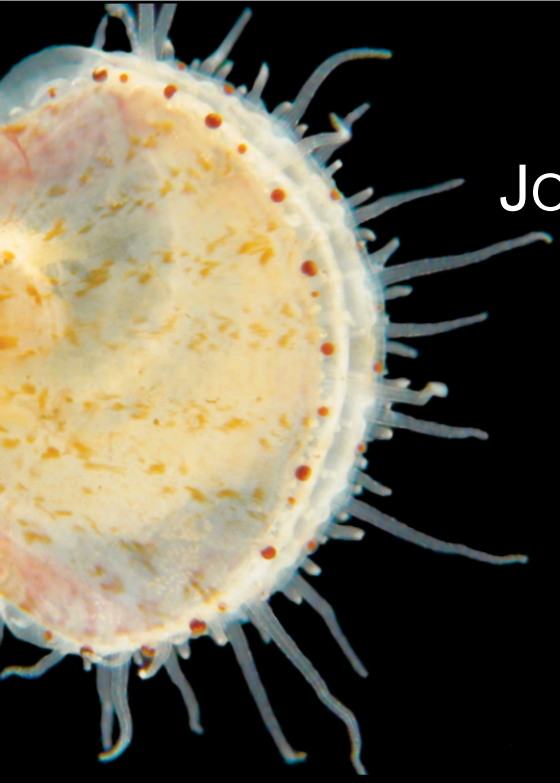
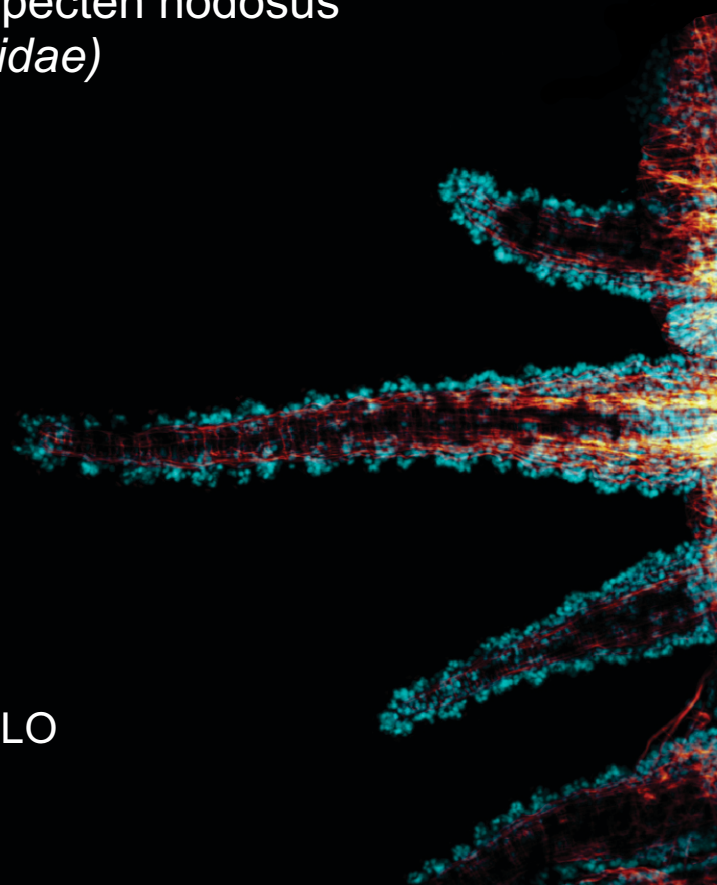


JORGE ALVES AUDINO

Anatomia e morfogênese da margem do manto da vieira *Nodipecten nodosus* (L. 1758) (Bivalvia: Pectinidae)

Anatomy and morphogenesis of the mantle margin in the scallop Nodipecten nodosus *(L. 1758) (Bivalvia: Pectinidae)*



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

Orientadora: Profa. Dra. Sônia Godoy Bueno Carvalho Lopes
Co-orientador: Prof. Dr. José Eduardo Amoroso Rodriguez Marian

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COMISSÃO JULGADORA

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As coisas tangíveis
tornam-se insensíveis
à palma da mão.

Mas as coisas findas,
muito mais que lindas,
essas ficarão.

Carlos Drummond de Andrade, *Memória*
(Antologia Poética)

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INTRODUÇÃO

INTRODUÇÃO GERAL

1. O FILO MOLLUSCA COM ÊNFASE NA CLASSE BIVALVIA

O filo Mollusca representa um dos mais diversificados filos de metazoários com cerca de 200.000 espécies descritas para a fauna atual e 70.000 para o registro fóssil (Haszprunar *et al.*, 2008). O grupo teve sua origem no final do Pré-Cambriano a partir de um provável ancestral de simetria bilateral, vermiforme, e cuja presença de uma cutícula dorsal secretora de espículas calcárias é a principal evidência na sua reconstituição como o "primeiro molusco" (Salvini-Plawen & Steiner, 1996). Atualmente, assumem-se como principais sinapomorfias do filo a presença de cutícula dorsal com secreções calcárias (espículas ou conchas sólidas), pé conspícuo, manto, rádula e brânquias pectinadas, sendo essas duas últimas características sujeitas a diversas alterações e perdas secundárias (Haszprunar *et al.*, 2008; Ponder & Lindberg, 2008).

Os moluscos atuais são tradicionalmente divididos em oito classes (estimativa do número de espécies atuais indicada entre parênteses): Solenogastres (Neomeniomorpha) (250), Caudofoveata (Chaetodermomorpha) (150), Polyplacophora (1.000), Monoplacophora (25), Scaphopoda (600), Bivalvia (20.000), Gastropoda (100.000) e Cephalopoda (1.000) (Haszprunar *et al.*, 2008). Neomeniomorpha e Chaetodermomorpha correspondem ao agrupamento tradicionalmente denominado Aplacophora, incluindo moluscos vermiformes desprovidos de concha, mas portadores de uma cutícula dorsal secretora de espículas calcárias (Todt *et al.*, 2008; Todt & Wanninger, 2010). Os quítons (Polyplacophora) são moluscos que apresentam oito placas dorsais que se sobrepõem (Todt *et al.*, 2008). Os monoplacóforos são moluscos de águas profundas, pouco diversos na fauna atual, e que possuem concha formada por uma única peça abrigando órgãos seriados (Haszprunar, 2008). As demais quatro classes correspondem aos mais comumente conhecidos e diversos moluscos: Gastropoda, Cephalopoda, Scaphopoda e Bivalvia. Os gastrópodes são caracterizados pela torção do corpo e pelo enrolamento espiral da concha, enquanto os cefalópodes são distintos pelo pé modificado em tentáculos, bem como por uma série de alterações morfofisiológicas relacionadas à conquista do ambiente pelágico (Aktipis *et al.*, 2008; Nishiguchi *et al.*, 2008). A classe Scaphopoda reúne moluscos com concha de aspecto semelhante às presas de um elefante, além de captáculos, estruturas tentaculares únicas do grupo (Reynolds & Steiner, 2008). Os bivalves são moluscos que perderam a rádula e que apresentam a concha dividida em duas valvas. Juntamente com os gastrópodes,

são os moluscos com maior número de espécies, além de ampla diversidade ecológica e morfológica (Giribet, 2008).

Com registro fóssil do início do Cambriano (542-521 Ma), aceita-se que os primeiros bivalves fossem comedores seletivos de depósitos, cujo pé muscular desenvolvido estaria relacionado à escavação e obtenção de partículas de alimento (Harper *et al.*, 2000; Parkhaev, 2008; Elicki & Gursu, 2009). Na história evolutiva de Bivalvia, é notável a irradiação e ocupação de diferentes zonas adaptativas pelos representantes da classe, o que está possivelmente relacionado à expressiva diversificação morfológica do grupo (Morton, 1996). Apesar de extensos esforços taxonômicos, a atual diversidade dos bivalves ainda não é consenso, mas estimativas apontam para 20.000 espécies viventes (Haszprunar *et al.*, 2008). Durante as últimas décadas, um progressivo aumento no número de pesquisas incluindo dados morfológicos, moleculares e paleontológicos permitiu profundo avanço em hipóteses filogenéticas para classe; não obstante, muitas questões ainda permanecem desconhecidas ou conflitantes (*e.g.*, Steiner & Hammer. 2000; Giribet & Wheeler, 2002; Giribet & Distel, 2003; Giribet, 2008; Bieler *et al.*, 2013; Bieler *et al.*, 2014).

Associada às principais linhagens dentro de Bivalvia (Anexo 1) (Bieler *et al.*, 2010), a morfologia das brânquias é aparentemente um caráter profundamente relacionado a mudanças ecológicas, tanto no aspecto alimentar, quanto na ocupação do sedimento (Morton, 1996). Protobranchia é o agrupamento considerado mais basal em Bivalvia por apresentar muitas das condições ancestrais, tais como ctenídio bipectinado (protobrânquia) e alimentação por ação ciliar do pé e palpos labiais (Davenport, 1988; Bieler & Mikkelsen, 2006). Já a subclasse Autobranchia é o grupo mais diversificado, caracterizado por um arranjo branquial do tipo lamelibrânquia, mais complexo e em forma de W (Bieler & Mikkelsen, 2006). Por meio de ação ciliar, este tipo de ctenídio é responsável não somente pela limpeza e respiração como também pela obtenção de partículas de alimento por filtração (Morton, 1996).

O grande clado Heteroconchia compreende a maior diversidade de bivalves, com representantes epifaunais e principalmente infaunais, além de muitos outros hábitos de vida, como perfurador e comensal. Neste grupo, assume-se que a presença de pés escavadores e sifões desenvolvidos esteja associada às condições que permitiram a ocupação de diferentes zonas nos sedimentos em ampla variedade de ambientes (Giribet, 2008). Em Paleoheterodonta, a diversidade de espécies reunidas na ordem Unionoida representa o maior exemplo de diversificação de bivalves em águas doces, com distribuição global (Graf & Cummings, 2006). Dentre os Heterodonta, bivalves marinhos tipicamente infaunais,

destacam-se superfamílias megadiversas como Mactroidea, Veneroidea, Tellinoidea, e Lucinoidea, esta última contendo o grupo mais diverso de moluscos associados a quimiossimbiontes (Mikkelsen *et al.*, 2006; Taylor & Glover, 2006; Taylor *et al.*, 2007). Representantes epifaunais também estão presentes, como os bivalves gigantes da família Tridacnidae, que possuem associação com algas fotossintetizantes (Yonge, 1982). No clado Anomalodesmata, dentre vasta diversidade morfológica, destaca-se a evolução de hábitos carnívoros em bivalves septibrânquios (Harper *et al.*, 2006).

Em Pteriomorphia, grupo-irmão de Heteroconchia, a condição ancestral de escavadores superficiais foi amplamente substituída pelo hábito de vida epifaunal na maioria das linhagens (Stanley, 1972). Em Mytilida, ordem representada pelos mexilhões, a fixação por meio do bisso é a condição predominante, ainda que o hábito perfurador e semi-infaunal tenha evoluído em alguns grupos (Distel, 2000; Owada, 2007). No diverso agrupamento das arcas (Arcida), ocorrem bivalves endobissados ou epibissados em substratos não-consolidados, e até mesmo escavadores livres (Oliver & Holmes, 2006). Já em Pteriida, as ostras (Ostreidae) cimentam uma das valvas sobre substratos consolidados, enquanto muitos Pteriidae vivem bissados em diferentes tipos de substrato, assim como Pinnidae (Tëmkin, 2006). Em Pectinida, a evolução do hábito epifaunal livre é notável, incluindo espécies natantes e vágeis em Pectinidae e Limidae, embora haja casos secundários de cimentação, como em Spondylidae (Waller, 2006).

2. MARGEM DO MANTO

Apesar de poucos estudos detalharem a margem palial entre as classes de moluscos, a presença de três pregas na região é considerada a condição generalizada do grupo (Stasek & McWilliams, 1973). Aceita-se que, no molusco ancestral, a borda do manto estaria relacionada à produção de uma cutícula secretora, a qual sofreu diversas modificações de forma e função ao longo da história evolutiva do filo (Stasek & McWilliams, 1973). A secreção da concha é a função mais comumente associada à margem palial e, resumidamente, é realizada por células especializadas na deposição de carbonato de cálcio (na forma de calcita ou aragonita) ao longo da superfície externa da prega externa do manto (Timmermans, 1969; Taylor, 1973; Wilbur & Saleuddin, 1983). O perióstraco, revestimento proteico da concha formado principalmente por conchiolina, é secretado entre as pregas externa e mediana da margem palial (Taylor, 1973).

Os aplacóforos possuem a borda do manto reduzida, sendo considerada uma condição basal a presença de pequenas dobras na diminuta cavidade palial posterior de

Caudofoveata (Stasek & McWilliams, 1973). Os quítons possuem três pregas distintas na margem do manto, sendo a interna e a externa reduzidas, e a mediana bastante desenvolvida (Stasek & McWilliams, 1973). Essa hipertrofia da região mediana origina o cinturão, estrutura típica de Polyplacophora, que recobre parcialmente as valvas, além de apresentar superfície cuticular com espículas (Stasek & McWilliams, 1973). Em Conchifera, agrupamento que reúne Monoplacophora, Gastropoda, Cephalopoda, Scaphopoda e Bivalvia, a borda do manto possui diversas variações estruturais e funcionais, e a compreensão de sua evolução é considerada obscura (Stasek & McWilliams, 1973). Apesar de gastrópodes e escafópodes geralmente apresentarem a margem palial simples, ou seja, sem pregas evidentes, alguns gastrópodes possuem a prega mediana mais desenvolvida que as demais (Stasek & McWilliams, 1973; Steiner, 1991). Já *Neopilina galathea* (Monoplacophora) possui três pregas paliais distintas, com um sulco do perióstraco conspícuo (Lemche & Wingstrand, 1959). De modo semelhante, dentre os cefalópodes viventes, o manto de *Nautilus pompilius* é divido em três pregas bem definidas (Westermann *et al.*, 2005). Em bivalves, a condição de três pregas no manto é dominante, incluindo projeções desenvolvidas e especializadas, bem como o marcante deslocamento do sulco do perióstraco para sob a borda da valva (Stasek & McWilliams, 1973; Yonge, 1983).

Em Bivalvia, a borda do manto representa um ponto chave na compreensão da irradiação evolutiva do grupo, pois está aparentemente relacionada à ocupação de diferentes nichos e aos hábitos de vida. A condição bivalve implica em um animal cuja maior parte do corpo, senão ele todo, está envolto e protegido por duas valvas. Na história evolutiva do grupo, o encerramento do corpo pelas valvas, a redução da região cefálica e a alimentação por filtração são considerados importantes fatores relacionados ao estabelecimento de alterações significativas na borda do manto, como função sensorial e controle da circulação de água (Yonge, 1983). Desse modo, a margem palial representa uma importante estrutura de interação com o ambiente externo, apresentando diversas modificações associadas à evolução dos hábitos de vida em Bivalvia (Yonge, 1983). A função de secreção do perióstraco e das diferentes camadas da concha bivalve está relacionada, assim como nos demais moluscos, com a prega externa (Taylor, 1973). A prega mediana genericamente concentra funções sensoriais, enquanto a prega interna é amplamente muscular (Yonge, 1983). Entretanto, esse padrão não contempla a vasta diversidade de formas e funções da margem palial em bivalves, tema este ainda carente de estudos sob perspectivas anatômicas, funcionais e evolutivas. Por exemplo, configurações atípicas da margem palial ocorrem em Veneridae, com quatro pregas descritas (Ansell, 1961; Hillman & Shuster, 1966; Sartori *et*

al., 2008), e em bivalves da família Arcidae, onde apenas duas pregas estão presentes (externa e mediana), sendo a externa duplicada (Waller, 1980; Morton & Peharda, 2008).

Uma das especializações mais marcantes na evolução de Bivalvia é a fusão das pregas do manto. Em diferentes graus, a fusão palial permitiu notável irradiação evolutiva na ocupação de nichos infaunais, principalmente devido à formação de sifões em Heteroconchia (Yonge, 1948, 1957). Nestes bivalves, as fusões das pregas da borda do manto formam sifões inalante e exalante que auxiliam na circulação de água enquanto o animal ocupa o sedimento (Yonge, 1948, 1957, 1983). Deve-se ressaltar que os sifões são muito variados morfologicamente quanto ao grau de fusão entre si, simetria, extensão, função sensorial e presença de microestruturas diversas (Yonge, 1957, 1983). Em *Tridacna* e *Hippopus* (Tridacnidae), as dobras internas são hipertrofiadas e estão fundidas, formando, além do sifão, uma extensa superfície onde estão abrigados dinoflagelados fotossintetizantes, enquanto as pregas mais externas auxiliam na proteção do manto e na penetração no substrato (Yonge, 1982). Em bivalves especializados como os teredos (Teredinidae), perfuradores de madeira, as valvas são muito reduzidas e o corpo vermiforme possui a margem do manto fundida ao longo de sua extensão, formando, na extremidade posterior, os sifões e também as paletas calcárias que fecham a abertura de sua galeria (Lopes & Narchi, 1998).

Em muitos bivalves, mesmo naqueles infaunais, a margem palial pode encontrar-se livre, isto é, com redução ou ausência de pontos de fusão. Nestas condições, especializações morfofuncionais são comuns, geralmente associadas a interações entre o animal e o meio externo (Yonge, 1983). Na prega mediana de representantes da família Limidae ocorrem peculiares tentáculos não-retráteis relacionados à locomoção do animal, bem como à sua proteção (Guilmour, 1963). Tentáculos com função protetora também ocorrem de modo convergente em espécies de Galeommatidae cujas valvas são reduzidas (Morton, 1973). Glândulas paliais especializadas estão presentes em diversos grupos de bivalves, atuando na lubrificação, limpeza e adesão do manto (Beedham & Owen, 1965; Morton, 1987). Em espécies de *Lithophaga* (Mytilidae), a prega mediana se estende para além da concha e auxilia na perfuração de rochas ou corais por meio de secreções (Morton & Scott, 1980). Em famílias como Ostreidae, Pectinidae e Spondylidae, a prega interna é intensamente muscular e forma o velum, ou véu palial, responsável por delimitar as regiões inalante e exalante (Nelson, 1938; Yonge, 1983). Os bivalves da família Pectinidae são notáveis pela complexidade da margem do manto, o que está possivelmente associado a diversificação de diferentes hábitos epifaunais livres (Dakin, 1909; Alejandrino *et al.*, 2011). Além dos

aspectos ecológicos e comportamentais, merecem destaque os característicos tentáculos sensoriais e olhos paliais complexos que há tempos despertam o interesse dos zoólogos (Morton, 2008; Serb & Eernisse, 2008). Apesar do extenso acúmulo de conhecimento nas áreas de sistemática filogenética, aquicultura e ecologia, os pectinídeos ainda carecem de investigações sobre desenvolvimento e anatomia, principalmente quanto à margem do manto e demais órgãos internos.

2.1. Órgãos sensoriais da margem do manto

As principais estruturas da margem palial associadas à função sensorial em Bivalvia correspondem aos tentáculos, sifões e órgãos fotossensíveis (Yonge, 1983). Os tentáculos são estruturas sensoriais numerosas presentes em bivalves de diferentes famílias, compreendendo ampla variedade morfológica. A origem dos tentáculos na borda do manto é variada, ocorrendo na prega interna, associados aos sifões ou mesmo na prega mediana (Yonge, 1983). A presença de conjuntos ciliares e microestruturas variadas confere aos tentáculos as funções de mecano e quimiorrecepção, de modo que estas estruturas representam um importante meio de contato com o ambiente (Fishelson, 2000).

Quando as margens do manto se encontram fundidas formando sifões, estruturas tentaculares estão comumente presentes nas aberturas inalante e exalante (*e.g.*, Mouëza & Frenkiel, 1974; Fishelson, 2000; Sartori *et al.*, 2008). Além de atuarem como órgãos sensoriais, os tentáculos sifonais também impedem a entrada de partículas de tamanhos maiores para dentro dos canais paliais formados pelos sifões (Narchi, 1972; Hodgson & Fielden, 1984; Passos & Domaneschi, 2004). Em bivalves cujas margens do manto se encontram livres, isto é, sem pontos de fusão, é comum a presença de tentáculos e papilas ao longo de sua extensão. Por exemplo, curtos tentáculos ocorrem na prega interna e mediana de representantes das famílias Ostreidae e Pteriidae (Tëmkin, 2006), enquanto bivalves da família Galeommatidae são notáveis por seus numerosos tentáculos paliais de tamanhos variados (Lützen & Nielsen, 2005). Em Limidae, longos tentáculos septados distribuem-se pela margem palial, associados à autotomia e secreção de muco, possivelmente para evitar predadores (Owen & McCrae, 1979). Os tentáculos que ocorrem em Pectinidae são variados, incluindo pequenos tentáculos na prega interna e diferentes tipos tentaculares na prega mediana, organizados em tentáculos exploratórios, marginais e oculares (Dakin, 1909).

Órgãos fotossensíveis são amplamente distribuídos em bivalves, representando uma importante classe de receptores sensoriais no grupo (Anexo 2). Em Mollusca, os bivalves apresentam a maior diversidade de estruturas fotorreceptoras associadas à borda do manto

(Serb, 2008). Nessa classe, a percepção luminosa varia de acordo com a localização da estrutura receptora, de seu mecanismo óptico e da complexidade de elementos envolvidos (Serb, 2008; Serb & Eernisse, 2008). A fotopercepção pode ocorrer por receptores simples do manto ou mesmo por olhos paliais que variam quanto à prega do manto que os origina (Morton, 2008). Especula-se que estruturas mais elaboradas como os olhos paliais teriam surgido diversas vezes de forma independente em diferentes famílias de bivalves ao longo da evolução do grupo (Morton, 2008). Em Arcidae, numerosos olhos compostos estão distribuídos na prega externa do manto, formados por unidades semelhantes à omatídios e recobertas pelo perióstraco (Waller, 1980; Nilsson, 1994). Em Limidae, olhos paliais ocorrem na prega mediana como taças pigmentares que envolvem os fotorreceptores e a lente (Morton, 2000). Uma condição mais atípica ocorre em Tridacnidae, cuja prega palial interna é hipertrofiada e abriga numerosos ocelos onde fotossimbiontes associados possivelmente atuam como refletores, aumentando a eficiência da recepção de luz (Wilkens, 1986).

Olhos paliais complexos ocorrem nas famílias Pectinidae e Spondylidae, cuja estrutura ocular contém córnea, lente, retina dupla e camada refletora (Morton, 2008; Serb, 2008). Diferentes aspectos da anatomia e fisiologia de tais órgãos vêm sendo estudados desde o início do século 20 (*e.g.*, Dakin, 1910; Land, 1965; Barber *et al.*, 1967; Speiser & Johnsen, 2008, Serb *et al.*, 2013; Malkowsky & Götze, 2014). A luz que entra pela abertura ocular atravessa a córnea e a lente, sendo refletida no fundo do olho pela camada refletora, de modo que os feixes ópticos convergem na retina, resultando em uma imagem de baixa resolução (Land, 1965; Nilsson, 2013). Especula-se que o papel funcional dos olhos nestes animais esteja relacionado à resposta de fuga de predadores em espécies com capacidade de natação, embora haja controvérsias nessa hipótese (Wilkens, 2006). Funções relacionadas à orientação do animal no ambiente e substrato também foram sugeridas a partir de experimentos comportamentais (Hamilton & Koch, 1996). Notavelmente, em alguns representantes da família Laternulidae e Cardiidae, estão presentes na prega mediana olhos paliais muito semelhantes e tão complexos quanto aos de Pectinidae (Adal & Morton, 1973). Contudo, a grande distância filogenética presente entre essas famílias, pertencentes inclusive a subclasses distintas, revela ampla plasticidade da margem palial e alto grau de convergência em formação de sistemas visuais em bivalves (Morton, 2008). A evolução de olhos complexos, particularmente daqueles presentes em Pectinidae, representa um grande desafio de compreensão. Diversos aspectos dessas estruturas ainda carecem de estudos, de modo que diferentes técnicas e abordagens são necessárias para responder questões

relacionadas à evolução, função e anatomia desses órgãos (Serb & Eernesse, 2008). Além dessas questões, a formação e diferenciação de tentáculos e olhos paliais ainda representam uma significativa lacuna no conhecimento sobre desenvolvimento na família.

3. Desenvolvimento em Bivalvia

Os moluscos bivalves compartilham desenvolvimento indireto, geralmente formado por estádios larvais de trocófora, seguidos por larvas do tipo véliger, metamorfose e formação do indivíduo adulto (Raven, 1958; Moor, 1983). O embrião origina em poucas horas uma larva trócofora, caracterizada pela presença de prototróquio ciliar, formando uma faixa pré-oral, e tufo apical na região distal (Raven, 1958; Moor, 1983). Eventualmente, outros componentes ciliares podem estar presentes na trocófora, como o metatróquio pós-oral e o telotróquio na extremidade inferior (Raven, 1958; Hodgson & Burke, 1988). Com exceção dos bivalves protobrânquios, cuja fase larval é caracterizada pela larva pericálima (Gustafson & Reid, 1986), a formação do estádio de véliger nos demais bivalves ocorre a partir do desenvolvimento das valvas e do futuro manto por glândulas na região do escudo da concha, na superfície posterior e dorsal da larva trocófora. A formação da prodissoconcha I e II é característica da larva véliger, apresentando organização e composição típicas (Waller, 1981). Também é notável a formação do véu, com diversos grupos ciliares, responsável pela natação ativa das larvas e pela filtração de partículas de alimento (Moor, 1983). Em muitos bivalves, como no caso de Pectinidae e outras famílias, o véu regride e o pé se desenvolve originando o estádio denominado pedivéliger, caracterizando a transição do hábito larval planctônico para o bentônico (Moor, 1983; Cragg & Crisp, 1991). Uma vez associada ao substrato, a larva sofre metamorfose, conduzindo à gênese dos órgãos internos (como os ctenídios), dissoconcha e demais estruturas componentes do animal adulto (Raven, 1958; Moor, 1983; Cragg & Crisp, 1991).

Nas últimas décadas, extensos esforços foram realizados para compreensão de diversas questões relacionadas ao desenvolvimento em Mollusca, principalmente quanto à caracterização dos processos de morfogênese e às implicações na história evolutiva dos grupos. Particularmente em Bivalvia, estudos de morfogênese são escassos, e com base na revisão bibliográfica realizada, identificaram-se lacunas do conhecimento e perspectivas para pesquisas futuras com a classe. A grande maioria das investigações com larvas de bivalves é voltada para a caracterização geral dos estádios larvais e sua morfologia. Neste caso, o material de estudo corresponde principalmente a espécies comerciais de mexilhões (Mytilidae), ostras (Ostreidae), vieiras (Pectinidae) e berbigões (Veneridae) (*e.g.*, Waller,

1981; Hodgson & Burke, 1988; Moueza *et al.*, 1999). Apesar dos inúmeros estudos com Pectinidae voltados à aquicultura, em que são analisadas taxas de crescimento, assentamento e maturação, além de características de resistência e produtividade, esses trabalhos não têm por objetivo o detalhamento anatômico, por isso não serão citados aqui

O uso de Microscopia Eletrônica de Varredura permitiu grandes avanços nos estudos de embriogênese e anatomia larval, porém, tradicionalmente, as análises estiveram restritas à formação das valvas, charneira, ligamento e véu (*e.g.*, Doroudi & Southgate, 2003; Silberfeld & Gros, 2006; Wassnig & Southgate, 2012). Já os órgãos e demais estruturas internas, como trato digestivo e ctenídios, foram brevemente consideradas em poucos estudos de anatomia geral (*e.g.*, Cole, 1938; Allen, 1961; Sastry, 1965; Le Pennec, 1974; Elston, 1980; Hodgson & Burke, 1988; Bellolio *et al.*, 1993).

Recentemente, trabalhos que empregaram técnicas integradas de microscopia (microscopia de luz, eletrônica e confocal), além de métodos em marcação celular e expressão gênica, apresentaram resultados satisfatórios e inovadores na compreensão da ontogênese de invertebrados (Wanninger *et al.*, 2008). Atualmente, a abordagem da evo-devo, incluindo as frentes de estudos ontogenéticos e moleculares (Müller, 2012), representam a perspectiva mais promissora para compreensão da origem e das implicações evolutivas do desenvolvimento nos diferentes grupos de moluscos (Ponder & Lindberg, 2008). No caso de Mollusca, a maioria dos estudos de desenvolvimento e anatomia utilizou representantes de diferentes classes como modelos de estudo, obtendo evidências importantes para a proposição ou revisão de hipóteses acerca da evolução do grupo e de seus sistemas (Wanninger *et al.*, 2008). A investigação da miogênese e neurogênese em aplacóforos e poliplacóforos forneceram, entre outros aspectos, informações cruciais à discussão sobre sistemas seriados em moluscos (*e.g.*, Haszprunar & Wanninger, 2000; Wanninger & Haszprunar, 2002a; Scherholz *et al.*, 2013). Dados relevantes também foram obtidos para Scaphopoda (Wanninger & Haszprunar, 2002b), porém os gastrópodes provavelmente representam o grupo mais bem estudado em abordagens morfogenéticas, incluindo vasto registro de padrões e variações (*e.g.*, Wollesen *et al.*, 2008).

Como apontado por Wanninger *et al.* (2008), análises do desenvolvimento de Mollusca que empreguem técnicas de microanatomia são essenciais para melhor compreensão da evolução e ontogênese dos grupos, principalmente daqueles clados que ainda foram pouco estudados. Considerando a ampla diversidade de Bivalvia, e sua profunda importância em estudos comparativos entre moluscos, a carência de informações sobre morfogênese no grupo é acentuada. O conhecimento sobre desenvolvimento muscular e

nervoso em bivalves está principalmente resumido a informações obtidas com *Mytilus trossulus* (Voronezhskaya *et al.*, 2008; Dyachuk & Odintsova, 2009). Por sua vez, a morfogênese e diferenciação dos ctenídios foram estudadas em detalhes para *Mytilus edulis* (Cannuel *et al.*, 2009). Sendo assim, estudos de morfogênese de diferentes sistemas larvais em diferentes grupos de bivalves se mostram fundamentais para compreensão da variação na classe e suas implicações morfofuncionais.

