	0010321001	0002010001	000nnn1120	000?11000?	01
Mytilimeria nuttallii	0/320000nn1	1111211100	0000321001	0002010001	000nnn1120	000?1?0000	02
Pandora inaequivalvis	020001101	1101001000	0010301000	0000010001	000nnn1101	0002100000	01

Appendix 2. Data matrix (continue)

	000000000	**1111111111**	**2222222222**	**3333333333**	**4444444444**	**5555555555**	**66**
	123456789	**0123456789**	**0123456789**	**0123456789**	**0123456789**	**0123456789**	**01**
Frenamya ceylanica	020001101	1101001000	0010301000	0000010001	000nnn1121	0002100000	01
Myadora striata	020001011	1011011100	0000301000	0000010001	000nnn1120	0004110000	00
Myochama anomioides	020001011	1011001100	00000nnn00	n000010001	000nnn1120	0004110100	02
Offadesma angasii	021100nn1	2110111100	0000201000	0000010001	000nnn1121	0003100000	01
Cochlodesma praetenue	011100nn1	2111001100	0000301000	0000010001	000nnn1120	000?100000	01
Laternula truncata	011100nn1	2111301110	1021301010	0001010001	000nnn1121	0002020000	01
Laternula elliptica	011100nn1	210?301110	102???100?	000?010001	000nnn112?	000?120000	01
Trigonothracia jinxingae	210000nn1	2111001200	0001301000	0000020001	000nnn1121	0003100000	01
Thracia meridionalis	210000nn1	2121011100	0001301000	0000010001	000nnn1120	0004110000	01
Parvithracia lukini	210001011	211??11200	00?1301000	0000010001	000nnn1120	000?1?0000	01
Clistoconcha insignis	212100nn1	2012001110	1021301000	0000020001	000nnn1120	0002010000	01

CAPÍTULO 3

The use of Micro-Computed Tomography as a non-invasive tool for anatomical study of bivalves (Mollusca: Bivalvia)

FABRIZIO MARCONDES MACHADO & FLÁVIO DIAS PASSOS

ABSTRACT

The X-ray microtomograph is a non-invasive technique capable of generating 2D images and 3D tomographic reconstructions of the small-sized bivalves without altering or destroying the specimens. Bivalvia outstands as its second largest class of Mollusca, comprising about 8,000 Recent species, for which anatomical data have been fundamental to understand the various aspects related to their taxonomy, phylogeny and biology. Among Bivalvia, the Anomalodesmata Dall 1886 stands out for harbouring the rarest and most specialized marine species, being considered a key group for understanding the phylogenetic backbone of this class. The difficulty in accessing anomalodesmatans makes that group remains one of the least known and least understood groups of Bivalvia, with 80% of their species without any anatomical information. In order to explore and evaluate the use of X-ray microtomography as a new tool for the anatomical description of marine bivalves and to increase the knowledge about the anomalodesmatans, fleshy fixed and museum species of seven different families of Anomalodesmata were scanned. Herein, tomographic anatomical descriptions of Pandora pinna, Lyonsia alvarezii, Allograma formosa, Trigonulina ornata, Poromya rostrata, Cetoconcha spinosula, Cetoconcha smithii and Cuspidaria glacialis were provided, highlighting the main internal features for the systematics of Anomalodesmata. The tomographic images allow the description of important anatomical structures as mantle, ctenidia, labial palps, siphons and details about the digestive, reproductive and nervous systems, expanding the knowledge on the taxonomy of the group as whole. The re-discussion of some organs of A. formosa, as well as, the reallocation of Poromya spinosula into the genus Cetoconcha also was possible through a tomographic approach. This non-destructive tool is, therefore, very efficient for the anatomical description of marine bivalves allowing a complete and precise interpretation of the internal tissues via high resolution tomographic images. The potential of this tool allied to the constant development of new scanners, brings new perspectives for the anatomical study of bivalves, making of this technique a potential tool for the development of a new taxonomic era.

INTRODUCTION

Among the Mollusca, the Bivalvia outstands as its second largest class, comprising about 8,000 known Recent species (Rosenberg 2014, Gofas 2017), for which anatomical data have been fundamental to understand the various aspects related to their taxonomy, phylogeny and biology, such as the mode of feeding and locomotion, and the details of the reproduction and development (e.g., Purchon 1959, 1960, 1963, Cox 1969, Morse & Zardus 1997, Morton *et al*. 1998, Giribet 2008). Traditionally, the knowledge about the anatomy of bivalves has been constructed through observations made by dissections, histology and electron microscopy (e.g., Pelseener 1911, Atkins 1937, Stasek 1963, Knudsen 1970, Yonge 1982, Morton 1985, Purchon 1987, Passos & Machado 2014, Morton *et al*. 2016a), techniques which commonly result in permanently change of the original sample, and for this collectively called as “invasive”. Through these studies, the animal is sectioned (for histology), or modified by a metallic coating (for SEM), or completely destroyed (in the case of dissections), this representing a particular problem when a limited number of specimens or just one specimen of the species are available (Candás *et al*. 2016).

In the last years, by using micro-computed X-ray tomography (µCT or micro-CT), the acquisition of images both in 2D and 3D has greatly improved anatomical studies, allowing observations without altering or destroying the specimens (Golding *et al*. 2009, Faulwetter *et al*. 2013a, Fernández *et al*. 2014, Parapar *et al*. 2017). The Micro-CT is also faster and capable of generating sections in three planes (sagittal, frontal, transverse) from a single specimen, impossible *via* histological approach (Golding *et al*. 2007, Candás, 2016). Although this novel non-invasive tool also has limitations, as for example, the difficult to distinguish organs with a similar density, it expands the possibilities for the study of internal tissues, consequently, bringing a new perspective for the anatomical studies of small and rare marine bivalves.

Among the six monophyletic groups that make up Bivalvia, Anomalodesmata stands out for harbouring the rarest species, since many occur in deeper waters and are usually restricted to highly specialized niches (Morton 1985, Harper *et al*. 2000, Allen 2008, Machado *et al*. 2017). In addition, the difficulty in accessing the species of this group, both in the field and in museums, makes that Anomalodesmata remains one of the least known and least understood groups of Bivalvia (Harper *et al*. 2006). According to Machado *et al*. (2018, submitted), just about 20% of all species of Anomalodesmata have any information about their internal tissues.

In this context, the main goal of this paper is to explore and evaluate the use of X-ray microtomography as a non-destructive tool for the anatomical description of marine bivalves. For this purpose, fleshy fixed and museum species of seven different families of Anomalodesmata were selected, aiming to increase the anatomical knowledge of this group and to elaborate brief anatomical descriptions highlighting the main internal features used for taxonomy and systematics of this group.

MATERIAL AND METHODS

Specimens

Eight bivalve specimens from eight different species were used for µCT scanning, all belonging to the clade Anomalodesmata (Bivalvia): *Pandora pinna* (Montagu, 1803) and *Cuspidaria glacialis* (Sars G. O., 1878) collected in the 60's decade, stored in the MCZ (Museum of Comparative Zoology- Harvard University) – USA; *Poromya rostrata* Rehder, 1843, *Cetoconcha smithii* Dall, 1808 collected in the 90's; and *Lyonsia alvarezii* d'Orbigny, 1846, *Poromya spinosula* Thiele, 1912, *Allograma formosa* (Jeffreys, 1882) and *Trigonulina ornata* d'Orbigny, 1853 in the 2000s (freshly fixed specimens), deposited in the ZUEC (Museu de Zoologia da Universidade Estadual de Campinas) – Brazil. Table 1 provides an overview of the specimens employed for imaging, as well as GPS coordinates for the localities, depth and dimensions.

Contrast solution

The specimens were stained using a solution containing 0.3% phosphotungstic acid (PTA), which also included 3% dimethyl sulfoxide (DMSO) to increase cell membrane permeability, in ethanol (at 95% concentration) (protocol adapted of Faulwetter *et al*. 2013b and Fernández *et al*. 2014). However, to compare the effectiveness of contrast in the internal tissues and the immersion time of the samples in the solution, two different PTA contrast solutions were tested: (i), elaborated from an initial concentration of 99.995% of acid (pure concentration) and (ii), from an already diluted solution as a concentration of 10% of PTA. Once stained the specimens were placed into small plastic tubes of 0.5 ml (for small specimens) and 1.5 ml (for large specimens) submerged in clean ethanol (95% EtOh) and closed at the top end using Parafilm (Pechiney Plastic Packaging Co., Chicago, Illinois, USA) to be scanned. All specimens were scanned in their full-length prioritizing the visualization of all internal structures, important for the anatomical description and taxonomy of these bivalves.

Table 1. List of specimens used for µCT scanning.

Family	**Species**	**Specimen data**	**Specimen dimensions**	**Staining information**	**Scanning parameters**
Pandoridae	*Pandora pinna* (Montagu, 1803)	MCZ 375576; collected July 1967; Bay of Biscay, Atlantic Ocean; GPS 47°40'N, 5°0'W; fixed in formalin, stored in 80% EtOh	1 mm diameter, 9 mm length	0.3% PTA-10% + 3% DMSO for 38 days.	*SkyScan 1173*, 106 Kv, 60 µA, 41 min 9 s, 6.31 µm
Lyonsiidae	*Lyonsia alvarezii* d'Orbigny, 1846	ZUEC 7005; collected July 2014; Araçá Bay, São Paulo, Brazil; GPS 23º49′20″S, 45º24′10″W, 20 m; fixed in glutaraldehyde, stored in 95% EtOh	2.1 mm diameter, 6 mm length	0.3% PTA-99,9% + 3% DMSO for 3 days	*SkyScan 1272*, 37 Kv, 140 µA, 3h 45 min 11 s, Al 1mm, 4.24 µm
Lyonsiellidae	*Allograma formosa* (Jeffreys, 1882)	ZUEC 7003; collected September 2013 on the continental slope, Argentina; GPS 37°49.661'S, 54°7.943'W, 1395 m; fixed and strored in 95% EtOh	5.8 mm diameter, 12.6 mm length	0.3% PTA-10% + 3% DMSO for 38 days	*SkyScan 1173*, 123 kV, 63 µA, 1h 2 min 14 s, 6.37µm
Verticordiidae	*Trigonulina ornata* d'Orbigny, 1853	ZUEC 7004; collected February 2009; Campos Basin, Rio de Janeiro, Brazil; GPS 22º55'7,5''S, 42º0'49,2''W, 29 m; fixed in formalin, stored in 70%	1 mm diameter, 3.2 mm length	0.3% PTA-99.9% + 3% DMSO for 3 days	*SkyScan 1272*, 35 Kv, 140 µA, 5h 4 min 43 s, 4 µm
Poromyidae	*Poromya rostrata* (Rehder, 1843)	ZUEC 2243; collected January 1998; São Paulo, Brazil; GPS 25º43'90''S,45º09'50''W, 511 m; fixed in formalin, stored in 70% EtOh	2.2 mm diameter, 6.5 mm length	0.3% PTA-99.9% + 3% DMSO for 3 days	*SkyScan 1272*, 70 Kv, 142 µA, 3h 40 min 17 s, Al 0.5 mm, 4.66 µm.
Cetoconchidae	*Cetoconcha spinosula* (Thiele, 1912)	ZUEC 7002; collected September 2013 on the continental slope, Argentina; GPS 37°49.661'S, 54°7.943'W, 1395 m; fixed and strored in 95% EtOh	4.6 mm diameter, 13.8 mm length	0.3% PTA-99.9% + 3% DMSO for 3 days	*SkyScan 1173*, 35 Kv, 140 µA, 2h 46 min 40 s, 7.08 µm
Cetoconchidae	*Cetoconcha* aff. *smithii* Dall, 1808	ZUEC 2235; collected Setember 1998; São Paulo, Brazil; GPS 24º20'53''S, 43º46'76''W, 505 m; fixed in formalin, stored in 70% EtOh	2.7 mm diameter, 7.1 mm length	0.3% PTA-99.9% + 3% DMSO for 3 days	*SkyScan 1272*, 70 Kv, 142 µA, 3h 37 min 23 s, 5 µm
Cuspidariidae	*Cuspidaria glacialis* (Sars G. O., 1878)	MCZ 388260; collected June 1961; Gulf of Maine, Atlantic Ocean; GPS 43°0'N, 69°45'W, 3300 m; stored in 80% EtOh	3.5 mm diameter, 13 mm length	0.3% PTA-10% + 3% DMSO for 35 days	*SkyScan 1173*, 120 Kv, 61 µA, 40 min 36 s, 8.14 µm

Micro-computed tomography (image acquisition)

Imaging was performed using two µCT scanners (Bruker MicroCT, Kontich, Belgium), SkyScan 1173 equipped with X-ray source of 40-130 Kv, Flat Panel sensor of 2240x2240 pixels and maximum detectability of 5 µm; and SkyScan 1272 equipped with X-ray source of 20-100 kV, Flat Panel sensor of 14450x14450 pixels and maximum detectability of 0,3-0,4 µm. Scanning parameters for these two µCT scanners were as follows: SkyScan 1173, source voltage = 35–123 kV, source current = 60-140 mA, exposure time = 350- 3,200 ms, frames averaged = 4–6, random movement = 10, filter= no, binning = no, flat field correction= on, and scanning time = about 40–166 min; and

SkyScan 1272 source voltage = 35–70 kV, source current = 140-142 mA, exposure time = 658-3,751 ms, frames averaged = 3–5, random movement = 10, filter= Al, binning = no, flat field correction= on, and scanning time = about 217–304 min. Images were reconstructed using the software Nrecon and processed with the software CTAnalyzer. DataViewer software was used for the visualization and interpretation of 2D serial tomographic sections. The plates of 2D images were made using inverse background to improve the sharpness and consequently the visualization of the virtual sections. CTVox software was used to perform the 3D volume rendering and to assist in the virtual dissections. Density-based false-colour renderings were applied to the data in order to facilitate the visualization of the internal structures.

Anatomical description

The mantle, siphons, ctenidia, labial palps, musculature and the organs of the digestive, reproductive and nervous system were specially emphasized and described, when possible. Some shell features of these species (outline, thickness, outer sculpture and ligament) also had been provided using stereomicroscope (Fig. 1). In order to put these species in a taxonomic context, a summary about the most accepted classification for these species was also provided before each description (see Bieler *et al.* 2014). At the end of each description there is a list containing the main references that helped us in the identification of the species and interpretation of the tomographic images. In addition, it worth noting that among the bivalves studied, just *Allograma formosa* (Lyonsiellidae) already had detailed anatomical information available in the literature (see Morton 1984b), while the other seven species were hitherto unknown as to their anatomical aspects.

RESULTS

Herein, the 3D tomographic reconstructions were able to reproduce with good quality all large anatomical structures (siphons, ctenidia, labial palps, etc) of the eight specimens analysed, being therefore extremely useful for the visualization and interpretation of these structures. Specifically, for some minute organs of the visceral mass (small nervous ganglia, kidney, heart) the three-dimensional reconstructions were not very effective, being that some of them could not be easily visualized. For these minute organs, therefore, visualization was only possible through the 2D tomographic sections. Anatomical structures measuring less than 4µm, as for example, mantle margin glands, statocysts and gametic cells were not possible to be observed through µCT scanners used in this study.

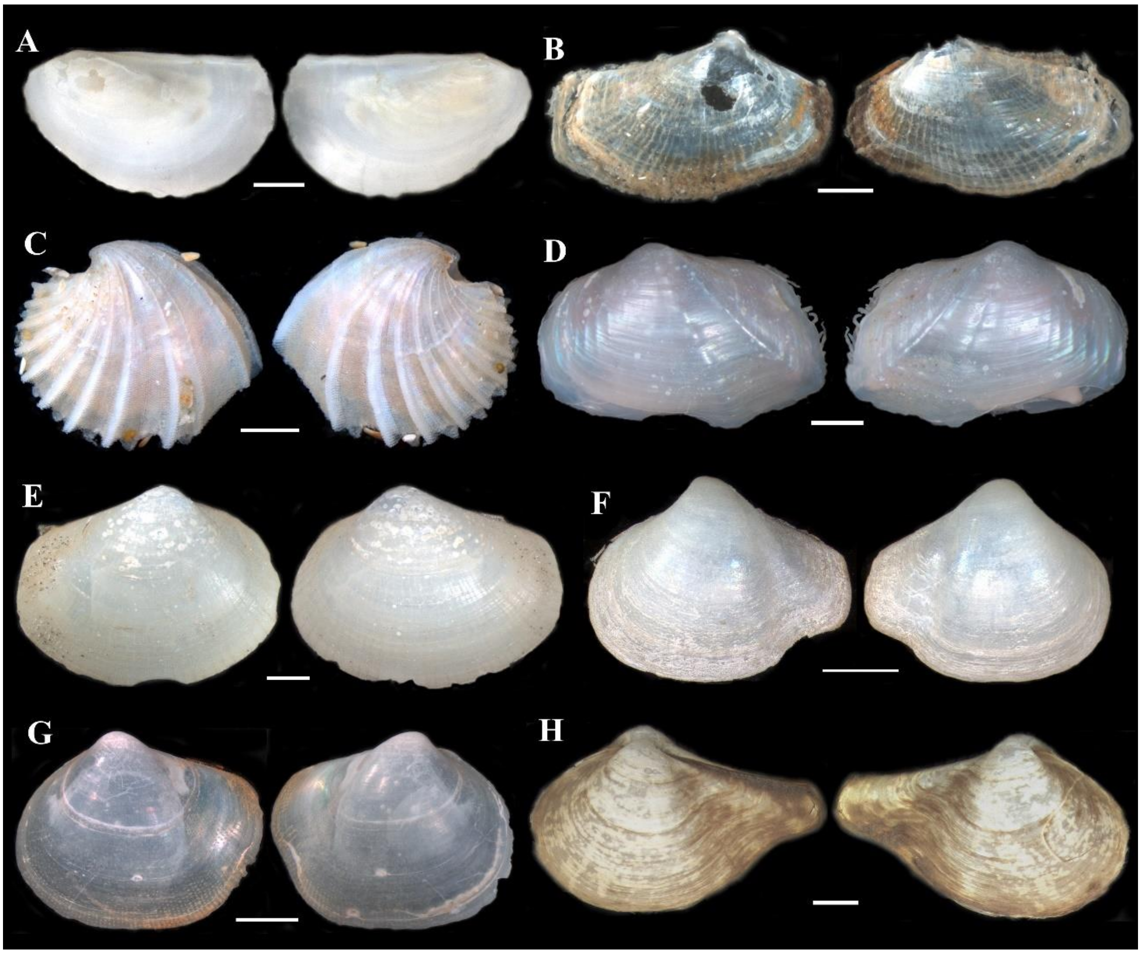

Figure 1. Shell photomicrographys of the specimens scanned. A. *Pandora pinna* (MCZ 375576), B. *Lyonsia alvarezii* (ZUEC 7005), C. *Trigonulina ornata* (ZUEC 7004), D. *Allograma formosa* (ZUEC 7003), E. *Cetoconcha spinosula* (ZUEC 7002), F. *Poromya rostrata* (ZUEC 2243), G. *Cetoconcha* aff. *smithii* (ZUEC 2235), H. *Cuspidaria glacialis* (MCZ 388260). Scale bars: A, B, D-H = 2 mm, C = 1 mm.

Below follow the anatomical descriptions for these bivalves plus two anatomical plates for each species: (i) 2D tomographic images, with transverse, sagittal and frontal sections, (ii) 3D renderings, showing the virtual dissections.

Systematics

Class Bivalvia Linnaeus, 1758
Subclass Heterodonta Neumayr, 1884
Infraclass Euheterodonta Giribet & Distel, 2003

Superorder Anomalodesmata Dall, 1889

Family Pandoridae Rafinesque, 1815

Genus *Pandora* Bruguière, 1797

Pandora pinna (Montagu, 1803)

(Figs. 2, 3)

Description

Shell: subquadrate to subovate, robust, very compressed, strongly inequivalve with left valve inflated and right valve flat or slightly concave, lithodesma absent. *Mantle*: mantle margin fused forming a short anterior pedal aperture and posteriorly forming short and very similar siphons; fourth pallial aperture absent. *Siphons*: inhalant and exhalant siphons similar in size and outline, simple tube-shaped, fused almost to the tip but not covered with periostracum; apertures of the inhalant and exhalant fringed with a ring of, respectively, ~18 and ~16 short tentacles. *Ctenidia*: eulamellibranch and plicate; complete with a large inner demibranch and a much reduced outer demibranch consisting only of reflected descending lamellae; presence of a marginal food groove on the inner demibranch. *Labial palps*: large, wide, lamellate with sorting ridges, complete and symmetrical (typically bivalve plan). *Musculature*: adductor muscles present, well developed, posterior slightly larger than anterior; presence of poor developed posterior and anterior pedal retractor muscles. *Foot*: well developed with a large pedal groove. *Digestive system*: presence of a tube-shaped mouth, long and thin oesophagus, small and rounded shape stomach with a short anterodorsal extension; stomach associated to the long crystalline style sac; crystalline style not visible in the specimen analysed; style sac conjoined with the mid gut; hind gut/rectum penetrates the heart and kidney to pass over the posterior adductor muscle and the end in an anus. *Organs of the visceral mass*: heart + kidney apparently supported by a thin accessory muscle. *Reproductive system*: located in the dorsal portion of the visceral mass, the ovarian is closely associated to the digestive diverticulum; although congeneric species are generally described as hermaphrodites, testes were not observed here. *Nervous system*: presence of circumesophagic and visceral ganglia; pedal ganglia and statocysts not visible.

Main references: Pelseneer (1911), Allen (1954), Thomas (1994), Morton (1984a), Güller & Zelaya (2016).

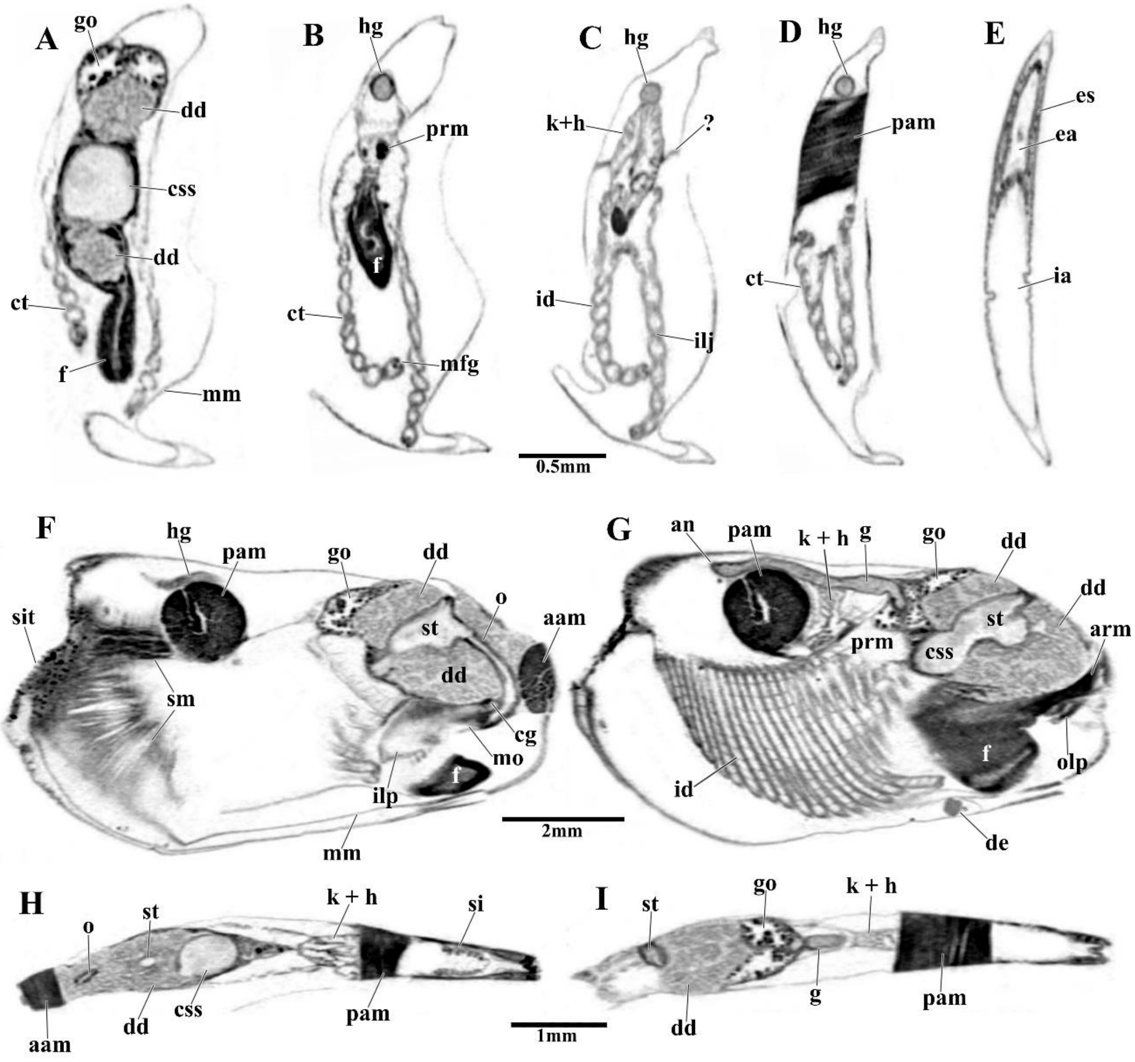

Figure 2. Selected virtual 2D sections through the µCT dataset of a PTA-stained specimen of ***Pandora pinna***. Transverse (A-E), sagittal (F, G) and frontal (H, I) sections. Abbreviations: an, anus; aam, anterior adductor muscle; arm, anterior retractor muscle; cg, circumesophagic ganglia; ct, ctenidia; css, crystalline style sac; dd, digestive diverticulum; de, debris; ea, exhalant siphonal aperture; es, exhalant siphon; f, foot; g, gut; go, gonad; hg, hind gut; ia, inhalant siphonal aperture; id, inner demibranch; ilj, interlamellar junctions; ilp, inner labial palp; k + h, kidney + heart; mfg, marginal food groove; gut; mo, mouth; mm, mantle margin; o, oesophagus; olp, outer labial palp; pam, posterior adductor muscle; prm, posterior retractor muscle; st, stomach; ?, unknown structure (maybe an accessory muscle). Scale bars: A-E – 0.5mm; F, G – 2mm; H, I – 1mm.

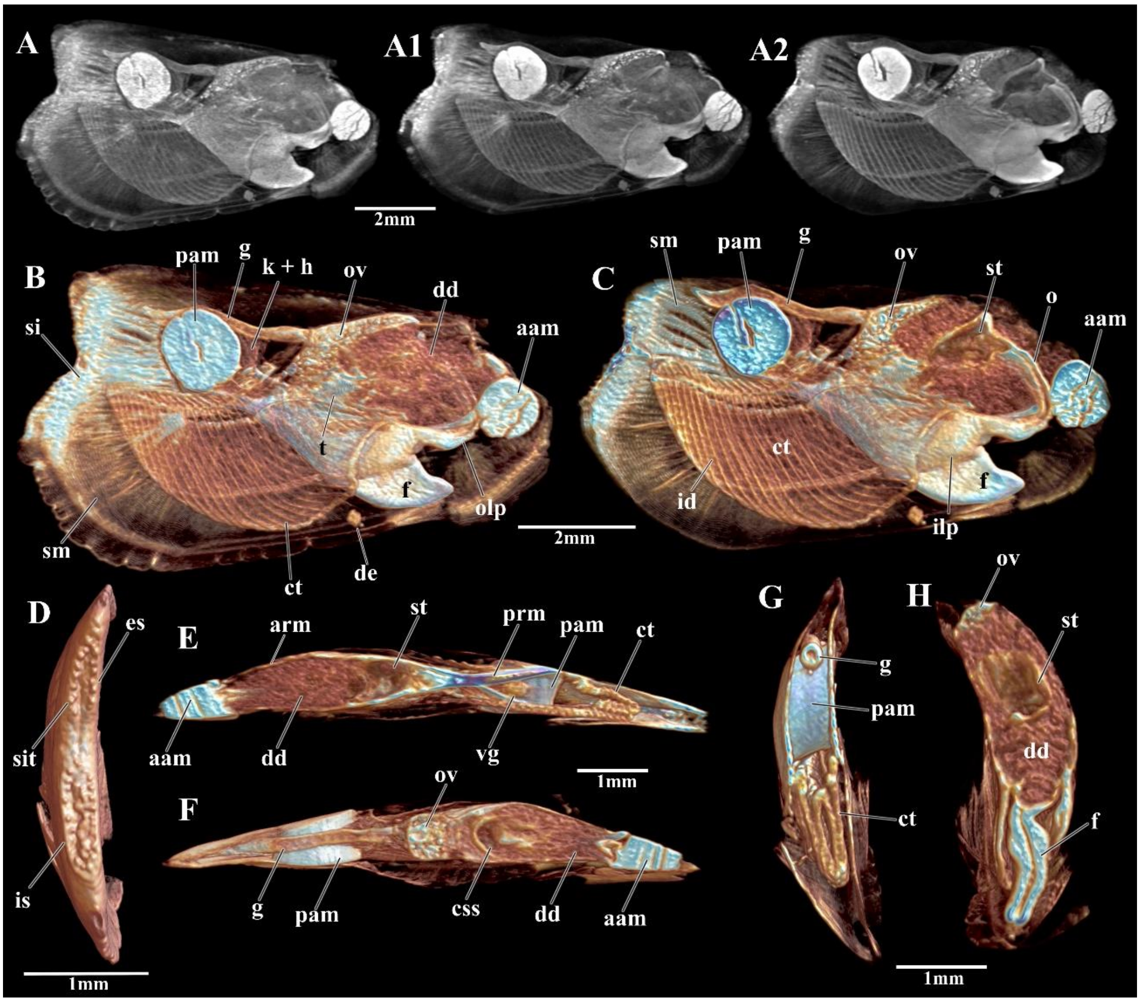

Figure 3. 3D volume rendering based on the µCT dataset of ***Pandora pinna***. Dissection sequence in original tomographic color (A-A2); false-color volume rendering (B-H) and virtual dissections (A1, A2, C-H). Abbreviations: aam, anterior adductor muscle; arm, anterior retractor muscle; ct, ctenidia; css, crystalline style sac; dd, digestive diverticulum; de, debris; es, exhalant siphon; f, foot; g, gut; hg, hind gut; ia, inhalant siphonal aperture; id, inner demibranch; ilp, inner labial palp; is, inhalant siphon; k + h, kidney + heart; mo, mouth; mm, mantle margin; o, oesophagus; olp, outer labial palp; ov, ovarian; pam, posterior adductor muscle; prm, posterior retractor muscle; si, siphons; sit, siphonal tentacle; sm, siphonal musculature; st, stomach; t, testis; vg, visceral ganglia. Scale bars: A-C – 2mm; D, H – 1mm.

Family Lyonsiidae P. Fischer, 1887

Genus *Lyonsia* Turton, 1822

Lyonsia alvarezii d'Orbigny, 1846

(Figs. 4, 5)

Description

Shell: subovate-elongated, thin, delicate, translucid, moderately inflate in the anterior part, posterior end laterally compressed; external sculpture formed by thin radial ridges with sand grains attached by the entire extension of the valves; presence of a large lithodesma. *Mantle*: this specimen had an important part of the mantle lost during the removal of the shell, turning the analysis incomplete; presence of a fourth pallial opening closer to the inhalant aperture. *Siphons*: inhalant and exhalant siphons similar in size and outline, short, simple tube-shaped and separated; papillate tentacles covering the inhalant siphon; apertures of the inhalant and exhalant fringed with a ring of, respectively, ~32 and ~45 short tentacles. *Ctenidia*: eulamellibranch and plicate; complete with a large inner demibranch bigger than outer; free edge of inner demibranch with a deep marginal food groove. *Labial palps*: large, wide, lamellate with sorting ridges, symmetrical (typically bivalve plan) and coiling. *Musculature*: adductor muscles present, heteromyarian, with the posterior adductor well-developed and the anterior reduced; presence of well-developed posterior and a reduced anterior pedal retractor muscle. *Foot*: elongated with a long pedal groove; presence of byssal thread. *Digestive system*: the tube-shaped mouth opens into a long oesophagus that enters into the anterior portion of the stomach; with an elongated shaped, the stomach is connected to the also elongated crystalline style sac; style sac conjoined with an anterior coiled mid gut; hind gut/rectum penetrates the heart; kidney not visible. *Reproductive system*: hermaphrodite, ovarian located in the dorsal portion of the visceral mass, closely associated to the digestive diverticulum; testis small and poor visible located in the anterior portion of visceral mass. *Nervous system*: nervous ganglia not visible.

Main references: Yonge (1952), Narchi (1968), Morgan & Allen (1976), Morton (1987a), Thomas (1994).