A margem do manto dos bivalves é provavelmente um dos temas que recebeu menor atenção quanto à sua morfogênese. Especificamente para Bivalvia, segundo Cragg & Crisp (1991), a borda do manto possui muitas questões em aberto, especialmente quanto à sua organização e relação entre os distintos estádios ao longo do desenvolvimento. Tradicionalmente, considera-se que a larva bivalve possui apenas duas pregas paliais, enquanto o adulto, três (Moor, 1983; Cragg & Crisp, 1991). Contudo, pouco se sabe sobre a origem das pregas paliais e suas modificações ao longo do desenvolvimento larval e pós-larval. Poucos trabalhos descrevem a organização dessa região nos estádios larvais e, dentre as esparsas contribuições ao tema, destaca-se a descrição dos cílios da borda do manto do pectinídeo *Argopecten purpuratus*, observados sob microscopia eletrônica de varredura (Bellonio *et al.*, 1993), e estudos histológicos da margem palial da ostra *Ostrea edulis* (Cranfield, 1974). Em suma, o desenvolvimento da margem do manto em bivalves, incluindo a formação e diferenciação das pregas, permanece pouco compreendido. Mais notável ainda é a ausência de estudos que relacionem as estruturas larvais com a formação do adulto, ou seja, que coloquem a margem do manto sob a perspectiva de sua morfogênese. Neste contexto, espécies da família Pectinidae representam um potencial modelo de estudo para a investigação de tais questões. Dentre os motivos, destacam-se o longo histórico de estudos sobre a margem palial das vieiras e informações disponíveis sobre sua anatomia larval. Além do mais, espécies comerciais da família, assim como para outros grupos de bivalves, facilitam a obtenção de amostras em diferentes estádios do ciclo de vida e em quantidades satisfatórias, o que se revela muito vantajoso para estudos detalhados de desenvolvimento.

4. OBJETIVOS

Considerando o contexto aqui apresentado, o presente estudo visou preencher as diversas lacunas no conhecimento relacionado à morfogênese da margem do manto em Pectinidae, utilizando como modelo a espécie *Nodipecten nodosus* (Linnaeus, 1758), popularmente conhecida como vieira, veria pata-de-leão, ou *coquille Saint-Jacques*. Mais especificamente, a investigação visou contemplar os seguintes objetivos:

1. Descrever a anatomia da margem palial quanto à musculatura, epitélio, tecido conjuntivo, inervação e estruturas sensoriais (olhos, tentáculos e cílios) nos estádios de véliger, pedivéliger, pós-larva, juvenil e adulto;

2. Analisar a formação e diferenciação das pregas paliais ao longo do desenvolvimento da espécie por meio do emprego de diferentes técnicas de análise morfológica;

3. Analisar o desenvolvimento e anatomia das estruturas associadas à margem palial, como tentáculos, olhos paliais e véu palial, empregando técnicas integradas de microscopia;

4. Confrontar as informações obtidas para *Nodipecten nodosus* com os demais dados provenientes de outras espécies e famílias de bivalves, a fim de possibilitar uma abordagem comparativa;

5. Com base nas evidências obtidas, propor um modelo de desenvolvimento que explique a morfogênese da margem do manto em Pectinidae, bem como de órgãos associados, contribuindo para compreensão da anatomia e desenvolvimento dessa região em Bivalvia.

5. METODOLOGIA GERAL

Amostras de *N. nodosus* em estádios de véliger, pedivéliger, pós-larva, juvenil e adulto foram obtidas no Instituto de Ecodesenvolvimento da Baía de Ilha Grande, IED-BIG (Angra dos Reis, Rio de Janeiro). Os diferentes estádios foram amostrados ao longo dos meses de setembro de 2012, março, maio e setembro de 2013. Os espécimes larvais e juvenis foram removidos de tanques artificiais, onde eram mantidos sob condições controladas de temperatura, salinidade, aeração e alimentação. Indivíduos adultos foram coletados na baía, onde sistemas de lanterna oferecem substrato e abrigo artificial para criação de vieiras.

Os estudos com espécimes vivos, bem como a preparação para fixação, foram conduzidos nas instalações do Centro de Biologia Marinha da Universidade de São Paulo, CEBIMar-USP (São Sebastião, São Paulo). Os estudos de microscopia de luz e eletrônica foram realizados nas dependências do Instituto de Biociências da Universidade de São Paulo, IB-USP (São Paulo). Os estudos de microscopia confocal e reconstrução tridimensional foram conduzidos na Faculdade de Ciências da Vida, Universidade de Viena (Viena, Áustria).

5.1. ESTUDO DE ESPÉCIMES VIVOS

Após a obtenção do material de pesquisa no instituto de cultivo (IED-BIG), indivíduos de *N. nodosus* em diferentes estádios de desenvolvimento foram transferidos ao Centro de Biologia Marinha da Universidade de São Paulo (CEBIMar-USP). As amostras foram mantidas por curto período de tempo (entre 2 a 3 dias) até sua fixação para estudos de microscopia. Aspectos de comportamento e morfologia dos espécimes vivos foram observados e devidamente registrados por meio de fotografias e filmagens, com auxílio de estereomicroscópio e microscópio óptico.

5.2. FIXAÇÃO DO MATERIAL PARA ESTUDOS DE MICROSCOPIA

Antes da fixação, os indivíduos foram anestesiados em solução de cloreto de magnésio a 7,5% adicionada à água do mar (proporção 1 anestésico: 3 água do mar), sob refrigeração, por um período de até 3 horas (conforme o tamanho dos indivíduos). Esse procedimento foi realizado com o objetivo de se reduzir as contrações musculares observadas durante a fixação. Indivíduos até o estádio juvenil foram fixados inteiramente, enquanto que vieiras adultas tiveram sua margem palial dissecada e posteriormente fixada. Três protocolos de fixação foram empregados. Parte das amostras foi fixada em solução de paraformaldeído a 4% em tampão PBS a 1,0 M (com osmolaridade ajustada para 1000 mOsm e pH 7.2) por três horas, sendo em seguida lavadas e mantidas em tampão PBS. Outro conjunto de amostras foi fixada em solução de Karnovsky modificada (paraformaldeído a 2% e glutaraldeído a 2,5% em solução tampão cacodilato de sódio a 0,1 M, com osmolaridade ajustada para 1000 mOsm e pH 7.4) por três horas, sendo em seguida lavadas e mantidas em tampão cacodilato de sódio a 0,1 M. Todas as amostras utilizadas para microscopia eletrônica foram fixadas em solução de Karnovsky, enquanto os estudos histológicos foram realizados tanto com amostras fixadas em Karnovsky quanto com aquelas fixadas em paraformaldeído a 4% em PBS. Finalmente, as amostras submetidas ao procedimento de microscopia confocal foram fixadas em paraformaldeído a 4%, lavadas em tampão PB, e mantidas sob refrigeração em tampão PB contendo NaN_3 a 0.1%.

5.3. HISTOLOGIA E HISTOQUÍMICA

Espécimes inteiros e porções da margem palial foram utilizados para análises histológicas a partir da metodologia de fixação descrita acima. Amostras larvais e juvenis foram descalcificadas em solução de ácido ascórbico a 3% *overnigh*t. Após desidratação em série alcoólica ascendente até etanol a 100%, as amostras foram incluídas em resina à base

de glicol-metacrilato da marca "Leica" ("Leica Historesin Kit"), seguindo-se instruções do fabricante. Foram obtidos cortes de 3 µm de espessura para amostras de juvenis e adultos, e cortes de 2 µm para larvas e pós-larvas. Os cortes seriados foram corados com Hematoxilina de Mayer e Eosina, ou com Azul de Toluidina e Fucsina Básica. Ensaios histoquímicos foram realizados utilizando espécimes dos estádios de véliger, juvenil e adulto. As amostras foram processadas como descrito anteriormente e incluídas em historresina. Cortes de 3-4 µm de espessura foram corados com os seguintes métodos: Ácido Periódico e Reativo de Schiff (PAS), para detecção de polissacarídeos; Azul de Alcian, para detecção de polissacarídeos ácidos; Azul de Bromofenol, para detecção de proteínas; e Alizarina Sódica, para detecção de depósitos de cálcio. Todas as lâminas produzidas foram finalmente montadas com resina apropriada ("Entellan") e lamínula.

5.4. Microscopia Eletrônica de Varredura (MEV)

Amostras fixadas em solução de Karnovsky foram lavadas em tampão e submetidas à segunda fixação com tetróxido de ósmio a 1% em solução tampão de cacodilato de sódio a 0.1 M durante 30 minutos. Subsequentemente, foram lavadas com ácido tânico em solução tampão por 15 minutos e, então, mantidas em nova solução de OsO_4 por mais 15 minutos. Larvas e pós-larvas foram descalcificadas segundo a metodologia descrita anteriormente. Todas as amostras foram desidratadas até etanol a 100% em série alcoólica ascendente, submetidas ao ponto crítico com CO_2 como fluido transicional, montadas em *stubs*, metalizadas com ouro e analisadas no Microscópio Eletrônico de Varredura.

5.5. Microscopia Eletrônica de Transmissão (MET)

Amostras fixadas em solução de Karnovsky foram lavadas em tampão, submetidas à segunda fixação com OsO_4 a 1% em solução tampão de cacodilato de sódio a 0.1 M durante uma hora e lavadas em tampão. Subsequentemente, foram desidratadas até etanol a 100% em série alcoólica ascendente e incluídas em resina Epoxi. Cortes ultrafinos (50-70 nm) foram obtidos, montados em telas de cobre, contrastados com acetato de uranila e citrato de chumbo, e analisados no Microscópio Eletrônico de Transmissão.

5.6. Microscopia Confocal de Varredura a Laser (MCVL)

Para marcação dos filamentos de actina F, as amostras foram mantidas em solução tampão PBT (PB contendo Triton-X 100 a 2%) *overnight* e incubadas em Alexa Flour 488 Phalloidin (Molecular Probes) em PBT, com diluição de 1:40, por 24 horas no escuro. Para

marcação neuronal, as amostras foram incubadas em PBT contendo soro de cabra a 6% (block-PBT) *overnight*. Em seguida, anticorpos primários, *i.e.*, anti-serotonina, anti-FMRFamida e anti-α-tubulina, foram aplicados em concentração de 1:400 em solução de block-PBT por 24 horas. As amostras foram lavadas repetidas vezes em block-PBT antes da incubação com anticorpo secundário conjugado a fluorocromo (Alexa Fluor 488 e 633, Molecular Probes) em concentração de 1:200, por 24 horas no escuro. Núcleos foram corados com 1 µl de 4', 6-diamidino-2-fenilindol (DAPI, Invitrogen). Todas as amostras foram devidamente lavadas em solução tampão PBS, montadas em lâminas de microscopia utilizando Fluoromount G como meio de montagem, e mantidas sob refrigeração antes da análise no Microscópio Confocal de Varredura a Laser. Planos ópticos foram registrados a cada 0,3 µm ao longo do eixo z, e digitalmente agrupados pelo método de projeção de máxima intensidade. Reconstruções tridimensionais foram geradas a partir do conjunto de planos ópticos varridos, utilizando o software Imaris.

6. ORGANIZAÇÃO DA DISSERTAÇÃO

Além da presente "Introdução" e das "Considerações Finais", a Dissertação está organizada em mais três capítulos estruturados como artigos científicos e redigidos em inglês. Precedendo cada capítulo, há um breve resumo em português, contendo as principais ideias e contribuições do artigo. As respectivas figuras são apresentadas independentemente ao final de cada capítulo. Embora estejam organizados para publicação, nenhum dos manuscritos está submetido, publicado ou no prelo. Dessa forma, todas as sugestões, correções e críticas dos membros da Banca poderão ser incorporadas aos mesmos antes de sua publicação.

O capítulo 1, "*Mantle margin morphogenesis in Nodipecten nodosus (Mollusca: Bivalvia): new insights into the development and roles of bivalve pallial folds*", descreve detalhadamente o desenvolvimento da margem do manto em *N. nodosus*, por meio de técnicas combinadas de análise anatômica, e apresenta um modelo de desenvolvimento da região para Pectinidae. Esse manuscrito será submetido ao periódico *Journal of Morphology*.

O capítulo 2, "*Anatomy of tentacular organs from the mantle margin of the scallop Nodipecten nodosus (Bivalvia: Pectinidae)*", compreende a formação e anatomia dos órgãos tentaculares presentes na margem palial de *N. nodosus*, amplamente discutidos com base no conhecimento sobre estruturas similares nos demais bivalves. Esse manuscrito será submetido ao periódico *Zoologischer Anzeiger*.

O capítulo 3, "*Development of the pallial eye in Nodipecten nodosus (Mollusca: Bivalvia): insights into early visual performance in scallops*", expõe meticulosamente a sequência de mudanças anatômicas durante a diferenciação dos olhos paliais de *N. nodosus* e sua contribuição para a compreensão de estruturas visuais em Pectinidae. Esse manuscrito será submetido ao periódico *Zoomorphology*.

Ao final da dissertação, o anexo 1 apresenta a relação taxonômica dos principais grupos recentes de bivalves, baseada na literatura científica. O anexo 2, "S*íntese do conhecimento sobre a diversidade de sistemas visuais em Mollusca, com ênfase em Bivalvia*", compreende uma extensão revisão sobre o tema, incluindo informações sobre a morfologia e evolução de estruturas fotorreceptoras no grupo. O manuscrito foi submetido ao periódico nacional *Papéis Avulsos de Zoologia*.

REFERÊNCIAS BIBLIOGRÁFICAS

Adal, M. N. & Morton, B. 1973. The fine structure of the pallial eyes of *Laternula truncata* (Bivalvia: Anomalodesmata: Pandoracea). Journal of Zoology 170: 533–556.

Aktipis, S. W.; Giribet, G.; Lindberg, D. R. & Ponder, W. F. 2008. Gastropoda, an overview and analysis, p. 201-270. In: Ponder, W. F. & Lindberg, D. R. (Ed.). Phylogeny and Evolution of the Mollusca. California, University of California Press.

Alejandrino, A.; Puslednik, L. & Serb, J. M. 2011. Convergent and parallel evolution in life habit of scallops (Bivalvia: Pectinidae). BMC Evolutionary Biology 11: 164.

Allen, J. A. 1961. The Development of *Pandora inaequivalvis* (Linné). Journal of Embryology & Experimental Morphology 9: 252–268.

Ansell, A. D. 1961. The functional morphology of the British species of Veneracea (Eulamellibranchis). Journal of the Marine Biological Association of the United Kingdom 41: 489–515.

Barber, V. C.; Evans, E. M. & Land, M. F. 1967. The fine structure of the eye of the mollusk *Pecten maximus*. Zeitschritt fur Zellforschung 76: 295–312.

Beedham, G. E. & Owen, G. 1965. The mantle and shell of *Solemya parkinsoni* (Protobranchia: Bivalvia). Proceedings of the Zoological Society of London 145(3): 405–430.

Bellolio, G.; Lohrmman, K. & Dupré, E. 1993. Larval morphology of the scallop *Argopecten purpuratus* as revealed by scanning electron microscopy. The Veliger 36: 322–342.

Bieler, R. & Mikkelsen, P. M. 2006. Bivalvia – a look at the branches. Zoological Journal of the Linnean Society 148: 223–235.

Bieler, R.; Carter, J. G. & Coan, E.V. 2010. Classification of bivalve families. Malacologia 52: 113–133.

Cannuel, R.; Beninger, P. G.; McCombie, H. & Boudry, P. 2009. Gill development and its functional and evolutionary implications in the blue mussel *Mytilus edulis* (Bivalvia: Mytilidae). Biological Bulletin 217: 173–188.

Cole, H. A. 1938. The fate of the larval organs in the metamorphosis of *Ostrea edulis*. Journal of the Marine Biological Association of the United Kingdom 22(2): 469–484.

Cragg, S. M. & Crisp, D. J. 1991. The biology of scallop larvae. In: S. E. Shumway & Parsons, G. J. (Ed.). Scallops: Ecology, Biology and Aquaculture. Amsterdam, Elsevier.

Cranfield, H. J. 1974. Observations on the morphology of the mantle folds of the pediveliger of *Ostrea Edulis* L. and their function during settlement. Journal of the Marine Biological Association of the United Kingdom 54: 1–12.

Dakin, W. J. 1909. *Pecten*. In: Liverpool Marine Biology Committee. Memoirs on typical British marine plants & animals. XVII. London, Williams & Norgate.

Dakin, W. J. 1910. The eye of *Pecten*. Quarterly Journal of Microscopical Science 55:49–112.

Davenport, J. 1988. The Feeding Mechanism of *Yoldia* (= Aequiyoldia) *eightsi* (Courthouy). Proceedings of the Royal Society of London. Series B, Biological Sciences 232: 431–442.

Distel, D. L. 2000. Phylogenetic relationships among Mytilidae (Bivalvia): 18s rRNA data suggest convergence in mytilids body plans. Molecular Phylogenetic Evolution 15(1): 25–33.

Dorouldi, M. S. & Southgate, P. C. 2003. Embryonic and larval development of *Pinctada margaritifera* (Linnaeus, 1758). Molluscan Research 23: 101–107.

Dyachuk, V. A. & Odintsova, N. A. 2009. Development of the larval muscle system in the mussel *Mytilus trossulus* (Mollusca, Bivalvia). Development, Growth & Differentiation 51: 69–79.

Elicki, O. & Gursu, S. 2009. First record of *Pojetaia runnegari* Jell, 1980 and *Fordilla barrande*, 1881 from the Middle East (Taurus Mountains, Turkey) and critical review of Cambrian bivalves. Palaeontologische Zeitschrift 83: 267–291.

Elston, R. 1980. Functional anatomy, histology and ultrastructure of the soft tissues of the larval American oyster, *Crassostrea virginica*. Proceeding of the National Shellfish Association 70: 65–93.

Fishelson, L. 2000. Comparative morphology and cytology of siphons and siphonal sensory organs in selected bivalve molluscs. Marine Biology 137: 497–509.

Giribet, G. & Wheeler, W. 2002. On bivalve phylogeny: a high-level analysis of the Bivalvia (Mollusca) based on combined morphology and DNA sequence data. Invertebrate Biology 121(4): 271–324.

Giribet, G. & Distel, D. L. 2003. Bivalve phylogeny and molecular data, p. 45-90. In: C. Lydeard & Lindberg, D. R. (Eds). Molecular Systematics and Phylogeography of Mollusks. Washington, D.C., Smithsonian Books.

Giribet, G. 2008. Bivalvia, p. 105-141. In: Ponder, W. F. & Lindberg, D. R. (Ed.). Phylogeny and Evolution of the Mollusca. California, University of California Press.

Graf, D. L. & Cummings, K. S. 2006. Palaeoheterodont diversity (Mollusca: Trigonioida + Unionoida): what we know and what we wish we knew about freshwater mussel evolution. Zoological Journal of the Linnean Society 148: 343–394.

Guilmour, T. H. J. 1963. A note on the tentacles of *Lima hians* (Gmelin) (Bivalvia). Journal of Molluscan Studies 35(2-3): 82–85.

Gustafson, R. G. & Reid, R. G. B. 1986. Development of the pericalymma larva of *Solemya reidi* (Bivalvia: Cryptodonta: Solemyidae) as revealed by light and electron microscopy. Marine Biology 93: 411–427.

Hamilton, P. V. & Koch, K. M. 1996. Orientation toward natural and artificial grassbeds by swimming bay scallops, *Argopecten irradians* (Lamarck, 1819). Journal of Experimental Marine Biology and Ecology 199: 79–88.

Harper, E. M.; Taylor, J. D. & Crame, J. A. 2000. The evolutionary biology of the Bivalvia. Geological Society special publication 177. The Geological Society, London, United Kingdom.

Harper, E. M.; Dreyer, H. & Steiner, G. 2006. Reconstructing the Anomalodesmata (Mollusca: Bivalvia): morphology and molecules. Zoological Journal of the Linnean Society 148: 395–420.

Haszprunar, G. 2008. Monoplacophora (Tryblidia), p. 97-104. In: Ponder, W. F. & Lindberg, D. R. (Ed.). Phylogeny and Evolution of the Mollusca. California, University of California Press.

Haszprunar, G. & Wanninger, A. 2000. Molluscan muscle systems in development and evolution. Journal of Zoological Systematics and Evolutionary Research 38: 157–163.

Haszprunar, G.; Schander, C. & Halanych, K. M. 2008. Relationships of higher molluscan taxa, p. 19-32. In: Ponder, W. F. & Lindberg, D. R. (Ed.). Phylogeny and Evolution of the Mollusca. California, University of California Press.

Hillman, R. E. & Shuster, C. N. 1966. A comment on the origin of the fourth fold in the mantle of the Quahog, *Mercenaria mercenaria*. Chesapeake Science 7(2): 112–113.

Hodgson, C. A. & Burke, R. D. 1988. Development and larval morphology of the Spiny Oyster, *Chlamys hastata*. Biological Bulletin 174: 303–318.

Hodgson, A. N. & Fielden, L. J. 1984. The structure and distribution of peripheral ciliated receptors in the bivalves molluscs *Donax serra* and *D. sordidus*. Journal of Molluscan Studies 50(2): 104–112.

Land, M. F. 1965. Image formation by a concave reflector in the eye of the scallop, *Pecten maximus*. The Journal of Physiology 179: 138–153.

Lemche, H. & Wingstrand, K. G. 1959. The anatomy of *Neopilina galatheae*, 1957 (Molllusca, Tryblidiacea). Galathea Report 3: 9–71.

Le Pennec, M. 1974. Morphogenèse de la coquille de *Pecten maximus* (L.) élevé au laboratoire. Cahiers de Biologie Marine 15 475–482.

Lopes, S. G. B. C. & Narchi, W. 1998. Functional anatomy of *Nausitora fusticula* (Jeffreys, 1860) (Bivalvia: Teredinidae). The Veliger 41(3): 274–288.

Lützen, J. & Nielsen, C. 2005. Galeommatid bivalves from Phuket, Thailand. Zoological Journal of the Linnean Society 144: 261–308.

Malkowsky, Y. & Götze, M. C. 2014. Impact of habitat and life trait on character evolution of pallial eyes in Pectinidae (Mollusca: Bivalvia). Organisms Diversity and Evolution 14: 173–185.

Mikkelsen, P. M.; Bieler, R.; Kappner, I. & Rawlings, T. A. 2006. Phylogeny of Veneroidea (Mollusca: Bivalvia) based on morphology and molecules. Zoological Journal of the Linnean Society 148: 439–521.

Moor, B. 1983. Organogenesis. In: K. M. Wilbur (Ed.). The Mollusca: Development. Florida, Academic Press.

Morton, B. 1973. Dymantic display in *Galeomma polita* Deshayes (Bivalvia: Leptonacea). Journal of Conchology 28: 365–369.

Morton, B. 1987. The mantle margin and radial mantle glands of *Entodesma saxicola* and *E. inflata* (Bivalvia: Anomalodesmata: Lyonsiidae). Journal of Molluscan Studies 53: 139–151.

Morton, B. 1996. The evolutionary history of the Bivalvia. In: J. D. Taylor (Ed.). Origin and Evolutionary radiation of the Mollusca. Londres, Oxford University Press.

Morton, B. 2000. The pallial eyes of *Ctenoides floridanus* (Bivalvia: Limoidea). Journal of Molluscan Studies 66: 449–455.

Morton, B. 2008. The evolution of eyes in the Bivalvia: new insights. American Malacological Bulletin 26(1/2): 35–45.

Morton, B. & Peharda, M. 2008. The biology and functional morphology of *Arca noae* (Bivalvia: Arcidae) from the Adriatic Sea, Croacia, with a discussion on the evolution of the bivalve mantle margin. Acta Zoologica 89: 19–28.

Morton, B. & Scott, P. J. B. 1980. Morphological and functional specializations of the shell, musculature and pallial glands in the Lithophaginae (Mollusca: Bivalvia). Journal of Zoology of London 192: 179–203.

Mouëza, M. & Frenkiel, L. 1974. Contribution a l'etude des structures palleales des Tellinacea. Morphologie et structure du manteaux de *Donax trunculus* L. Journal of Molluscan Studies 41(1): 1–19.

Mouëza, M.; Gros, O. & Frenkiel, L. 1999. Embryonic, larval, and post-larval development of the tropical clam *Anomalocardia brasiliana* (Mollusca: Bivalvia, Veneridae). Journal of Molluscan Studies 65: 73–88.

Müller, G. B. 2007. Evo-devo: extending the evolutionary synthesis. Nature Reviews Genetics 8: 943–949.

Narchi, W. 1972. Comparative study of the functional morphology of *Anomalocardia brasiliana* (Gmelin, 1791) and *Tivela mactroides* (Born, 1778) (Bivalvia, Veneridae). Bulletin of Marine Science 22(3): 643–670.

Nelson, T. C. 1938. The feeding mechanism of the oyster. I. On the pallium and the branchial chambers of *Ostrea virginica, O. edulis* and *O. angulata*, with comparisons with other species of the genus. Journal of Morphology 63: 1–61.

Nilsson, D. 1994. Eyes as optical alarms systems in fan warms and ark clams. Philosophical Transactions of the Royal Society B 346: 195–212.

Nilsson, D. 2013. Eye evolution and its functional basis. Visual Neuroscience 30: 5–20.

Nishiguchi, M. K. & Mapes, R. H. 2008. Cephalopoda. 163-199. In: Ponder, W. F. & Lindberg, D. R. (Ed.). Phylogeny and Evolution of the Mollusca. California, University of California Press.

Oliver, P. G. & Holmes, A. M. 2006. The Arcoidea (Mollusca: Bivalvia): a review of the current phenetic-based systematics. Zoological Journal of the Linnean Society 148: 237–251.

Owada, M. 2007. Functional morphology and phylogeny of the rock-boring bivalves *Leiosolenus* and *Lithophaga* (Bivalvia: Mytilidae): a third functional clade. Marine Biology 150: 853–860.

Owen, G. & McCrae, J. M. 1979. Sensory cell/gland cell complexes associated with the pallial tentacles of the bivalve *Lima hians* (Gmelin), with a note on specialized cilia on the pallial curtains. Philosophical Transactions of the Royal Society of London B 287/1017: 45–62.

Parkhaev, P. Y. 2008. The Early Cambrian radiation of Mollusca, p. 33–69. In: Ponder, W. F. & Lindberg, D. R. (Ed.). Phylogeny and Evolution of the Mollusca. California, University of California Press.

Passos, F. D. & Domaneschi, O. 2004. Biologia e anatomia funcional de *Donax gemmula* Morrison (Bivalvia, Donacidae) do litoral de São Paulo, Brasil. Revista Brasileira de Zoologia 21(4): 1017–1032.

Ponder, W. F. & Lindberg, D. R. 2008. Molluscan evolution and phylogeny, p. 1-17. In: Ponder, W. F. & Lindberg, D. R. (Ed.). Phylogeny and Evolution of the Mollusca. California, University of California Press.

Raven, C. P. 1958. Morphogenesis: the Analysis of Molluscan Development. New York, Pergamon Press.

Reynolds, P. D. & Steiner, G. 2008. Scaphopoda, p. 143-161. In: Ponder, W. F. & Lindberg, D. R. (Ed.). Phylogeny and Evolution of the Mollusca. California, University of California Press.

Salvini-Plawen, L. & Steiner, G. 1996. Synapomorphies and plesiomorphies in higher classification of Mollusca. In: J. D. Taylor (Ed.). Origin and Evolutionary Radiation of the Mollusca. Oxford, Oxford University Press.

Sartori, A. F.; Printrakoon, C.; Mikkelsen, P. & Bieler, R. 2008. Siphonal structure in the Veneridae (Bivalvia: Heterodonta) with an assessment of its phylogenetic application and a review of venerids of the Gulf of Thailand. The Raffles Bulletin of Zoology 18: 103–125.

Sastry, A. N. 1965. The development and external morphology of pelagic larval and post-larval stages of the bay scallop, *Aequipecten irradians concentricus* Say, reared in the laboratory. Bulletin of Marine Science, 15(2): 417–35.

Scherholz, M.; Redl, E.; Wollesen, T.; Todt, C. & Wanninger, A. Aplacophoran mollusks evolved from ancestors with polyplacophoran-like features. Current Biology 23: 2130–2134.

Serb, J. M. 2008. Toward developing models to study the disease, ecology and evolution of the eye in Mollusca. American Malacological Bulletin 26: 3–18.

Serb, J. M. & Eernisse, D. J. 2008. Charting evolution´s trajectory: using molluscan eye diversity to understanding parallel and convergent evolution. Evolution: Education and Outreach 1: 439–447.

Serb, J. M.; Porath-Krause, A. J. & Pairett, A. N. 2013. Uncovering a gene duplication of the photoreceptive protein, opsin, in scallops (Bivalvia: Pectinidae). Integrative and Comparative Biology 53: 68–77.

Silberfeld, T. & Gros, O. 2006. Embryonic development of the tropical bivalve *Tivela mactroides* (Born, 1778) (Veneridae: subfamily Meretricinae): a SEM study. Cahiers de Biologie Marine 47: 243–251.

Speiser, D. I. & Johnsen, S. 2008. Comparative morphology of the concave mirror eyes of scallops (Pectinoidea). American Malacological Bulletin 26: 27–33.

Stanley, S. M. 1972. Functional morphology and evolution of byssally attached bivalve mollusks. Journal of Paleontology 46(2): 165–212.

Stasek, C. R. & McWilliams, W. R. 1973. The comparative morphology and evolution of molluscan mantle edge. The Veliger 16: 1–19.

Steiner, G. 1991. Observations on the anatomy of the scaphopod mantle, and the description of a new family, the Fustiariidae. American Malacological Bulletin 9: 1–20.

Steiner, G. & S. Hammer. 2000. Molecular phylogeny of the Bivalvia inferred from 18S rDNA sequences with particular reference to the Pteriomorphia, p. 11-29. In: E.M. Harper, J.D. Taylor & J.A. Crame (Eds.). The Evolutionary Biology of the Bivalvia. London, The Geological Society of London.

Taylor, J. D. 1973. The structural evolution of the bivalve shell. Palaeontology 16: 519–534.

Taylor, J. D. & Glover, E. A. 2006. Lucinidae (Bivalvia) – the most diverse group of chemosymbiotic molluscs. Zoological Journal of the Linnean Society 148: 421–438.

Taylor, J. D.; Williams, S. T.; Glover, E. A. & Dyal, P. 2007. A molecular phylogeny of heterodont bivalves (Mollusca: Bivalvia: Heterodonta): new analyses of 18S and 28S rRNA genes. Zoologica Scripta 36: 587–606.

Tëmkin, I. 2006. Morphological perspective on the classification and evolution of recent Pterioidea (Mollusca: Bivalvia). Zoological Journal of the Linnean Society 148: 253–312.

Timmermans, L. P. M. 1969. Studies on shell formation in molluscs. Netherlands Journal of Zoology 19: 417–523.