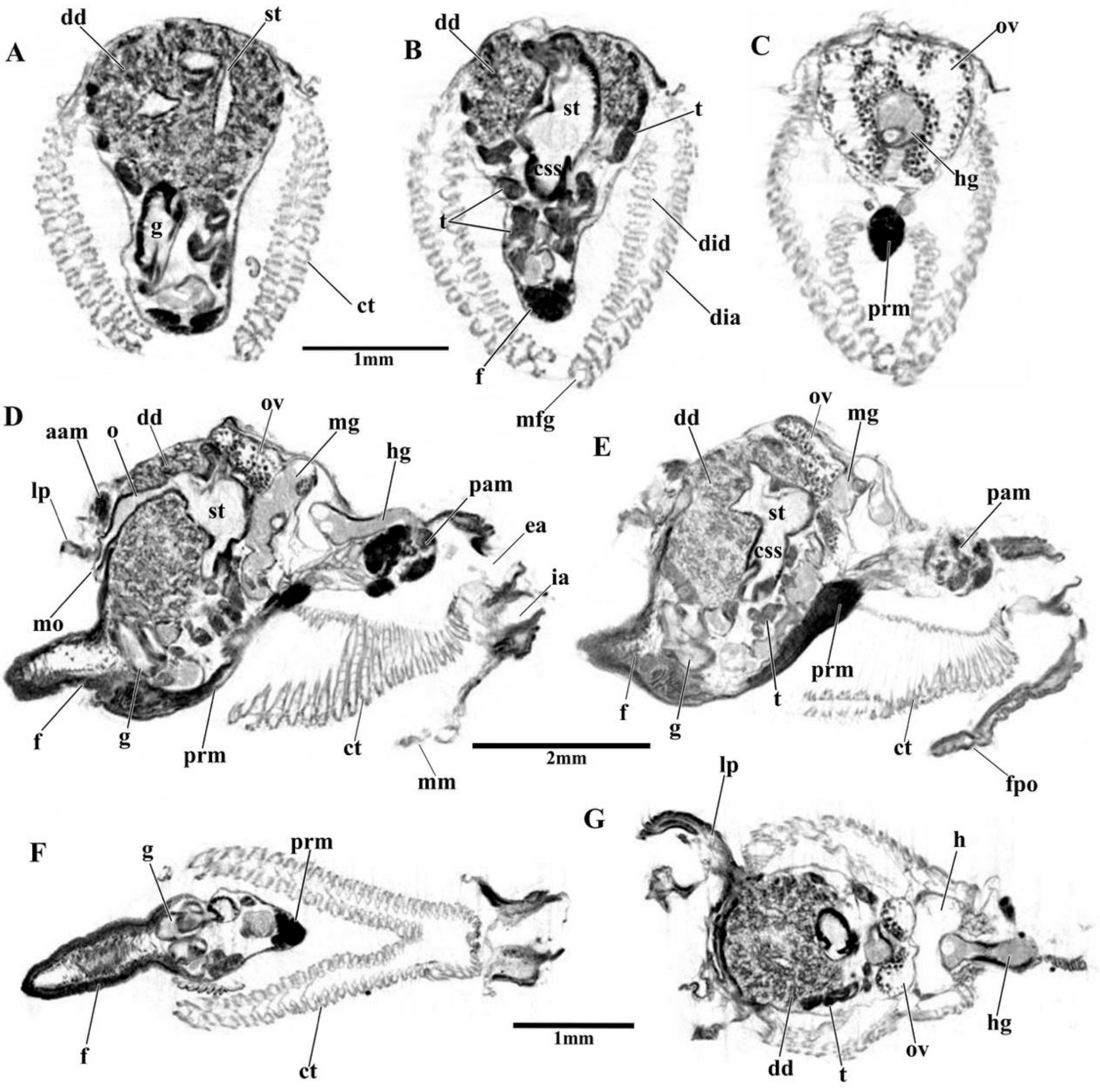

Figure 4. Selected virtual 2D sections through the µCT dataset of a PTA-stained specimen of ***Lyonsia alvarezii***. Transverse (A-C), sagittal (D, E) and frontal (F, G) sections. Abbreviations: aam, anterior adductor muscle; ct, ctenidia; css, crystalline style sac; dd, digestive diverticulum; did/dia, descending and ascending lamella of the inner demibranch; ea, exhalant siphonal aperture; f, foot; fpo, fourth pallial opening; g, gut; h, heart; hg, hind gut; ia, inhalant siphonal aperture; k, kidney; lp, labial palp; mfg, marginal food groove; mg, mid gut; mo, mouth; mm, mantle margin; o, oesophagus; ov, ovarian; pam, posterior adductor muscle; prm, posterior retractor muscle; st, stomach; t, testis. Scale bars: A-C, F, G – 1mm; D, E – 2mm.

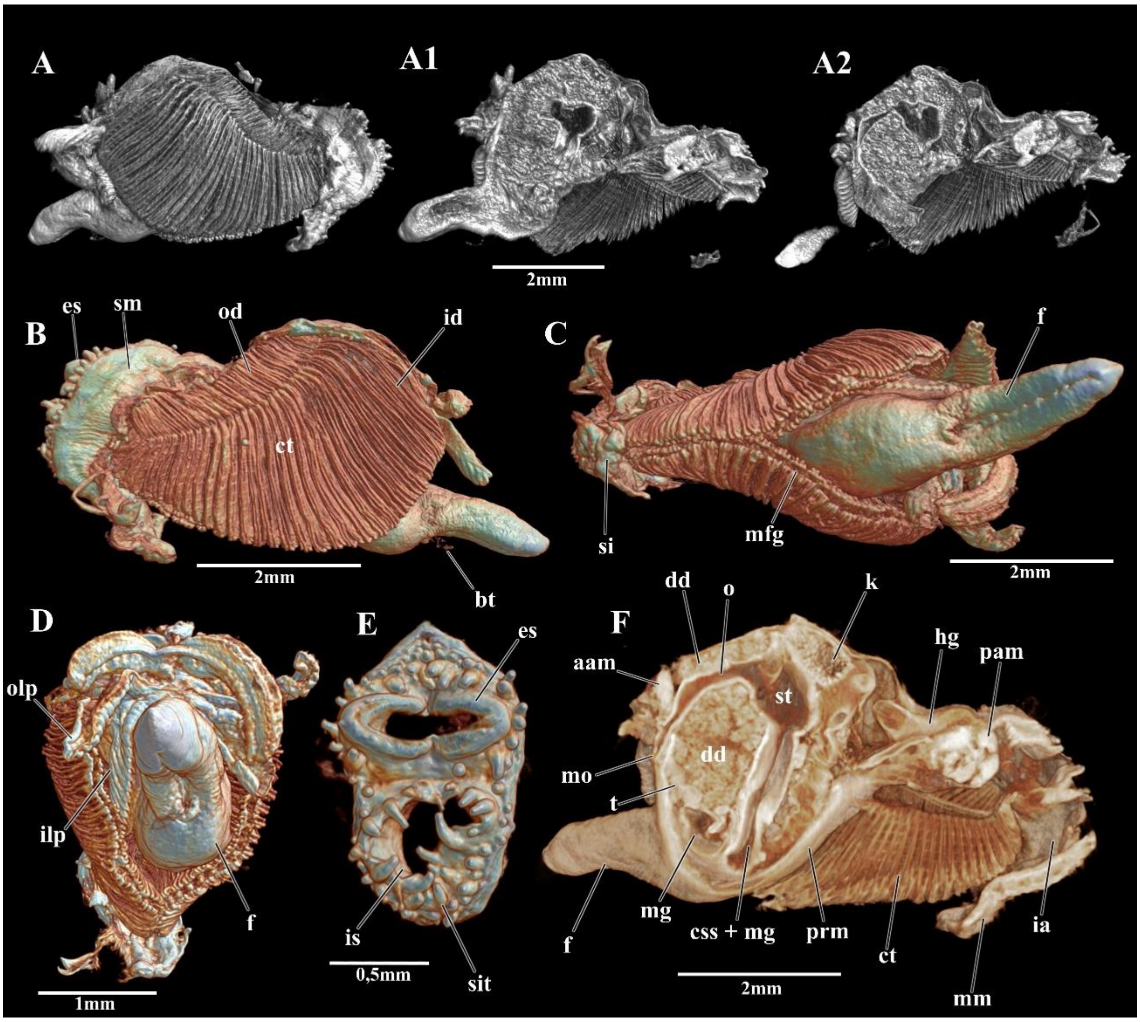

Figure 5. 3D volume rendering based on the µCT dataset of ***Lyonsia alvarezii***. Dissection sequence in original tomographic color (A-A2); false-color volume rendering (B-F) and virtual dissections (A1, A2, D-F). Abbreviations: aam, anterior adductor muscle; bt, byssal thread; ct, ctenidia; css + mg, crystalline style sac + mid gut; dd, digestive diverticulum; es, exhalant siphon; f, foot; g, gut; hg, hind gut; ia, inhalant aperture; id, inner demibranch; ilp, inner labial palp; is, inhalant siphon; k, kidney; mfg, marginal food groove; mg, midgut; mm, mantle margin; mo, mouth; o, oesophagus; od, outer demibranch; olp, outer labial palp; pam, posterior adductor muscle; prm, posterior retractor muscle; si, siphons; sm, siphonal musculature; st, stomach; sit, siphonal tentacles, t, testis. Scale bars: A-C, F – 2mm; D – 1mm; E – 0.5mm.

Family Lyonsiellidae Dall, 1895

Genus *Allogramma* Dall, 1903

Allogramma formosa (Jeffreys, 1882)

(Figs. 6, 7)

Description

Shell: subquadrate, inflated, equivalve and inequilateral; extremely fragile, pearl glow; outer sculpture well marked with two long radial ridges from the umbo until the posterior ventral margin of the valves, small spines in the posterodorsal portion of the shell; presence of lithodesma. *Mantle*: mantle margin with two fused points, anteriorly forming a wide pedal gape and posteriorly forming the siphons; postero-ventrally mantle margin fusion formed by inner and middle folds (Type B) (Yonge, 1982); absence of a fourth pallial aperture. *Siphons*: separated, different in size and outline; inhalant siphon, large, modified in a raptorial appendice, usually contracted into the pallial cavity; exhalant siphon short, tube-shaped; both surrounded in the base by a ring of approximately 50 smaller tentacles closest to the apertures and by ~60 bigger tentacles peripherally located. *Ctenidia*: eulamellibranch very reduced, non-plicate and horizontal aligned; complete, with two demibranchs. *Labial palps*: non-lamellate, extremely complex, outer and inner palps medially fused forming two pouches or buccal sacs, one small anterior and another large posterior; unfused tips forming narrow fluted funnels laterally. *Musculature*: posterior and anterior adductor muscles present and isomyarian; presence of posterior and anterior pedal retractor muscle; absence of taenioid muscle. *Foot*: large and elongated; absence of byssal thread. *Digestive system*: the funnel-shaped mouth opens into a thick and muscular oesophagus that enters into the anterodorsal portion of the stomach, a sphincter is present between the oesophagus and the stomach; the stomach is small, rounded, with internal longitudinal grooves in the dorsal wall, connected to the large crystalline style sac and surrounded dorsally and anteriorly by gonads and digestive diverticulum; style sac conjoined with an anterior coiled mid gut; presence of crystalline style; hind gut/rectum penetrates the heart and the kidney. *Organs of visceral mass*: a lacunar system formed by haemocoel spaces is present in the posterior portion of visceral mass associated with kidney and heart. *Reproductive system*: hermaphrodite, ovarian and testis well visible closely associated to the digestive diverticulum; ovarian dorsally located and testis in the anteroventral portion of visceral mass penetrating partially into the foot. *Nervous system*: presence of circumesophagic, pedal and visceral ganglia; nervous bundles of visceral ganglia branching into the siphons, visceral mass and ctenidia.

Main references: Knudsen (1967, 1970), Allen & Turner (1974), Morton (1984b), Poutiers & Bernard (1995).

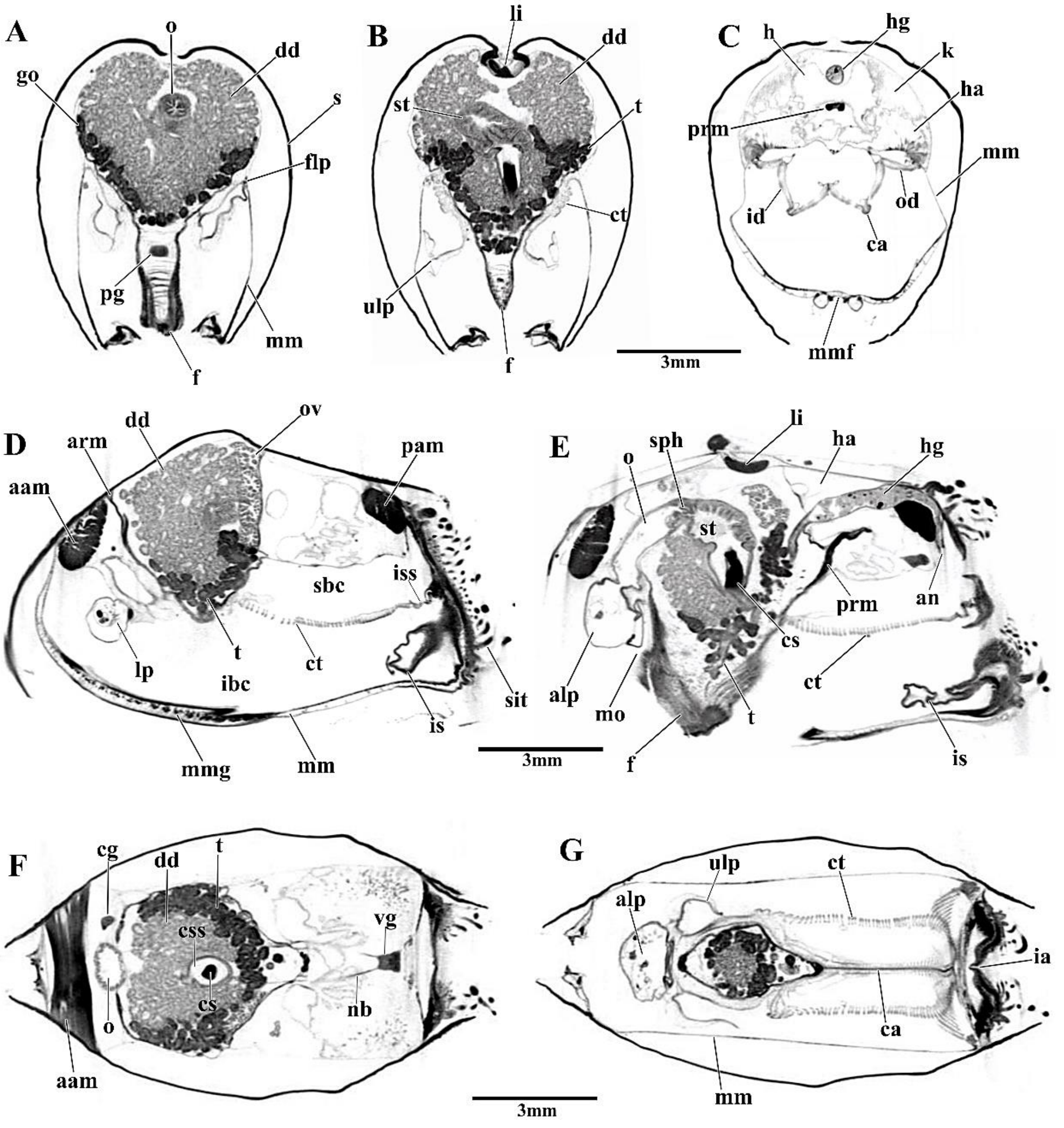

Figure 6. Selected virtual 2D sections through the µCT dataset of a PTA-stained specimen of ***Allograma formosa***. Transverse (A-C), sagittal (D, E) and frontal (F, G) sections. Abbreviations: an, anus; aam, anterior adductor muscle; alp, anterior labial palp or anterior labial pouch; arm, anterior retractor muscle; ca, ctenidia axis; cg, circumesophagic ganglia; ct, ctenidia; cs, crystalline style; css, crystalline style sac; dd, digestive diverticulum; f, foot; flp, fusion of the labial palp; go, gonad; h, heart; ha, haemocoel space; hg, hind gut; ibc, infra-branchial chamber; id, inner demibranch; is, inhalant siphon; iss, inter-siphonal septum; ibc, infra-brachial chamber; k, kidney; li, lithodesma (calcified ligament); lp, labial palp; mm, mantle margin; mmf, mantle margin

fused; mmg, mantle margin glands; mo, mouth; nb, nervous bundles; o, oesophagus; od, outer demibranch; ov, ovaria; pam, posterior adductor muscle; pg. pedal ganglia; prm, posterior retractor muscle; s, shell; sbc, supra-branchial chamber; sit, siphonal tentacle; sph, sphincter; st, stomach; t, testis; ulp, unfused labial palps, vg, visceral ganglia. Scale bars: A-G – 3mm.

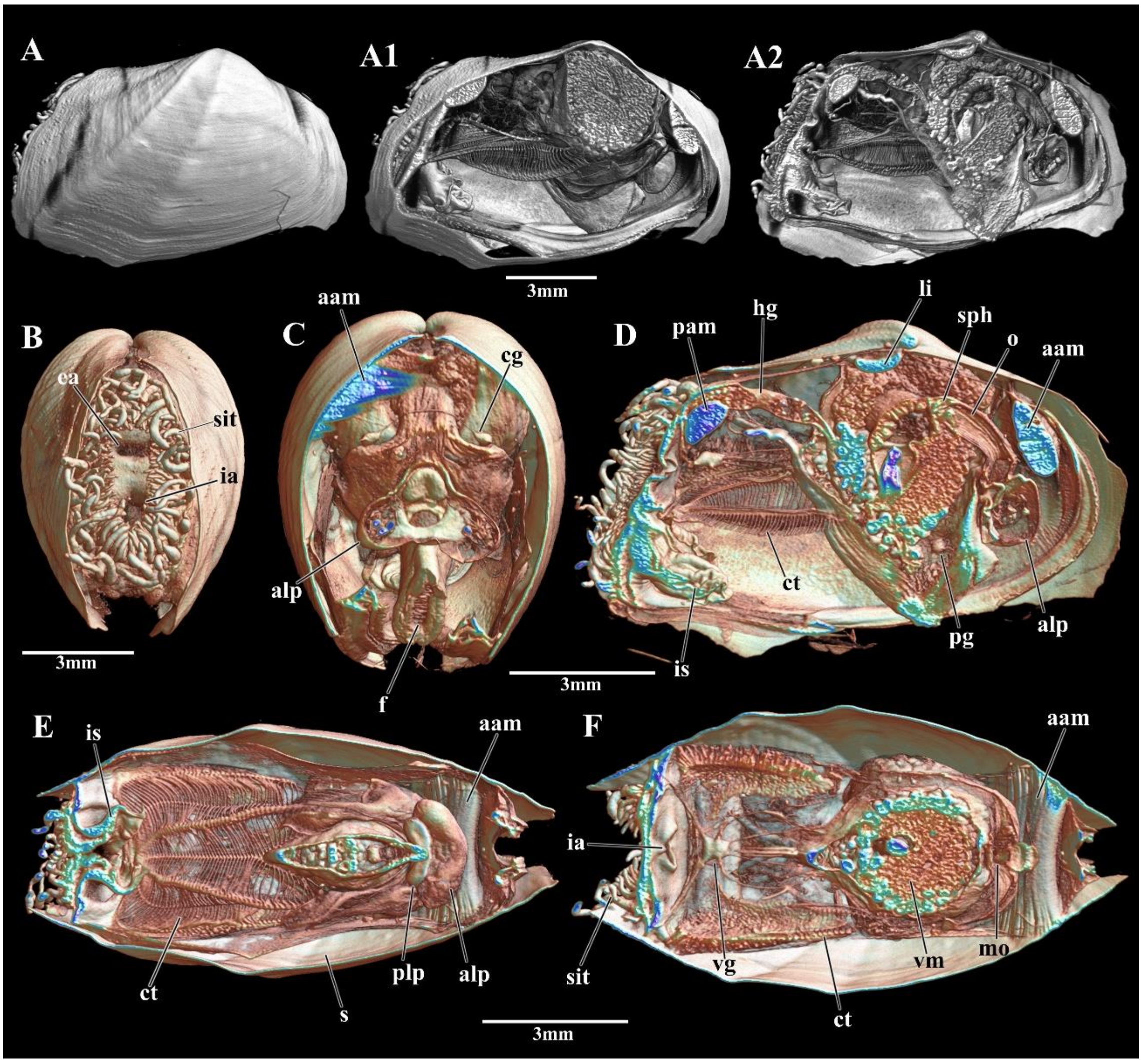

Figure 7. 3D volume rendering based on the µCT dataset of ***Allograma formosa***. Dissection sequence in original tomographic color (A-A2); false-color volume rendering (B-F) and virtual dissections (A1, A2, C-F). Abbreviations: aam, anterior adductor muscle; alp, anterior labial palp or anterior labial pouch; cg, circumesophagic ganglia; ct, ctenidia; ea, exhalant aperture; f, foot; hg, hind gut; ia, inhalant aperture; is, inhalant siphon; li, lithodesma (calcified ligament); mo, mouth; o, oesophagus; pam, posterior adductor muscle; pg, pedal ganglia; plp, posterior labial palp or posterior labial pouch; s,

shell; sit, siphonal tentacles; sph, sphincter; vg, visceral ganglia; vm, visceral mass. Scale bars: A-F – 3mm.

Family Verticordiidae Stoliczka, 1870

Genus *Trigonulina* d'Orbigny, 1853

Trigonulina ornata d'Orbigny, 1853

(Figs. 8, 9)

Description

Shell: oval, compressed, robust; external sculpture formed by prominent and irregularly spaced radial ribs; lunule deeply impressed; debris attached by the entire length of outer surface of the valves; lithodesma present. *Mantle*: mantle margin completely unfused anteriorly forming a large pedal aperture; posteriorly there is a ventral fusion forming the siphons; absence of a fourth pallial opening. *Siphons*: separated; inhalant siphon, small, muscular, half cone-shaped, inverted into pallial cavity; exhalant, very small, probably contracted, not visible; siphonal apertures surrounded in their base by siphonal tentacles, ~20 around the inhalant and 3 around the exhalant aperture. *Ctenidia*: very reduced, non-plicate and horizontal aligned; complete, with inner and reduced outer demibranchs, extended from the mouth to the ventral side of the exhalant aperture. *Labial palps*: absent *Musculature*: adductor muscles present, isomyarian; presence of posterior and anterior pedal retractor muscle. *Foot*: small, short; pedal groove and byssal thread absent. *Digestive system*: the funnel-shaped mouth opens into a thick and muscular oesophagus that enters into the anterodorsal portion of the stomach; oesophagus large with the presence of longitudinal internal grooves throughout its all length; the stomach is rounded, large, probably dilated by the presence of huge preys (maybe ostracods); stomach connected to the short and reduced crystalline style sac with a typhlosole stomach and the crystalline sac open; the stomach is surrounded dorsally and anteriorly by gonads and digestive diverticulum; style sac conjoined with the mid gut; crystalline style absent; hind gut/rectum penetrates the heart. *Organs of visceral mass*: haemocoel spaces can be observed closed to the siphons. *Reproductive system*: hermaphrodite, ovarian located in the dorsal portion of the visceral mass, closely associated to the digestive diverticulum; testis located in the anterior portion of visceral mass. *Nervous system*: presence of circumesophagic, pedal and visceral ganglia.

Main references: Allen & Turner (1974), Morton (1987b).

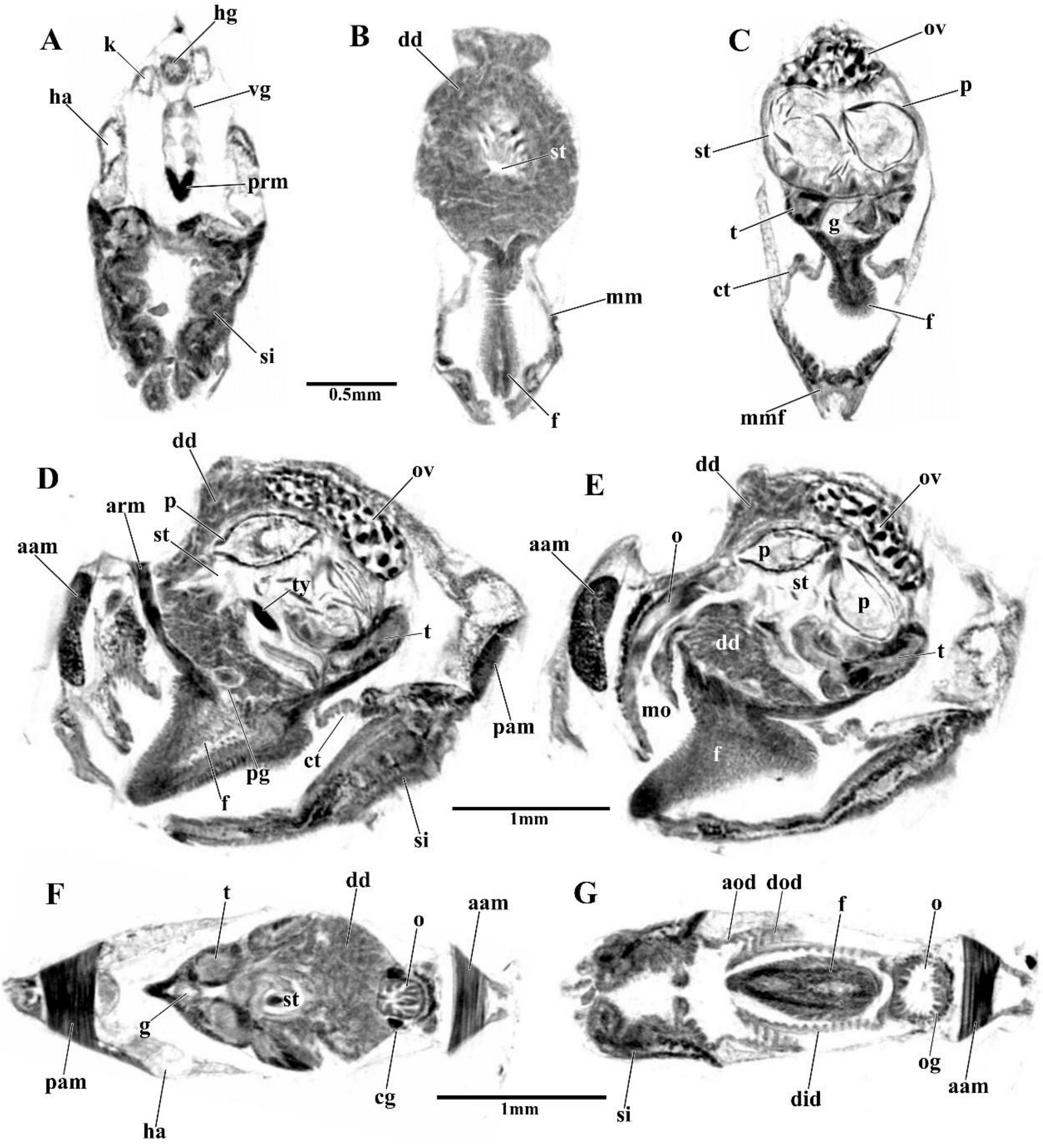

Figure 8. Selected virtual 2D sections through the µCT dataset of a PTA-stained specimen of ***Trigonulina ornata***. Transverse (A-C), sagittal (D, E) and frontal (F, G) sections. Abbreviations: aam, anterior adductor muscle; aod, ascending lamellae of outer demibranch; arm, anterior retractor muscle; cg, circumesophagic ganglia; ct, ctenidia; dd, digestive diverticulum; did/dod, descending lamella of the inner and outer demibranch; f, foot; g, gut; ha, haemocoel space; hg, hind gut; k, kidney; mo, mouth; mm, mantle margin; mmf, mantle margin fusion; o, oesophagus; ; og, oesophagus grooves; ov, ovarian; p, prey inside stomach; pam, posterior adductor muscle; pg, pedal ganglia; prm, posterior retractor muscle; si, siphons; st, stomach; t, testis; ty, typhlosole; vg, visceral ganglia. Scale bars: A-C – 0.5mm; D-G – 1mm.

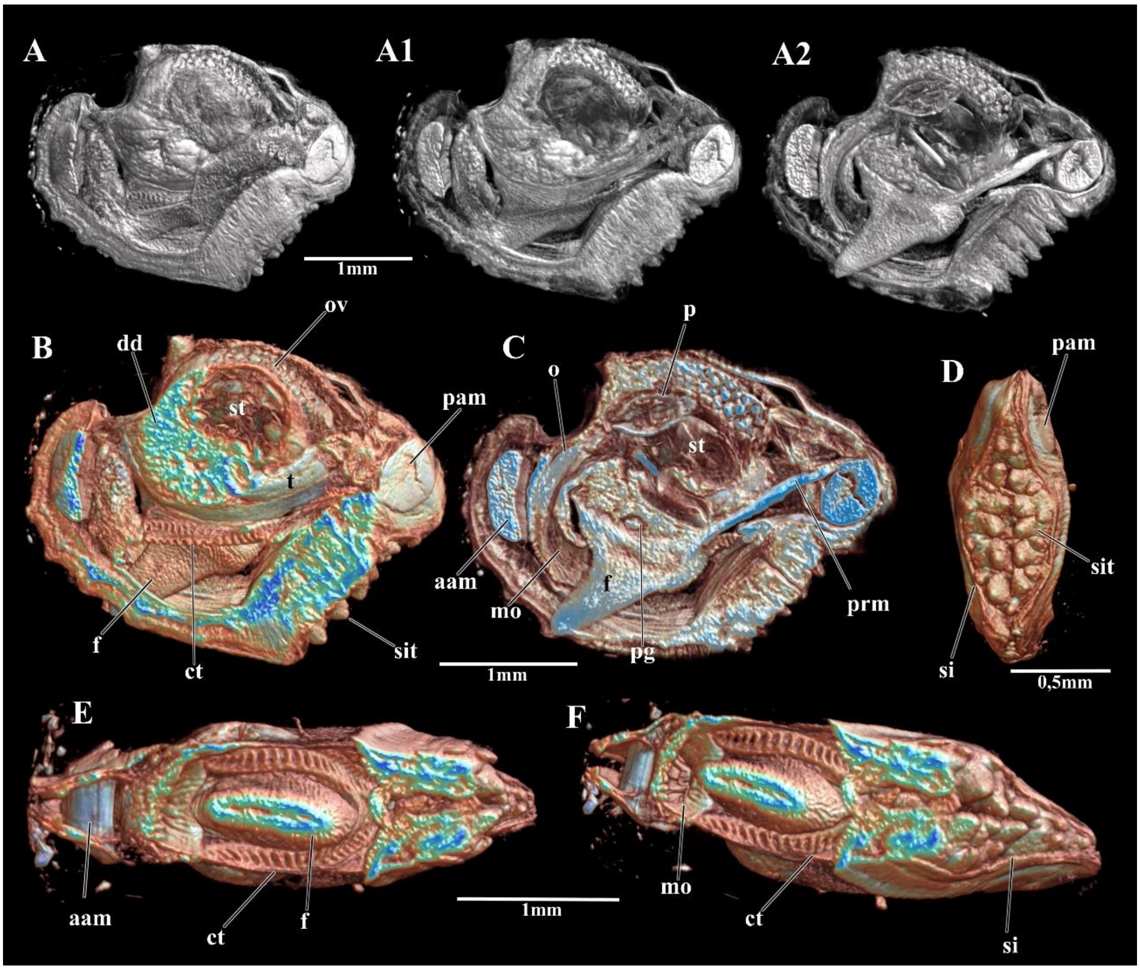

Figure 9. 3D volume rendering based on the µCT dataset of ***Trigonulina ornata***. Dissection sequence in original tomographic color (A-A2); false-color volume rendering (B-F) and virtual dissections (A1, A2, B-F). Abbreviations: aam, anterior adductor muscle; ct, ctenidia; dd, digestive diverticulum; f, foot; mo, mouth; o, oesophagus; ov, ovarian; p, prey inside stomach; pam, posterior adductor muscle; pg, pedal ganglia; prm, posterior retractor muscle; si, siphons; sit, siphonal tentacle; st, stomach, t, testis. Scale bars: A-F – 1mm.

Family Poromyidae Dall, 1886

Genus *Poromya* Forbes, 1844

Poromya rostrata (Rehder, 1943)

(Figs. 10, 11)

Description

Shell: ovate-trigonal, inflated, thin, whitish, inequilateral, slightly inequivalve; posterior margin with a short rostrum; outer sculpture granulated with micro pustules over the entire shell; absence of lithodesma. *Mantle*: ventral mantle margin with one anteriorly wide pedal gape, extending from the anterior adductor until the inhalant siphon; absence of a fourth pallial aperture. *Siphons*: separated, different in size and outline; inhalant siphon, large, modified in a raptorial appendice, typically retracted into the infra-septal chamber; exhalant siphon short, cone-like and everted in this specimen; both surrounded in the base by a ring of large siphonal tentacles, 12 around the inhalant and 3 around exhalant; debris are also attached in the siphonal walls. *Septum*: thin, ventral surface with well defined two paired groups of slit-like branchial apertures, with no interfilamentar connections; anterior group with four and posterior with five slits. Presence of a small and lobulate hollow sac in the posterior inner floor of the septum. *Labial palps*: non-lamellate, flattened and asymmetrical with anterior labial palp large and posterior labial palp small. *Musculature*: posterior and anterior adductor muscles present and isomyarian; presence of posterior and anterior pedal and septal retractor muscles; lateral septal muscles not visible in this specimen; taenioid muscle absent. *Foot*: large and elongated; absence of pedal groove and byssal thread. *Digestive system*: the funnel-shaped mouth opens into a thick, short and muscular oesophagus that enters into the anterodorsal portion of the stomach; presence of a sphincter between oesophagus and the stomach opening; stomach large, rounded, internal grooves not visible into the gastric chamber; stomach connected to the short and small crystalline style sac located on the median portion of the stomach floor; no preys were observed inside of stomach of this specimen; stomach surrounded dorsally and anteriorly by gonads and digestive diverticulum; two gastric caeca were also observed from the digestive diverticulum; crystalline style sac conjoined with the anterior portion of the mid gut; crystalline style present; hind gut/rectum penetrates the heart and pass above the kidney. *Reproductive system*: hermaphrodite, ovarian and testis well visible; ovarian is closely associated to the digestive diverticulum cover the roof and the posterior wall of the stomach; the testis lie ventral to the ovary and consist of a pair of large lobulate sacs. *Nervous system*: presence of circumesophagic, pedal and visceral ganglia.

Main references: Pelsenner (1911), Yonge (1928), Allen & Morgan (1981), Morton (1981, 1987b).