Todt, C.; Okusu, A.; Schander, C. & Schwabe E. 2008. Solenogastres, Caudofoveata, and Polyplacophora, p. 71-96. In: Ponder, W. F. & Lindberg, D. R. (Ed.). Phylogeny and Evolution of the Mollusca. California, University of California Press.

Todt, C. & Wanninger, A. 2010. Of tests, trochs, shells, and spicules: Development of the basal mollusk *Wirenia argentea* (Solenogastres) and its bearing on the evolution of trochozoan larval key features. Frontiers in Zoology 7: 6.

Voronezhskaya, E. E.; Nezlin, L. P.; Odintsova, N. A.; Plummer, J. T. & Croll, R. P. 2008. Neuronal development in larval mussel *Mytilus trossulus* (Mollusca: Bivalvia). Zoomorphology 127: 97–110.

Waller, T. R. 1980. Scanning electron microscopy of shell and mantle in the order Arcoida (Mollusca: Bivalvia). Smithsonian Contributions to Zoology 313.

Waller, T. R. 1981. Functional morphology and development of veliger larvae of the European oyster, *Ostrea edulis*. Smithsonian Contributions to Zoology, 328: 70.

Waller, T. R. 2006. Phylogeny of families in the Pectinoidea (Mollusca: Bivalvia): importance of the fossil record. Zoological Journal of the Linnean Society 148: 313–342.

Wanninger, A. & Haszprunar, G. 2002a. Chiton myogenesis: perspectives for the development and evolution of larval and adult muscle systems in molluscs. Journal of Morphology 251: 103–113.

Wanninger, A. & Haszprunar, G. 2002b. Muscle development in *Antalis entalis* and its significance for scaphopod relationships. Journal of Morphology 254: 53–64.

Wanninger, A.; Koop, D.; Moshel-Lynch, S. & Degnan, B. M. 2008. Molluscan evolutionary development, p. 427–445. In: W. Ponder & D. Lindberg (Ed.). Phylogeny and Evolution of the Mollusca. California, University of California Press.

Wassnig, M. & Southgate, P. C. 2012. Embryonic and larval development of *Pteria penguin* (Röding, 1798) (Bivalvia: Pteriidae). Journal of Molluscan Studies 78: 134–141.

Westermann, B.; Schmidtberg, H. & Beuerlein, K. 2005. Functional morphology of the mantle of *Nautilus pompilius* (Mollusca, Cephalopoda). Journal of Morphology 264: 277–285.

Wilbur, K. M. & Saleuddin, A. S. M. 1983. Shell formation, p. 235-287. In: Saleuddin, A. S. M. & Wilbur, K. M. (Eds.). The Mollusca. Academics, New York.

Wilkens, L. A. 1986. The visual system of the giant clam *Tridacna*: behavioral adaptations. Biological Bulletin 170: 393–408.

Wilkens, L. A. 2006. Neurobiology and behaviour of the scallop. In: Shumway, S. E. & Parsons, G. J. Scallops: Biology, Ecology and Aquaculture. Amsterdam, Elsevier.

Wollesen, T.; Wanninger, A. & Klussmann-Kolb, A. 2008. Myogenesis in *Aplysia californica* (Cooper, 1863) (Mollusca, Gastropoda, Opisthobranchia) with special focus on muscular remodeling during metamorphosis. Journal of Morphology 269: 776–789.

Yonge, C. M. 1948. Formation of siphons in Lamellibranchia. Nature 161: 198–199.

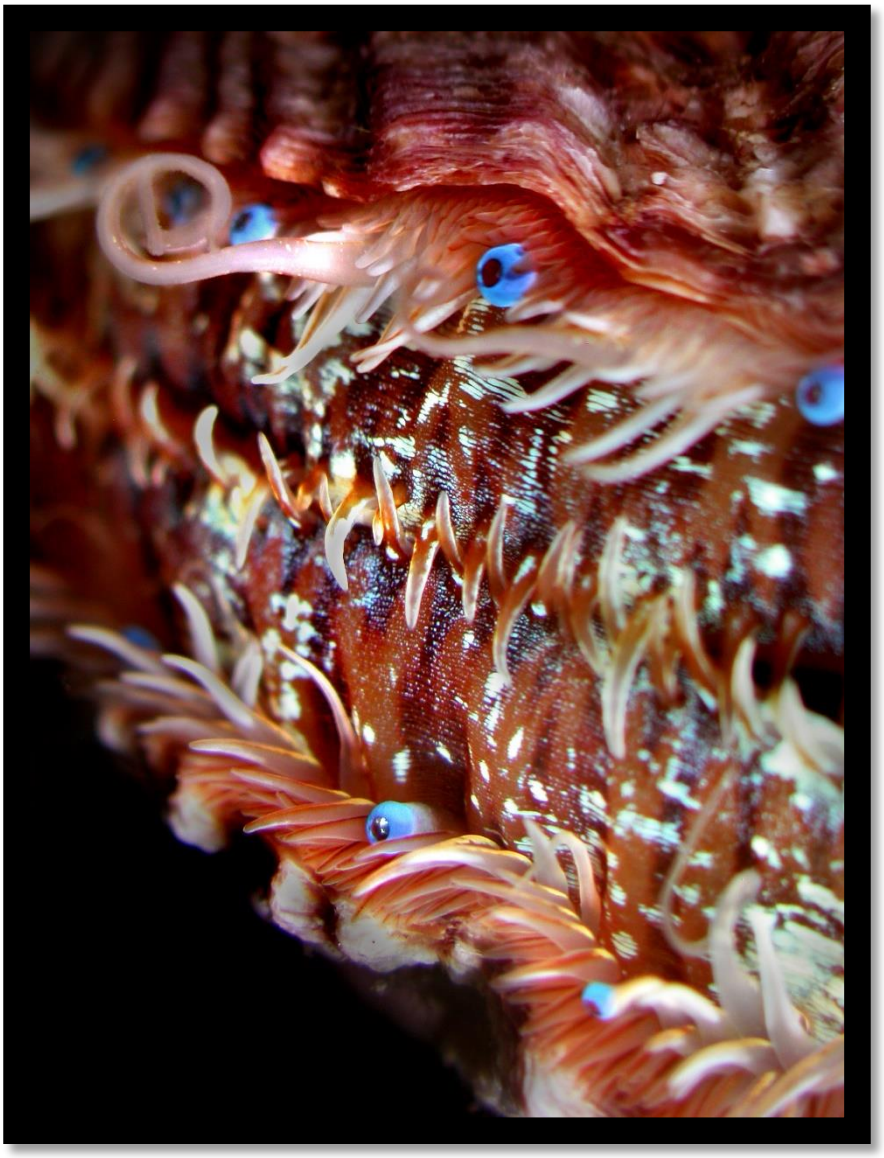
Yonge, C. M. 1957. Mantle fusion in the Lamellibranchia. Pubblicazioni della Stazione Zoologica di Napoli 29: 151–171.

Yonge, C. M. 1982. Functional morphology and evolution in the Tridacnidae (Mollusca: Bivalvia: Cardiacea). Records of the Australian Museum 33(17): 735–777.

Yonge, C. M. 1983. Symmetries and role of mantle margins in the bivalve Mollusca. Malacological Review 16: 1–10.

CHAPTER 1

MANTLE MARGIN MORPHOGENESIS IN *NODIPECTEN NODOSUS* (MOLLUSCA: BIVALVIA): NEW INSIGHTS INTO THE DEVELOPMENT AND ROLES OF BIVALVE PALLIAL FOLDS



CAPÍTULO 1

DESENVOLVIMENTO DA MARGEM PALIAL EM *NODIPECTEN NODOSUS* (BIVALVIA: PECTINIDAE)

RESUMO

Apesar do extenso conhecimento acumulado para a margem do manto em bivalves, diversas questões relacionadas ao seu desenvolvimento, anatomia e diversidade morfológica permanecem pouco compreendidas. Por exemplo, a formação das pregas paliais e sua subsequente diferenciação ainda são obscuras. Bivalves da família Pectinidae (vieiras) são particularmente promissores para esclarecer essa questão, pois eles possuem uma complexa margem palial e seus diferentes estádios de desenvolvimento podem ser obtidos a partir da maricultura. A margem do manto da vieira *Nodipecten nodosus* (L. 1758) foi investigada por meio de técnicas integradas de microscopia (*i.e.*, histologia, microscopia eletrônica de varredura e transmissão, além de imunocitoquímica aplicada à microscopia confocal). Uma minuciosa descrição da margem palial é fornecida ao longo da ontogenia da espécie, com ênfase em aspectos conservados e profundas modificações. Inicialmente sem pregas, a margem palial é dividida em regiões proximal e distal pela zona de formação do perióstraco. O surgimento da musculatura palial, combinada à inervação do manto, são etapas cruciais durante o desenvolvimento larval. No estádio de pediveliger, a margem se torna pregueada, resultando na condição bilobada, com um sulco do perióstraco e a formação de diferentes tipos ciliares. Após a metamorfose, um segundo evento de evaginação é responsável pela formação da prega palial mediana a partir da região interna da prega interna. Assim que a condição de três pregas paliais estabelece-se, as características do manto adulto rapidamente se desenvolvem. Com base em diferentes linhas de evidências, nós propomos um modelo para explicar a formação da margem do mando em Pectinidae. Os resultados obtidos devem fornecer potencial contribuição ao desenvolvimento da margem palial em Bivalvia, além de base para futuros estudos comparativos na classe.

Palavras-chave: borda do manto, microscopia integrada, ontogenia, Pectinidae, sulco do perióstraco.

O manuscrito a seguir contendo as informações detalhadas será submetido ao periódico internacional *Journal of Morphology*.

CHAPTER 1

MANTLE MARGIN MORPHOGENESIS IN *NODIPECTEN NODOSUS* (MOLLUSCA: BIVALVIA): NEW INSIGHTS INTO THE DEVELOPMENT AND ROLES OF BIVALVE PALLIAL FOLDS

ABSTRACT

Even though extensive amount of knowledge of bivalve mantle margin is available in the literature, several issues concerning its development, anatomy and morphological diversity remain unclear. For example, the formation of bivalve mantle folds and their further differentiation are still obscure. Bivalves from the family Pectinidae (scallops) are particularly promising to cast some light into this question, for they exhibit a complex mantle margin, and their developmental stages are easily obtained due to scallop farming. We investigated the mantle margin of the scallop *Nodipecten nodosus* (L. 1758) during larval and postmetamorphic stages, by means of integrative microscopy techniques (*i.e.* histology, scanning and transmission electron microcopy, and immunocytochemistry combined with confocal microscopy). A thorough description of the mantle edge is provided throughout the species ontogeny, with emphasis on conservative features and major changes. Initially unfolded, the pallial margin is divided into distal and proximal regions by the periostracum forming zone. The emergence of the pallial musculature, combined with mantle innervation, are crucial steps during larval development. By the late pediveliger stage, the margin becomes folded, resulting in a bilobed condition, with a periostracal groove and the development of different types of cilia. After metamorphosis, a second folding process is responsible for developing the middle mantle fold from the inner surface of the inner fold. Once the three-folded condition is established, the general adult features are rapidly developed. Based on several lines of evidence, we propose a model to explain mantle margin formation in the Pectinidae. Our data should provide insights into pallial margin development in other Bivalvia, as well as a basis for future comparative studies within the class.

Key words: integrative microscopy, mantle edge, ontogeny, Pectinidae, periostracal groove

INTRODUCTION

The molluscan mantle margin, which corresponds to the free portion of the mantle, exhibits great diversity of form and function. The presence of three pallial folds in this region is claimed to be the general condition for conchiferan molluscs; nevertheless, structural diversity in the pallial margin is very pronounced among and within molluscan classes (Stasek and McWilliams, 1973). Even though gastropods and scaphopods most often display a simple, swollen projection in the mantle rim, in a few gastropods the mantle margin is molded into distinctive folds (Stasek and McWilliams, 1973; Steiner, 1991). The monoplacophoran *Neopilina galathea* displays three folds in the mantle margin, and a periostracal groove is placed between the outer and middle folds (Lemche and Wingstrand, 1959). Similarly, among living cephalopods, the mantle of *Nautilus pompilius* also exhibits the three-folded pattern (Westermann *et al.*, 2005).

In Bivalvia, the mantle margin is divided into three pallial folds, each with a specific function: the secretory outer fold, the sensorial middle fold, and the muscular inner fold (Yonge, 1957, 1983). The periostracum is formed in a deep groove between the outer and middle folds, while the shell layers are secreted by the outer mantle epithelium (Taylor, 1973; Wilbur and Saleuddin, 1983). However, exceptions in this three-folded pattern do exist, *e.g.*, four pallial folds are conventionally described in the Veneridae (Ansell, 1961; Hillman and Shuster, 1966; Sartori *et al.*, 2008), and two pallial folds occur in the Arcidae (with the outer one duplicated; Waller, 1980; Morton and Peharda, 2008).

The bivalve mantle margin displays several adaptive traits associated with bivalve's lifestyles, resulting in the huge morphological diversity observed within the class. Different levels of fusion in the mantle margin and siphon formation are among the key features associated with the evolutionary radiation of infaunal bivalves (Yonge, 1948, 1957, 1982). Specialized, secretory glands from the mantle margin are widespread in many groups performing a variety of roles, *e.g.*, cleansing, adhesion, lubrication, and boring (Beedham and Owen, 1965; Morton and Scott, 1980; Morton, 1987). Pallial tentacular structures are also present in some families, such as Limidae, Pectinidae and Galeommatidae, performing sensorial, defensive and secretory functions (Gilmour, 1967; Morton, 1973; Moir, 1977; Mikkelsen and Bieler, 2003). In addition, photoreceptors and pallial eyes have evolved independently in the mantle margin of several unrelated Bivalvia taxa (Morton, 2008; Serb and Eernisse, 2008). Even though extensive amount of knowledge of bivalve mantle margin

has been produced, these studies have mainly focused on adult anatomy, several developmental and functional issues remaining largely unclear (Cragg, 2006).

Bivalve organogenesis is poorly understood compared to other molluscan groups, such as gastropods, and further investigations applying techniques of microanatomy are vital to evaluate the functional ontogeny in the Bivalvia (Wanninger *et al.*, 2008). Morphogenesis of the bivalve mantle margin has been scarcely studied so far, and further developmental evidence is still necessary to understand the changes and mechanisms underlying fold differentiation. Apart from the detailed study on mantle anatomy of *Ostrea edulis* larvae carried out by Cranfield (1974), details on larval mantle are fragmentary, and restricted to descriptions on general larval morphology (Raven, 1958; Waller, 1981; Moor, 1983; Carriker, 1990; Cragg, 1996). In contrast, the development of other organs and structures, such as gills, foot, velum, and, most of all, shell, has been thoroughly documented for several bivalve representatives (*e.g.*, Cole, 1938; Ansell, 1962; Gruffydd *et al.*, 1975; Hodgson and Burke, 1988; Moueza *et al.*, 1999; Carriker, 2001; Weiss *et al.*, 2002; Cannuel *et al.*, 2009).

Scallops (bivalves of the family Pectinidae) are particularly promising to cast some light into the origin and differentiation of the bivalve mantle margin. Due to their economical importance, developmental stages are easily obtained in scallop farming areas. In addition, pectinids bear an especially complex mantle margin, displaying distinct organs and pallial structures, such as elaborate eyes and tentacles. Scallops are a well-studied group, and large background knowledge of their behavior, taxonomy, phylogeny and farming is available (*e.g.*, Alejandrino *et al.*, 2011; Beninger and Le Pennec, 2006; Waller, 2006; Wilkens, 2006). Their pallial eyes have been extensively studied due to their intricate structure and optical performance (Dakin, 1910; Land, 1965; Morton, 2001; Malkowsky and Jochum, 2014). The inner mantle fold is a muscular curtain responsible for the regulation of the water flow into and out of the mantle cavity, especially during clapping movements and swimming behavior (Buddenbrock, 1911; Yonge, 1936; Moore and Trueman, 1971). Although there is a plethora of studies on scallop pallial structures, there is no specific information concerning anatomy and developmental changes of their mantle margin.

The present study aimed at analyzing the morphogenesis of the mantle margin in the scallop *Nodipecten nodosus* (Linnaeus, 1758). We provide a thorough description of this region throughout its development, from veliger larvae to mature adults, by means of integrative microscopy techniques, *i.e.* light, electron and confocal microscopy.

MATERIAL AND METHODS

Specimens of *Nodipecten nodosus* at different developmental stages were obtained in the scallop farm *Institute of Eco-Development from Baía da Ilha Grande* (IED-BIG), Rio de Janeiro, Brazil. Veligers (about 100 μm length) correspond to larvae two weeks after fertilization, while pediveliger (around 150 μm length) were three weeks old, and settled individuals (about 400 μm length) reached metamorphosis slightly before the fourth week. Juveniles (maximum of 4 mm length), around a few weeks after metamorphosis, and adult mature individuals (about 8 cm length) were obtained as well. The specimens were removed from artificial hatcheries and, in case of larvae, observed under the light microscope to check if they exhibited healthy morphology and behavior. Then, samples were anesthetized by gradual addition of drops of 7.5% $MgCl_2$ for 2 hours prior to fixation.

HISTOLOGICAL PROCEDURES

Specimens were fixed for 3 hours at 4°C in a modified Karnovsky solution (2% paraformaldehyde + 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer at pH 7.4 and 1000 mosm adjusted with sucrose). Larvae, postlarvae and juveniles were decalcified for 12 hours at room temperature in 3% ascorbic acid in distilled water, while adult specimens were dissected after anesthesia to remove fragments from the pallial margin. Then, specimens were dehydrated in a graded ethanol series and embedded in glycol-methacrylate resin (Leica Historesin Kit). Serial sections of 2-3 μm were produced on a Leica RM2255 microtome and stained with hematoxylin and eosin (HE) or toluidine blue and acid fuchsin (TB). In order to evidence possible secretory cells in the mantle, histochemical methods were applied using Periodic acid-Schiff stain (PAS) and Alcian blue (AB) for neutral and acid mucopolysaccharide staining, respectively. Digital images were captured using a Nikon eclipse 80i microscope equipped with a Nikon DS-Ri1 camera.

SCANNING AND TRANSMISSION ELECTRON MICROSCOPY

For Scanning Electron Microscopy (SEM), samples were previously fixed in modified Karnovsky solution. Post-fixation was performed for 30 minutes in 1% OsO_4 in buffer solution (sodium cacodylate buffer at pH 7.4), followed by 15 minutes in 1% tannic acid in buffer solution, and more 15 minutes in new solution of 1% OsO_4 at 4°C. Then, specimens were decalcified as previously described for histological procedures and dehydrated in graded ethanol series. Samples were critical point dried using CO_2 as a

transitional fluid in a Balzers CPD 030, mounted on stubs, coated with gold in a Balzers SCD 050 sputter coater, and observed in a Zeiss DSM 940. For Transmission Electron Microscopy (TEM), post-fixation was performed for 1 hour in 1% OsO_4 in buffer solution. Larval samples were embedded in Epoxi resin; ultrathin sections (50-70 nm) were cut using a Leica Ultracut UCT microtome, mounted on copper slot-grids, contrasted with uranyl acetate and lead citrate, and analyzed using a Zeiss EM 900 electron microscope.

Immunocytochemistry, Confocal Laser Scanning Microscopy and 3D Reconstruction

Specimens were fixed in 4% paraformaldehyde in 0.1 M phosphate buffered (PB) for 1 hour, followed by four rinses with buffer solution. Until further preparation, all samples were properly stored in 0.1 M PB containing 0.1% NaN_3 at 4°C. Prior to staining procedures, larval and postmetamorphic individuals were decalcified in 0.05 M EGTA for 1 hour. Juvenile specimens were dissected in order to remove small fragments of the mantle margin for Confocal Laser Scanning Microscopy (CLSM).

For F-actin staining, specimens were permeabilized in PB containing 2% Triton-X 100 (PBT) overnight and then incubated in a 1:40 dilution of Alexa Fluor 488 Phalloidin (Molecular Probes) in PBT, for 24 hours at room temperature, in the dark. For neuronal staining, larval specimens were incubated in 6% normal goat serum in PBT (block-PBT) overnight at room temperature. Subsequently, primary antibodies (*e.g.*, anti-serotonin raised in rabbit and anti-α-tubulin raised in mouse) were applied at a concentration of 1:400 in block-PBT for 24 hours. Then, specimens were rinsed several times in block-PBT prior to application of a secondary fluorochrome-conjugated antibody (goat anti-rabbit Alexa Fluor 488 and goat anti-mouse Alexa Flour 633, Molecular Probes) in block-PBT at a concentration of 1:200 for 24 hours in the dark. Nuclei were stained by adding a 1 µl drop of 4', 6-diamidino-2-phenylindole (DAPI) (Invitrogen, 3 µg mL^{-1}) in conjunction with secondary antibody or Phalloidin incubation. Then, all samples were washed three times in PBS for about 30 minutes and mounted in Fluoromount G (Southern-Biotech, Alabama) on standard microscope slides, which were stored in freezer prior to analysis.

Analysis and image acquisition were performed on a Leica TCS SP5 II confocal laser scanning microscope equipped with the software Leica Application Suite Advanced Fluorescence (LAS AF), Version 2.6.0 (Leica Microsystems, Wetzlar, Germany). Confocal image stacks were recorded with 0.3 µm step size along the z-axis and digitally merged as

maximum intensity projections. 3D reconstructions were created from selected confocal stacks using the imaging software Imaris, Version 4.1 (Bitplane, Zürich, Switzerland). Images were further processed with Photoshop CS3 (Adobe Systems, San Jose, USA) to adjust contrast and brightness, and digital complementary drawings were created with Corel Draw X5 (Corel Corporation, Ottawa, Canada).

RESULTS

In veliger larvae of *Nodipecten nodosus*, the mantle comprises two lobes that enclose the animal body and define a small pallial cavity where the larval velum is located (Fig. 1D, E). The mantle margin runs along the shell edge from each side of the mantle isthmus (*i.e.*, dorsal region where the hinge is situated). The pallial margin of live larvae cannot be properly visualized by means of conventional light microscopy because of its transparency and location underneath the shell edge, being also partly obliterated by the large, anterior-ventrally located ciliated velum (Fig. 1A-C). Histological cross sections from decalcified veligers show the mantle margin as a slender mantle projection connected to the velum by a short membrane (Fig. 1D, E). A schematic illustration of the larval mantle margin is represented in Figure 2. The margin, which becomes thinner towards the distal region, is unfolded, and no particular structure is present. As revealed by scanning electron microscopy, the periostracum is a fine layer originating on the inner surface of the pallial margin, close to the distal region (Fig. 1F, 2). The secretion occurs in a well-defined site, the periostracum forming zone (PFZ), marked by a line, parallel to the margin, between the mantle epithelium and the newly formed sheath (Fig. 1G). From this region, the periostracum begins as a wrinkled band, but becomes smooth when covering the rest of the distal mantle margin (Fig. 1H). In the proximal region, immediately before the PFZ, the exposed inner epithelium contains short microvilli on its surface, and no cilia are present (Fig. 1G).

By the early pediveliger stage, larvae of *N. nodosus* grow and develop an extensible foot (Fig. 3A). The swimming habit prevails, although the crawling behavior becomes gradually common upon available hard surfaces. The general morphology of the pallial margin in pediveligers closely resembles that observed in veligers, including an unfolded projection (Fig. 3B) with an evident PFZ. However, a major difference is present in both mantle margins: the emergence of a row of cilia along all the periostracum forming zone (Fig. 3C, 4A, 4B). These cilia exhibit the classical microtubular arrangement "9 + 2" (Fig. 4C, D). From the PFZ, the periostracum covers the rest of the inner distal pallial margin (Fig.

4C, E) and extends to cover the shell. The proximal portion of the mantle margin is characterized by the presence of several secretory cells, particularly in the inner surface (Fig. 4C, F), as well as well-developed rough endoplasmic reticula around nuclei (Fig. 4C, G). The content of those vesicles are granular, electron-lucent and seems to be released on the inner surface (Fig. 4F). Despite the presence of secretory activity in this region, no evidence for mucopolysaccharides was detected applying standard histochemical techniques (Fig. 3D).

Even though the pediveliger's mantle margin is quite similar to the veliger's, crucial anatomical changes arise during this period, contributing to pallial system morphogenesis. In scallop veligers, no muscle or nerve were detected associated to the mantle margin. On the other hand, distinctive serotonergic fibers are found in the mantle of pediveligers, where a conspicuous nerve runs along the entire edge (Fig. 5A), likely corresponding to the developing circum-pallial nerve. Whereas the mantle is anteriorly innervated by projections from the apical-cerebral ganglia, the posterior portion receives fibers from the visceral ganglion (Fig. 5B). A distinctive pallial musculature is also developed in pediveliger larvae, containing smooth and striated myofibers (Fig. 5C, D). While some of them are parallel to the edge, other bundles are branched retractors, attached to the shell, and running towards the mantle margin (Fig. 5D).

In late pediveliger larvae, when the foot is completely developed and the velum gradually shrinks, another crucial modification takes place in the mantle margin. A folding process gradually occurs where the ciliary row is located, exactly at the level of the periostracum forming zone, resulting in a lateral evagination of the proximal region (Fig. 6A-C). As a result, two projections are eventually present in the mantle margin with the PFZ between them (Fig. 6C). This folding process is responsible for the appearance of an inner pallial extension and, at the same time, for positioning the periostracum forming zone (and respective ciliary row) at the bottom of the newly formed groove, *i.e.*, the periostracal groove (Fig. 6C). The distal region of the mantle margin, permanently covered by the periostracum, corresponds to the outer pallial fold, while the proximal region is modified into a projection, henceforward named inner pallial fold.

Metamorphosis produces intense changes in larval anatomy and behavior. The settled larva secretes the dissoconch, while internal organs are reorganized. The larval velum completely degenerates and the first gill filaments grow rapidly (Fig. 7A, B). Whereas the larval mantle margin is inconspicuous and not visible in live specimens, the mantle edge in postlarvae is evidenced by the presence of a well-developed curtain-like inner fold bearing

cilia (Fig. 7B, C). The mantle lobes become much longer, extending the free margin far from the visceral organs, providing space for the numerous developing gills within the pallial cavity (Fig. 7D, E). The pallial musculature is enlarged, bundles grow thicker, and the combined arrangement of margin-parallel bundles and retractor muscles persists (Fig. 7F, G).

The mantle margin in postmetamorphic scallops comprises the outer and inner folds (Fig. 8), as in late pediveligers. The outer fold is usually reduced, covered by the periostracum, while the curtain-like inner fold is pronounced (Fig. 9A, B, C). Two types of cilia arrangement are present on the inner fold. The first type, present along the rim of the inner fold, corresponds to ciliary tufts sparsely scattered over the epithelium, containing few cilia (Fig. 9D). The second type comprises a ciliary band, in which long cilia are densely distributed on the inner surface of the inner fold. This later type, however, is restricted to the anterior portion of the left mantle lobe (Fig. 7B, 9B, C, E). In live animals, intense ciliary beating from this later type is produced in this region. In the dorsal region of postmetamorphic individuals, the inner folds are fused both anteriorly and posteriorly, the outer fold and periostracal groove remaining intact (Fig. 9F, G). Such fusion occurs near the mantle isthmus, where the right and left mantle lobes are originated.

In juvenile individuals of *N. nodosus*, most of general adult morphological features are displayed (Fig. 10A). A few weeks after metamorphosis, the pallial margin now comprises three folds, including the newly formed middle fold, which contains numerous eyes and tentacles (Fig. 10B, C, D). The scallop three-folded condition is represented in figure 11. The epithelium of the outer surface of the middle fold (*i.e.*, the one facing the periostracal groove) exhibits densely distributed cilia continuous with the cilia of the periostracal groove (Fig. 10E). The inner fold, usually referred as "velum" for pectinid bivalves, is a muscular curtain-like fold bearing sparsely distributed ciliary tufts (Fig. 10C, D). Whereas mucin cells were not detected in the mantle of. *N. nodosus* larvae, they were observed in juvenile individuals. In those specimens, gland cells producing acid mucopolysaccharides are spread in the outer surface of the mantle and outer fold, suggesting intense mucous secretion in the extra-pallial cavity, *i.e.*, the space between the mantle and the shell (Fig. 10F).

In juveniles, the pallial musculature becomes evident in histological sections due to the increase of thickness and number of bundles (Fig. 12A). Within the mantle margin, numerous, long muscles are radially distributed, with striated and smooth fibers mixed in parallel bundles running from the pallial line to the mantle margin (Fig. 12B, C). Within the

inner fold, besides radial muscles, densely organized striated muscle bundles running parallel to the mantle margin are present (Fig. 12A, B). These bundles are more closely arranged in the distal region of this fold (Fig. 12D).

The nervous system of the mantle margin comprises numerous radial nerves that run towards the distal region and exhibit strong immunoreactivity for α-tubulin (Fig. 13A, E). In addition, the serotonergic pallial system includes a reticulate pattern distribution over the entire mantle margin (Fig. 13B). The very prominent circum-pallial nerve is responsible for innervation of the pallial margin (Fig. 13C, D, F). Unlike other nerves, completely formed by fibrous projections combined with glia cells, the circum-pallial nerve displays a typical ganglionic structure. The central portion (*i.e.*, the neuropil) is composed exclusively by neuronal fibers, while the periphery (*i.e.*, the cortical region) contains the cell bodies (Fig. 13D).

Adult individuals of *Nodipecten nodosus* (Fig. 14A) display a very prominent mantle margin, with numerous tentacles, eyes, and a pigmented velum (Fig. 14B). At the auricular region (dorsal ears), the inner fold remains fused (Fig. 14C). The mantle is proportionally larger and thicker than in juveniles, and the outer fold is quite smaller compared to the remaining folds (Fig. 14D). The pallial musculature is provided with large bundles of striated and smooth fibers, which form two major muscle groups that run close to the outer and inner epithelium, respectively (Fig. 15A). Small transversal muscles are also present (Fig. 15A). Embedded within the connective tissue, pallial nerves reach the circum-pallial nerve at the level of the pallial folds (Fig. 15B). The circum-pallial nerve preserves its ganglionic organization, *i.e.*, with neuronal bodies distributed at the periphery, and numerous axons merged at the core (Fig. 15C, D).