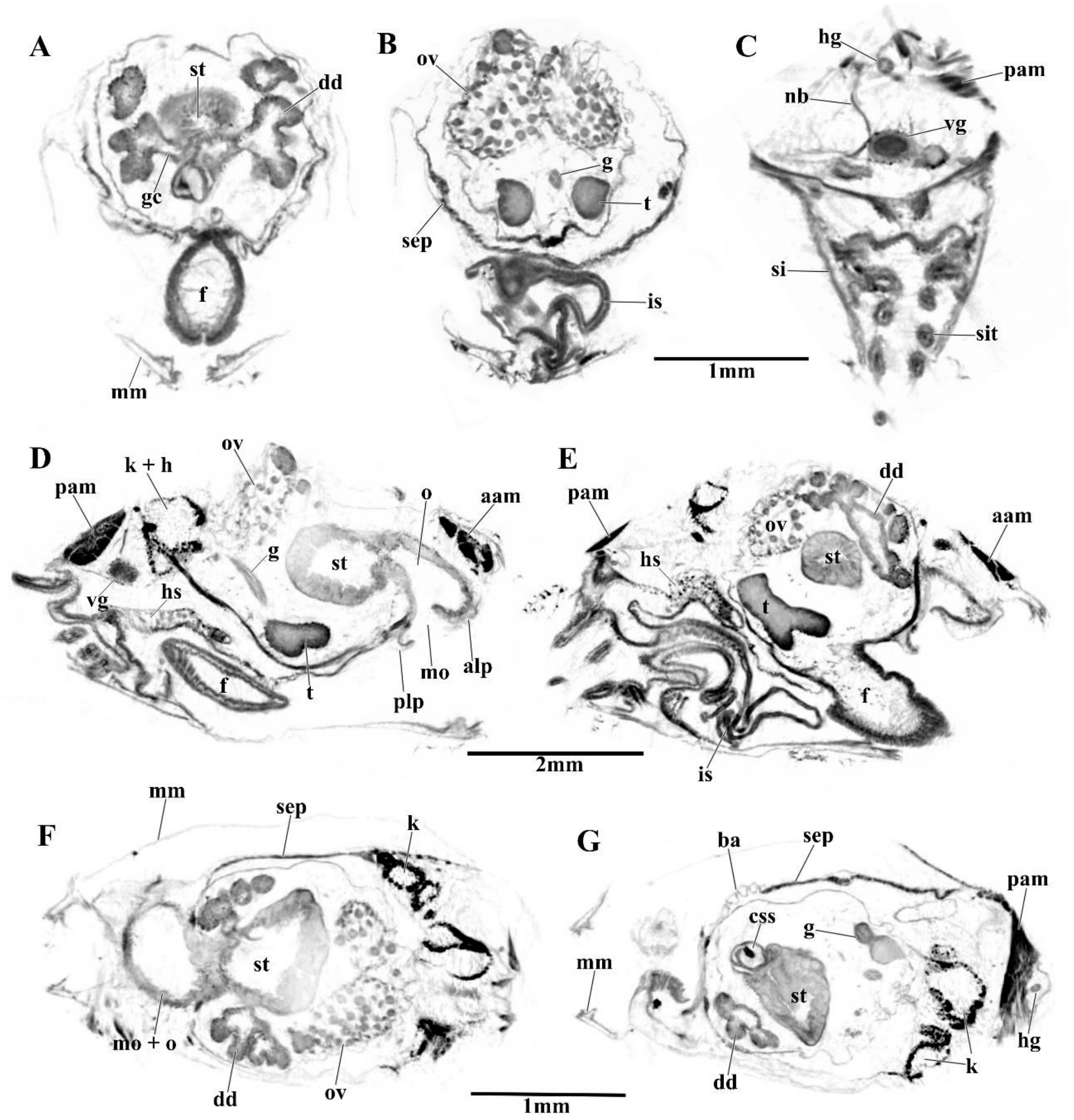

Figure 10. Selected virtual 2D sections through the µCT dataset of a PTA-stained specimen of ***Poromya rostrata***. Transverse (A-C), sagittal (D, E) and frontal (F, G) sections. Abbreviations: aam, anterior adductor muscle; alp, anterior labial palp; arm, anterior retractor muscle; bf, branchial filament; bs, branchial apertures; css, crystalline style sac; dd, digestive diverticulum; f, foot; g, gut; gc, gastric caecum; hg, hind gut; hs, hollow sac; is, inhalant siphon; k, kidney; mm, mantle margin; mo, mouth; mo + o, mouth + oesophagus; nb, nervous bundles; o, oesophagus; ov, ovarian; pam, posterior adductor muscle; plp, posterior labial palp; sep, muscular septum; sit, siphonal tentacles; st, stomach; t, testis; vg, visceral ganglia. Scale bars: A-C, F, G – 1mm; D, E – 2mm.

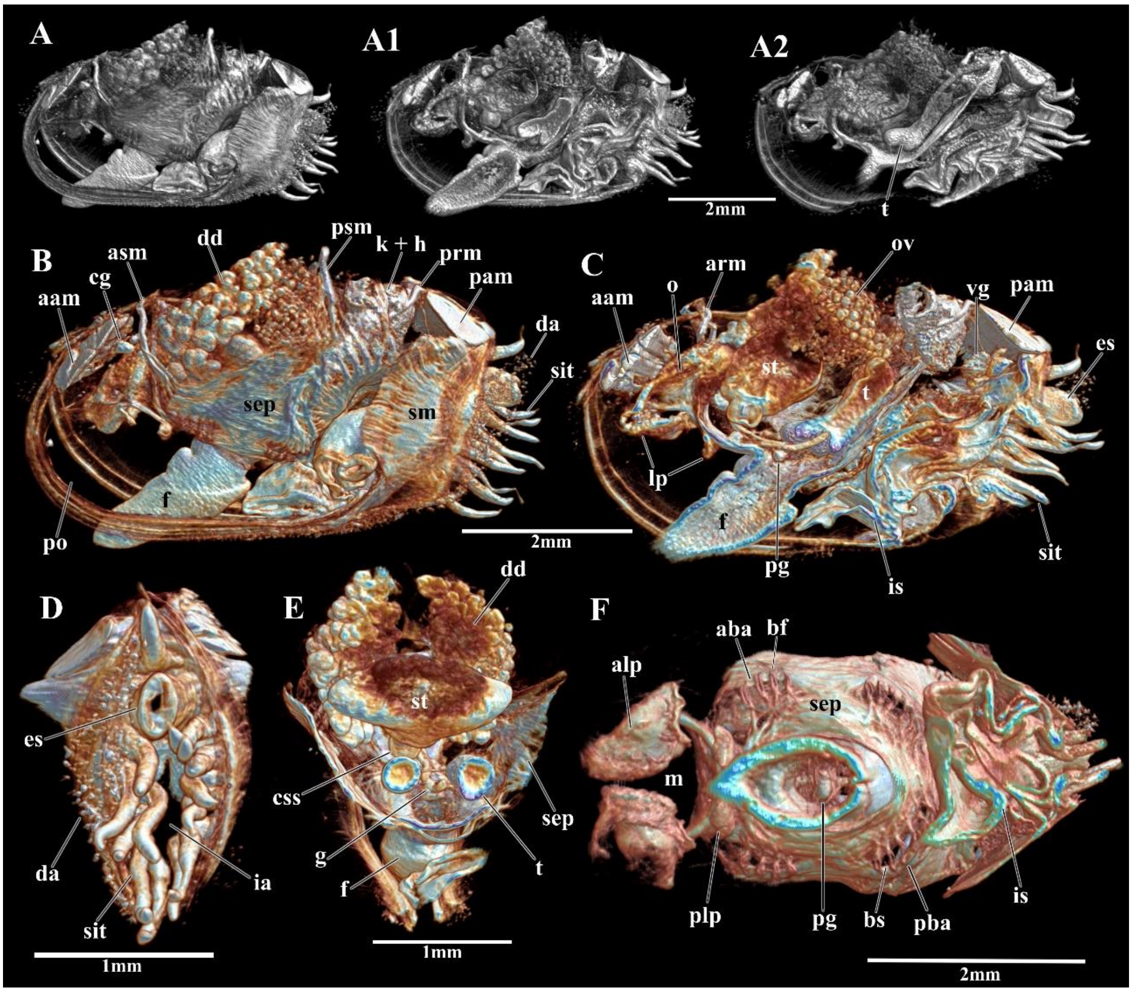

Figure 11. 3D volume rendering based on the µCT dataset of ***Poromya rostrata***. Dissection sequence in original tomographic color (A-A2); false-color volume rendering (B-F) and virtual dissections (A1, A2, C-F). Abbreviations: aam, anterior adductor muscle; aba, anterior branchial apertures; alp, anterior labial palp; arm, anterior retractor muscle; asm, anterior septal muscle; bf, branchial filament; bs, branchial sieve slit; da, debris attached; dd, digestive diverticulum; cg, circumesophagic ganglia; css, crystalline style sac; dd, digestive diverticulum; es, exhalant siphon; f, foot; g, gut; ia, inhalant aperture; ilp, inner labial palp; is, inhalant siphon; k + h, kidney + heart; lp, labial palp; mm, mantle margin; m, mouth; o, oesophagus; olp, outer labial palp; ov, ovarian; pam, posterior adductor muscle; pba, posterior branchial apertures; plp, posterior labial palp; pg, pedal ganglia; po, pedal opening; prm, posterior retractor muscle; psm, posterior septal muscle; sit, siphonal tentacle; sm, siphonal musculature; sep, septum; st, stomach; t, testis. Scale bars: A-C, F – 2mm; D, E – 1mm.

Family Cetoconchidae Dall, 1886

Genus *Cetoconcha* Forbes, 1844

Cetoconcha spinosula (Thiele, 1912)

(new combination for *Poromya spinosula sensu* Thiele)

(Figs. 12, 13)

Description

Shell: ovate, relatively thick, inflated, equivalve and approximately equilateral; presence of a brownish periostracum and sand grains attached covering the shell; absence of lithodesma. *Mantle*: ventral mantle margin with one anteriorly and wide pedal gape, extending from the anterior adductor until 2/3 of the full length of ventral margin; postero-ventrally mantle margin fusion formed by inner folds (Type A) (Yonge, 1982); absence of a fourth pallial aperture. *Siphons*: separated, different in size and outline; inhalant siphon, huge, modified in a raptorial appendice, typically retracted into the infra-septal chamber when the living animal is at rest or in museum specimens due to the alcohol contraction; the inverted inhalant siphon can also be referred to in the literature as siphonal cowl (hood) or branchial valve, the latter considered a misinterpretation (Pelsenner 1911, Yonge 1928, Bernard 1974, Morton 1981); exhalant siphon short, cone-like; siphons surrounded in the base by a ring of large siphonal tentacles, 10 around the inhalant and 3 around exhalant; presence of ~15 siphonal papillae between the siphonal tentacles. *Septum*: thin, transparent, perforated by three pairs of ostial perforations withouth interfilamentar connections, comprising six perforations anteriorly, five to six in the middle and four posteriorly. Presence of a swollen, hollow and bilobate sac in the posterior inner floor of the septum. *Labial palps*: non-lamellate and asymmetric with anterior labial palp, well-developed, large, cup-shaped and posterior palp reduced, almost imperceptible in the 3D reconstructions. *Musculature*: posterior and anterior adductor muscles present, isomyarian; presence of posterior and anterior pedal and septal retractor muscles; lateral septal muscles and taenioid muscle absent. *Foot*: large and elongated; absence of pedal groove and byssal thread. *Digestive system*: the funnel-shaped mouth opens into a thick, short and muscular oesophagus that enters into the anterodorsal portion of the stomach; no sphincter was observed between oesophagus and the stomach opening; stomach large, rounded, with longitudinal deep grooves in the posterodorsal and anteroventral walls; presence of preys inside of the gastric chamber (maybe ostracod and copepods); stomach connected to the short and small crystalline style sac located on the ventroanterior portion of the stomach floor; stomach surrounded dorsally and anteriorly by gonads and digestive diverticulum; crystalline style sac conjoined with an anterior mid gut; crystalline style not visible; hind gut/rectum penetrates the heart and pass above the kidney. *Organs of visceral mass*: haemocoel spaces are present in the dorsoposterior portion of visceral mass. *Reproductive system*: hermaphrodite; ovarian and testis well visible closely associated to the digestive diverticulum; ovarian dorsally located and testis in the anteroventral portion of visceral mass close to the lateral wall of the stomach. *Nervous system*: presence of circumesophagic, pedal and visceral ganglia.

Main references: Pelsenner (1911), Thiele (1912: plate 18, fig. 26), Yonge (1928), Bernard (1974), Allen & Morgan (1981), Morton (1981, 1987b), Dell (1990: fig. 107), Aldea & Troncoso (2010: fig. 245).

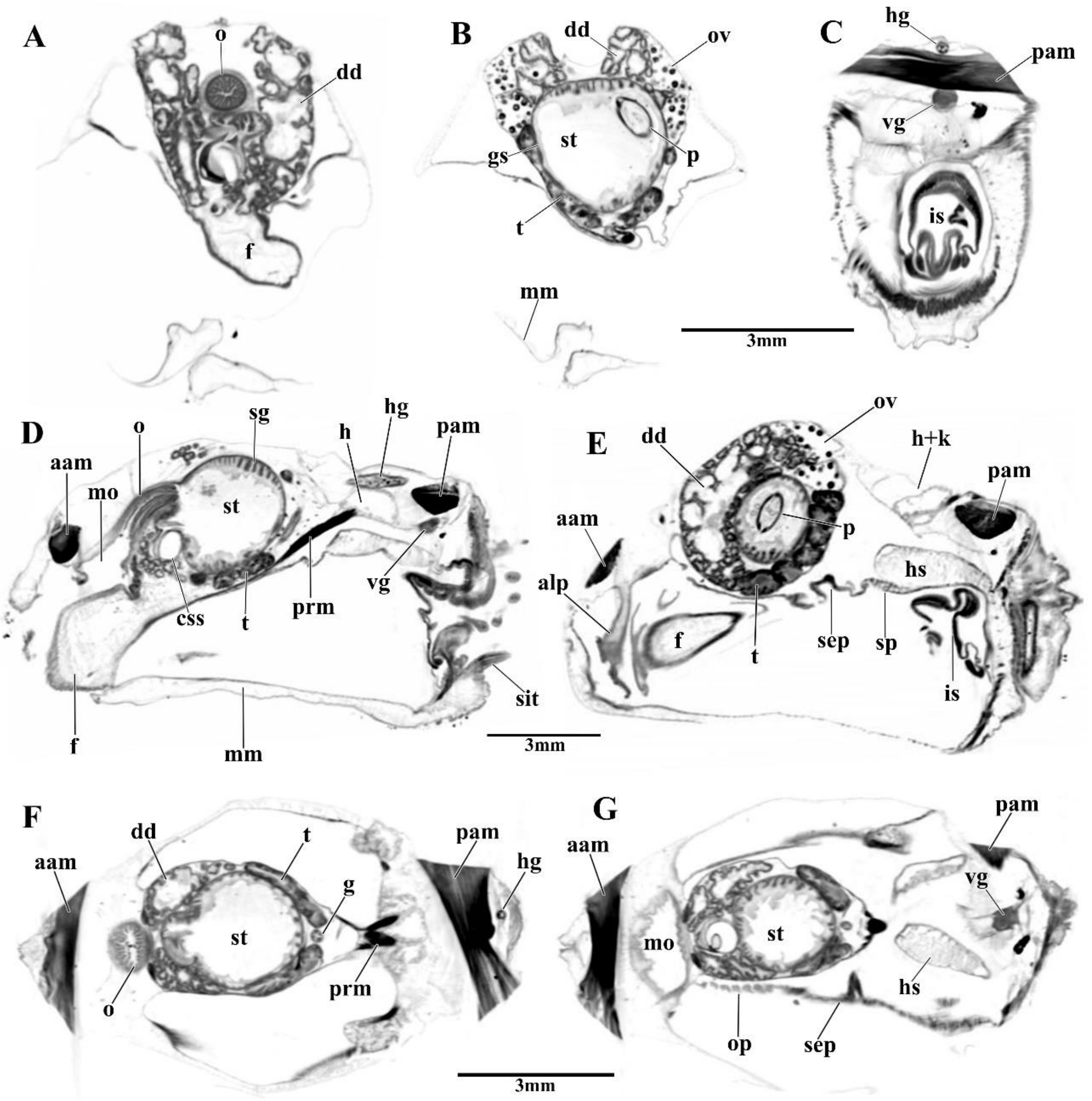

Figure 12. Selected virtual 2D sections through the µCT dataset of a PTA-stained specimen of ***Cetoconcha spinosula***. Transverse (A-C), sagittal (D, E) and frontal (F, G) sections. Abbreviations: aam, anterior adductor muscle; alp, anterior labial palp; ao, anal opening; arm, anterior retractor muscle; css, crystalline style sac; ct, ctenidia; dd, digestive diverticulum; f, foot; gs, gastric shield; h, heart; h + k, heart + kidney; hg, hind gut; hs, hollow sac; is, inhalant siphon; k, kidney; mm, mantle margin; mo, mouth; nb, nervous bundles; o, oesophagus; op, ostial perforations; ov, ovarian; p, prey inside stomach; pam, posterior adductor muscle; prm, posterior retractor muscle; sep, muscular septum; sg, stomach grooves; st, stomach; t, testis; vg, visceral ganglia. Scale bars: A-G – 3mm.