Simple, cuboidal cells are found in the inner surface of the outer fold, in the middle fold epithelium and in the inner pallial surface. Simple, columnar cells are common in the inner fold, in the outer pallial surface, as well as in the outer surface of the outer fold. The adult inner mantle margin surface is covered by sparsely distributed tufts of cilia (Fig. 15E), and few epithelial mucous cells with neutral mucopolysaccharide content (Fig. 15F). The outer mantle margin epithelium differs from the inner one by the presence of short microvilli (Fig. 15G), and numerous secretory cells containing exclusively acid mucopolysaccharide vesicles (Fig. 15H). This condition remains the same at the outer surface of the outer fold (Fig. 16A). However, the inner epithelium of the outer fold possesses two types of secretory cells, related to acid and neutral mucopolysaccharide secretion (Fig. 16B). At the periostracal groove, a pair of glandular folds is responsible for periostracum secretion (Fig. 16C, D).

The adult middle fold epithelium is intensively ciliated on its outer surface (Fig. 17A). The middle fold tentacles contain mucous and pigment cells (Fig. 17B, C), while the pallial eyes display many ocular components (Fig. 17D). The inner fold is the largest structure of the adult pallial margin, bearing small tentacles at its extremity (Fig. 14B). The inner fold musculature becomes more prominent and regionalized in adults (Fig. 18A-D). Radial muscles come from the pallial musculature and extend through the entire fold (Fig. 18A), running from the base of the velum to the marginal end (Fig. 18A, D). The margin-parallel muscles seen in juveniles are now organized in bundles that become more numerous and larger as they approach the middle and distal portions (Fig. 18C, D). During this gradual transition, the radial musculature is laterally compressed against the base of the epithelium, providing space for the margin-parallel fibers (Fig. 18D).

DISCUSSION

LARVAL AND POSTMETAMORPHIC ANATOMY OF THE MANTLE MARGIN

Larval mantle folds and periostracum forming zone

The larval mantle margin in *N. nodosus* is unfolded, comprising a single projection divided into distal and proximal regions by the periostracum-forming zone. Nevertheless, many authors have adopted the term "fold" to designate such division at early larval stages, which may have led to possible misinterpretations of larval morphology, particularly concerning mantle margin development. Two folds were reported at the mantle rim in larvae of *Pecten maximus* by Cragg (2006): the "outer fold" being covered by the periostracum, and the epithelium of the "inner fold" being exposed and covered by short microvilli. However, the schematic representation of the mantle margin provided by Cragg (2006) exhibits no folds, but proximal and distal regions, quite similar to the larval pallial organization of *N. nodosus* described herein. Other similar examples include the mantle margin of *Crassostrea virginica* (Elston, 1960), *Cardium edule* (Creek, 1998), *Nucula delphinodonta* (Drew, 1901), and *Lasaea adansonii* (Altnöder and Haszprunar, 2008), and *Ostrea edulis* (Waller, 1981), where the figures (illustrations or photographs based on light or electron microscopy) provided by the authors all clearly show an unfolded larval margin. Considering the results obtained in the present study combined with the above-mentioned

information, it seems more suitable to use the terms "distal" and "proximal" regions rather than "fold" to designate specific areas of the larval mantle margin.

Although it is assumed that the presence of two pallial folds corresponds to the typical condition of bivalve larvae (Cranfield, 1974), most of the available information for larval mantle margin is restricted to observations of a single larval stage, generally with no data on previous stages (*e.g.*, Cranfield, 1974). In a developmental perspective, this could suggest that early mantle traits are unknown for some species. Therefore, it is reasonable to consider that, at least for some bivalves, such as scallops, the larval mantle margin may count with an unfold step in early development, with subsequent division into two mantle folds.

In *N. nodosus*, the larval periostracum begins as a wrinkled band before covering the distal margin and shell, a condition also detected for *O. edulis* by Cranfield (1974) (referred by him as "convoluted periostracum") and Waller (1981). According to Cranfield (1974), this organic sheath is comprised by three layers formed within a groove between the outer and inner folds of bivalve larvae. However, periostracum secretion is started before the mantle folds are developed, *i.e.*, when real groove is not present. In this case, the secretory activity occurs on a band/line at the distal region of the inner mantle margin surface (Cragg, 2006; Altnöder and Haszprunar, 2008), as confirmed by the herein TEM observations on *N. nodosus*. The name "periostracum forming zone" was proposed to designate the site where the organic sheath is produced in the unfolded mantle margin of *L. adansonii* larvae by Altnöder and Haszprunar (2008), and we agree with the appropriateness of this term.

Larval mantle margin anatomy

The covering of the inner surface of the mantle margin by microvilli seen in *N. nodosus* was previously observed in *P. maximus* (Cragg, 2006) and *O. edulis* (Cranfield, 1974, Waller, 1981). The presence of cilia has been reported for the larval mantle margin of several bivalves, but this information is quite always imprecise. For instance, cilia were described along the pallial margin of veligers of the scallop *Aequipecten irradians*, but no details about type or distribution were obtained (Sastry, 1965). In *N. nodosus*, cilia are not present until the pediveliger stage, when a row of long cilia is formed adjacent to the PFZ. According to Cragg (1996), five ciliary types are present on the mantle margin of *P. maximus* larvae. Type 1 represents few cilia located in small depressions; type 2 is a row of long cilia in line, close to the distal region of the edge; type 3 includes tufts of numerous cilia scattered on the posterior margin; type 4 is a line of single cilia around the posterior edge; and type 5 is a row of short cilia in a protuberance dorsal to the anus. Even though not all these ciliary

types were detected in larvae of *N. nodosus*, the row of cilia adjacent to the periostracum forming zone likely matches type 2.

When the mantle margin becomes two-folded in late pediveligers of *N. nodosus*, new ciliary groups arise, and after metamorphosis, they become more prominent. Within the periostracal groove, dense rows of cilia are distributed on the outer surface of the inner fold. Such ciliary band was also detected in *O. edulis* by Cranfield (1974), but not by Waller (1981). Epithelial cells containing several electron-lucent vesicles were found in *P. maximus*, close to the ciliated cells at the PFZ (Cragg, 2006). In addition, great abundance of rough endoplasmic reticulum was detected in mantle margin cells of *C. virginica* (Elston, 1980). In general, these ultrastructural characteristics seem in accordance with the present data for *N. nodosus*. On the edge of the inner fold, tufts of cilia are scattered along the margin, exactly like those classified as type b in pediveligers of *Argopecten. purpuratus* (Bellolio *et al.*, 1993), and type 3 in *P. maximus* (Cragg, 2006). Long cilia, similar to those present in the ciliary band from the inner surface of the inner mantle fold of *N. nodosus*, also occur in tufts along the inner larval fold of *Mytilus edulis* (Bayne, 1971), *O. edulis* (Cranfield, 1974) and *Pinna carnea* (Allen, 2010). However, no cilia were detected in the mantle margin of *Pandora inaequivalvis* larvae prior or after metamorphosis (Allen, 1961).

The larval anatomy of bivalve pallial musculature and nervous system remains largely unknown, given that mantle muscles and innervation received little attention in previous studies. Some muscle fibers were described for the inner fold of some Pinnidae species (Allen, 2010), but a detailed comparison with the present data is prevented, since no image was provided in that study. Smooth fibers are supposed to be present near the mantle folds of *P. maximus*, based on larval descriptions (Cragg, 2006). In a detailed anatomical study conducted with pediveligers of *O. edulis*, Waller (1981) suggested that radial muscles must be present in the larval mantle margin based on his observations of mantle contractions in live specimens. The present investigation with *N. nodosus* provided clear evidence for the emergence of pallial musculature during the pediveliger stage, including retractor and margin-parallel bundles. The formation of pallial muscles in mid-stage larvae was also observed in *A. adansonii*, where both muscle types are formed by smooth fibers and spread over the mantle margin (Altnöder and Haszprunar, 2008). Regarding pallial innervation, the mantle of *N. nodosus* seems to become assisted by the peripheral nervous system only by the pediveliger stage, when neuronal serotonergic projections extend to the mantle forming a nerve that run parallel to the margin. Besides Cragg (1996), who suggested the possible presence of neuronal cells adjacent to the mantle margin, a serotonergic nerve was detected

running along the mantle in *M. edulis* pediveligers, and catecholaminergic cells in *Placopecten magellanicus* (Croll *et al.*, 1977).

Postmetamorphic mantle margin anatomy

The mantle of adult bivalves has been extensively studied, mainly regarding aspects of shell secretion and siphon anatomy (*e.g.*, Kellog 1892; Yonge, 1983; Carriker, 2001; Sartori *et al.*, 2008). The bivalve mantle epithelium generally comprises cuboidal to columnar cells, with numerous secretory cells spread on both surfaces. The mantle lobes may exhibit free margins partially united at specific regions, as commonly observed in several protobranchs and pteriomorphian groups, as well as different degrees of marginal fusion, including a variety of siphons, such as those present in infaunal bivalves (Yonge, 1948, 1957; Stanley, 1968). At the auricular region of *N. nodosus*, the inner folds from both lobes are dorsally fused, characterizing a very restricted fusion in contrast to the wide free ventral margins. In general, reduced fusion or the lack thereof are common features in the mantle margin of epifaunal bivalves from the Pteriomorphia clade (Allen, 2010).

Whereas the outer mantle fold remains unaltered and covered by the periostracum after metamorphosis, the inner fold of *N. nodosus* becomes greatly pronounced into the typical curtain-like fold ("velum") observed in grown scallops (Drew, 1906). In this respect, "pallial curtain" is the name applied to the hypertrophied marginal projection of the inner fold that controls the passage of water into and out of the pallial cavity (Nelson, 1938). Pallial curtains are also found in Ostreidae and Pteridae, where the inner mantle fold is enlarged (Yonge, 1957).

As demonstrated herein, the middle fold emerges after metamorphosis, giving origin to tentacles and eyes in *N. nodosus*. Such organs were described as papillary projections and pigmented protuberances that arise in the mantle margin after metamorphosis of *A. irradians* (Sastry, 1965). The pallial organs from the scallop middle fold have been extensively studied by several authors since the beginning of the last century (*e.g.*, Drew, 1906; Dakin, 1909; Ciocco, 1998).

The late larval musculature of *N. nodosus* mantle seems to be preserved after metamorphosis. Notwithstanding, little is known about the anatomy of bivalve mantle musculature shortly after metamorphosis. In *M. trossulus*, the postlarval muscles of the mantle margin comprise margin-parallel bundles emerged during the pediveliger stage (Dyachuk and Odintsova, 2009). A rapid development of mantle retractor muscles was also observed after metamorphosis in pinnids (Allen, 2010), but it is possible that mantle muscles

were present in late larvae, but may not have been detected due to methodological limitations. In *N. nodosus*, the inner fold is the most muscular region of the mantle margin, being composed mainly of numerous margin-parallel bundles of striated fibers, as well as mantle retractors. In contrast, striated muscles are not present in mussels and other bivalves after metamorphosis, resulting in an entire muscular system reorganized by smooth myofibers (Dyachuk & Odintsova, 2009). Bivalve muscular development is poorly understood, especially in larvae, although they could be very insightful for many anatomical, functional and developmental approaches, as confirmed in the present investigation with Pectinidae and in other molluscan taxa (*e.g.*, Wanninger and Haszrpunar, 2002; Wollesen *et al.*, 2008).

The innervation of the mantle margin in adult scallops is relatively well-known for some species, the studies covering several anatomical and physiological topics (Drew 1906, Dakin, 1928; Spagnolia and Wilkens, 1983; Ciocco, 1998; Wilkens, 2006). Nevertheless, little information is available on its development. The circum-pallial nerve is the most significant nerve in the scallop pallial margin, running parallel to the margin's entire extension, and being responsible for the innervation of the pallial folds and organs (Drew, 1906). The anterior dorsal portion of the nerve is formed by fibers from the cerebral-ganglia, while the ventral and posterior regions are innervated by long fibers from the visceral ganglion (Drew, 1907; Dakin, 1928). Strong serotonergic immunoreactivity was detected in the circum-pallial nerve of juvenile *N. nodosus*, as well as in projections towards mantle organs. Considering that these characteristics were also detected in the nervous system of pediveligers, it seems reasonable to conclude that the nerve along the larval mantle margin is, in fact, the circum-pallial nerve (formed, therefore, even before marginal folding). Such information is vital to support hypotheses of early emergence and subsequent development of neuronal activity in the pallial margin. The presence of a neuropil and a cortex in the circum-pallial nerve is regarded by some authors as an anatomical evidence for the crucial role of this nerve to the mantle margin, which was even considered a marginal ganglion by Drew (1907).

MANTLE MARGIN DEVELOPMENT

General development of the mantle margin

A hypothesis for the development of the pectinid mantle margin (summarized in Figure 19) is proposed based on the data obtained herein for *N. nodosus* combined with

revisited information from literature (*e.g.*, Cole, 1938; Creek, 1960; Elston, 1960; Cranfield, 1974; Waller, 1981; Bellolio *et al.*, 1993; Cragg, 2006, Altnöder and Haszprunar, 2008). Even though the developmental sequence includes some unique characters from scallops, such as the pallial eyes from the middle fold, the morphogenesis is represented in order to provide general insights into mantle margin development in the Bivalvia.

The bivalve mantle is formed shortly after the appearance of the shell field on the dorsal surface of late trocophore larvae (Moor, 1983; Moueza *et al.*, 1999). The epithelium of the shell gland then extends, giving rise to the mantle which will permanently underlie the valves (Raven, 1958; Silberfeld and Gros, 2006). In scallops, the veliger exhibits an unfolded mantle margin, *i.e.*, a single projection beneath the shell margin with no distinct folds (Fig. 19). The PFZ marks the division between the distal region of the mantle margin, which is permanently covered by the periostracum, and the proximal region (Fig. 19). During larval development, the transition to the pediveliger stage is marked by the emergence of a row of cilia in the proximal region, adjacent to the PFZ (Fig. 19). In addition, the pallial musculature arises, as well as the mantle innervation (Fig. 19). The unfolded condition is conserved until slightly before metamorphosis, when the first of two major folding processes takes place. An evagination of the proximal region near the row of cilia produces the inner mantle fold, the distal region now corresponding to the outer fold (Fig. 19). Consequently, the PFZ is confined within a groove, and the row of cilia, previously present on the surface of the proximal mantle region, comes to lie on the outer surface of the inner mantle fold. Metamorphosis produces a variety of anatomical changes, the inner fold becoming a prominent pallial curtain-like fold (Fig. 19). Also, at this stage the second folding process occurs, forming the middle fold through an evagination at the base of the outer surface of the inner fold (Fig. 19). Consequently, the ciliated epithelium of the periostracal groove is now located on the outer surface of the middle fold (Fig. 19). Once the three-folded condition is achieved, further development includes the growth of sensorial organs (middle fold tentacles and eyes first appearing at the juvenile stage), and further growth of the pallial curtain (Fig. 19).

Implications for mantle fold developmental hypotheses

The present investigation on the development of the mantle margin in *N. nodosus* responds and casts new questions to old issues in anatomy and development of bivalves. Firstly, the unfolded condition of the mantle margin is indeed an initial condition, at least for some bivalves. Whereas two folds are achieved in late pediveliger of *N. nodosus*,

apparently such a condition may occur early in other bivalve species (*e.g.*, Cole, 1938; Cranfield, 1974). In addition, the groups of cilia scattered on the larval inner mantle fold of some species (*e.g.*, Cranfield, 1974; Waller, 1981) only arise in scallops after metamorphosis. Despite common pallial features in those bivalves, the timing of emergence of cilia and mantle folds seems to vary. This could be explained by different rates of differentiation during ontogeny, leading to heterochronical alterations across Bivalvia groups (Smith, 2001; McNamara, 2012). Heterochrony has been argued as the reason for modifications in developmental time of bivalve larvae, and for significant anatomical and evolutionary changes (Yonge, 1962; Stanley, 1972).

In adult Arcoida, the outer mantle fold is usually subdivided into two distinctive folds, while the remaining folds are more variable. Whereas species of *Arca* and *Glycymeris* exhibit no middle fold (Waller, 1980), the inner fold in *Philobrya*, *Bathyarca*, *Barbatia* and *Trisidos* is duplicated, exhibiting variation in size and shape (Morton, 1982, 1987). Based on epithelial differences observed in the single inner fold of *Arca noae*, Morton and Peharda (2007) suggested that both middle and inner folds are present in ark clams, although they are combined in one single fold. In addition, the two-folded mantle margin of adults of some arks (Waller, 1980; Morton, 1982) and the presence of two folds in oyster pediveligers (Waller, 1981) led Morton and Peharda (2008) to suggest that the inner surface of the inner mantle fold would be the putative middle fold, not ontogenetically differentiated into a distinctive structure. The present investigation with *N. nodosus* has provided the first developmental evidence to support the origin of the middle fold from the inner surface of the inner fold, reinforcing Morton and Peharda's (2008) hypothesis.

The two-folded arrangement was claimed to be a primitive condition in the Bivalvia (Waller, 1980; Morton and Peharda, 2008). There are, however, few comparative studies on the bivalve mantle margin, particularly in Pteriomorphia, and none of them was analyzed under a cladistic perspective, which prevents further inferences on character evolution. We agree with Smith (2002) that analysis of developmental sequence make no *a priori* assumptions on the conservation of developmental stages or about how sequence should evolve, so we are not able to ascertain if the two-folded mantle margin is a primitive condition, or a highly derived trait of some species (*e.g.*, in which the middle fold would not be differentiated).

Shell secretion is generally regarded to be the main function of the mantle, which exhibits different secretory cells spread on the outer epithelium adjacent to the outer fold (*e.g.*, Wada, 1964; Wilbur, 1964; Timmermans, 1969; Wilbur and Saleuddin, 1983; Furihashi *et al.*, 2009). Biomineralization has been extensively studied in bivalve molluscs, including mechanisms of calcification and shell microstructure (*e.g.*, Taylor, 1973; Addadi *et al.*, 2006). During development, shell formation is initiated during the late trocophore stage, when mantle gland cells are involved in secretion of the larval prodissoconch I and II, and subsequently, the postmetamorphic dissoconch (Carriker, 2001; Weiss *et al.*, 2002). Apart from shell secretion, functional studies on larval mantle margin are very scarce, although some efforts have been made to elucidate the roles of chitin secretion in bivalve larvae (Weiss and Schonitzer, 2006). PAS-reactive vesicles and acid mucopolysaccharide-protein complex content were detected in cells from the outer and inner mantle folds of *O. edulis* pediveligers (Cranfield, 1974). In contrast, the results obtained with *N. nodosus* have provided no evidence for such secretory roles at least until metamorphosis. Further studies are still necessary to investigate the developmental origin and possible changes in mantle secretory activity during development.

In juvenile and adults of *N. nodosus*, secretory cells are present in all three pallial folds, as well as in the remaining inner and outer mantle epithelia. The columnar cells of the outer surface of the outer fold gradually become the cuboidal epithelium observed on the inner surface of the same fold and in the rest of the mantle. Reduction of mantle epithelial thickness throughout the outer fold was reported for other bivalves, such as *M. edulis*, *Cardium edule*, *Nucula sulcata* (Bubel, 1773), and *Gomphina veneriformis* (Lee *et al.*, 2007), and related to possible differences in secretory activities (Beedham, 1958). Similar to the gland cells observed in the outer mantle fold of *N. nodosus*, positive reaction for PAS and Alcian Blue was obtained in secretory cells from the outer fold of *Pinctada fulcata* (Fang *et al.*, 2008) and *Cerastoderma edule* (Richardson *et al.*, 1981), suggesting intense secretion of neutral and acid mucopolyssacharides in this region. In the extra-pallial cavity of the outer mantle fold, the calcified shell is deposited in an organic matrix formed by proteins, mucopolyssacharides, glycoproteins and lipids (Beedham, 1958; Wada, 1964; Wilbur and Saleuddin, 1983). The importance of the organic matrix to shell formation may explain the role of the several mucous-secreting cells concentrated in the outer mantle epithelium observed in these bivalves. Additionally, it has also been suggested that the products of mucous cells in this region may be concerned with mantle lubrication (Beedham, 1958;

Timmermans, 1969). Mantle secretory cells of *P. margaritifera* exhibit mucous or granular content in all three mantle folds, although the number of cells responding to PAS-Alcian blue decreases in the middle one (Jabbour-Zahab *et al.*, 1992), a condition also observed in *C. edule* (Richardson *et al.*, 1981). In contrast, numerous cells secreting acid mucopolysaccharides were detected in the middle fold tentacles of *N. nodosus*. Although PAS-positive and Alcian Blue-positive gland cells are present in both middle and outer folds, we observed some differences within each group when applying hematoxylin to stain nuclei (different purple to bluish tones), suggesting that more types of mucous secretion are present.

In several bivalves, including scallops, the inner mantle fold and the inner pallial surface commonly display mucous cells, usually associated with ciliated cells (Richardson *et al.*, 1981; Jabbour-Zahab *et al.*, 1992; Beninger *et al.*, 1999; Beniner and Le Pennec, 2006). Cilia and mucocyte distribution on the inner mantle surface of *N. nodosus* are in accordance with previous observations on *P. magellanicus*, where mantle cilia are suspected not to contribute much to the water flow or particle transportation inside the mantle cavity, due to their sparse distribution (Beninger *et al.*, 1999). Furthermore, the high viscosity of the acid mucopolysaccharides secretions in scallop mantle surface, in addition to the absence of a specialized mantle ciliary transportation, suggest that this mucous secretion might facilitate the passage of water over the mantle surface (Beninger *et al.* 1997; Beninger and St-Jean 1997; Beninger and Le Pennec, 2006).

The tufts of cilia in *P. maximus* larvae are assumed to perform sensory tasks, likely acting as mechanoreceptors, and no water current formation seems to be produced (Cragg, 2006). Similarly, the tufts of cilia in the inner fold of oyster pediveligers are related to sensory cells, while the wide ciliary band may display cleansing functions (Cranfield, 1974). However, in the case of *N. nodosus* postlarvae, the wide ciliary band is restricted to the anterior portion of the inner fold, within the inhalant region of the pallial cavity. In live postlarvae, those cilia exhibit intense beating, which may suggest a potential contribution to water flow into the mantle cavity, instead of a sensorial function.

The emergence of the pallial musculature in pediveligers indicates effective retraction of the mantle margin into the mantle cavity, which has been suggested by previous study with pectinids (Waller, 1981). By the same time, the development of the circum-pallial nerve along the mantle margin may contribute to innervation of this region, allowing sensory functions and muscular control. For instance, serotonin is known to play a vital role in regulation of cilia activity in bivalve and other molluscan larvae (Gosselin, 1961; Beiras & Widdows, 1995; Kuang & Goldberg, 2001). In addition, the larval development of mantle

muscles and innervation demonstrate how anatomical organization anticipates fold establishment during mantle margin morphogenesis. After metamorphosis, further modifications in both systems are deeply associated with specialization of mantle folds and structures.

By the juvenile stage, the scallop mantle margin is completely organized in three pallial folds containing specialized structures and organs. The typical functions concerning each bivalve mantle fold are present in the Pectinidae, including a secretory outer mantle fold, a sensorial middle fold and a muscular inner fold (Yonge 1957, 1987). The concentration of chemical, mechanical and optical receptors in the middle mantle fold stimulated extensive studies on functional properties. The pallial eyes, remarkable by their complexity and debatable evolutionary significance, have been deeply studied (Dakin, 1909, 1928; Ciocco, 1998; Morton, 2001; Speiser and Johnsen, 2008; Malkowsky and Jochum, 2014), and recent molecular investigations have cast some light into the origin and diversification of such organs (Pairett and Serb, 2013; Serb *et al.*, 2013).

Finally, within an evolutionary context, issues concerning the development and diversification of mantle folds are still unclear. According to Waller (1980), the evolution of the bivalve mantle margin might have been associated with the emergence of new functions, new pallial structures, and fold specialization. Nevertheless, general hypotheses concerning both phylogenetic and developmental diversification of the bivalve mantle margin are still challenging. Even though our current knowledge of mantle margin diversity is very fragmentary, the plasticity of this region is a certainty (Morton and Peharda, 2008). The mantle margin is a complex system, and its evolution should be regarded as anatomical and developmental innovations combined to selective forces leading to diversification of form and function.

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REFERENCES

Addadi L, Joester D, Nudelman F, Weiner S. 2006. Mollusk shell formation: a source of new concepts for understanding biomineralization processes. Chemistry A European Journal 12:980–987.

Alejandrino A, Puslednik L, Serb JM. 2011. Convergent and parallel evolution in life habit of the scallops (Bivalvia: Pectinidae). BMC Evolutionary Biology 11:164.

Allen JA. 1961. The Development of *Pandora inaequivalvis* (Linné). Journal of Embryology & Experimental Morphology 9:252–268.

Allen JA. 2011. On the functional morphology of *Pinna* and *Atrina* larvae (Bivalvia: Pinnidae) from the Atlantic. Journal of the Marine Biological Association of the UK 91:823–829.

Altnöder A, Haszprunar G. 2008. Larval morphology of the brooding clam *Lasaea adansonii* (Gmelin, 1791) (Bivalvia, Heterodonta, Galeommatoidea). Journal of Morphology 269:762–774.

Ansell AD. 1961. The functional morphology of the British species of Veneracea (Eulamellibranchis). Journal of the Marine Biological Association of the United Kingdom 41:489–515.

Ansell AD. 1962. The functional morphology of the larva, and the post-larval development of *Venus striatula* (da Costa). Journal of the Marine Biological Association of the United Kingdom 42:419–443.

Bayne BL. 1971. Some morphological changes that occur at the metamorphosis of the larvae of *Mytilus edulis*. D.J. Crisp (Ed.), Fourth European Marine Biology Symposium. Cambridge University Press, Cambridge, pp. 259–280.

Beedham GE. 1958. Observations on the mantle of the Lamellibranchia. Quarterly Journal of Microscopical Science s3-99, 181-197.

Beedham GE, Owen G. 1965. The mantle and shell of *Solemya parkinsoni* (Protobranchia: Bivalvia). Proceedings of the Zoological Society of London 145(3):405–430.

Beiras R, Widdows J. 1995. Effects of the neurotransmitters dopamine, serotonin and norepinephrine on the ciliary activity of mussel (*Mytilis edulis*) larvae. Marine Biology 122:597–603.

Bellolio G, Lohrmman K, Dupré E. 1993. Larval morphology of the scallop *Argopecten purpuratus* as revealed by scanning electron microscopy. The Veliger 36:322–342.

Beninger PG, Veniot A, Poussart Y. 1999. Principles of pseudofeces rejection on the bivalve mantle: integration in particle processing. Marine Ecology Progress Series 178:259–269.

Beninger PG, Lynn, JW, Dietz TH, Silverman H. 1997. Mucociliary transport in living tissue: the two-layer model confirmed in the mussel *Mytilus edulis* L. The Biological Bulletin 193:4–7.

Beninger PG, St-Jean SD. 1997. The role of mucus in particle processing by suspension-feeding marine bivalves: unifying principles. Marine Biology 129:389–397.

Benninger PG, Le Pennec. 2006. Structure and function in scallops. .In Shumway SE, Parsons GJ (Editors). Scallops: biology, ecology and aquaculture. Amsterdam, Elsevier, pp. 123–227.

Bubel A. 1973. An electron-microscope investigation of the cells lining the outer surface of the mantle in some marine molluscs. Marine Biology 21:245–255.

Buddenbrock W Von. 1911. Untersuchungen iiber die Schwimmbewegungen und die Statocysten der Gattung *Pecten*. Sitzber. Heidelberg. Akad. Wiss. Bd 28, S. l–24.

Cannuel R, Beninger PG, McCombie H, Boudry P. 2009. Gill development and its functional and evolutionary implications in the blue mussel *Mytilus edulis* (Bivalvia: Mytilidae). Biological Bulletin 217(2):173–188.

Carriker MR. 1990. Functional significance of the pediveliger in bivalve development. In: B Morton (Ed.). The Bivalvia: Proceedings of a Memorial Symposium in Honour of Sir Charles Maurice Yonge. Hong Kong, Hong Kong University Press.

Carriker MR. 2001. Embryogenesis and organogenesis of veligers and early juveniles. In Kraeuter JN, Castagna M (Editors). Biology of the hard clam. Gulf Professional Publishing, Elsevier.

Ciocco NF. 1998. Anatomía de la vieira tehuelche, *Chlamys tehuelcha* (d´Orb). IV. Sistema nervioso y estructuras sensoriales (Bivalvia, Pectinidae). Revista de Biologia Marina y Oceanografía 33(1):25–42.

Cole HA. 1938. The fate of the larval organs in the metamorphosis of *Ostrea edulis*. Journal of the Marine Biological Association of the United Kingdom 22(2):469–484.

Cragg SM. 1996. The phylogenetic significance of some anatomical features of bivalve veliger larvae. In Taylor J. (ed), Origin and evolutionary radiation of the Mollusca. Oxford University Press, New York.

Cragg SM. 2006. Development, physiology, behavior and ecology of scallop larvae. In Shumway SE, Parsons GJ (Editors). Scallops: biology, ecology and aquaculture. Elsevier, pp. 45–122.

Cranfield HJ. 1974. Observations on the morphology of the mantle folds of the pediveliger of *Ostrea edulis* L. and their function during settlement. Journal of the Marine Biological Association of the United Kingdom 54:1–12.

Creek GA. 1960. The development of the lamellibranch *Cardium edule* L. Proceedings of the Zoological Society of London 135(2):243–260.

Croll RP, Jackson DL, Voronezhskaya EE. 1997. Catecholamine-containing cells in larval and post-larval bivalve molluscs. Biological Bulletin 193:116–124.

Dakin WJ. 1909. *Pecten*. In: Liverpool Marine Biology Committee. Memoirs on typical British marine plants & animals. XVII. London, Williams & Norgate.

Dakin WJ. 1910. The eye of *Pecten*. Quarterly Journal of Microscopical Science 55:49–112.

Dakin WJ. 1928. The anatomy and phylogeny of *Spondylus*, with a particular reference to the Lamellibranchiata nervous system. Proceedings of the Royal Society of London 103:337–354.

Drew GA. 1901. The life-history of *Nucula delphinodonta* (Mighels). Quarterly Journal of Microscopical Science 44:313–391.

Drew GA. 1906. The habits, anatomy, and embryology of the giant scallop (*Pecten tenuicostatus*, Mighels). University of Maine.

Drew GA. 1907. The circulatory and nervous systems of the giant scallop (*Pecten tenuicostatus*, Mighels), with remarks on the possible ancestry of the Lamellibranchiata, and on a method for making series of anatomical drawings. Biological Bulletin 12(4):225–258.

Dyachuk VA, Odintsova NA. 2009. Development of the larval muscle system in the mussel *Mytilus trossulus* (Mollusca, Bivalvia). Development Growth & Differentiation 51:69–79.

Dyachuk V, Wanninger A, Voronezhskaya E. 2012. Innervation of bivalve larval catch muscles by serotonergic and FMRFamidergic neurons. Acta Biologica Hungarica 63 (Suppl. 2):221–229.