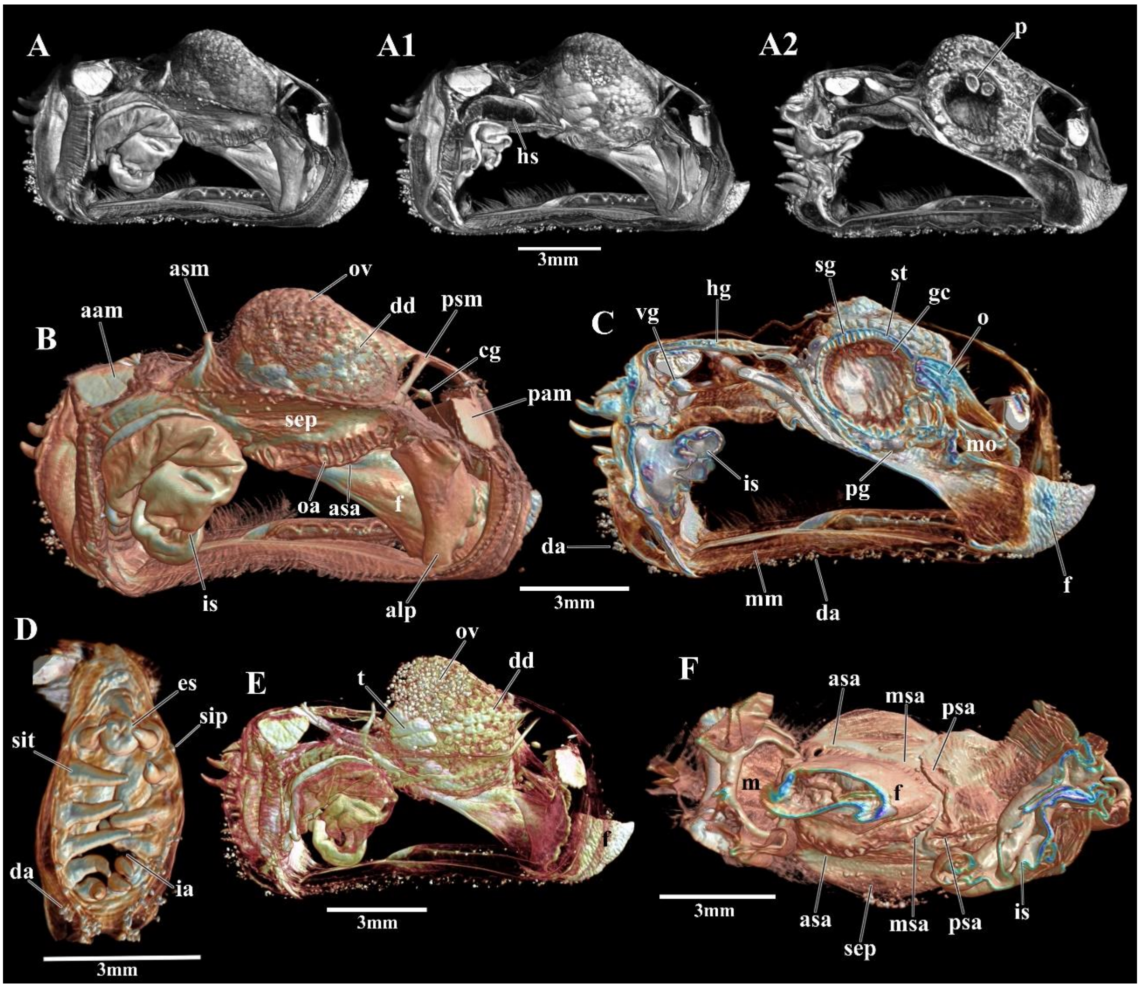

Figure 13. 3D volume rendering based on the µCT dataset of ***Cetoconcha spinosula***. Dissection sequence in original tomographic color (A-A2); false-color volume rendering (B-F) and virtual dissections (A1, A2, C-F). Abbreviations: aam, anterior adductor muscle; alp, anterior labial palp; asa, anterior septal apertures; asm, anterior septal muscle; da, debris attached; cg, circumesophagic ganglia; dd, digestive diverticulum; es,

exhalant siphon; f, foot; gc, gastric chamber; hg, hind gut; hs, hollow sac; ia, inhalant aperture; is, inhalant siphon; mm, mantle margin; m, mouth; msa, middle septal apertures; o, oesophagus; oa, ostial aperture; ov, ovarian; p, prey inside stomach; pam, posterior adductor muscle; psa, posterior septal apertures; psm, posterior septal muscle; sg, stomach grooves; si, siphons; sip, siphonal papillae; sit, siphonal tentacle; sep, septum; st, stomach; t, testis; vg, visceral ganglia. Scale bars: A-F – 3mm.

Family Cetoconchidae Ridewood, 1903

Genus *Cetoconcha* Dall, 1886

Cetoconcha* aff. *smithii Dall, 1908

(Figs. 14, 15)

Description

Shell: ovate-trigonal, thin, inflated, slightly translucid, prominent umbones, inflated; sculpture of radial lines of micro pustules for entire ventral surface of the valves; absence of lithodesma. *Mantle*: ventral mantle margin with one anteriorly wide pedal gape extending from the anterior adductor until the inhalant siphon; absence of a fourth pallial aperture. *Siphons*: separated, different in size and outline; inhalant siphon, large, modified in a raptorial appendice, typically retracted into the infra-septal chamber; exhalant siphon short, cone-like and everted in this specimen; both surrounded in the base by a ring of siphonal tentacles, ~10 around the inhalant and ~3 around exhalant. *Septum*: thin, perforated by three rows of grouped pores, the two anterior groups lie in a similar position to those in *Cetoconcha spinosula* and the third and smallest group lies behind the posterior septal muscles; absence of a hollow sac in the posterior inner septal floor. There are nine pairs of pores in the anterior group, five in the middle and three in the posterior group. *Labial palps*: non-lamellate and asymmetrical with anterior labial palp, large, thin (probably contracted), cup-shaped and the posterior labial palp small. *Musculature*: posterior and anterior adductor muscles present and isomyarian; presence of posterior and anterior pedal and septal retractor muscles; prominent lateral septal muscles are present in this specimen; taenioid muscle absent. *Foot*: large, pedal groove not observed, absence of byssal thread. *Digestive system*: the funnel-shaped mouth opens into a thick and muscular oesophagus that enters into the anterodorsal portion of the stomach; stomach large, rounded, with longitudinal deep internal grooves in the dorsal wall, connected to the short and small crystalline style sac located on the median portion of the stomach floor; preys were observed inside of stomach (ostracod: fig. 14A2); stomach surrounded dorsally and anteriorly by gonads and digestive diverticulum; crystalline style sac not visible in this specimen. *Reproductive system*: maybe dioecious, only ovarian is visible in this specimen; ovarian closely associated to the digestive diverticulum cover the roof of the stomach. *Nervous system*: only the visceral ganglia was observed.

Main references: Knudsen (1970), Allen & Morgan (1981), Krylova (1991), Coan & Valentich-Scott (2012: plate 323).

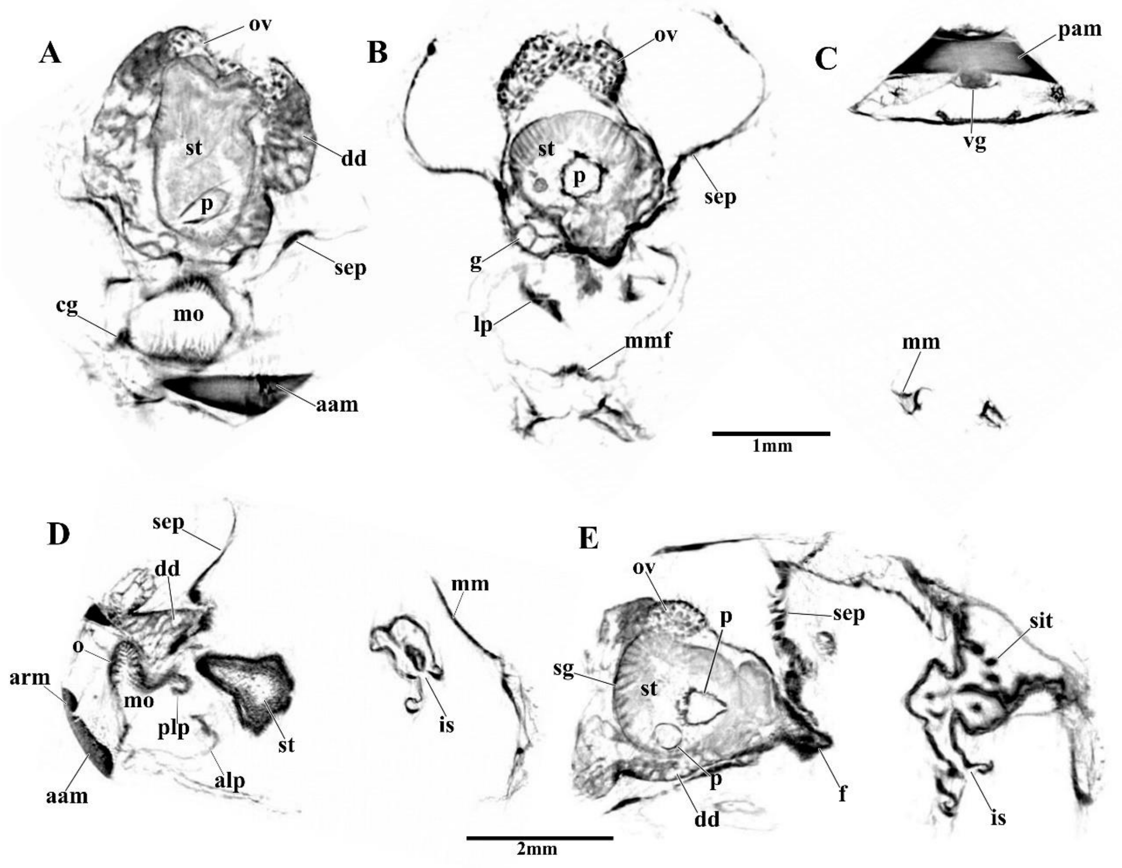

Figure 14. Selected virtual 2D sections through the μCT dataset of a PTA-stained specimen of ***Cetoconcha* aff. *smithii***. Transverse (A-C), frontal slightly oblique (D, E) sections. Abbreviations: aam, anterior adductor muscle; alp, anterior labial palp; arm, anterior retractor muscle; cg, circumesophagic ganglia; dd, digestive diverticulum; f, foot; g, gut; is, inhalant siphon; lp, labial palp; mm, mantle margin; mmf, mantle margin fused; mo, mouth; o, oesophagus; ov, ovarian; p, prey inside stomach; pam, posterior adductor muscle; plp, posterior labial palp; sep, septum; sg, stomach grooves; sit, siphonal tentacles; st, stomach; vg, visceral ganglia. Scale bars: A-C – 1mm; D, E – 2mm.

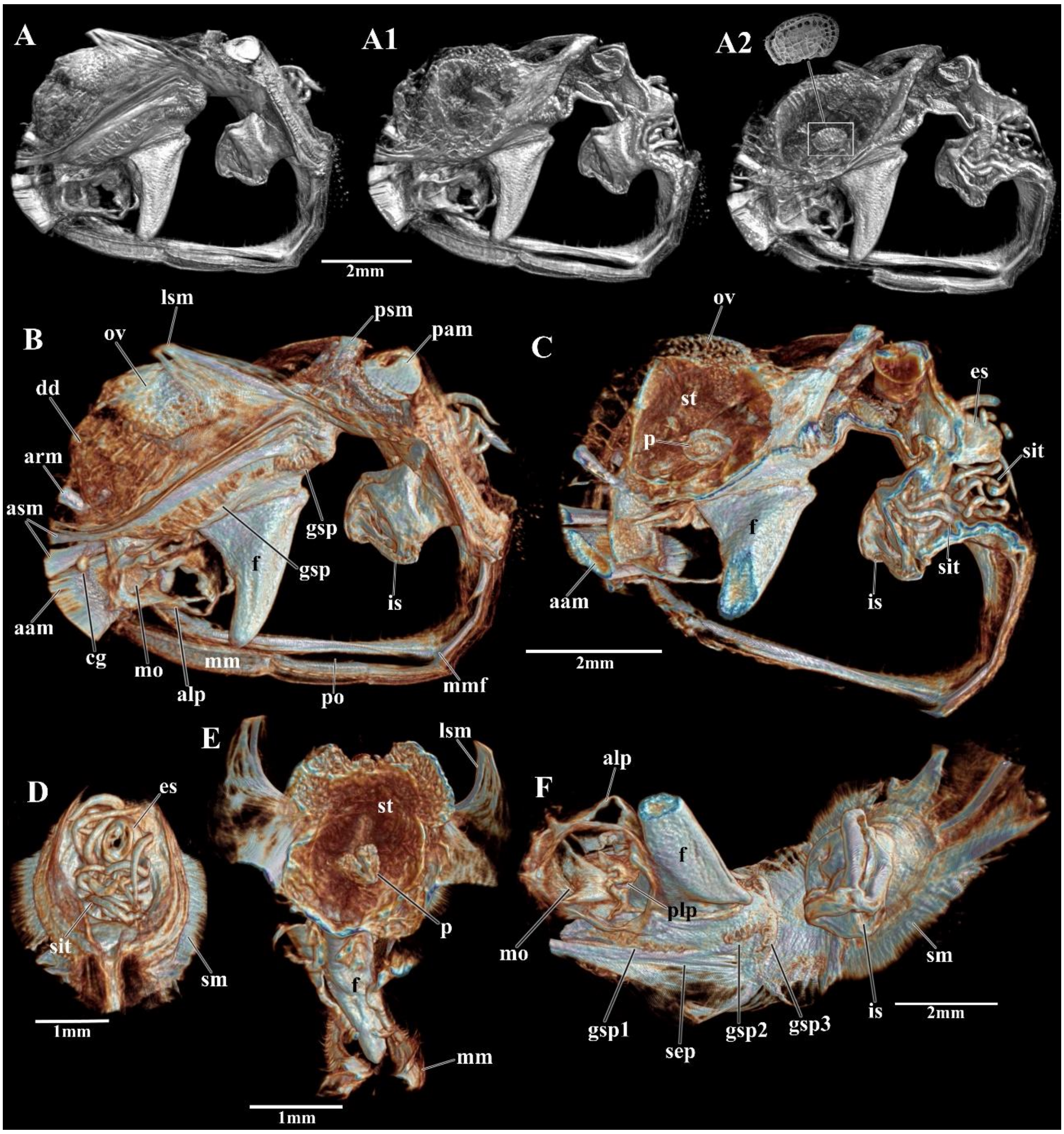

Figure 15. 3D volume rendering based on the µCT dataset of ***Cetoconcha* aff. *smithii***. Dissection sequence in original tomographic color (A-A2); false-color volume rendering (B-F) and virtual dissections (A1, A2, C-F). Abbreviations: aam, anterior adductor muscle; alp, anterior labial palp; arm, anterior retractor muscle; asm, anterior septal muscle; cg, circunesophagic ganglia; dd, digestive diverticulum; es, exhalant siphon; f, foot; gsp1-3, grouped septal pores; is, inhalant siphon; lsm, lateral septal muscle; mm, mantle margin; mmf, mantle margin fused; mo, mouth; o, oesophagus; ov, ovarian; p, prey inside stomach; pam, posterior adductor muscle; plp, posterior labial palp; psm, posterior septal muscle; sit, siphonal tentacles; sep, muscular septum; sm, siphonal musculature; st, stomach. Scale bars: A-C, F – 2mm; D, E – 1mm.

Family Cuspidariidae Dall, 1886

Genus *Cuspidaria* Nardo, 1840

Cuspidaria glacialis (Sars G. O., 1878)

(Figs. 16, 17)

Description

Shell: elongated, robust, inequilateral, equivalve and slightly inflated; presence of a prominent and long rostrum in the posterior portion of the shell; smooth, valves covered by a dehiscent, thick and light brown periostracum; presence of lithodesma. Mantle: mantle margin with one anteriorly large pedal gape, extending from the anterior adductor until almost the beginning of the inhalant siphon; posteroventral mantle margin fusion formed by inner and middle folds (Type B) (Yonge 1982); absence of a fourth pallial aperture. *Siphons*: the detailing of this structure was not possible due to the state of contraction of the specimen analysed. *Septum*: presence of a well-developed horizontal muscular septum (‘septibranch condition’) dividing the mantle cavity into infra and supraseptal chambers; the septum is long and wide, perforated ventrally by 5 pairs of isolated septal pores. *Labial palps*: poor developed, non-lamellate, slightly asymmetrical, with the anterior palp bigger than posterior palp; anterior labial palp attached to the ventroanterior mantle margin, close to the anterior adductor muscle. *Musculature*: posterior and anterior adductor muscles present and isomyarian; presence of a well-developed pedal and septal retractor muscles, both bifurcated in the end portion before insertion into the shell; lateral septal muscle is also present being more concentrated posteriorly; taenioid muscle absent. *Foot*: large, with a long pedal groove; byssal thread absent. *Digestive system*: a funnel-shaped mouth opens into a thick and muscular oesophagus that enters into the anterodorsal portion of the stomach; stomach large, rounded, with a dorsal short projection, internal grooves not visible; stomach connected to the short and small crystalline style sac located on the median portion of the stomach floor; crystalline style present; large preys were observed inside of stomach (gastropod and ostracod: figs. 15B, F; 16D, E); stomach surrounded dorsally and anteriorly by gonad and digestive diverticulum. *Reproductive system*: dioecious, only ovarian is visible in this specimen; ovarian closely associated to the digestive diverticulum cover the roof of the stomach. *Nervous system*: only the visceral ganglia had been observed.

Main references: Yonge (1928), Knudsen (1970), Reid & Reid (1974), Allen & Morgan (1981), Machado *et al*. (2017).