Elston R. 1980. Functional anatomy, histology and ultrastructure of the soft tissues of the larval American oyster, *Crassostrea virginica*. Proceeding of the. National Shellfish Association 70:65–93.

Fang Z, Feng Q, Chi Y, Xie L, Zhang R. 2008. Investigation of cell proliferation and differentiation in the mantle of *Pinctada fulcata* (Bivalve, Mollusca). Marine Biology 153:745–754.

Furuhashia T, Schwarzingerb C, Miksikc I, Smrzc M, Berand A. 2009. Molluscan shell evolution with review of shell calcification hypothesis. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology 154(3):351–371.

Guilmour THJ. 1967. The defensive adaptations of *Lima hians* (Mollusca, Bivalvia). Journal of the Marine Biological Association of the United Kingdom 47:209-221.

Gosselin RE. 1961. The cilioexcitatory activity of serotonin. Journal of Cellular and Comparative Physiology Volume 58:17–25.

Gruffydd LD, Lane DJW, Beaumont AR. 1975. The glands of the larval foot in *Pecten maximus* L. and possible homologues in other bivalves. Journal of the Marine Biological Association of the United Kingdom 55(02):463–476.

Hillman RE, Shuster CN. 1966. A comment on the origin of the fourth fold in the mantle of the Quahog, *Mercenaria mercenaria*. Chesapeake Science 7(2):112–113.

Hodgson CA, Burke RD. 1988. Development and larval morphology of the Spiny Oyster, *Chlamys hastata*. Biological Bulletin 174:303–318.

Jabbour-Zahab R, Chagot D, Blanc F, Grizel H. 1992. Mantle histology, histochemistry and ultrastructure of the pearl oyster *Pinctada margaritifera* (L.). Aquatic Living Resources 5:287–298.

Kellogg JL. 1892. A contribution to our knowledge of the morphology of the lamellibranchiate mollusks. Bulletin of the United States Bureau of Fisheries 10, 389.

Kuang S, Goldberg JI. 2001. Laser ablation reveals regulation of ciliary activity by serotonergic neurons in molluscan embryos. Journal of Neurobiology 47:1–15.

Land MF. 1965. Image formation by a concave reflector in the eye of the scallop *Pecten maximus*. Journal of Physiology 179:138–153.

Lee JS, Joo JY, Park JJ. 2007. Histology and ultrastructure of the mantle epidermis of the equilateral venus, *Gomphina veneriformis* (Bivalvia: Veneridae). Journal of Shellfish Research 26(2):413–421.

Lemche H, Wingstrand KG. 1959. The anatomy of *Neopilina galatheae*, 1957 (Molllusca, Tryblidiacea). Galathea Report 3:9–71.

Malkowsky Y, Jochum A. 2014. Three-dimensional reconstructions of pallial eyes in Pectinidae (Mollusca: Bivalvia). Acta Zoologica (Stockholm) 0:1–7.

McNamara KJ, 2012. Heterochrony: the Evolution of Development. Evolution: Education and Outreach 5:203–218.

Moir AJG. 1977. Ultrastructural studies on the ciliated receptors of the long tentacles of the Giant Scallop, *Placopecten magellanicus* (Gmelin). Cell and Tissue Research 184:367–380.

Moor B. 1983. Organogenesis. In: K. M. Wilbur (Ed.). The Mollusca: Development. Florida, Academic Press.

Moore GD, Trueman ER. 1971. Swimming of the scallop, *Chlamys opercularis* (L.). Journal of Experimental Marine Biology and Ecology 6:179–185.

Morton B. 1973. Dymantic display in *Galeomma polita* Deshayes (Bivalvia: Leptonacea). Journal of Conchology 28:365–369.

Morton B. 1982. Functional morphology of *Bthyarca pectunculoides* (Bivalvia: Arcacea) from a deep Norwegian fjord with a discussion of the mantle margin in the Arcoida. Sarsia 67:269–282.

Morton B. 1987. The mantle margin and radial mantle glands of *Entodesma saxicola* and *E. inflata* (Bivalvia: Anomalodesmata: Lyonsiidae). Journal of Molluscan Studies 53:139–151.

Morton B. 2001. The function of pallial eyes within the Pectinidae, with a description of those present in *Patinopecten yessoensis*. Geological Society, London, Special Publications 177:247–255.

Morton B. 2008. The evolution of eyes in the Bivalvia: new insights. American Malacological Bulletin 26(1-2):35–45.

Morton B, Peharda M. 2008. The biology and functional morphology of *Arca noae* (Bivalvia: Arcidae) from the Adriatic Sea, Croatia, with a discussion on the evolution of the bivalve mantle margin. Acta Zoologica (Stockholm) 89:19–28.

Morton B, Scott PJB. 1980. Morphological and functional specializations of the shell, musculature and pallial glands in the Lithophaginae (Mollusca: Bivalvia). Journal of Zoology 192:179–203.

Mouëza M, Gros O, Frenkiel L. 1999. Embryonic, larval, and post-larval development of the tropical clam *Anomalocardia brasiliana* (Mollusca: Bivalvia, Veneridae). Journal of Molluscan Studies 65:73–88.

Nelson TC. 1938. The feeding mechanism of the oyster. I. On the pallium and the branchial chambers of *Ostrea virginica*, *O. edulis* and *O. angulata*, with comparisons with other species of the genus. Journal of Morphology 63:1–61.

Pairett AN, Serb JM. 2013. De novo assembly and characterization of two transcriptomes reveal multiple light-mediated functions in the scallop eye (Bivalvia: Pectinidae). PLoS ONE 8(7): e69852.

Raven CP. 1958. Morphogenesis: the Analysis of Molluscan Development. New York, Pergamon Press.

Richardson CA, Runham NW, Crisp DJ. 1981. A histological and ultrastructural study of the cells of the mantle edge of a marine bivalve, *Cerastoderma edule*. Tissue & Cell 13:715–730.

Sartori AF, Printrakoon C, Mikkelsen P, Bieler R. 2008. Siphonal structure in the Veneridae (Bivalvia: Heterodonta) with an assessment of its phylogenetic application and a review of venerids of the Gulf of Thailand. The Raffles Bulletin of Zoology 18:103–125.

Sastry AN. 1965. The development and external morphology of pelagic larval and postlarval stages of the bay scallop, *Aequipecten irradians concentricus* Say, reared in the laboratory. Bulletin of Marine Science, 15(2):417–435.

Silberfeld T, Gros O. 2006. Embryonic development of the tropical bivalve *Tivela mactroides* (Born, 1778) (Veneridae: subfamily Meretricinae): a SEM study. Cahiers de Biologie Marine 47:243–251.

Serb JM, Eernisse DJ. 2008. Charting evolution's trajectory: using molluscan eye diversity to understand parallel and convergent evolution. Evolution Education Outreach 1:439–447.

Serb JM, Porath-Krause AJ, Pairett AN. 2013. Uncovering a gene duplication of the photoreceptive protein, opsin, in scallops (Bivalvia: Pectinidae). Integrative and Comparative Biology 53:68–77.

Smith KK. 2001. Heterochrony revisited: the evolution of developmental sequences. Biological Journal of the Linnean Society 73:169–186.

Spagnolia T, Wilkens LA. 1983. Neurobiology of the scallop. II. Structure of the parietovisceral ganglion lateral lobes in relation to afferent projections from the mantle eyes. Marine Behaviour and Physiology 10:23–55.

Speiser DI, Johnsen S. 2008. Comparative morphology of the concave mirror eyes of scallops (Pectinoidea). American Malacological Bulletin 26:27–35.

Stanley SM. 1972. Functional morphology and evolution of byssally attached bivalve mollusks. Journal of Paleontology 46:165–212.

Stasek CR, McWilliams WR. 1973. The comparative morphology and evolution of molluscan mantle edge. The Veliger 16:1–19.

Steiner G. 1991. Observations on the anatomy of the scaphopod mantle, and the description of a new family, the Fustiariidae. American Malacological Bulletin 9:1–20.

Taylor JD. 1973. The structural evolution of the bivalve shell. Palaeontology 16:519–534.

Timmermans LPM. 1969. Studies on shell formation in molluscs. Netherlands Journal of Zoology 19:417–523.

Wada K. 1964. Studies on the mineralization of the calcified tissue in molluscs. VII. Histological and histochemical studies of organic matrices in shells. Bulletin of the National Pearl Research Laboratory 9:1078–1086.

Waller TR. 1980. Scanning electron microscopy of shell and mantle in the order Arcoida (Mollusca: Bivalvia). Smithsonian Contributions to Zoology 313.

Waller TR. 1981. Functional morphology and development of veliger larvae of the European oyster, *Ostrea edulis* Linné. Smithsonian Contributions to Zoology 328.

Waller TR. 2006. New phylogenies of the Pectinidae (Mollusca: Bivalvia): Reconciling morphological and molecular approaches. In Shumway SE, Parsons GJ (Editors). Scallops: biology, ecology and aquaculture. Amsterdam, Elsevier, pp 1–44.

Wanninger A, Haszprunar G. 2002. Muscle development in *Antalis entalis* (Mollusca, Scaphopoda) and its significance for scaphopod relationships. Journal of Morphology 254:53–64.

Wanninger A, Koop D, Moshel-Lynch S, Degnan BM. 2008. Molluscan evolutionary development. In: Ponder W and Lindberg W (Editors). Phylogeny and Evolution of the Mollusca. California, University of California Press.

Weiss IM, Tuross N, Addadi L, Weiner S. 2002. Mollusc larval shell formation: amorphous calcium carbonate is a precursor phase for aragonite. Journal of Experimental Zoology 293:478–491.

Weiss IM, Schönitzer V. 2006. The distribution of chitin in larval shells of the bivalve mollusk *Mytilus galloprovincialis*. Journal of Structural Biology 153:264–277.

Westermann B, Schmidtberg H, Beuerlein K. 2005. Functional morphology of the mantle of *Nautilus pompilius* (Mollusca, Cephalopoda). Journal of Morphology 264:277–285.

Wilbur KM. 1964. Shell formation and regeneration. In: Wilbur KM, Yonge CM (Editors). Physiology of Mollusca, vol. 1. Academic, New York, pp 243–282.

Wilbur KM, Saleuddin ASM. 1983. Shell formation. In: Saleuddin ASM, Wilbur KM (Editors). The Mollusca. Academics, New York, pp 235–287.

Wilkens LA. 2006. Neurobiology and behaviour of the scallop. In Shumway SE, Parsons GJ (Editors). Scallops: Biology, Ecology and Aquaculture. Amsterdam, Elsevier pp 317–356.

Wollesen T, Wanninger A, Klussmann-Kolb A. 2008. Myogenesis in *Aplysia californica* (Cooper, 1863) (Mollusca, Gastropoda, Opisthobranchia) with special focus on muscular remodeling during metamorphosis. Journal of Morphology 269:776–789.

Yonge CM. 1936. The evolution of the swimming habit in the Lamellibranchia. Mem. Mus. R. Hist. nato Belg. 3:77–100.

Yonge CM. 1948. Formation of siphons in Lamellibranchia. Nature 161:198–199.

Yonge CM. 1957. Mantle fusion in the Lamellibranchia. Pubblicazioni della Stazione Zoologica di Napoli 29:151–171.

Yonge CM. 1962. On the primitive significance of the byssus in the Bivalvia and its effects in evolution. Journal of the Marine Biological Association of the United Kingdom 42:113–125.

Yonge CM. 1982. Mantle margins with a revision of siphonal types in the Bivalvia. Journal of Molluscan Studies 48:102–103.

Yonge CM. 1983. Symmetries and role of mantle margins in the bivalve Mollusca. Malacological Review 16:1-10.

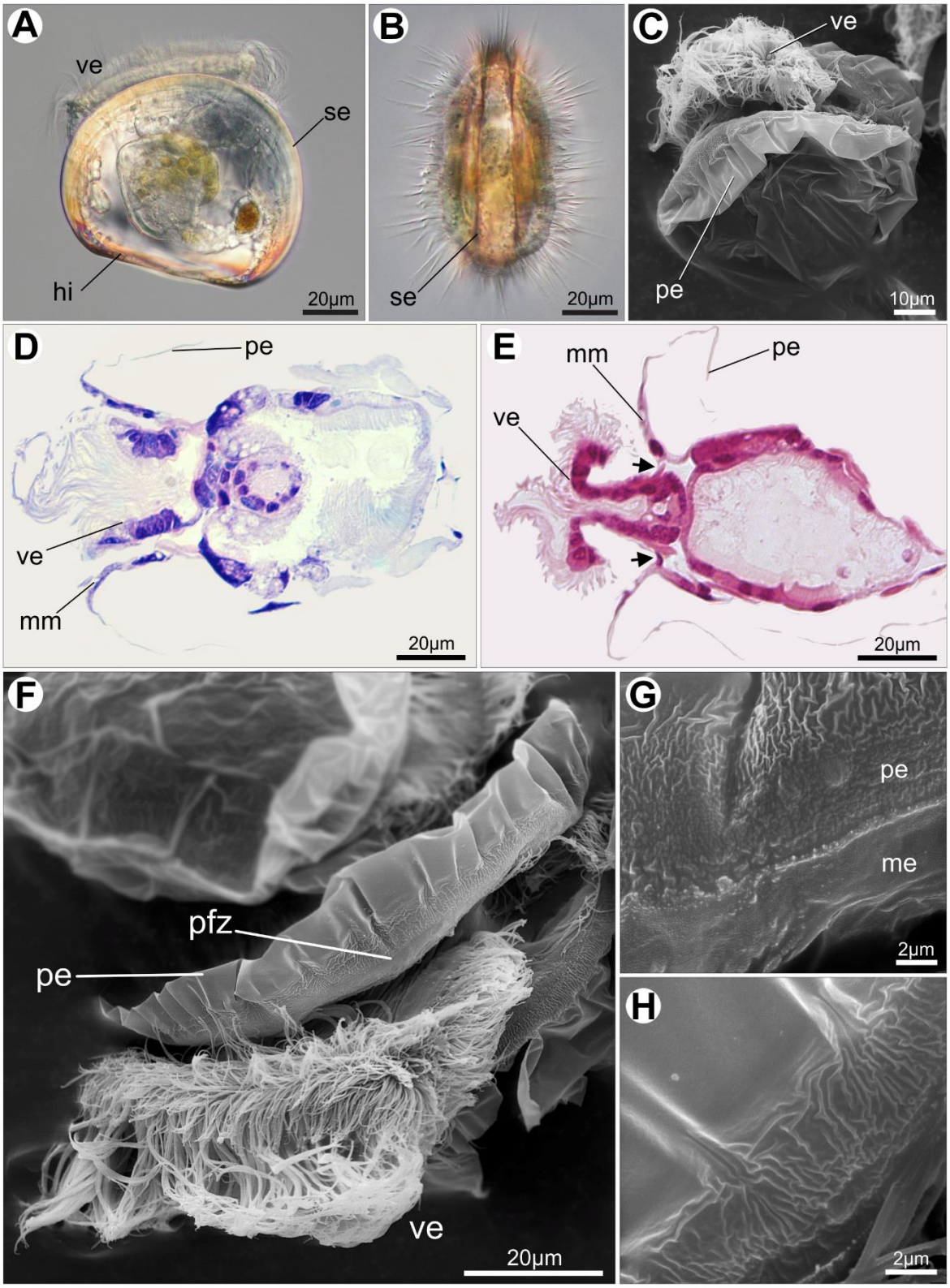

Figure 1. Veliger larvae of *N. nodosus*. **A.** Specimen observed by light microscopy applying differential interference contrast; lateral view. **B.** Same individual, ventral view. **C.** General morphology of a decalcified veliger as observed by SEM. **D.** Cross section of a veliger individual with the larval velum retracted. TB. **E.** Cross section of a veliger individual with the larval velum exposed. Arrow points to the membrane connecting the larval velum and the mantle margin. HE. **F.** Detail of the larval ventral view under SEM. **G.** Detail of the periostracum forming zone. **H.** Detail of the newly formed periostracum, with smooth and wrinkled zones. Abbreviations: *hi*, hinge line; *me*, mantle epithelium; *mm*, mantle margin; *pe*, periostracum; *pfz*, periostracum forming zone; *se*, shell edge; *ve*, larval velum.

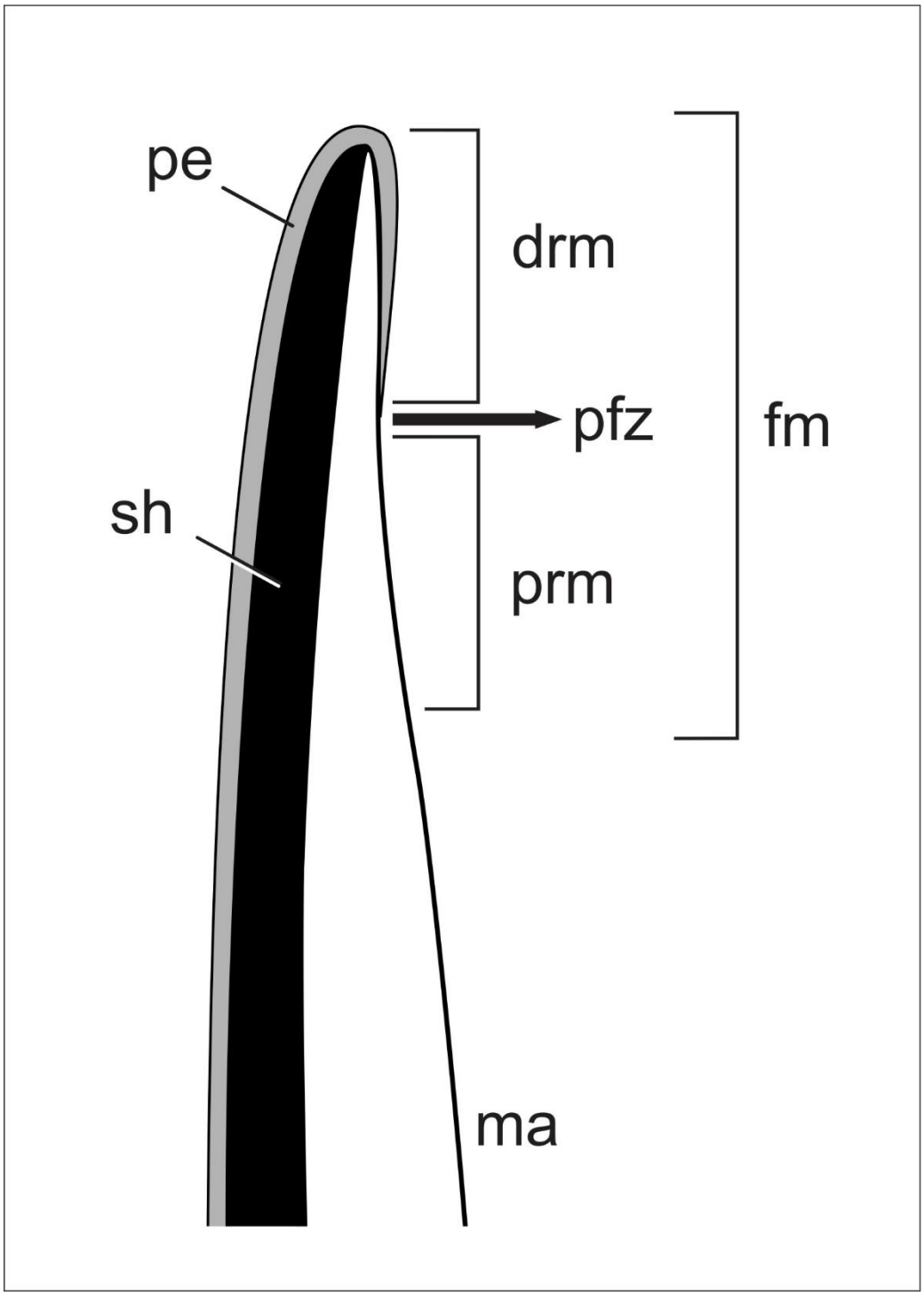

Figure 2. Schematic representation of the mantle margin in veliger larvae of *N. nodosus*. The unfolded mantle margin is divided into two regions, the distal and proximal ones, by the periostracum forming zone. Abbreviations: *drm*, distal region of the mantle edge; *fm*, free mantle margin; *ma*, mantle; *sh*, shell; *pe*, periostracum; *pfz*, periostracum forming zone; *prm*, proximal region of the mantle edge.

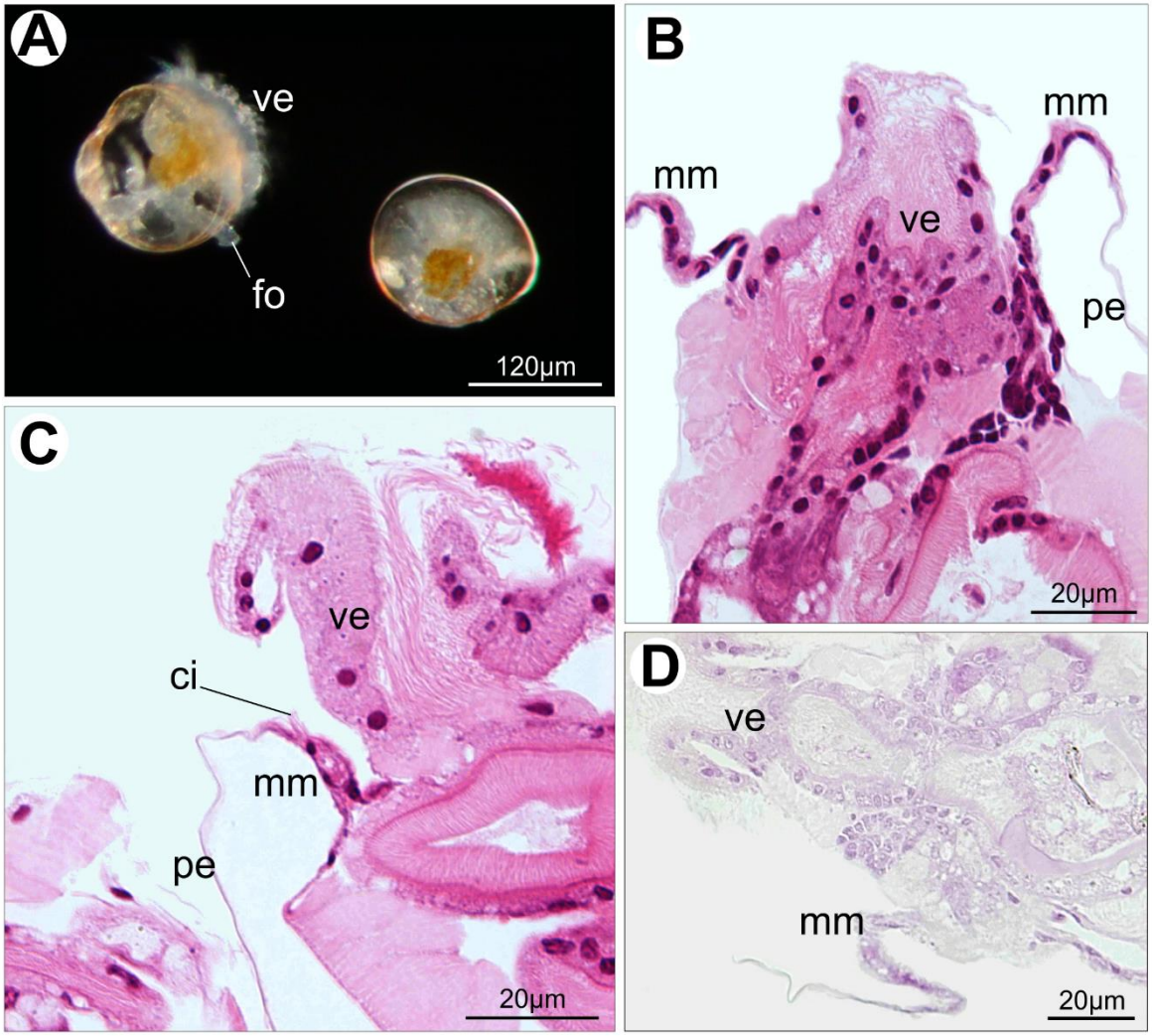

Figure 3. Pediveliger larvae of *N. nodosus*. **A.** Specimens observed by light microscopy applying differential interference contrast; lateral view. **B.** Cross section of the mantle margin and velum. HE. **C.** Cross section of the mantle margin showing cilia close to the periostracum forming zone. HE. **D.** Cross section of an individual showing no particular reaction to Periodic Acid-Schiff staining. Abbreviations: *ci*, cilia; *fo*, foot; *mm*, mantle margin; *pe*, periostracum; *ve*, larval velum.

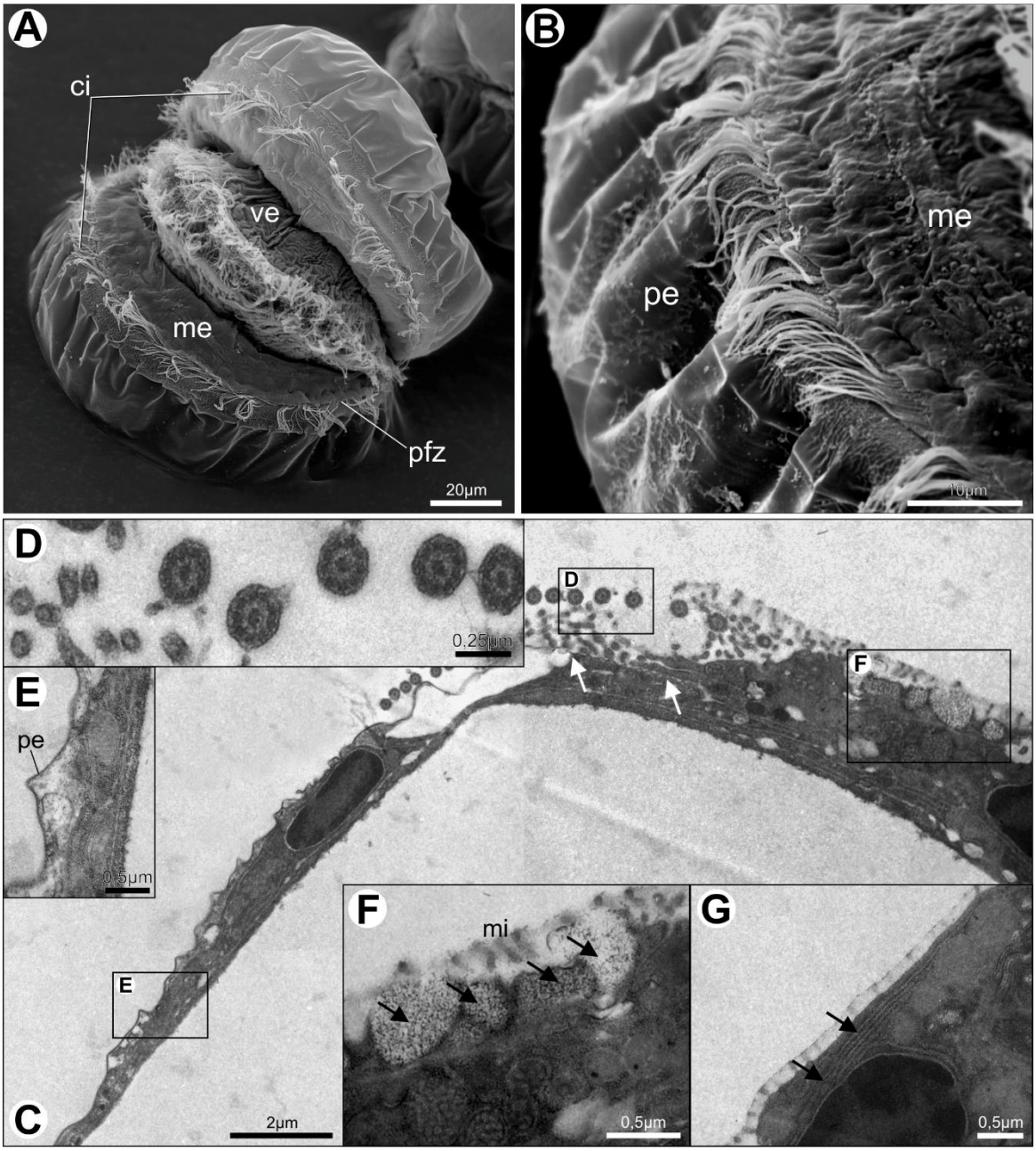

Figure 4. Pediveliger larvae of *N. nodosus* analyzed under scanning electron microscopy (A-B) and transmission electron microscopy (C-G). **A.** General morphology of the mantle margins exposed by the retracted larval velum, ventral view. **B.** Detail of the mantle margin showing the periostracum forming zone and the adjacent row of cilia. **C.** Mantle margin of a pediveliger specimen, proximal region to the right and distal region to the left; white arrows point to periostracum secretion. **D.** Cross section of cilia present in the periostracum forming zone, showing the classical microtubular arrangement "9 + 2". **E.** Periostracal layer covering the distal region of the mantle. **F.** Detail of electron-lucent vesicles (black arrows) present in the epithelium of the inner surface of the proximal region of the mantle, covered by short microvilli **G.** Detail of the rough endoplasmic reticulum around nucleus in a cell from the proximal region of the mantle margin. Abbreviations: *ci*, cilia; *me*, mantle epithelium, *mi*, microvilli; *pe*, periostracum; *pfz*, periostracum forming zone; *ve*, larval velum.

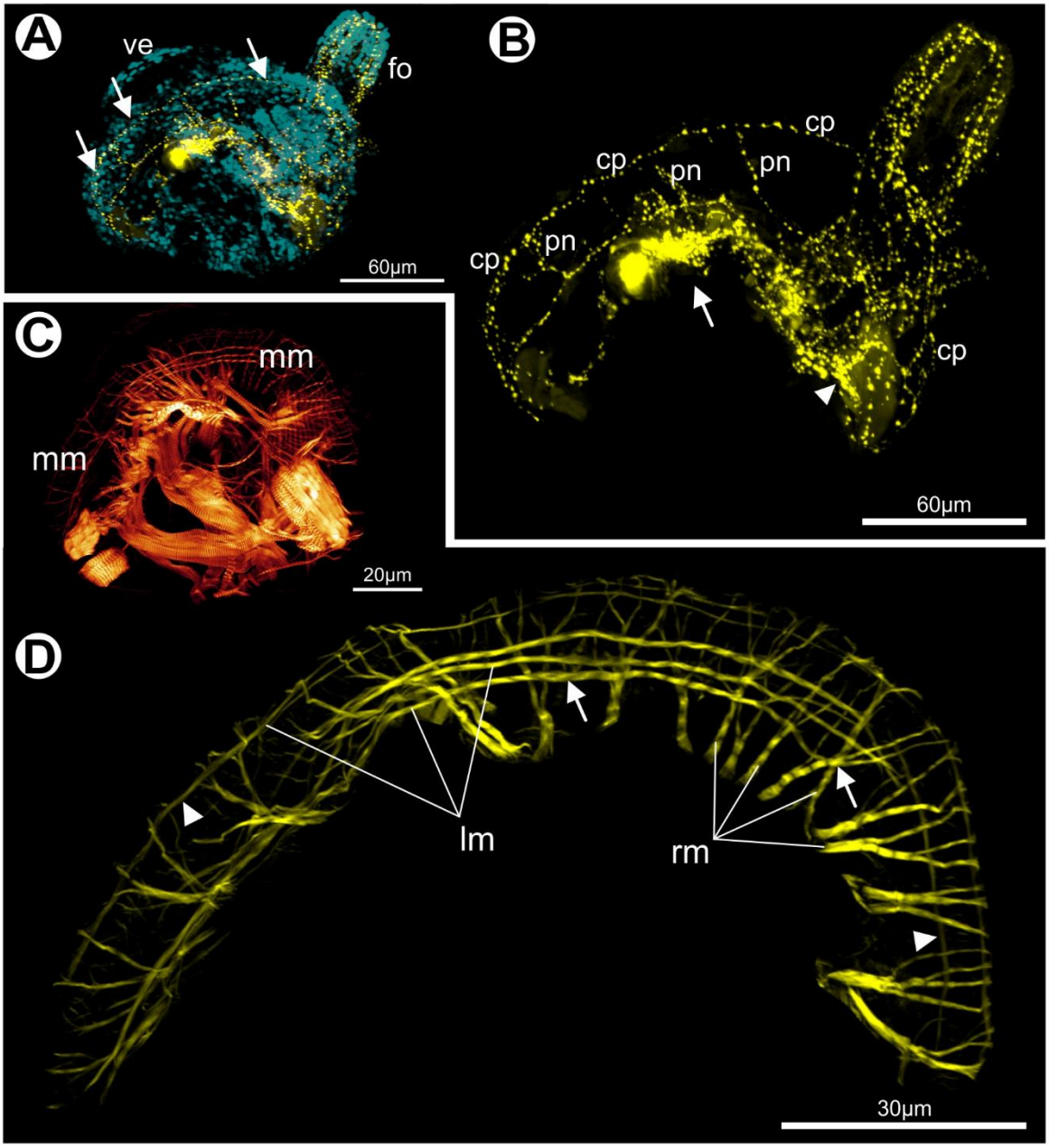

Figure 5. Pediveliger larvae of *N. nodosus* treated against serotonin (A-B) and stained with phalloidin (C-D), as revealed by confocal laser scanning microscopy (CLSM). **A.** Pediveliger showing strong serotonergic immunoreactivity (yellow) in the central and peripheral nervous system, particularly in the circum-pallial nerve (arrows) and foot nervous plexus. Lateral view, posterior region to the right. Nuclei are stained in blue by DAPI. **B.** 3D reconstruction of pediveliger nervous system based on CLSM image stack showing the mantle innervation in detail. Arrow points to the apical-cerebral ganglia and arrowhead to the visceral ganglion **C.** Larval musculature of half-individual, lateral view, posterior region to the right. **D.** 3D reconstruction of pediveliger pallial musculature, showing retractor and margin-parallel muscles; arrows point to striated fibers and arrowheads to non-striated fibers. Abbreviations: *cp*, circum-pallial nerve; *fo*, foot; *lm*, longitudinal muscles; *mm*, mantle margin; *pn*, pallial nerves; *rm*, retractor muscles; *ve*, larval velum.

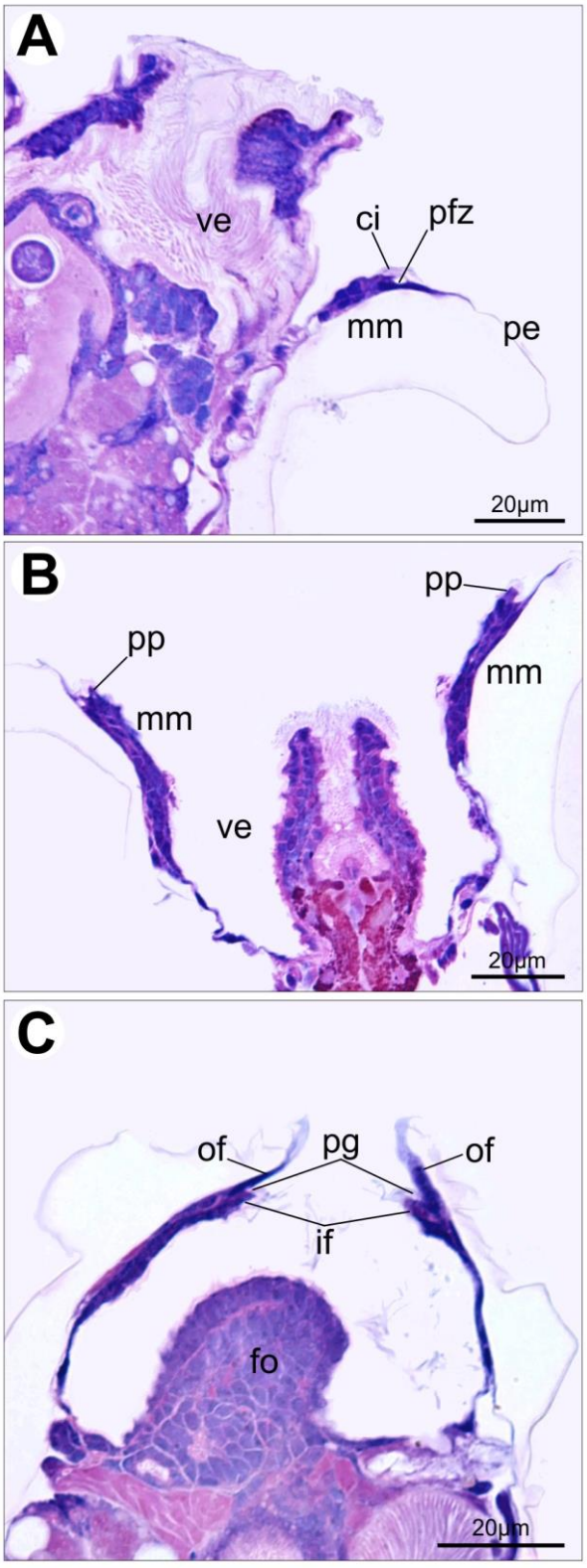

Figure 6. Cross sections from late pediveliger larvae of *N. nodosus.* TB. **A.** Unfolded mantle margin with cilia in the periostracum forming zone. **B.** Proximal region of the mantle slightly projected. **C.** Two-folded mantle margin with distinctive outer and inner folds. Abbreviations: *ci*, cilia; *fo*, foot; *if*, inner fold; *mm*, mantle margin; *of*, outer fold; *pe*, periostracum; *pfz*, periostracum forming zone; *pg*, periostracal groove; *pp*, projection of the proximal region of the mantle margin; *ve*, larval velum.

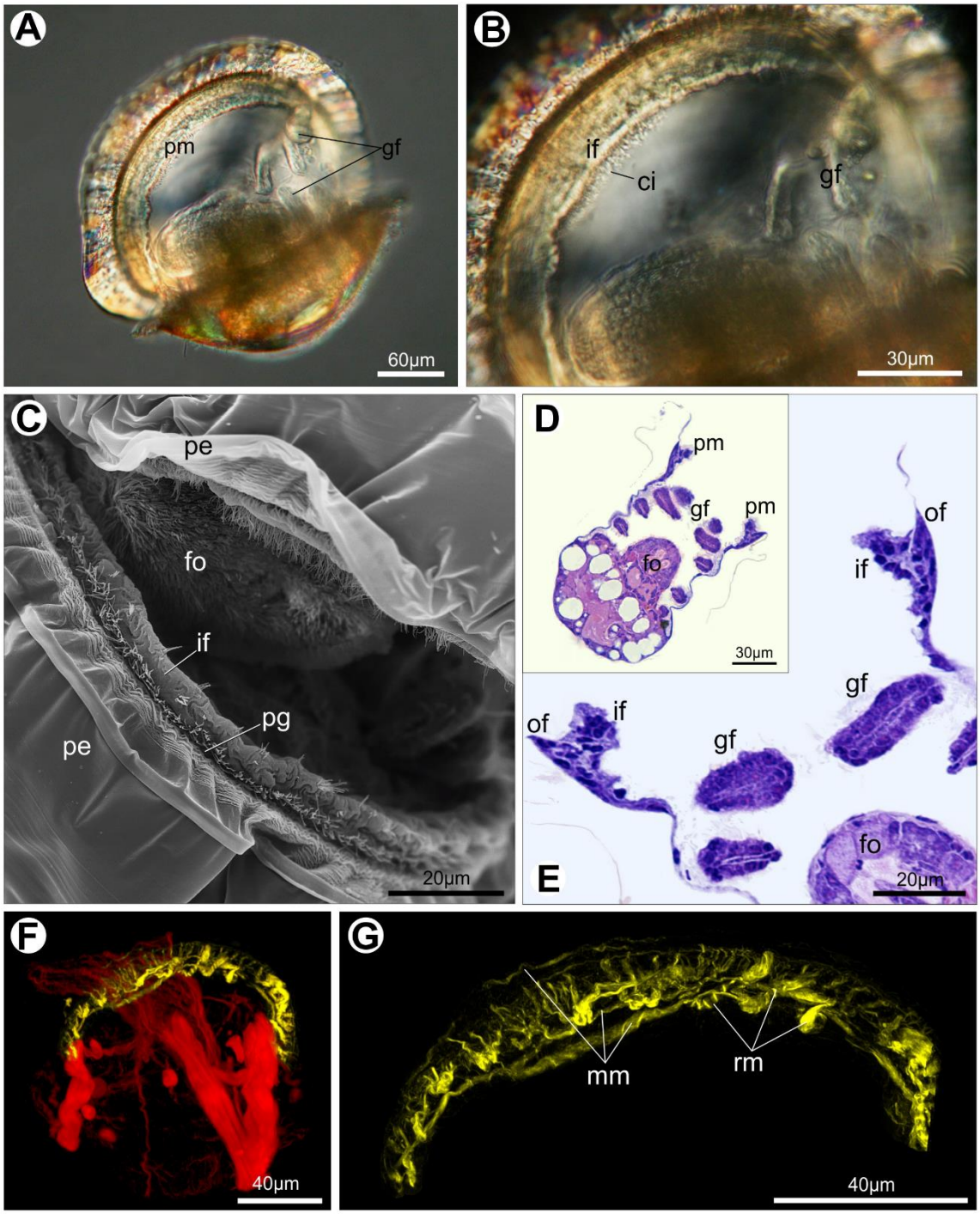

Figure 7. Postmetamorphic *N. nodosus* scallops. **A.** Specimen observed by light microscopy applying differential interference contrast; ventral view, posterior region to the right. **B.** Detail from the same individual, showing the inner curtain-like fold, with cilia on its edge. **C.** Mantle margin with inner fold and row of cilia on the periostracal groove, as revealed by SEM; ventral view, posterior region to the right. **D.** Cross section of an entire specimen showing extension of the mantle and pallial cavity where gill filaments are growing. TB. **E.** Detail of the pallial cavity and the two-folded mantle margins. TB. **F.** Postmetamorphic musculature stained with phalloidin and reconstructed based on CLSM image stack. Pallial muscles are presented in yellow; lateral view, posterior region to the right. **G.** Detail of the pallial musculature from F, showing retractor and margin-parallel muscles. Abbreviations: *ci*, cilia; *fo*, foot; *gf*, gill filaments; *if*, inner fold; *mm*, margin-parallel muscles; *of*, outer fold; *pe*, periostracum; *pg*, periostracal groove; *pm*, pallial margin, *rm*, retractor muscles.

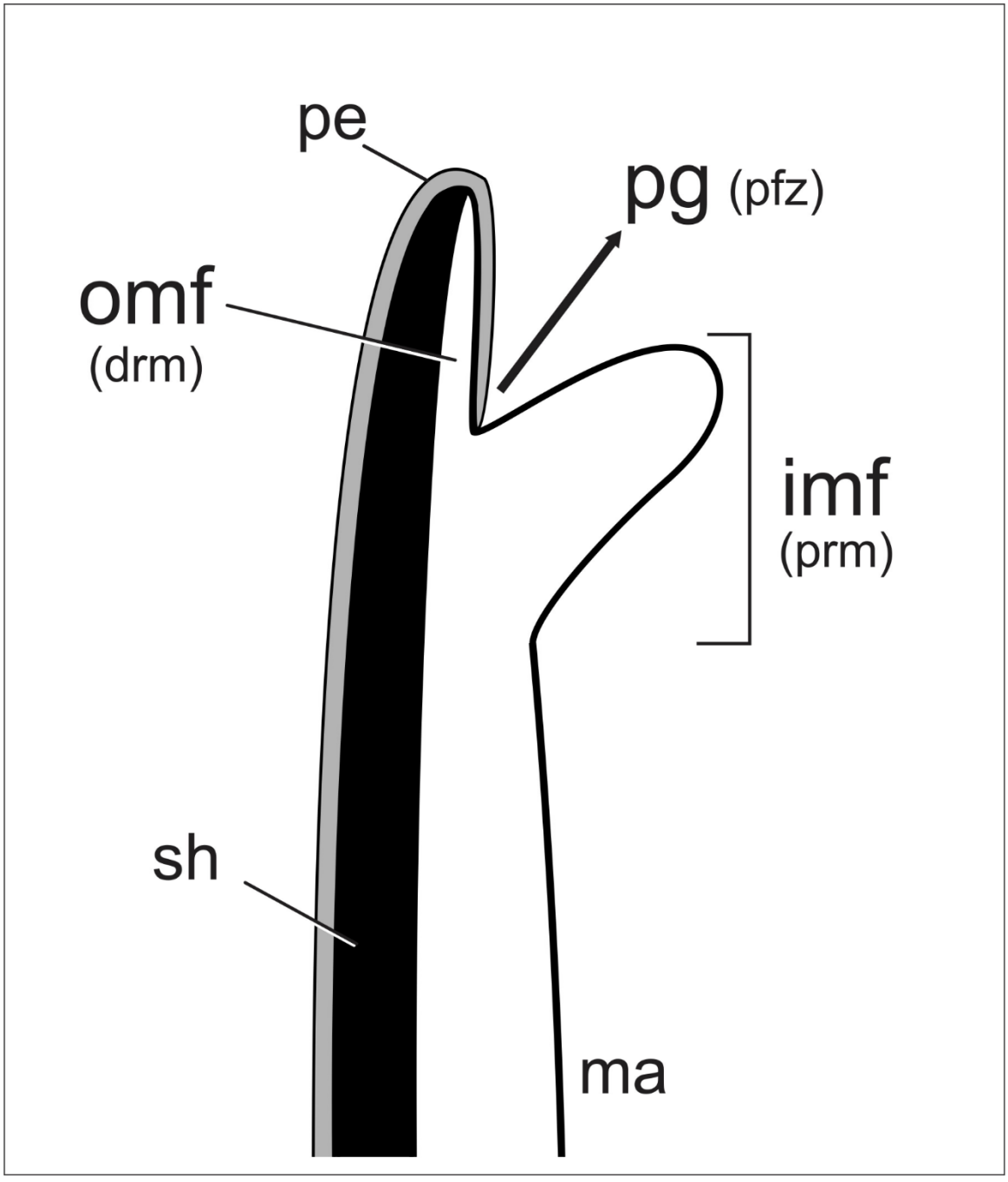

Figure 8. Schematic representation of the postmetamorphic mantle margin in *N. nodosus*. The two-folded condition includes the outer and inner folds, which correspond to the distal and proximal regions of the larval mantle margin, respectively. The periostracum forming zone ends up in the bottom of a groove between the folds. Abbreviations: *drm*, distal region of the mantle; *imf*, inner mantle fold; *ma*, mantle; *omf*, outer mantle fold; *pe*, periostracum; *pfz*, periostracum forming zone; *pg*, periostracal groove; *prm*, proximal region of the mantle; *sh*, shell.

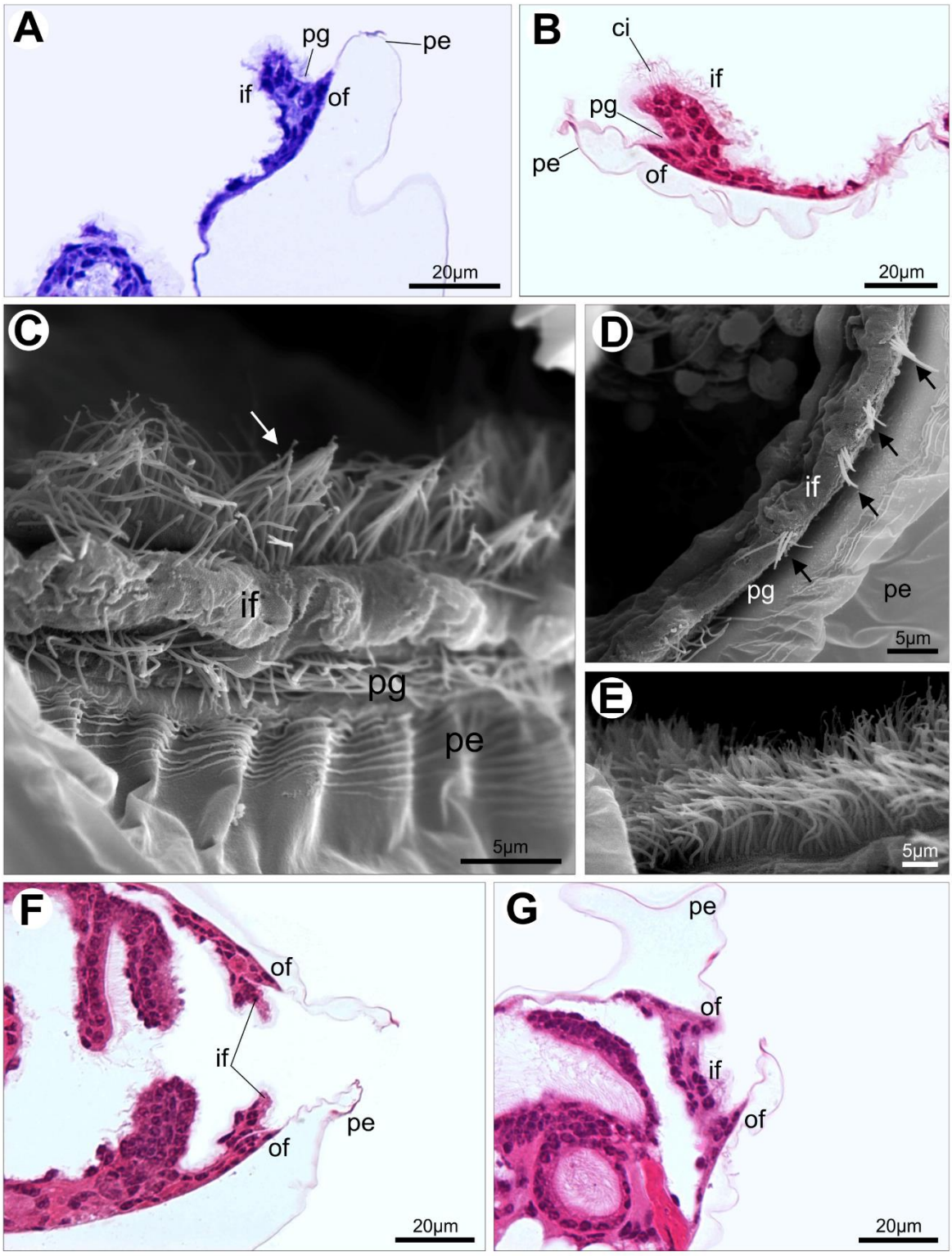

Figure 9. Postmetamorphic mantle margin in *N. nodosus*, as revealed by histological cross sections (A-B, F-G) and scanning electron microscopy (C-E). **A.** Mantle margin showing the two-folded condition and cilia present on the periostracal groove. TB. **B.** Anterior region of the left mantle margin, showing a developed inner fold with a dense ciliary band on the inner surface. HE. **C.** Detail of the anterior region of the left mantle margin, showing rows of cilia on the periostracal groove, scattered cilia on the rim of the inner fold, and dense ciliary band (arrow) on the inner surface of the inner fold. **D.** Tufts of cilia (arrows) scattered along the rim of the inner mantle fold. **E.** Detail of the dense ciliary band on the inner surface of the left inner fold. **F.** Free edges of the mantle margin in the ventral region. HE. **G.** Fused inner folds in the dorsal region; periostracal groove and outer fold remain intact. HE. Abbreviations: *ci*, cilia; *if*, inner fold; *of*, outer fold; *pe*, periostracum; *pg*, periostracal groove.

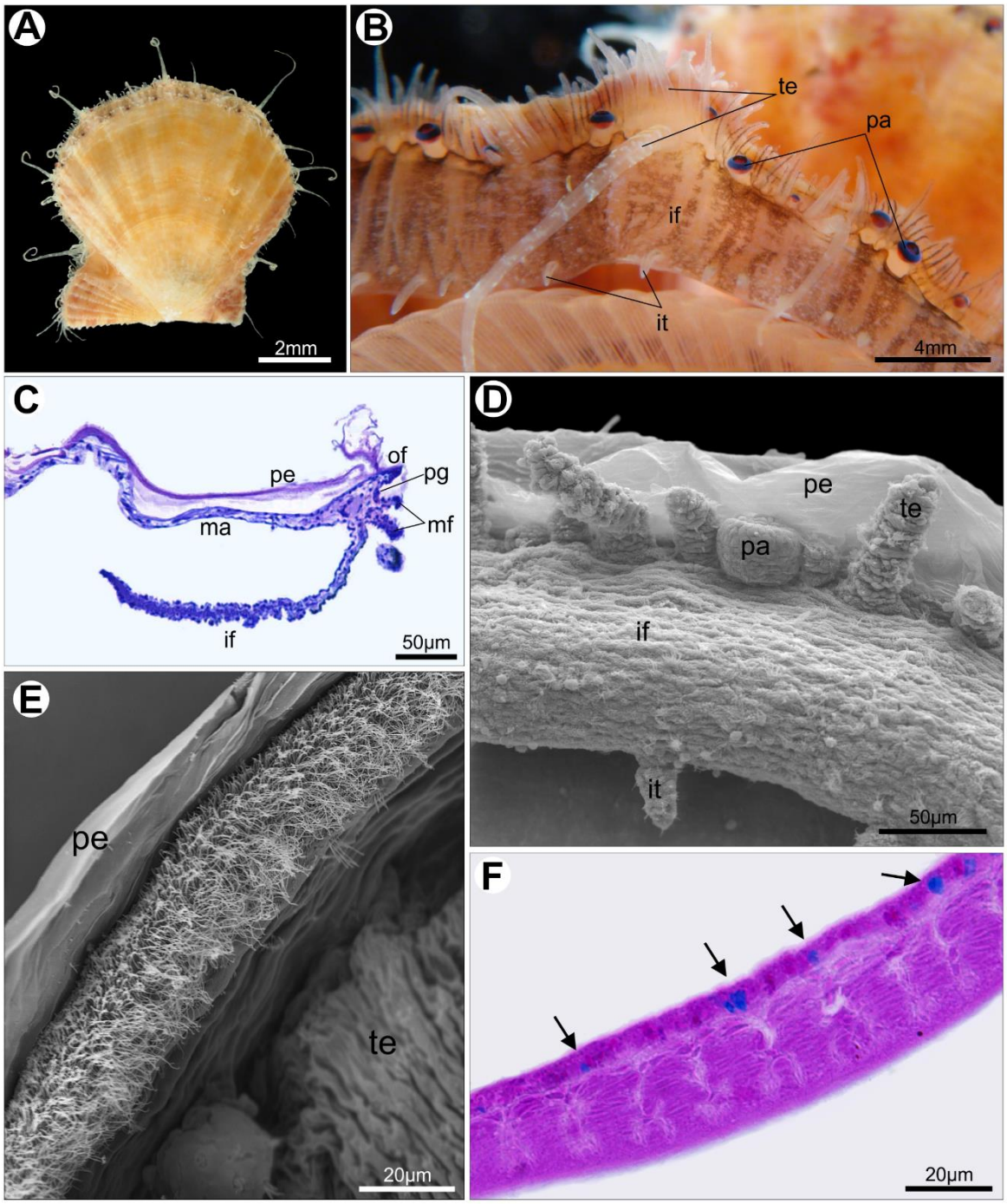

Figure 10. Juvenile scallops of *N. nodosus*, as revealed by magnifying lens (A, B), histological cross sections (C, F) and scanning electron microscopy (D, E). **A.** Juvenile individual few weeks after metamorphosis. **B.** Detail of the mantle margin with a large inner fold ("velum"), and numerous tentacles and eyes on the middle fold. **C.** Three-folded condition of the mantle margin, including the newly formed middle fold provided with tentacles. TB. **D.** Ciliated surface of the middle mantle fold, with eyes and tentacles, and inner fold, very enlarged and containing small tentacles on its edge. Arrow points to the view observed in E. **E.** Detail of the outer surface of the middle fold, showing dense cilia covering continuous with the periostracal groove. **F.** Secretory cells (arrows) in the mantle epithelium, containing acid mucopolysaccharides stained by Alcian Blue. Abbreviations: *if*, inner mantle fold; *it*, inner fold tentacles; *ma*, mantle; *mf*, middle fold; *of*, outer mantle fold; *pa*, pallial eye; *pe*, periostracum; *pg*, periostracal groove; *te*, middle fold tentacles.

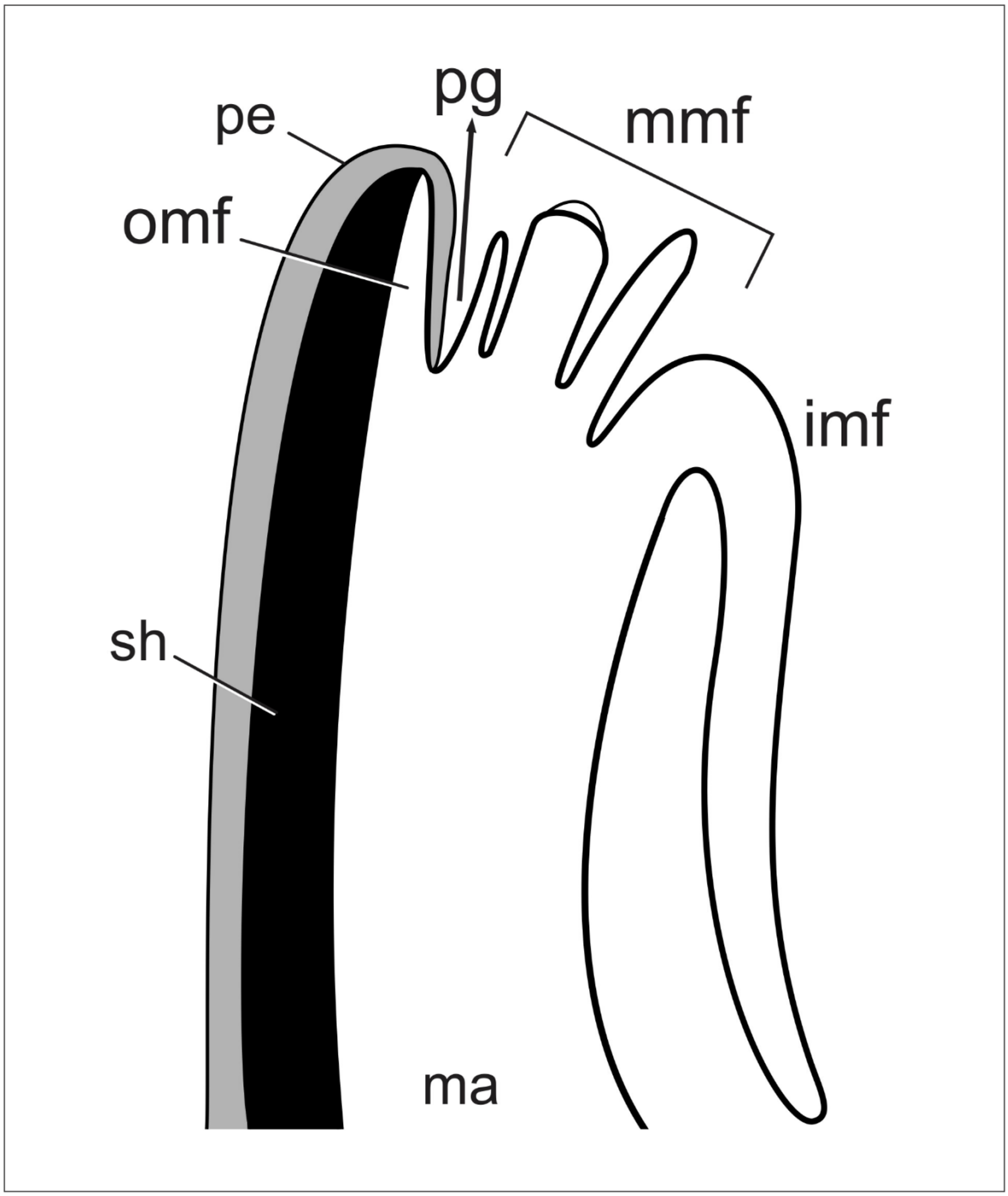

Figure 11. Schematic representation of the juvenile three-folded mantle margin in *N. nodosus*. The curtain-like inner mantle fold is greatly enlarged. The outer fold and the periostracal groove are similar to the postmetamorphic arrangement. The last fold to be formed, the middle one, is provided with numerous tentacles and pallial eyes. Abbreviations: *imf*, inner mantle fold; *ma*, mantle; *mmf*, middle mantle fold; *omf*, outer mantle fold; *pe*, periostracum; *pg*, periostracal groove; *sh*, shell.