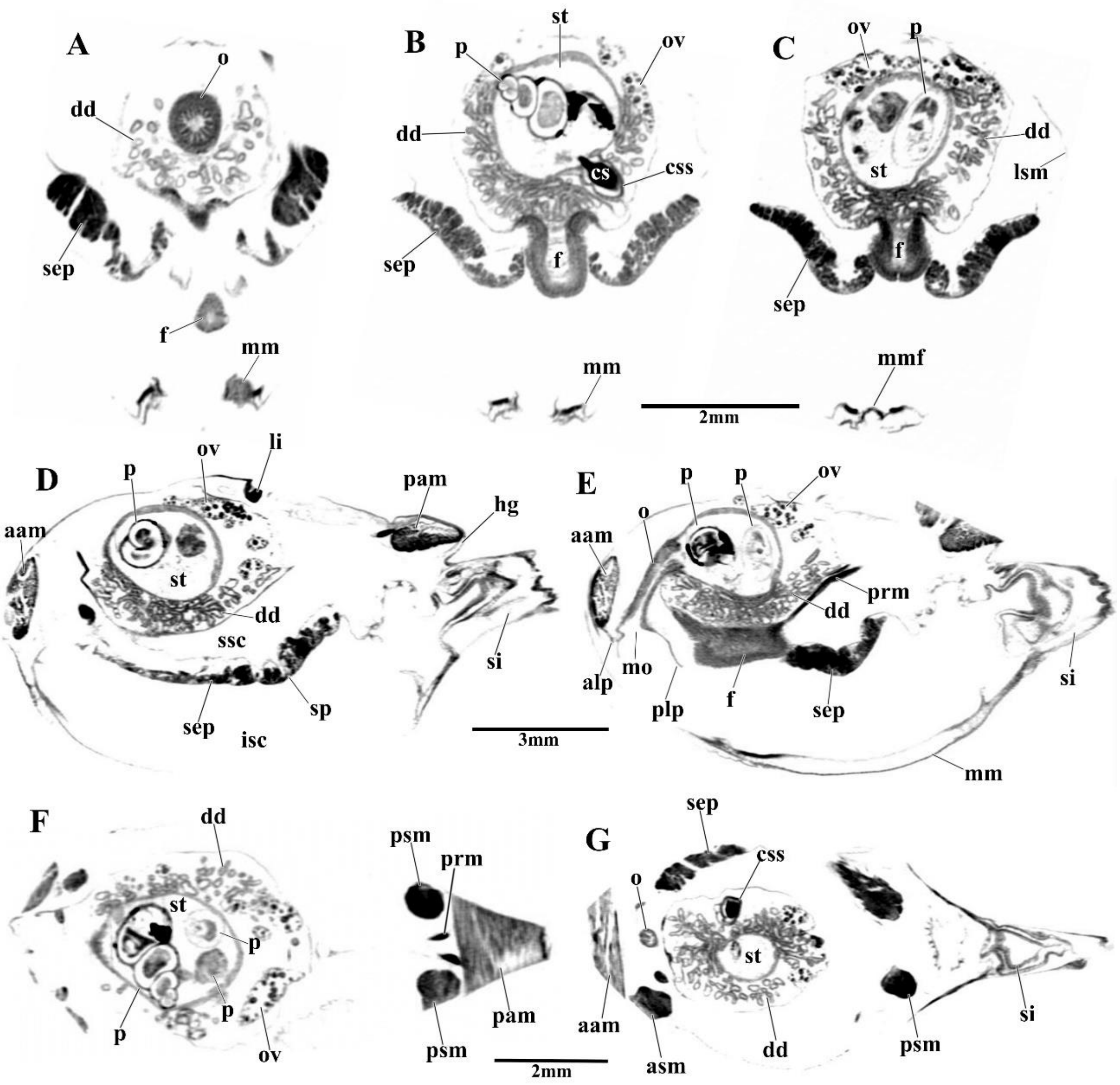

Figure 16. Selected virtual 2D sections through the µCT dataset of a PTA-stained specimen of ***Cuspidaria glacialis***. Transverse (A-C), sagittal (D, E) and frontal (F, G) sections. Abbreviations: aam, anterior adductor muscle; alp, anterior labial palp; asm, anterior septal muscle; cs, crystalline style; css, crystalline style sac; dd, digestive diverticulum; f, foot; hg, hind gut; isc, infraseptal chamber; li, lithodesma; lsm, lateral septal muscle; mm, mantle margin; mmf, mantle margin fused; mo, mouth; o, oesophagus; ov, ovarian; p, prey inside stomach; pam, posterior adductor muscle; plp, posterior labial palp; prm, posterior retratctor muscle; psm, posterior septal muscle; sep, septum; si, siphons; sp, septal pore; ssc, supraseptal chamber; st, stomach. Scale bars: A-C, F, G – 2mm; D, E – 3mm.

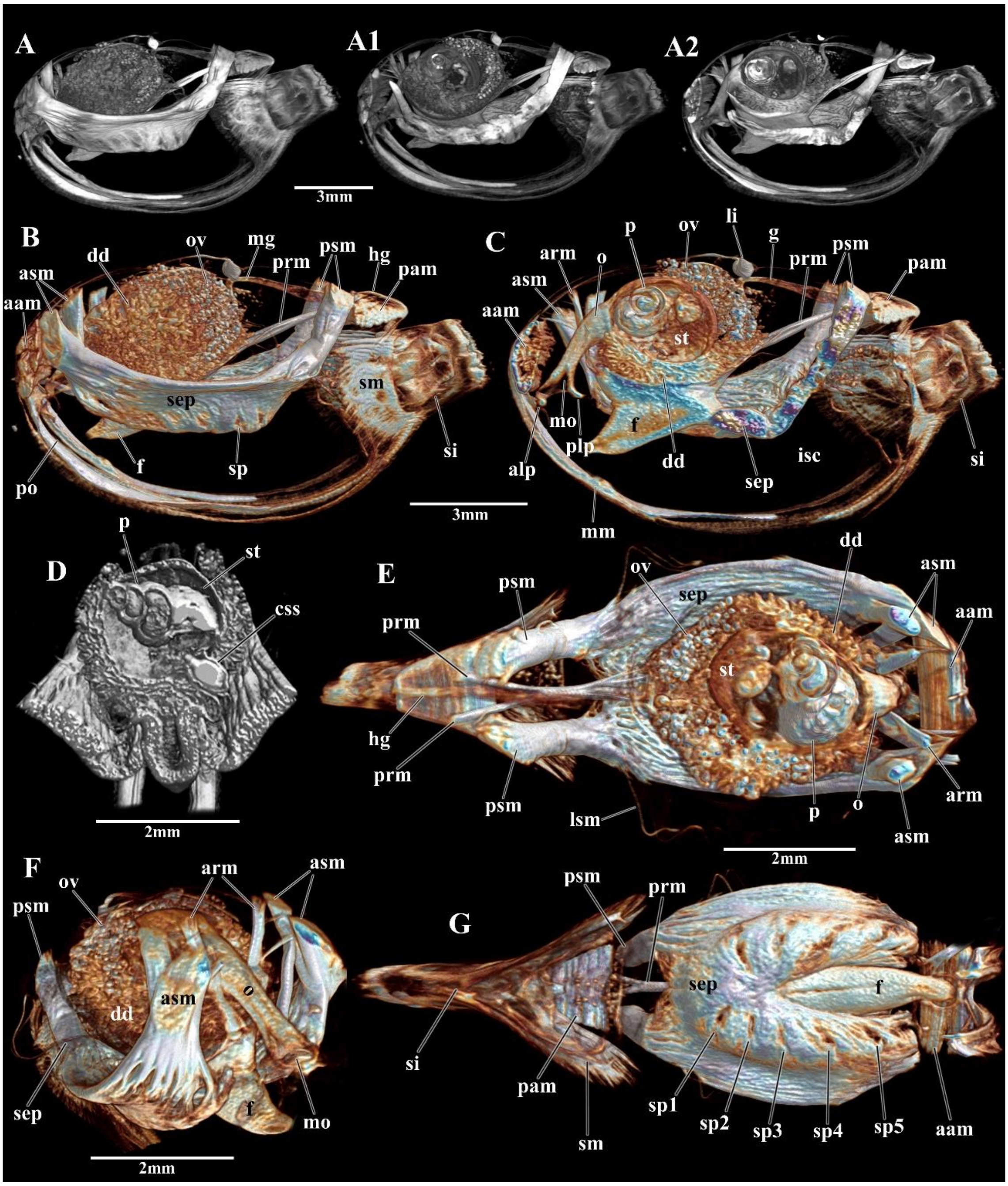

Figure 17. 3D volume rendering based on the µCT dataset of ***Cuspidaria glacialis***. Dissection sequence in original tomographic color (A-A2); false-color volume rendering (B-G) and virtual dissections (A1, A2, C-G). Abbreviations: aam, anterior adductor muscle; alp, anterior labial palp; arm, anterior retractor muscle; asm, anterior septal muscle; css, crystalline style sac; dd, digestive diverticulum; f, foot; g, gut; isc, infra septal chamber; li, lithodesma; lsm, lateral septal muscle; mg, mid gut; mm, mantle margin; mo, mouth; o, oesophagus; ov, ovarian; p, prey inside stomach; pam, posterior adductor muscle; plp, posterior labial palp; prm, posterior retractor muscle; psm, posterior septal

muscle; pg, pedal ganglia; si, siphons; sm, siphonal musculature; sep, muscular septum; sp1-5, septal pores; st, stomach. Scale bars: A-C – 3mm; D-G – 2mm.

DISCUSSION

Although some works have already used molluscs species as models to test the contrast and to evaluate the effectiveness of Micro-CT as tool to obtain anatomical information (Goulding *et al*. 2007, 2009, Alba-Tercedor *et al*. 2011, Faulwetter *et al*. 2013b, Candás *et al*. 2016, Pedrouzo *et al*. 2017), its application for the Bivalvia systematics is hitherto unknown. Therefore, the present study is the first to evaluate the potential of this non-destructive imaging method for the taxonomic and phylogenetic study of marine bivalves.

Our results show that the tomography is a very useful tool for the anatomical descriptions of marine bivalves, for both large (>10 mm) and small (<2 mm in length) or for freshly fixed and museum specimens, allowing not only a detailed analysis of the internal tissues but also a topographic visualization of the organs of the pallial cavity in 3D, in some cases replacing dissections and scientific drawings. The 2D tomographic sections also are of extreme importance since it provided an interpretation at the histological level of organs of the visceral mass as for example, the alimentary tract path, the differentiation between male and female gonads, nervous bundles, among others. Therefore, this non-destructive technique proved to be efficient not only to describe the anatomy but also as an important tool in the construction of the taxonomic and phylogenetic knowledge of Bivalvia.

A brief discussion about each internal morphological feature, important for the Anomalodesmata taxonomy is provided below, highlighting the effectiveness of Micro-CT for the visualization and interpretation of these structures.

Mantle

The mantle margins on Anomalodesmata are extensively fused, presenting three main openings: (i) a ventral and usually large pedal opening, and (ii), two posterior openings correspond to the inhalant and exhalant siphons. Besides, some Anomalodesmata species also presented a fourth pallial opening, as for example, *Brechites attrahens* (Lightfoot, 1786), *Humphreyia strangei* (A. Adams, 1854), *Lyonsia californica* Conrad, 1837, among others (Narchi 1968: fig. 1, Morton 1984c, 2002: fig. 8). This opening, located close to the base of the siphons, probably has the function of facilitating the elimination of pseudofaeces from the interior of the pallial cavity or functions as a pressure release valve through which some of the mantle water is ejected following rapid adduction in fast burrowing bivalves (Atkins 1937, Yonge 1952). It worth noting that among the eight species analysed, only *Lyonsia alvarezii* d'Orbigny, 1846 presented a fourth pallial aperture. The fourth aperture is also seen in representatives of a few non-anomalodesmatan taxa, for example, *Spisula*, *Lutraria* (Imparidentia, Mactridae), *Ensis* (Pharidae), *Tagelus* (Solecurtidae) and *Siliqua* (Solenidae), but the homology between these structures has never been tested (Morton 2010).

In Anomalodesmata, the mantle fusion involving different numbers of mantle folds and thus different species may be classified into three different types (A, B, C) according to Yonge (1982). Through the 2D tomographic sections some species had the ventral fusion of the mantle observed in detail as *Allograma formosa* (Jeffreys, 1882) and *Cuspidaria glacialis* (Sars G. O., 1878), Type B and *Cetoconcha spinosula* (Thiele, 1912) (new comb. to *Poromya spinosula*), Type A. For the other species, it was not possible to observe the mantle fusions.

Musculature

The µCT reconstructions were very effective for the visualization of the muscular structures in the species analysed, allowing a topographical interpretation of the main muscles present in Anomalodesmata. Herein, the structure, position and insertion of the adductor muscles, foot retractors and septal muscles were described. Taeniod muscles were not observed in any of the species analysed.

Here, it is also worth noting the absence of taenioid muscles in *A. formosa*. Usually associated to the predatory bivalves, these muscles are elongate siphonal retractors that have separate insertions on the shell valves. In *Parilimya fragilis* (Grieg, 1920), for example, they serve to pull the inhalant siphon into the mantle cavity assisting the bivalve in prey capture (Morton 1982). However, according to Morton (1984b) this musculature would be modified and reduced in *Allograma formosa* located within the fused ventral mantle margin. Through a detailed analysis of the 2D tomographic sections no muscle bundles were observed within the posteroventral portion of the mantle margin of *A.*

formosa, confirming, therefore, the absence of a taenioid muscle in the specimen analysed.

For the species without a muscular septum, as *Pandora pinna* (Montagu, 1803), *L. alvarezii*, *Trigonulina ornata* d'Orbigny, 1853 and *A. formosa*, adductor muscles with similar shape and size and poorly developed pedal retractor muscles were observed, except for *L. alvarezii*. The reduction of the pedal musculature is usually associated with the adoption of a passive burrowing or epifaunal lifestyle and had already observed to another anomalodesmatans as *P. fragilis*, *Cleidothaerus albidus* (Lamarck, 1819) and for some clavagellids (Morton 1974, 1982). Therefore, our results suggest that *P. pinna*, *T. ornata* and *A. formosa,* may also have a passive burrowing lifestyle. For *L. alvarezii*, specifically, the presence of a small byssal thread suggest a epifaunal lifestyle. In addition, the heteromyarian condition observed in *L. alvarezii* seems to be a feature common to other lyonsiids, due to the effects of byssal attachment (see Yonge 1952).

For the species that have a muscular septum, as *Cetoconcha spinosula*, *Poromya rostrata* Rehder, 1943, *Cetoconcha* aff. *smithii* Dall, 1908 and *Cuspidaria glacialis* (Sars G. O., 1878), the main differences are associated to the bifurcation of the retractor muscles of the foot and the septum, and the presence or absence of lateral septal muscles. These differences are mainly observed between some species of Poromyidae and Cetoconchidae, with the presence of a well developed lateral septal musculature in *C.* aff. *smithii* and absence in *Poromya rostrata*. In the same way, *C. glacialis* (Cuspidariidae) has a muscular pattern very different from the poromyids and cetoconchids due to the presence of a thick and wide septum, presenting a well-developed and bifurcated posterior and anterior pedal and septal retractor muscles.

In general, the musculature of Anomalodesmata is little used for the taxonomic and phylogenetic studies of this group, although are almost always described, and sometimes well detailed in works on the anatomy of the group (Allen & Turner 1974, Allen & Morgan, 1981). Our results showed that the Micro-CT can be very useful in the reconstruction of the musculature and that some patterns are easily observed through this technique.

Ctenidia

According to Harper *et al*. (2006) the anomalodesmatan ctenidia, if present, are complete, deeply plicate and heterorhabdic, defined as Type E by Atkins (1937). This type of eulamellibranch ctenidia is usually characterized by the presence of a complete inner demibranch and a reduced outer demibranch with only the descending lamellae. However, during the adaptive radiation of Anomalodesmata, the ctenidia were drastically reduced in the families Euciroidae, Lyonsiellidae and Verticordiidae; with remaining ctenidia filaments in Cetoconchidae and Poromyidae or entirely lost in Cuspidariidae and Spheniopsidae (Allen & Morgan 1974, 1981, Morton *et al*. 2016a, b).

Among the species analysed, just *P. pinna* and *L. alvarezii* present a Type E ctenidia. In *P. pinna*, for example, the µCT images show a deep plicate ctenidia and a much reduced outer demibranch with the presence of a marginal food groove at free edge of inner demibranch. For *L. alvarezii* the 3D reconstructions were important to visualize the disposition of demibranchs and a deep marginal groove. For both, the tomographic images were not able to show some gill details as the ctenidia filaments (shape, cilia) or the interfilamentar junctions.

For *A. formosa* (Lyonsiellidae) and *T. ornata* (Verticordiidae) the µCT images showed a reduced ctenidia without a dorsal attachment with the visceral mass, giving it a horizonal orientation into the pallial cavity. According to Morton (1984b) the horizontal position of the ctenidia in *A. formosa* allow that the ctenidia axis separated posteriorly from the visceral mass divide the pallial cavity in supra and infrabrachial chambers, a condition very similar to that observed in anomalodematans with a muscular septum. Still for *A. formosa*, important details as the ctenidia axis had been also observed via 2D tomographic sections.