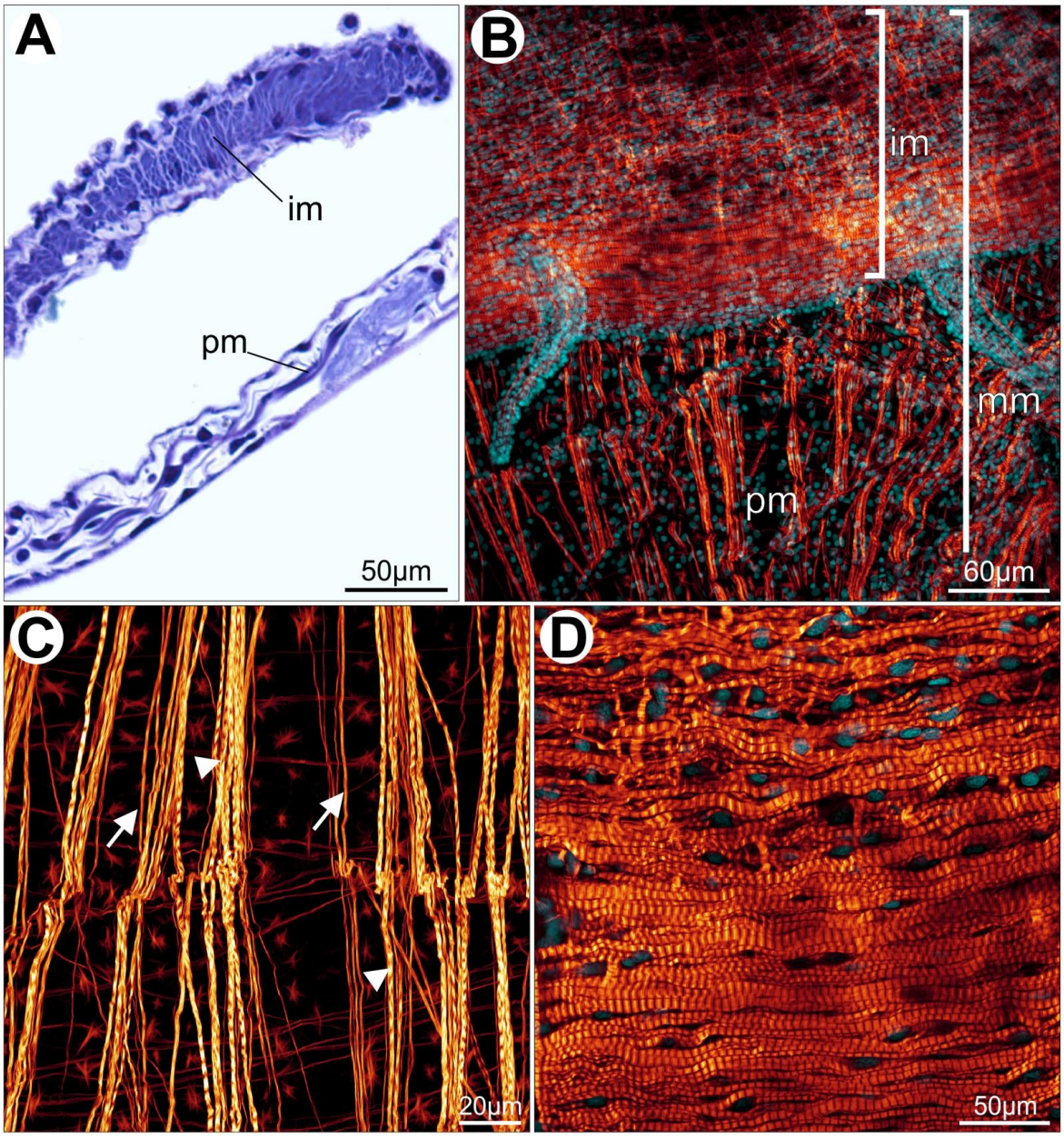

Figure 12. Pallial musculature in *N. nodosus* juveniles, as observed by histological sectioning (A) and stained with phalloidin combined with confocal laser scanning microscopy (B-D). **A.** Detail of muscle fibers in the mantle and dense muscular bundles in the inner mantle fold. TB. **B.** Overview of the mantle margin (distal region above, proximal region below), including the inner pallial fold (distal region below, proximal region above). Pallial muscles are radially distributed throughout the mantle margin, and margin-parallel muscles are present within the inner fold. Nuclei stained in blue by DAPI. **C.** Detail of the mantle margin radial musculature, showing a combination of non-striated (arrows) and striated myofibers (arrowheads). **D.** Detail of the arrangement of margin-parallel striated muscle fibers within the inner fold (proximal region above, distal region below), more densely arranged at the distal region. Nuclei stained in blue by DAPI. Abbreviations: *im*, inner mantle fold; *mm*, mantle margin; *pm*, pallial muscles.

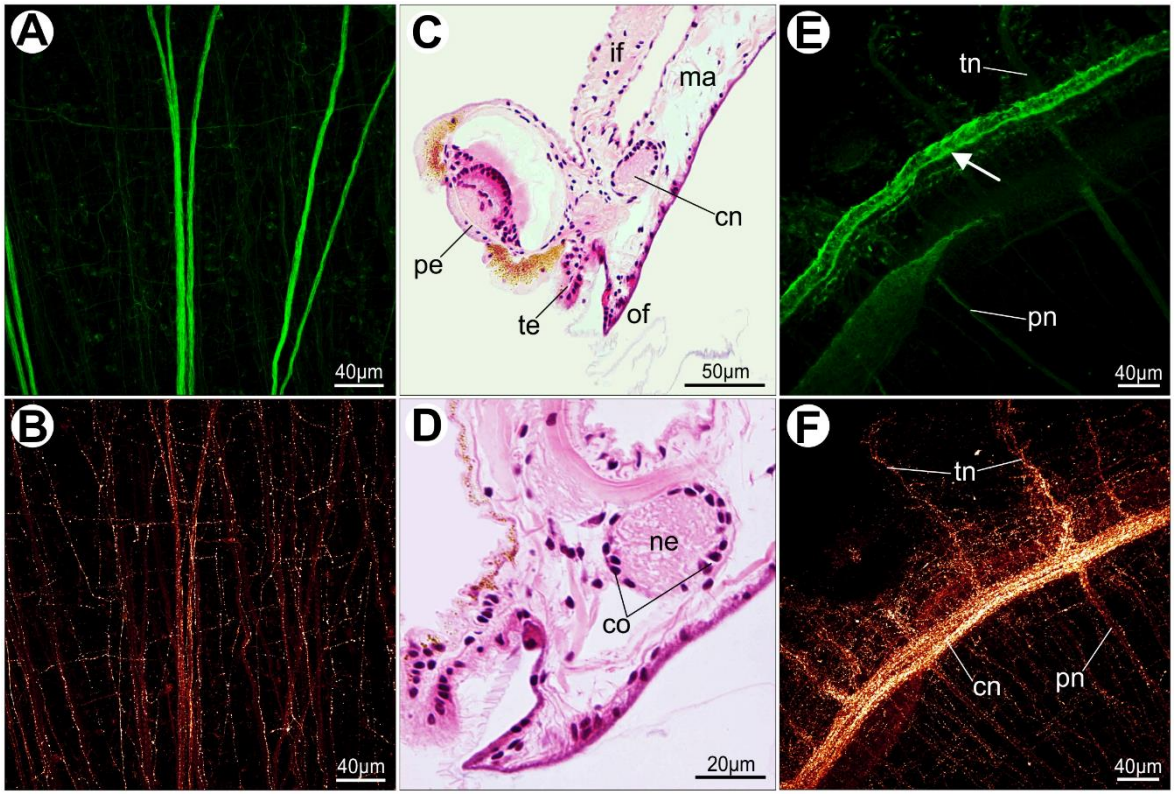

Figure 13. Innervation of the mantle margin in juveniles of *N. nodosus*, as revealed by histological cross sections (C, D), and immunoreactivity to serotonin (B, F) and α-tubulin (A, E) combined with confocal laser scanning microscopy. **A.** Radial nerves, shown in green, crossing the mantle towards its distal region. **B.** Serotonergic immunoreactivity in the mantle, exhibiting a reticulate pattern of innervation. **C.** Mantle margin showing the circum-pallial nerve and sensorial structures, such as tentacles and eyes. HE. **D.** Detail of the circum-pallial nerve, where a cortical region surrounds the neuropil. HE. **E.** Strong α-tubulin immunoreactivity in pallial nerves reaching the distal marginal region. Arrow points to the ciliated outer surface of the middle fold. **F.** Circum-pallial nerve and tentacles nerves showing strong serotonergic immunoreactivity. Abbreviations: *cn*, circum-pallial nerve; *co*, cortical layer; *if*, inner mantle fold; *ma*, mantle; *ne*, neuropil; *of*, outer mantle fold; *pe*, pallial eye; *pn*, pallial nerve; *te*, tentacle; *tn*, tentacle nerve.

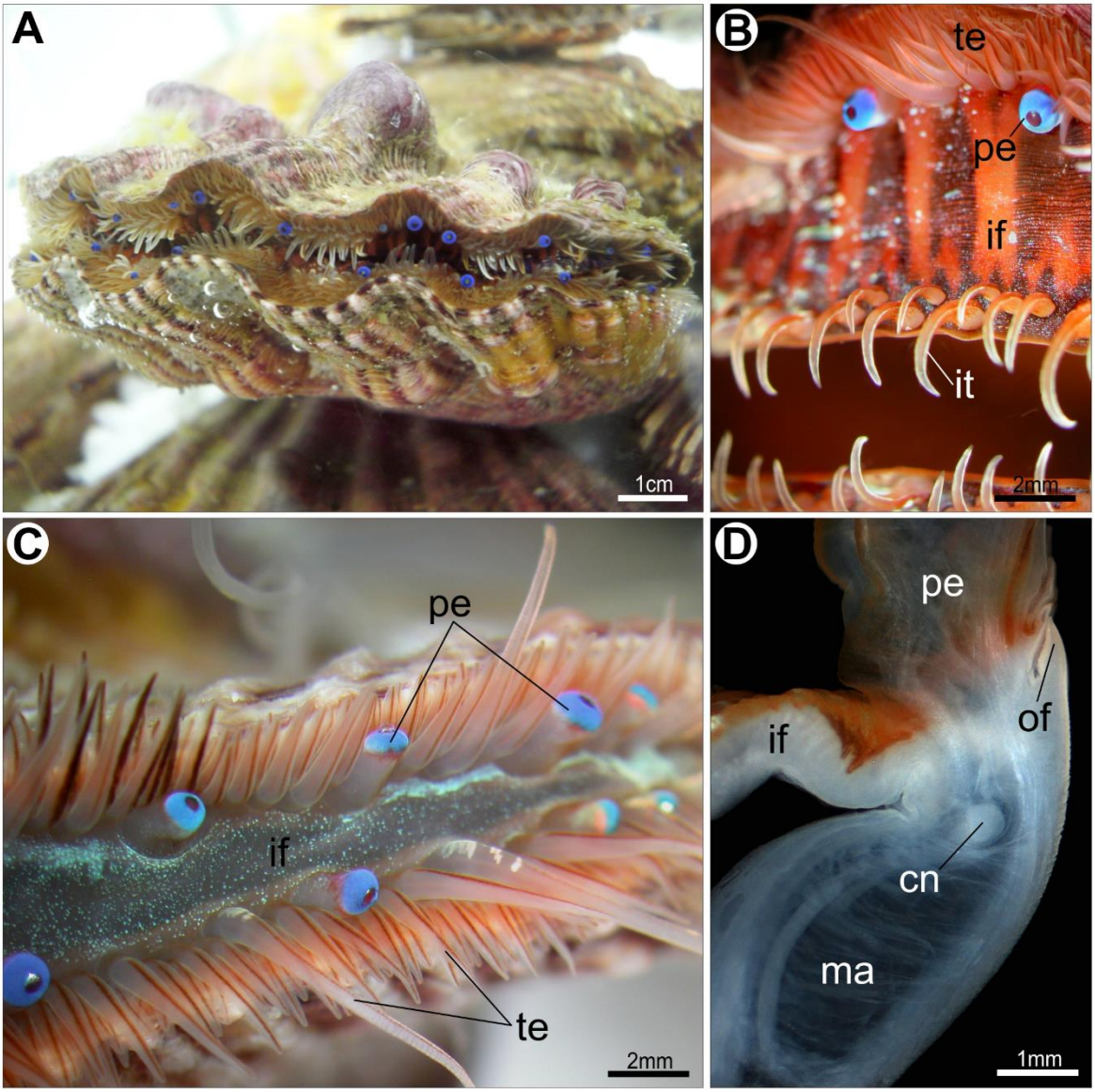

Figure 14. Mantle margin of adult *N. nodosus*. **A.** Living specimen with valves slightly opened, showing the pallial tentacles and eyes. **B.** Detail of the mantle margin in an anesthetized specimen, showing the large inner mantle fold with tentacles, and the middle fold with tentacles and eyes. **C.** Detail of the mantle margin at the auricular region, where the inner folds from both mantle lobes are fused. **D.** Cross dissection of the mantle margin. Abbreviations: *cn*, circum-pallial nerve; *if*, inner mantle fold; *it*, inner fold tentacle; *ma*, mantle; *of*, outer mantle fold; *pe*, pallial eye; *te*, middle fold tentacle.

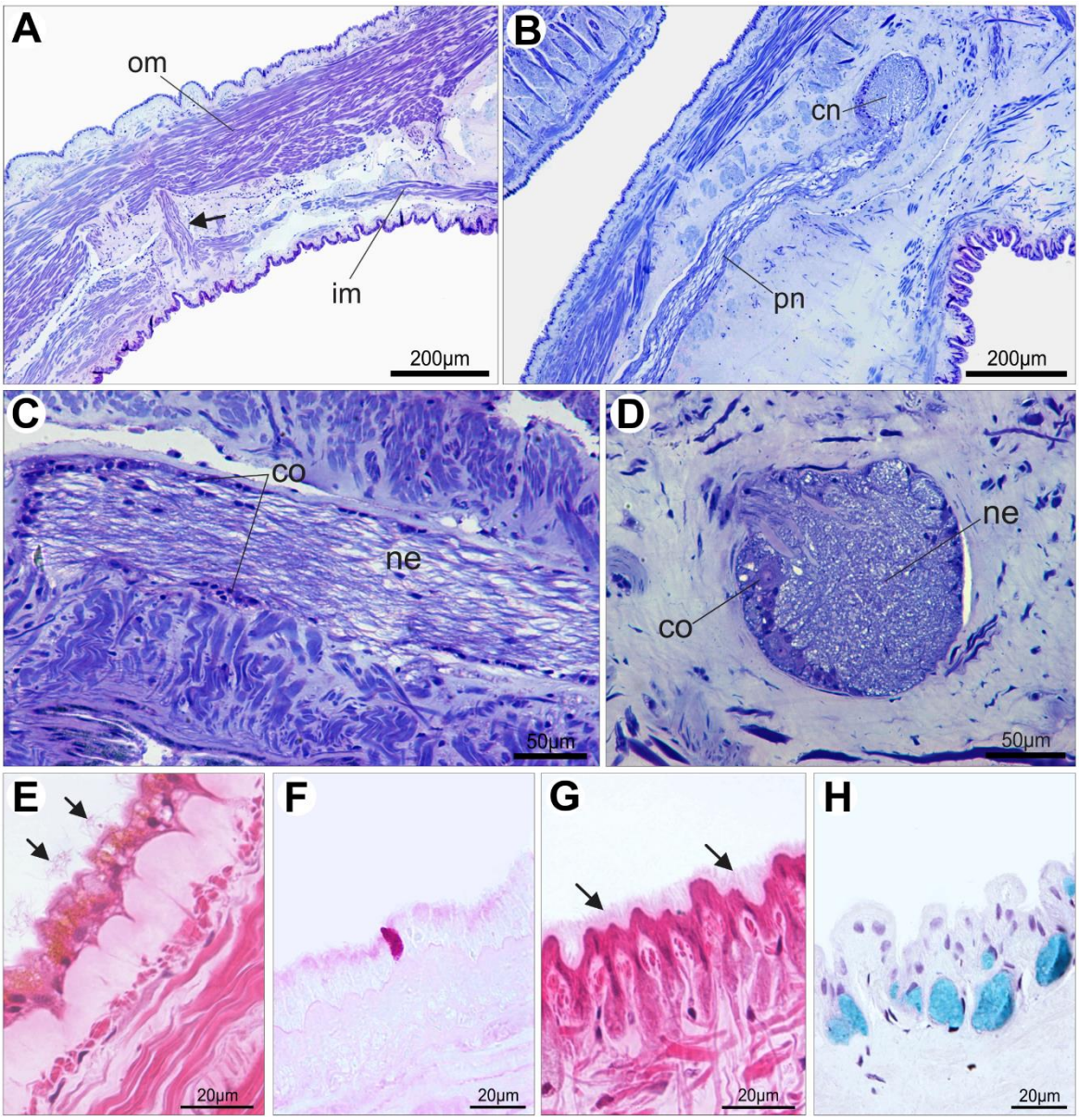

Figure 15. Histology of the adult mantle close to the folded margin. **A.** Adult pallial musculature composed of radial bundles (divided into outer and inner groups), and transversal bundles (arrow). TB. **B.** Long pallial nerve connecting to the circum-pallial nerve. TB. **C.** Longitudinal section of the circum-pallial nerve with cortical cellular bodies at the periphery. TB. **D.** Detail of a cross section through the circum-pallial nerve showing the cortical region and neuropil. TB. **E.** Inner mantle epithelium with cells bearing tufts of cilia (arrows). HE. **F.** Detail of neutral mucopolysaccharide secretion in a gland cell from the inner mantle epithelium. PAS. **G.** Outer mantle epithelium with long microvilli (arrows) covering the surface. HE. **H.** Subepithelial secretory cells with acid mucopolysaccharide content in the outer mantle epithelium. AB. Abbreviations: *cn*, circum-pallial nerve; *co*, cortex; *im*, inner radial muscles; *ne*, neuropil; *om*, outer radial muscles; *pn*, pallial nerve.

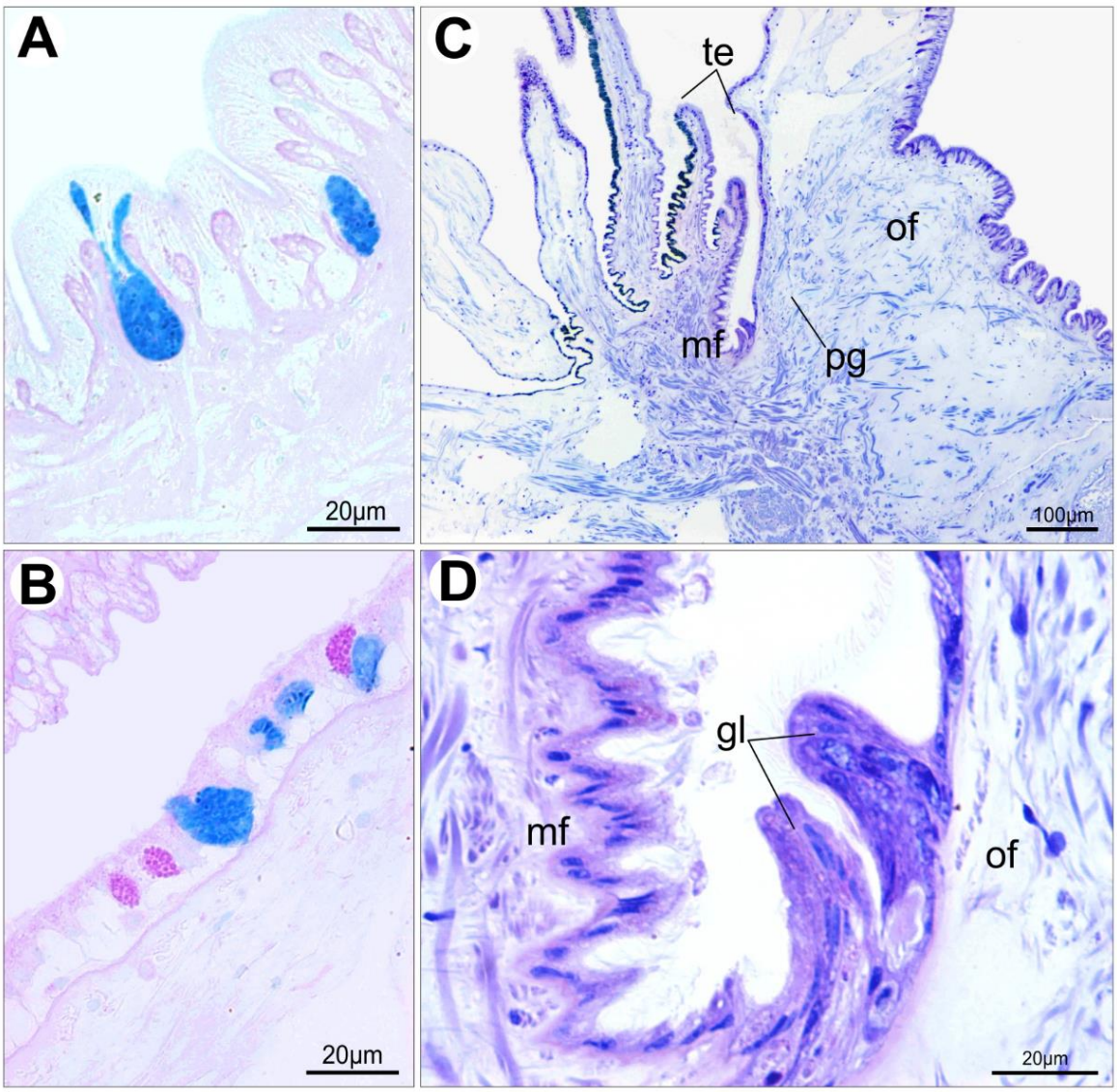

Figure 16. Histology of the outer mantle fold and periostracal groove of adult *N. nodosus*. **A.** Acid mucopolysaccharide-secreting cells spread over the outer mantle epithelium. AB. **B.** Two distinct types of secretory cells in the inner surface of the outer mantle fold: Alcian Blue-positive (acid mucopolysaccharides, AMS) and PAS-positive (neutral mucopolysaccharides; magenta color). **C.** Overview from the periostracal groove region, located between the middle and outer mantle folds. TB. **D.** Detail of the periostracal groove, with two glandular folds responsible for periostracum secretion at the bottom of the groove. TB. Abbreviations: *mf*, middle mantle fold; *gl*, periostracal glands; *of*, outer mantle fold; *pg*, periostracal groove; *te*, tentacles.

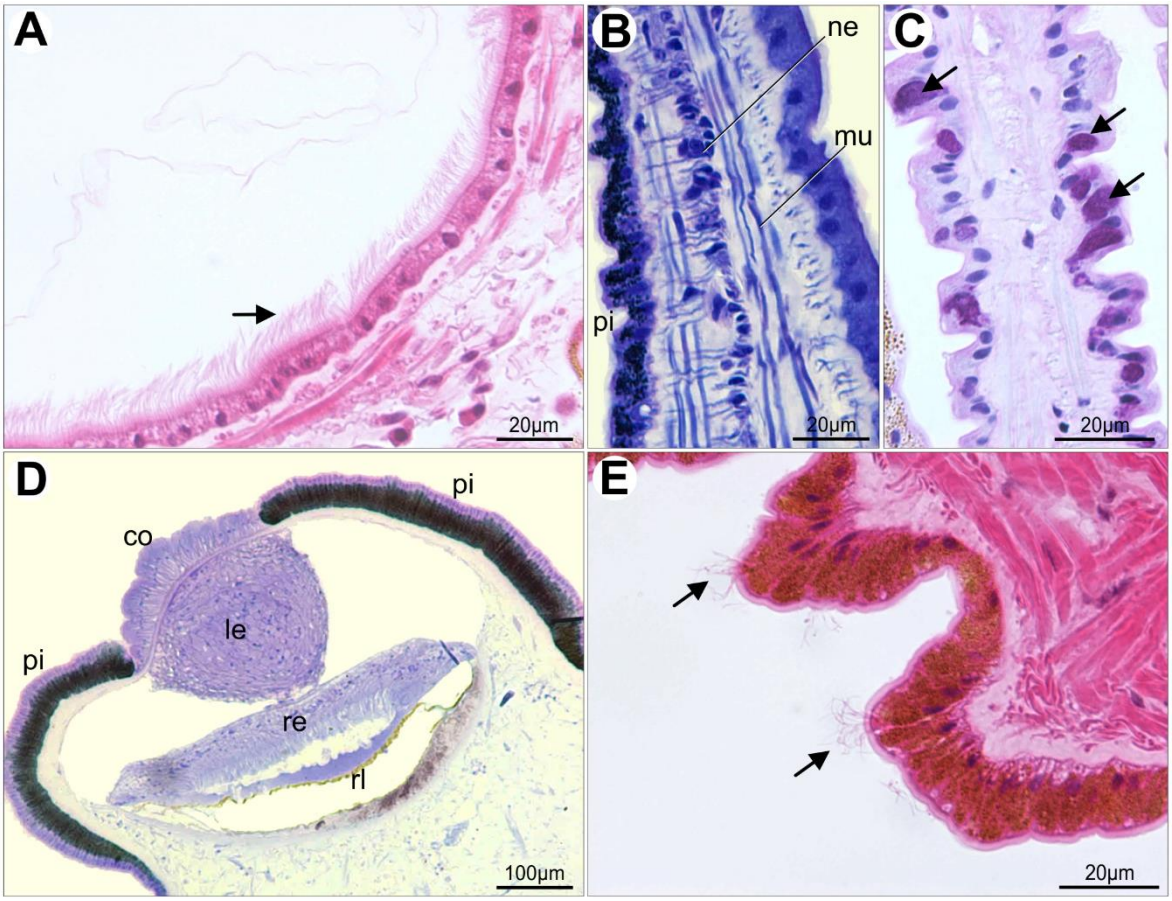

Figure 17. Histology of the middle and inner mantle folds of adult *N. nodosus*. **A.** Ciliated epithelium (arrow) of the outer surface of the middle mantle fold. HE. **B.** Middle fold tentacle displaying muscle and nervous fibers, and a pigmented epithelium. TB. **C.** PAS-positive mucous cells (arrows) in the middle fold tentacle. **D.** Section through the pallial eyes showing its basic structure. TB. **E.** Outer epithelium of the inner mantle fold with sparse tufts of cilia (arrows). HE. Abbreviations: *co*, cornea; *le*, lens; *mu*, muscles; *ne*, nerve; *pi*, pigmented epithelium; *re*, retina; *rl*, reflector layer.

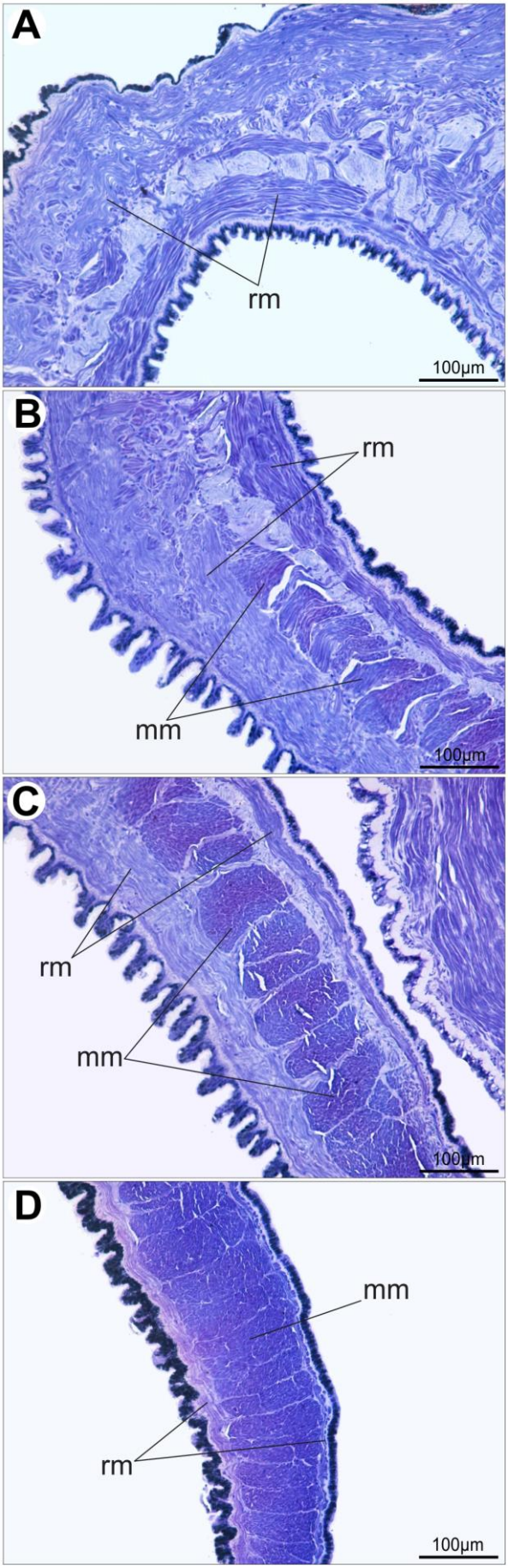

Figure 18. Sequence of sections through the inner mantle fold of *N. nodosus*, from the proximal to the distal region, showing gradual increase in margin-parallel musculature towards the distal region. TB. **A.** Radial muscles from the mantle extends into the inner fold's base. **B.** At intermediate levels of the inner fold, there is a combination of radial and margin-parallel muscles. **C.** Towards the margin, the connective tissue is reduced and margin parallel muscles increase in volume. **D.** At the distal region, the radial muscles are compressed and margin parallel muscles predominate. Abbreviations: *rm*, radial muscles; *mm*, margin parallel muscles.