The specimen of *T. ornata* scanned is the smallest among the species analysed, sized 3.2 mm in length, even so the 3D reconstructions were effective to visualize and consequently to understand the topography of the organs into the pallial cavity. The ctenidia of *T. ornata,* for example, is smaller and has less demibranch filaments when compared to the *A. formosa,* indicating a greater morphological proximity with the septibranch condition. Although it is apparently closer, it is also worth noting that *T. ornata* have just the ctenidia, i.e. this species does not have any type of muscular septum associated with their gill. Other verticordiids as, for example, *Spinosipella deshayesiana* (P. Fischer, 1862) and *Spinosipella costeminens* (Poutiers, 1981) have a ctenidia attached to a remaining septum (Simone & Cunha, 2008), showing an even greater proximity to this septal condition.

The muscular septum and its associated musculature are some of the anatomical structures best represented in our μCT images, allowing an excellent visualization of the different degrees of the ctenidia reduction. Therefore, it was also possible to distinguish thinner septa with remaining gill filaments (*Cetoconcha spinosula, C.* aff. *smithii, Poromya rostrata*) of a thick and well-developed septum without ctenidia filaments (*Cuspidaria glacialis*). Furthermore, the 3D reconstructions facilitated the descriptions about the position and quantity of branchial apertures and septal pores present in these muscular septa, consequently, producing very important information for the taxonomy of these families.

In general, the Poromyidae include species with septum perforated by two paired groups of branchial apertures, being divided into two subfamilies, the Poromyiinae, with no interfilamentar connections in the apertures (usually slit-like or ostial apertures) and the Cetomyinae, with such connections (usually sieve-like apertures) (Allen & Morgan 1981, Krylova 1997, 2001). The family Cetoconchidae, in turn, included species with three paired groups of branchial apertures (grouped pores) represented by only one genus, *Cetoconcha*, presenting species with or without interfilamentar connections, as for example, *Cetoconcha (Cribrosoconcha) alephtinae* and *Cetoconcha angolensis* Allen & Morgan, 1981, respectively (Allen & Morgan 1981, Krylova 1997, 2001). Based in this background, *Cetoconcha* aff. *smithii* and *Poromya rostrata* presented septal features corresponding to the diagnostic characteristics of their families, while the species previously identified as *Poromya spinosula* belongs to the geneus *Cetoconcha*, since it has three ostial septal apertures (grouped pores). Therefore, this paper suggests a new combination for the species *Poromya spinosula* Thiele, 1912, now designated as *Cetoconcha spinosula* (Thiele, 1912) and their consequent reallocation for the family Cetoconchidae.

Also associated to the septum, the presence of a bilobate hollow sac located in the posterior inner septal floor has been observed in *Cetoconcha spinosula* and *Poromya rostrata*. This structure is very similar to the 'haemocoelic compensation sac' observed in *Poromya granulata* (Nyst & Westendorp, 1839) (Morton 1981). According to the author this structure is associated to a complex mechanism of feed and ingestion in *P.*

granulata helping in the evertion of the inhalant siphon during the prey capture, through of fluids release (Morton 1981: fig.15). Although have not been observed alive, the presence of this structure in *C. spinosula* and *C. rostrata* suggests a similar prey capture mechanism to that described for *P. granulata*. This structure has not been observed in *C.* aff. *smithii*, maybe due to the contraction state of some organs of the posterior portion of the scanned specimen.

Finally, the Cuspidariidae have species with a thick and well-developed muscular septum (without ctenidia filaments) ventrally perforated by isolated pores. Usually presenting four pairs of pores, some genera can be 5-30 pores as for example, species of *Protocuspidaria* (5-30 pores) and *Halonympha* (8-20 pores) (Allen & Morgan 1981; Krylova 1994, 1995). The µCT reconstructions showed that *C. glacialis* have a huge muscular septum perforated by five pair of isolated pores. This number of pores is not common among species of *Cuspidaria* that usually presents four pairs, although this same configuration had already been observed in *Cuspidaria cuspidata* by Allen & Morgan (1981: pg. 453).

Labial palps

The palps are considered important feeding structures in Bivalvia, since it assists the ctenidia in the conduction of the food particles available into the pallial cavity until the mouth, to be ingested (Stasek 1963). In general, for Anomalodesmata, these labial palps can be present or absent, large or reduced, symmetrical or asymmetrical, lamellate with sorting ridges in the filter-feeding species (typical bivalve plan) or non-lamellate and usually modified in carnivorous species. Herein, the 3D reconstructions provided good quality images for the main characteristics of this structure and when present, outer and inner labial palps were described.

Among the species scanned, *T. ornata* is the only one that does not have labial palps. The suspension-feeders *P. pinna* and *L. alvarezii* have large, lamellate and symmetrical labial palps while the other five carnivorous species have a non-lamellate and modified outer and inner palps (asymmetrical).

The absence of labial palps is rare among the anomalodesmatans and appears to be exclusively associated with minute-sized carnivorous species as *T. ornata* and the spheniopsids *Grippina coronata* Machado & Passos, 2015 (1.67 mm in length) and

Spheniopsis brasiliensis Machado & Passos, 2015 (1.78 mm in length) (Machado & Passos 2015, Morton *et al*. 2016a, b). According to Morton *et al*. (2016b) in species without labial palps, the foot is probably used to push inhaled prey from the infra-septal chamber until the funnel-shaped mouth.

For the carnivorous species, *C. spinosula*, *P. rostrata* and *C.* aff. *smithii*, the anterior labial palps are larger than the posterior palps; *A. formosa* have extremely complex labial palps, while *C. glacialis* possess reduced labial palps. In species of Poromyiidae and Cetoconchidae, for example, the palp pattern observed (anterior larger and posterior reduced) is also reported for most species of these families as *Poromya australis* E. A. Smith, 1885, *Cetomya tornata* (Jeffreys, 1876), *Lissomya rotundula* Krylova, 1997, *C. angolensis* among others (Allen & Morgan 1981, Krylova 1997, 2001); being, therefore, an important characteristic for the systematic of this group.

The palps of *A. formosa* are complex, fused medially forming two globular flask-shaped buccal cavities below the mouth, very similar to the previous palp descriptions made by Morton (1984b) for the same species. The function for these palps is unknown.

For the Cuspidariidae the labial palps are generally small and reduced, can be classified as Type I, II and III (see Allen & Morgan,1981: pgs. 438, 439). The reduced palps of *C. glacialis*, specifically, also follow the same cuspidariid pattern, being similar to the palps observed for other *Cuspidaria* species and, therefore, classified as Type II by Allen & Morgan (1981).

Siphons

The siphons are muscular structures formed by the mantle margin fusion that perform the function of to connect the bivalves to the environment around it, allowing to explore the food resources, make gas exchange and release gametes. In Anomalodesmata these siphons can be, fused or separated, similar or different in size and outline, encased or not in a periostracal or in a tissue sheath, with or without sensory tentacles, inhalant siphon as a simple tube-shaped or highly modified, among others. Important for the taxonomy of Anomalodesmata, the siphons are also structures widely used in phylogenetic studies, presenting a good phylogenetic signal (Harper *et al*. 2000, Giribet & Wheller 2002, Bieler *et al*. 2014).

The µCT images, mainly 3D reconstructions, were very effective for visualizing of the inhalant and exhalant siphons, siphonal apertures, musculature associated and the number and position of sensory tentacles, allowing an accurate description of these structures. Except for *C. glacialis,* all species had their siphons and sensory tentacles characterized. The species *P. pinna* and *L. alvarezii* were the only ones to present siphons with the same size and outline (suspension-feeders), *T. ornata* have a short and half cone-shaped inhalant and a non-visible exhalant siphon, and for the other species the inhalant siphon is modified into a raptorial structure usually associated to the prey capture (predatory bivalves).

The raptorial inhalant siphons of *A. formosa*, *C. spinosula*, *P. rostrata* and *C.* aff. *smithii* are similar in size and outline, being usually visualized inverted into the pallial cavity. For these species, sensory tentacles always are present on the base of siphons, usually presenting a variation in relation to the shape, quantity and position. In *A. formosa*, for example, two different types of siphonal tentacles were identified, ~50 smaller tentacles resembling papillae and located closest to the siphonal apertures and ~60 bigger tentacles, with a finger-like shape peripherally located. According to Morton (1984b) the siphons of *A. formosa* are surrounded by a ring of ~48 tentacles arranged in two cycles; those of the outer cycle are larger and longer than those of the inner; results partially similar at those observed *via* 3D reconstructions, except for the number of tentacles.

For the Poromyidae, specifically, the arrangment (number and position of siphonal tentacles and papillae) are important for the taxonomy of this group (Krylova 2001: fig.17). Herein, the species *Poromya rostrata* (12 is, 3es + 0 sip) presented a configuration similar to that reported for *Poromya undosa* Hedley & Petterd, 1906 (10 is, 3es + 0 sip) and different to that observed for *Cetomya poutiersi* Krylova, 2001 (10 is, 3 es + 12 sip) and *Cetomya celsa* Krylova, 2001 (10 is, 3 es + 10 sip), indicating the potential of these siphonal arrangements to a differentiation to the genus-level.

The arrangement described for the cetoconchid *C. spinosula* (10 is, 3es + 15 sip) is diferrent to that observed in *C.* aff. *smithii* (10 is, 3 es + 0 sip), mainly due to the absence of siphonal papillae (sip) in the latter. On the other hand, the siphonal configuration of *C.* aff. *smithii* is similar to that reported for *C. angolensis* and *Cetoconcha braziliensis* by Allen & Morgan (1981), i.e. 15 tentacles around the siphons and no papillae. This paper shows for the first time the presence of siphonal papillae for a member of Cetoconchidae, indicating, therefore, a possible similarity between the siphonal arrangement of *C. spinosula* and some Poromyidae members.

Alimentary tract

Among the main structures that compose the digestive system, the stomach is the most important feature for the Bivalvia systematic, since the gastric chamber morphology generally reflects the feeding modes of species. For Anomalodesmata, specifically, two different types of stomach are reported, Type II and Type IV (see Purchon 1956, 1987). In general, the morphology of the stomach Type II is associated to the carnivorous habit characterized by a large rounded sac with thick muscular walls and an extensive scleroprotein linings which facilitate crushing prey; the stomach is combined with a reduced crystalline style sac (short crystalline style) plus a muscular oesophagus and a large mouth. Associated to the filter-feeder anomalodesmatans, the Type IV comprising a small and oval stomach associated to an elongated and non-muscularized oesophagus and a small mouth; and a gastric chamber elongated ventrally combined with the crystalline style sac and the presence of numerous gastric caeca (Purchon 1956, 1987, Harper *et al*. 2006, Mikkelsen & Bieler 2008).

The µCT images showed with a good quality the most of these structures, allowing the classification of the stomach type for the species analysed. Therefore, *T. ornata*, *C. spinosula*, *P. rostrata*, *C. glacialis* and *C.* aff. *smithii* have a stomach Type II, while *P. pinna* and *L. alvarezii* have the Type IV. *A. formosa* is the only one belonging to a traditionally carnivorous family (Lyonsiellidae) that presented an intermediate morphology between types II and IV, with a small and oval stomach associated to a well-developed muscular oesophagus. Although uncommon among the Bivalvia, the carnivorous *P. fragilis* (Parilimyidae) and the non-Anomalodesmata *Propeamussium jeffreysii* (E.A. Smith, 1885) (Propeamussiidae) also displays an intermediate morphology type between that of a filter feeding and that of a more specialized carnivore (Purchon 1897, 1990, Temkin & Strong 2013). It worth noting, that *A. formosa* also have a well-developed sphincter between oesophagus and stomach (oesophageal opening), very similar to the muscular sphincter reported for *Bathyneaera demistriata* (Allen & Morgan, 1981) (Cuspidariidae) by Temkin & Strong (2013).

In addition, most of Type II species present whole preys inside stomach, reinforcing its carnivorous habit. The tomographic images also showed for the first time a gastropod inside the stomach of *C. glacialis*, a type of prey never before reported for a cuspidariid member (Morton 1987b: table 1). Gastropods had already been found into the stomach of some poromyiids as *Cetomya butoni* (Prashad, 1932), *Cetomya bacata* Krylova, 2001 and *Cetomya celsa* Krylova, 2001 (Krylova 2001: table 1).

Other structures related to the alimentary system as the path of the gut, details about the mid gut, relationship between hind gut (rectum), kidney and heart were also possible to be visualized through the 2D tomographic images for all species scanned.

CONCLUSION

Our results demonstrate that micro-computed tomography is a tool of great potential for the study of the anatomy of small marine bivalves. The Micro-CT proved to be a fast and very precise tool, presenting 3D reconstructions and 2D tomographic sections of high quality. This paper provided, for the first time, anatomical data for seven Anomalodesmata species, *P. pinna*, *L. alvarezii*, *T. ornata*, *C. spinosula*, *P. rostrata*, *C.* aff. *smithii* and *C. glacialis*, re-discuss some characters of *A. formosa* and suggests the reallocation of *Poromya spinosula* Thiele, 1912 for the genus *Cetoconcha*; expanding, therefore, the taxonomic knowledge of this group *via* a tomographic approach. Although the Micro-CT scanners used in this study has presented some resolution limitations, especially for tiny structures (<3µm), new equipment as the Nano-CT and True-color Micro-CT are becoming more and more accessible allowing to increase the resolution and sharpness of the tomographic images, consequently, making of this technique a potential tool for the development of a new taxonomic era.

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CONSIDERAÇÕES FINAIS

Baseado em técnicas tradicionais como a dissecção, MEV e histologia, a descrição da morfologia funcional de *Cardiomya cleryana* trouxe de fato novos *insights* sobre o hábito carnívoro em Bivalvia, resultando no primeiro vídeo sobre o comportamento de uma espécie do grupo. Alguns resultados anatômicos como, por exemplo, orgãos internos bem visíveis e não contraídos, presença de um longo fio de bisso, glândulas arenofílicas protraídas, gânglios nervosos bem preservados, fácil diferenciação entre testículos e ovários, assim como detalhes sobre o comportamento dessa espécie, sugerem a importância da utilização de espécimes vivos em descrições desse tipo. Além disso, embora bivalves carnívoros sejam frequentemente descritos na literatura como raros e de águas profundas, a presença de *C. cleryana* em águas rasas e com uma certa abundância propõe uma nova perspectiva para o estudo desses bivalves, sugerindo que algumas espécies desse grupo sejam talvez mais comuns nesses ambientes do que se imaginava.

A nova filogenia apresentada no segundo capítulo dessa Tese amplia ainda mais o conhecimento sobre os bivalves carnívoros, reunindo pela primeira vez, em um mesmo teste cladístico, informações morfológicas sobre todas as famílias de hábito carnívoro, elucidando as relações filogenéticas de famílias pouco estudadas como Cetoconchidae, Euciroidae, Protocuspidariidae e Spheniopsidae. Além disso, as análises sugerem uma nova família de bivalves carnívoros (Bentholyonsiidae), formalmente descrita nesse trabalho. Nossos resultados também mostraram que o aumento da representatividade de taxa trouxe uma nova interpretação para as relações internas desse grupo, sugerindo que os Anomalodesmata, a despeito do que se imaginava em trabalhos anteriores, são compostos por quatro grupos monofiléticos, sendo três linhagens não-carnívoras reunindo 12 famílias, e uma única e complexa linhagem carnívora formada por 10 famílias.

Em relação ao escasso conhecimento sobre a anatomia dos Anomalodesmata, fato este que tem influenciado negativamente no entendimento sobre a evolução do grupo como um todo, o terceiro capítulo propõe uma alternativa para a ampliação desse conhecimento sem a necessidade de coletar novos espécimes e/ou destruir por meio de dissecções, exemplares raros depositados em museus. Os resultados apresentados nesse capítulo mostram que a tomografia de raios-x é uma ferramenta muito útil para a descrição dos tecidos internos de bivalves, propondo as primeiras descrições anatômicas de bivalves marinhos baseadas exclusivamente em imagens tomográficas e reconstruções 3D.

Embora estejamos em uma aparente Era molecular, onde estudos de genômica e proteômica são muito valorizados, os resultados apresentados aqui sugerem que uma abordagem morfológica, combinando taxonomia alfa e uma anatomia funcional bem detalhada, ainda pode e deve continuar a ser utilizada como uma ferramenta para o entendimento evolutivo de moluscos bivalves.

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Universidade Estadual de Campinas

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Em observância ao **§5º do Artigo 1º da Informação CCPG-UNICAMP/001/15**, referente a Bioética e Biossegurança, declaro que o conteúdo de minha Tese de Doutorado, intitulada "***DESVENDANDO A DIVERSIDADE DOS ANOMALODESMATA (MOLLUSCA: BIVALVIA): UMA ABORDAGEM MORFOLÓGICA E FILOGENÉTICA***", desenvolvida no Programa de Pós- Graduação em Biologia Animal do Instituto de Biologia da Unicamp, não versa sobre pesquisa envolvendo seres humanos, animais ou temas afetos a Biossegurança.

Assinatura:

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Nome do orientador: Prof. Dr. Flávio Dias Passos

Data: 30 de Janeiro de 2018.

Declaração

As cópias de artigos de minha autoria ou de minha co-autoria, já publicados ou submetidos para publicação em revistas científicas ou anais de congressos sujeitos a arbitragem, que constam da minha Dissertação/Tese de Mestrado/Doutorado, intitulada **DESVENDANDO A DIVERSIDADE DOS ANOMALODESMATA (MOLLUSCA: BIVALVIA): UMA ABORDAGEM MORFOLÓGICA E FILOGENÉTICA**, não infringem os dispositivos da Lei n.° 9.610/98, nem o direito autoral de qualquer editora.

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