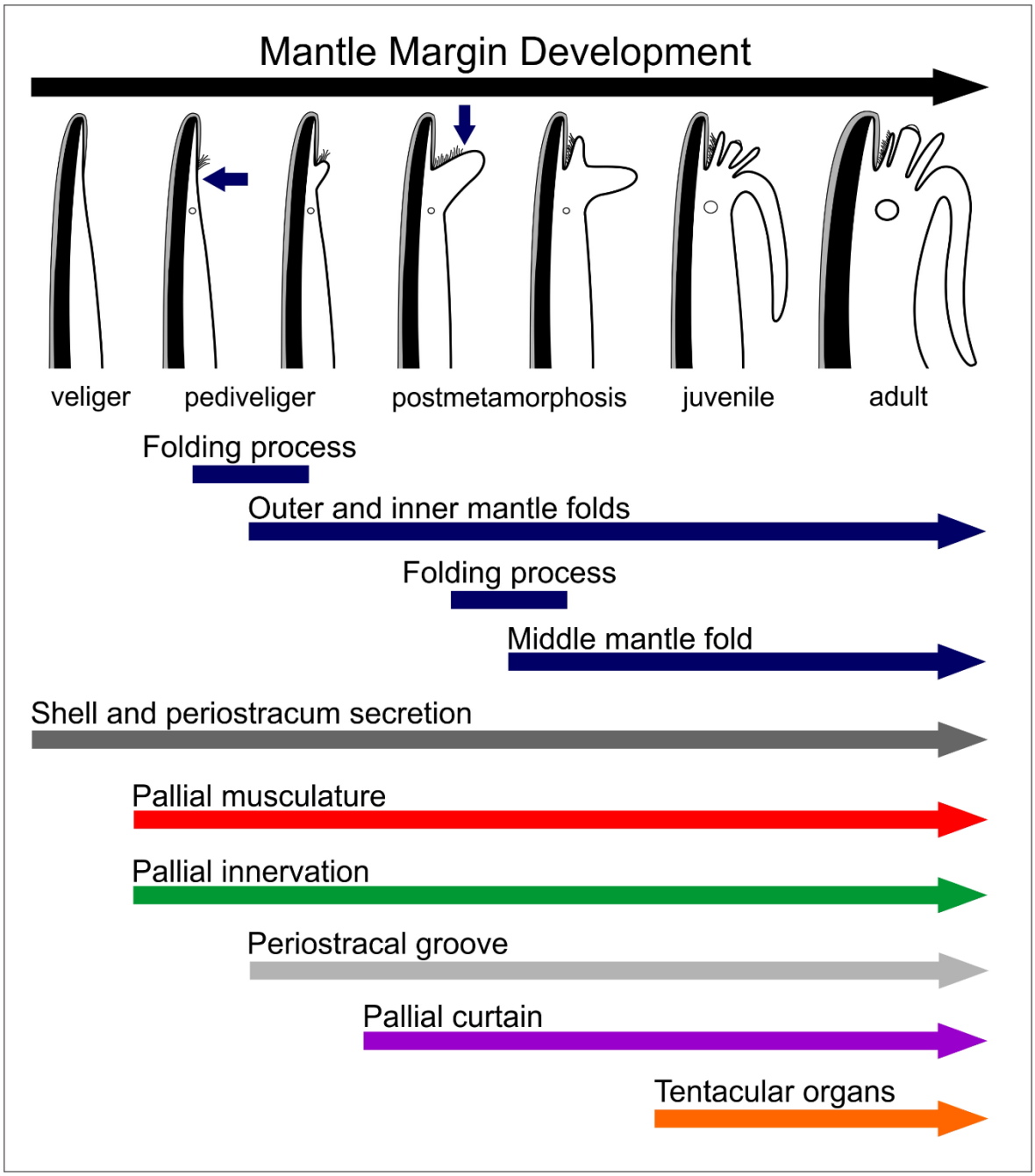

Figure 19. Schematic representation of the development of the mantle margin in *N. nodosus*, illustrating the developmental hypothesis to explain morphogenesis of the mantle folds. Processes are depicted in different colors for the same developmental sequence; blue arrows on the drawings indicate the point of origin of the inner and middle folds. Two folding processes are responsible for mantle fold formation (blue). The first one occurs in late pediveligers, forming the inner and outer mantle folds. After this process, the periostracum forming zone ends up situated between the folds, within a groove. The second folding process takes place after metamorphosis, originating the middle mantle fold from the inner surface of the inner fold. Periostracum and shell secretion (grey) is continuous throughout development since the formation of shell field in trocophores. Both muscle (red) and nervous systems (green) are initially formed by the early pediveliger stage, and further modifications are involved during folds' development. See text for more information.

CHAPTER 2

ANATOMY OF TENTACULAR ORGANS FROM THE MANTLE MARGIN OF THE SCALLOP *NODIPECTEN NODOSUS* (BIVALVIA: PECTINIDAE)

CAPÍTULO 2

ANATOMIA DOS ÓRGÃOS TENTACULARES DO MANTO DE *NODIPECTEN NODOSUS* (BIVALVIA: PECTINIDAE)

RESUMO

Órgãos tentaculares abrangem ampla variação de projeções corporais que desempenham funções especializadas em diversos filos de invertebrados. Em moluscos bivalves, tentáculos presentes na margem do manto possuem funções sensoriais e secretoras envolvidas na detecção de predadores e interação com o ambiente externo. Contudo, a diversidade morfológica, anatomia detalhada e papéis de tais estruturas foram pouco investigadas nesses moluscos. Bivalves da família Pectinidae são de amplo interesse neste contexto devido à sua diversidade de tentáculos paliais, incluindo distintos tipos tentaculares formados em diferentes pregas paliais, e até mesmo tentáculos portadores de olhos. Por meio de técnicas combinadas de microscopia, o presente estudo investigou a anatomia dos órgãos tentaculares em estádios pós-metamórficos da vieira *Nodipecten nodosus* (Linnaeus, 1758). Os tentáculos na espécie são formados após a metamorfose e, com exceção da pigmentação, eles crescem sem profundas modificações morfológicas. Os órgãos tentaculares de *N. nodosus* compreendem olhos paliais (tentáculos modificados), tentáculos curtos e longos da prega palial mediana e tentáculos da prega interna. Apesar dos todos os tipos mencionados possuírem uma mesma estrutura básica formada por epitélio ciliado, músculos periféricos e nervo central imerso em tecido conjuntivo, eles exibem acentuadas diferenças quanto à distribuição de cílios, atividade secretora epitelial e tipo de fibra muscular. A distribuição ciliar na extremidade distal de papilas sensoriais representa uma condição única dos longos tentáculos da prega mediana, e a secreção de muco é restrita aos tentáculos desta prega (com exceção dos pedúnculos oculares). Notavelmente, tentáculos da prega interna e da prega mediana exibem fibras musculares estriadas e não-estriadas, respectivamente. Finalmente, os dados obtidos são discutidos à luz da anatomia funcional da margem palial em bivalves.

Palavras-chave: bivalves, cílios, órgãos sensoriais, secreção, tentáculo.

O manuscrito a seguir contendo as informações detalhadas será submetido ao periódico internacional *Zoologischer Anzeiger*.

CHAPTER 2

ANATOMY OF TENTACULAR ORGANS FROM THE MANTLE MARGIN OF THE SCALLOP *NODIPECTEN NODOSUS* (BIVALVIA: PECTINIDAE)

ABSTRACT

Tentacular organs comprise a variety of body projections that play specialized functions in several invertebrate phyla. In bivalve molluscs, tentacles present on the mantle margin serve sensorial and secretory functions involved in predator detection and interactions with the surrounding environment. Nevertheless, their morphological diversity, detailed anatomy and functional roles have been scarcely investigated in these molluscs. Bivalves from the family Pectinidae are of particular interest in this context given the diversity of pallial tentacles, including distinct tentacle types arising on different mantle folds, and even eye-bearing tentacles. Combining several microscopy techniques, the present study investigated the anatomy of tentacular organs in postmetamorphic stages (juveniles and adults) of the scallop *Nodipecten nodosus* (Linnaeus, 1758). Scallop tentacles are formed shortly after metamorphosis, and except for pigmentation, they grow with no major morphological modifications. Tentacular organs of *N. nodosus* comprise eye-bearing and short and long tentacles from the middle mantle fold, and velar tentacles from the inner fold. Although all tentacle types share a common basic structure formed by a ciliated epithelium, peripheral muscle bundles, and a central nerve embedded in connective tissue, they exhibit marked differences in ciliary distribution, epithelial secretory activity, and type of muscle fibers. Cilia distribution at the distal tip of sensory papillae represents a unique condition for the long tentacles from the middle mantle fold, and mucous secretion is restricted to the middle fold tentacles (except for eyestalks). Strikingly, velar tentacles and middle fold tentacles exhibit striated and non-striated myofibers, respectively. These data are discussed in light of the functional anatomy of the bivalve mantle margin.

Keywords: bivalves, cilia, secretion, sensorial organs, tentacle

INTRODUCTION

Involved in a plethora of functions, tentacular organs are widespread in invertebrates, displaying extensive morphological diversity. For instance, oral tentacles play vital roles in protection and food acquisition in cnidarians and bryozoans, and ciliated tentacles and palps are commonly present in suspension- and deposit-feeder Polychaeta (*e.g.*, Gilmour, 1978; Dauer, 1985; Nielsen and Riisgard, 1998; Shimizu and Hiroshi Namikawa, 2009). In Mollusca, the cephalic and buccal tentacles found in gastropods are probably the most familiar example of tentacular organs (Künz and Haszprunar, 2001). Nevertheless, bivalve molluscs exhibit great diversity of tentacles on their mantle margin, usually associated with sensorial functions, but they have been scarcely explored so far.

Sensorial organs in Mollusca comprise not only complex structures, such as the camera-type eyes of cephalopods and rinophores of opistobranch gastropods (Young, 1971; Wertz *et al.*, 2006; Serb, 2008), but also a wide range of sensorial structures in bivalves, associated with the mantle margin, and acting as mechano-, chemo- or photoreceptors. Whereas some sense organs are sheltered inside the pallial cavity, *e.g.*, the abdominal organ of pteriomorphian bivalves (Haszprunar, 1985), pallial tentacles are exposed structures in direct contact with the surrounding environment (Yonge, 1983). Photoreceptive organs have independently evolved in tentacular organs of several unrelated Bivalvia taxa (Morton, 2008; Serb and Eernisse, 2008). Eye-bearing tentacles are known to perform a variety of optical tasks, usually associated to shadow or movement detection with consequent siphonal retraction, shell closure or escape response (Morton, 2008; Serb, 2008). Chemo- and mechanoreceptors are commonly found in the pallial epithelium of bivalves, also usually concentrated in the pallial tentacles (Fishelson, 2000). In fused mantle margins, tentacular structures are typically associated with the exhalant and inhalant apertures of siphons (*e.g.*, Moueza and Frenkiel, 1974; Fishelson, 2000; Sartori *et al.*, 2008). Besides sensorial functions, these siphonal tentacles prevent the entrance of large particles into the siphonal channel (Narchi, 1972; Hodgson and Fielden, 1984; Passos and Domaneschi, 2004). In a few species of razor clams (*e.g.*, *Solen dactylus*), a pair of extensible pallial tentacles is located at the dorsal end of the anterior pallial region, between the inner and middle folds, but their function is unknown (Saeedi *et al.*, 2013). In bivalves with free mantle edges, pallial tentacles may occur along its entire extension, but they exhibit considerable diversity in structure and distribution. In pinnids, for example, neither tentacles nor sensory structures occur on the middle mantle fold, but

in *Pinna carnea*, a row of short tentacles occur at the posterior region of the prominent inner fold (Yonge, 1957). In oysters and most pteriids, short tentacles are regularly distributed in both middle and inner folds, including branched tentacles in *Pinctada imbricata* (Waller, 1976; Tëmkin, 2006). Galeommatoid bivalves bear conspicuous pallial projections (Lützen and Nielsen, 2005), ranging from sensory ciliated papillae, like those observed on the middle mantle fold of *Mysella charcoti* (Passos *et al.*, 2005), to specialized tentacles, as reported for *Galeomma layardi* (Morton, 1973). In addition, secretory activity has been extensively reported for bivalve tentacles, representing an important aspect in the anatomy and roles of such structures (Yonge, 1983). Bivalves from the family Limidae are notorious by the presence of long and extensible tentacles, unable to be enclosed within the valves (Gilmour, 1967). Secretory cells are largely distributed in those organs, and associated with autotomy and release of distasteful mucous to avoid predators (Owen and McCrae, 1979).

Bivalves from the family Pectinidae (*i.e.*, scallops) have an enlarged mantle margin notable by its complex structures. Numerous tentacles are distributed along the entire margin, being divided into two categories: tentacles from the middle mantle fold and tentacles from the inner fold (Dakin, 1909). The first type includes numerous tentacles, some of them being differentiated into pallial eyes at irregular intervals. The second type consists of short tentacles located at the edge of the pallial curtain, *i.e.*, the inner mantle fold (or velum). Scallop tentacles are known to be involved in the perception of the substrate and detection of predators by mechanical and chemical stimulation, with subsequent triggering of behavioral responses (Wilkens, 2006). Considering the extensive diversity of tentacular form and function exhibited by pectinids, scallops may represent suitable models to investigations on tentacle structure and function in bivalves. Therefore, we investigated tentacle anatomy of the scallop *Nodipecten nodosus* (Linnaeus, 1758) to explore anatomical variation in tentacle types of Pectinidae, and to gain insight into their possible functions. Given the plethora of studies focused on the scallop optical system and eye anatomy, in the case of eye-bearing tentacles we restricted our analysis to the eyestalk.

MATERIAL AND METHODS

Specimens of *Nodipecten nodosus* were obtained in the scallop farm *Institute of Eco-Development from Baía de Ilha Grande* (IED-BIG), Rio de Janeiro, Brazil. Fragments from the mantle margin were removed from juvenile and adult individuals. Small scallop

juveniles (about 4 mm length) were removed from artificial tanks, while large mature individuals (about 8 cm length) were collected from artificial baskets. Prior to fixation, animals were anesthetized for 2 hours by gradual addition of drops of 7.5% $MgCl_2$. General morphology was observed using a Zeiss Stemi 2000-C stereomicroscope, and images were captured using a Zeiss AxioCam MRc. Digital microscopy images were further processed using Photoshop CS3 (Adobe Systems, USA) to adjust contrast and brightness, and drawings were created using Corel Draw X5 (Corel Corporation, Canada).

Histology. Specimens were fixed for 3 hours at 4 °C in a modified Karnovsky solution (2% paraformaldehyde + 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer at pH 7.4 and 1000 mosm adjusted with sucrose). Then, pallial margin fragments were dehydrated in a graded ethanol series and embedded in glycol-methacrylate resin (Leica Historesin Kit). Serial 3 μm sections were obtained using a Leica RM2255 microtome and stained with hematoxylin and eosin (HE) or toluidine blue and acid fuchsin (TB). Histochemical procedures were conducted with Alcian Blue (AB) and Periodic Acid-Schiff (PAS) staining. Digital images were captured using a Nikon eclipse 80i microscope equipped with a Nikon DS-Ri1 camera.

Scanning Electron Microscopy (SEM). After fixation in the modified Karnovsky solution, post-fixation was performed for 30 minutes in 1% OsO_4 in buffer solution (sodium cacodylate buffer at pH 7.4), followed by 15 minutes in 1% tannic acid in buffer solution, and more 15 minutes in new solution of 1% OsO_4 at 4°C. After complete dehydration in graded ethanol series, samples were critical point dried using CO_2 as a transitional fluid in a Balzers CPD 030, mounted on stubs, coated with gold in a Balzers SCD 050 sputter coater, and observed in a Zeiss DSM 940.

Immunocytochemistry, Confocal Laser Scanning Microscopy and 3D Reconstruction. Juvenile specimens were fixed in 4% paraformaldehyde in 0.1 M phosphate buffered (PB) for 1 hour, followed by four rinses with buffer solution. Individuals were dissected in order to remove small fragments of the mantle margin for Confocal Laser Scanning Microscopy (CLSM). For F-actin staining, specimens were permeabilized in PBS containing 2% Triton-X 100 (PBT) overnight and then incubated in a 1:40 dilution of Alexa Fluor 488 Phalloidin (Molecular Probes) in PBT, for 24 hours at room temperature, in the dark. For neuronal staining, samples were incubated in 6% normal goat serum in PBT (block-PBT) overnight at room temperature. Subsequently, primary antibodies, *i.e.*, anti-serotonin raised in rabbit and anti-α-tubulin raised in mouse, were applied at a concentration of 1:400 in block-PBT for 24 hours. Then, specimens were

rinsed several times in block-PBT prior to application of a secondary fluorochrome-conjugated antibody (goat anti-rabbit Alexa Fluor 488 and goat anti-mouse Alexa Flour 633, Molecular Probes) in block-PBT at a concentration of 1:200 for 24 hours in the dark. Nuclei were stained by adding a 1 µl drop of 4', 6-diamidino-2-phenylindole (DAPI) (Invitrogen, 3 µg mL^{-1}) in conjunction with secondary antibody or Phalloidin incubation. Then, samples were washed three times in PBS for about 30 minutes and mounted in Fluoromount G (Southern-Biotech, Birmingham, Alabama) on standard microscope slides. Analysis and image acquisition were performed on a Leica TCS SP5 II confocal laser scanning microscope equipped with the software Leica Application Suite Advanced Fluorescence (LAS AF), Version 2.6.0 (Leica Microsystems, Wetzlar, Germany). Confocal image stacks were recorded with 0.3 µm step size along the z-axis and digitally merged as maximum intensity projections. 3D reconstructions were created from selected image stacks using the software Imaris Version 4.1 (Bitplane, Switzerland).

RESULTS

Tentacle organs emerge as conspicuous, translucent projections of the middle and inner mantle folds in postmetamorphic juveniles of *Nodipecten nodosus* (Fig. 1A). Whereas middle fold tentacles are generally elongated with tapered ends, the eye-bearing ones are short and differentiate their distal regions into an ocular apparatus (Fig. 1B). The inner fold tentacles consist of short projections at the edge of the inner mantle fold (Fig. 1B). From the early emergence of the juvenile stage until the adult stage, tentacle anatomy remains similar throughout development, except for the increase in size, muscular density and pigmentation (Fig. 1B).

Middle mantle fold eyestalks. Pallial eyes are easily noticeable by their blue pigmentation in *N. nodosus* (Fig. 1B). While distal optic components comprise unique structures in scallop's anatomy, the eyestalk shares great similarity with adjacent middle-fold tentacles. The stalk is a short peduncle filled by connective tissue and a pair of longitudinal muscle bundles (Fig. 2A). The epithelium ranges from cuboidal to columnar cells, usually pigmented (Fig. 2A) and covered by microvilli (Fig. 2C), but without gland cells. The distal epithelium exhibits scarce tufts of cilia (Fig. 2B), while the proximal surface of the eyestalk is more densely ciliated, with several sparse ciliary tufts (Fig. 2D).

Middle mantle fold tentacles. Middle fold tentacles can be divided into two types: long and short. Long tentacles are extensible projections occurring at irregular intervals

along the middle mantle fold (Fig. 1A, B; 3A, B). These tentacles slowly extend to interact with the surrounding environment, and can rapidly contract when the animal is disturbed. The long tentacle's epithelium displays a gradual increase in ciliary density from proximal to distal regions (Fig. 3B). In the distal third of the tentacle, tufts of cilia occur at the tip of papillary projections (Fig. 3C). Ciliary tufts are also found interspersed between these projections (Fig. 3C). At the proximal surface, however, ciliary tufts are fewer and sparsely distributed (Fig. 3D). Short tentacles (Fig. 1B, 3E) are far more numerous and their length slightly exceeds the eye's stalk length. In this case, cilia are organized in dense tufts distributed over the entire tentacular surface (Fig. 3F).

Long and short tentacles share the same general histology. They bear several thin muscles and a central, branched nerve embedded within the connective tissue (Fig. 3G). Their epithelium have numerous secretory cells with large basal content with strong affinity for Alcian blue and PAS, which suggests intense acid mucopolysaccharide secretion (Fig. 3H, I). Both tentacle types are also innervated by long projections from the circum-pallial nerve. Tentacular nerves exhibit strong serotonergic (Fig. 4A, C) and α-tubulin (Fig. 4B, C) immunoreactivity, which permitted tracing their trajectory from the circum-pallial nerve at the mantle margin to the tentacular tip. The tentacular nerve is directly connected to the ciliated cells from the epithelium by thin neuronal projections, as revealed by α-tubulin and serotonin markers (Fig. 4A-C). Tentacular muscles originate from the pallial muscular system associated with the mantle margin (Fig. 5A). Surrounding the nervous axis, numerous bundles of longitudinal muscles run along the entire tentacle extension (Fig. 5A, B). Additionally, short transversal fibers are present as well (Fig. 5B). Both longitudinal and transverse muscle bundles in the middle fold tentacles are formed by non-striated myofibers (Fig. 5B).

Inner mantle fold tentacles. The tentacles distributed along the edge of the inner fold (Fig. 1B) are also referred to as "velar" or "guard tentacles" (*e.g.*, Beninger and Le Pennec, 2006). They are pigmented projections, of which general structure resembles that of the short middle fold tentacles, including a ciliated epithelium, and a central nerve and muscles embedded within the connective tissue (Fig. 3J). In addition, similarly to them, innervation exhibits strong serotonin and α-tubulin signals, with tentacular nerves passing through the inner fold from the circum-pallial nerve towards the velar tentacle distal tip (Fig. 4D, E). Nevertheless, there are some key differences between the tentacles from both folds. In velar tentacles, cilia are present in less dense and more sparsely distributed tufts than in the short tentacles of the middle fold (Fig. 3K). More strikingly, the velar tentacle

musculature is formed mainly by longitudinal fibers composed of striated myofibers, in contrast to the non-striated muscular structure exhibited by middle fold tentacles (Fig. 5C). Another important difference concerns the lack of secretory activity in velar tentacles, not detected by any staining method applied.

DISCUSSION

Tentacular organs of *N. nodosus* comprise eye-bearing and short and long tentacles from the middle mantle fold, and velar tentacles from the inner fold. While short tentacles are regularly present in the pectinid middle pallial fold, the extensible, long tentacles observed in *N. nodosus* were previously detected in other scallops, such as *Pecten maximus* (Dakin, 1909), although they were not reported for either *Argopecten irradians* (Gutsell, 1931) or *Placopecten magellanicus* (Drew, 1906). Such difference is intriguing, and deserves further studies to evaluate tentacle type and distribution in Pectinidae to understand functional roles and variation. Velar tentacles, similar to those described herein, are apparently more common, occurring on the edge of the inner mantle fold of *A. irradians* (Gutsell, 1931) and *P. magellanicus* (Moir, 1977). In Spondylidae, cemented bivalves phylogenetic close to Pectinidae (Waller, 2006), the mantle margin exhibits great similarity to the general pectinid condition, including the presence of pallial eyes, short tentacles and a pigmented velum. Nevertheless, neither long tentacles nor a row of velar tentacles are present (Dakin 1928; Viana and Rocha-Barreira, 2007). Short tentacles also occur on both middle and inner mantle folds of Ostreidae and Pteriidae (Tëmkin, 2006), which apparently suggests a convergent condition to tentacle organization in Pectinidae. Notwithstanding, tentacle variation and distribution is still poorly understood in more inclusive bivalve groups, so speculations on tentacle evolution should be treated with caution at this time.

Eye-bearing tentacles are not exclusive of Pectinidae, also occurring in the phylogenetically distant families Cardiidae and Laternulidae (Adal and Morton, 1973). The eyestalk of *Cerastoderma edule* shares great similarity with the scallop eyestalk, including an epithelium covered by microvilli, peripheral muscle bands and a central nerve (Barber and Wright, 1969). In contrast to the ciliated epithelium of scallop pallial eyestalks, ciliated receptor cells of *C. eduli* and *Cochlodesma praetenue* are restricted to the tip of the eye-bearing tentacles (Barber and Wright, 1969, Morton, 2008). Even though mechanisms of tentacle differentiation into pallial eyes have not been determined, the convergent

evolution of optic apparatus in tentacles highlights the potential for diversification and the high plasticity of tentacular organs in the Bivalvia.

The general anatomy of the scallop tentacle consists of a tentacular nerve surrounded by muscles and connective tissue. Tentacle extension is generated by hydrostatic pressure from the haemolymph, while retraction is produced by contraction of longitudinal muscles (Beninger and Le Pennec, 2006). To our knowledge, the present study is the first to detect differences in tentacle muscle types between middle and inner mantle folds. Whereas tentacles from the middle pallial fold of *N. nodosus* are formed by non-striated myofibers, velar tentacles possess striated fibers, similar to those observed in the musculature from the inner fold.

In a thorough description of central and peripheral nervous systems in bivalves, Duvernoy (1853) identified thin nerves from the circum-pallial nerve of *P. maximus* that reach tentacle structures of the middle mantle fold. According to the same author, those organs were named “tactile” and “visual pedicles”, due to their supposed sensory functions. The same innervation pattern was reported for *P. magellanicus* by Drew (1906). Dakin (1909) assumed that ciliated cells occurring on the epithelia of short and long tentacles of *P. maximus* were receptors, emphasizing the sensory role of such pallial structures. Serotonergic innervation was detected in the present study, confirming nervous activity in tentacles from the middle and inner folds, and a close connection of epithelial cilia and nerves by slender neuronal projections. Even though neuronal or muscular markers (combined with confocal microscopy) have never been used in studies with adult bivalves, this technique has already demonstrated great potential in understanding nervous organization in other molluscan taxa (*e.g.*, Wertz *et al.*, 2006; Wollesen *et al.*, 2009; Byern *et al.*, 2012). Consequently, our results represent a successful example applying such method to anatomical investigations in bivalves, especially concerning muscular and sensory organs.

The ultrastructure of the ciliated receptor cells spread over scallop tentacles have been studied in detail by Moir (1977). The long tentacles of *P. magellanicus* have their distal third organized in ciliated papillae, which radiate out from the central column (Moir, 1977). Similarly to *N. nodosus*, ciliary tufts are located on the distal tip of these papillae, where they are supposed to act as mechanoreceptors, while another ciliated cell type is present between the papillae (Moir, 1977). At the tentacle proximal region, ciliated groups of cell are irregularly spread over the surface and their function is still debatable (Moir, 1977, present study). In the mussel *Brachidontes pharaonis*, sensory ciliary tufts, similar to

those present in the short and velar tentacles of *N. nodosus*, are spread among short kinetic cilia in short tentacles (Fishelson, 2000). In infaunal bivalves, *e.g.*, from the families Veneridae and Donacidae, sensorial cilia are commonly distributed on siphonal tentacles, but the dimension, structure and number of cilia are remarkably different from ciliary types observed in scallops (Hodgson and Fielden 1984; Ansell *et al.*, 1999; Fishelson, 2000). Tentacle ciliated receptors have also been analyzed in detail in *Limaria hians*, in which different ciliary tufts are in close association with gland cells, acting as chemo- and mechanoreceptors (Owen and McCrae, 1979). In contrast to limid bivalves, no evidence has been reported in *N. nodosus* for mucous secretion associated to sensorial reception in tentacles. Sensorial performance of pectinid tentacles has been studied under many behavioral and physiological contexts in attempt to integrate stimulation and mantle margin response (*e.g.*, Gutsell, 1930; Stephens, 1978). These studies demonstrated that tentacle ciliated cells seem to serve as primary sensory receptors for tactile and chemical stimuli, triggering adjusts in pallial curtain movements, animal position, escape behavior and shell closure (Wilkens, 2006).

Our results suggest nervous anatomical similarity between velar tentacles and tentacles from the middle mantle fold in *N. nodosus*, including serotonergic innervation by a central nerve connected to the ciliated epithelial cells. Despite no evidence for differences in sensitivity between velar and long tentacles, Wilkens (2006) argues that both types would be able to detect tactile stimuli, but only the second one would detect substrate suitability and chemical stimulants from predators. Apart from sensorial functions, the secretory activity is clearly distinct between these tentacle types in *N. nodosus*. While short and long tentacles from the middle pallial fold exhibit intense mucous secretion, no secretory cell was detected in eyestalks and velar tentacles. Intense production of high-viscosity acid mucopolyssacharides was reported for the pallial epithelium of *Placopecten magellanicus*, probably facilitating the passage of water over the mantle surface (Beninger and St-Jean, 1997; Beninger and Le Pennec, 2006). Mucous secretion in scallop middle fold tentacles might be associated to lubrication and cleansing of the mantle margin (a role possibly lost during eyestalk evolution), the function of velar tentacles being possibly restricted to tactile detection. In the pearl oyster *Pinctada margaritifera*, the number of cells with affinity to PAS and Alcian blue decreases in the tentacles from the middle mantle fold in contrast to the inner fold (Jabbour-Zahab *et al.*, 1992), a condition also observed in *Cerastoderma edule* (Richardson *et al.*, 1981). Therefore, the abundance of

mucous-secreting cells in the tentacles of *N. nodosus* may represent a particular condition of Pectinidae in respect to the functional anatomy of tentacular organs.

Comparative analyses of tentacular features in bivalves are still scarce, evoking the necessity of more studies to evaluate and test possible homologies and convergent evolution of tentacular traits. Furthermore, investigations on tentacle functional anatomy in the Bivalvia are still necessary to cover structural diversity and to provide substantial evidence to explain tentacle evolution in the group. The present paper should stimulate and serve as a basis for further attempts in understanding tentacular functional morphology and evolution in the Bivalvia.

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REFERENCES

Adal, M.N. and Morton, B. 1973. The fine structure of the pallial eyes of *Laternula truncata* (Bivalvia: Anomalodesmata: Pandoracea). Journal of Zoology 170: 533-556.

Ansell, A.D.; Harvey, R. and Günther, C-P. 1999. Recovery from siphon damage in *Donax vittatus* (Da Costa) (Bivalvia: Donacidae). Journal of Molluscan Studies 65(2): 223-236.

Barber, V.C. and Wright D.E. 1968. The fine structure of the eye and optic tentacle of the mollusc *Cardium edule*. Journal of Ultrastructural Research 34: 515-528.

Beninger P.G. and St-Jean SD. 1997. The role of mucus in particle processing by suspension-feeding marine bivalves: unifying principles. Marine Biology 129: 389-397.

Benninger P.G. and Le Pennec, M. 2006. Structure and function in scallops. .In Shumway, S.E. and Parsons, G.J. (Eds). Scallops: biology, ecology and aquaculture. Amsterdam, Elsevier, pp. 123-227.

Byern, J.; Wani, R.; Schwaha, T.; Grunwald, I. and Cyran, N. 2012. Old and sticky—adhesive mechanisms in the living fossil *Nautilus pompilius* (Mollusca, Cephalopoda). Zoology 115: 1-11.

Dakin, W.J. 1909. *Pecten*. In: Liverpool Marine Biology Committee. Memoirs on typical British marine plants & animals. XVII. London, Williams & Norgate.

Dakin, W. J. 1928.The eyes of *Pecten*, *Spondylus*, *Amussium* and allied Lamellibranchs, with a short discussion on their evolution. Proceedings of the Royal Society of London B 103: 355-365.

Dauer, D.M. 1985. Functional morphology and feeding behavior of *Paraprionospio pinnata* (Polychaeta: Spionidae). Marine Biology 85: 143-151.

Drew, G.A. 1906. The habits, anatomy, and embryology of the giant scallop (*Pecten tenuicostatus*, Mighels). University of Maine.

Duvernoy, G.L. 1853. Mémoires sur le système nerveux des mollusques acéphales lamellibranches ou bivalves. Mémoires de l'Académie des sciences de l'Institut de France 24: 3-210.

Fishelson, L. 2000. Comparative morphology and cytology of siphons and siphonal sensory organs in selected bivalve molluscs. Marine Biology 137: 497-509.

Guilmour, T.H.J. 1963. A note on the tentacles of *Lima hians* (Gmelin) (Bivalvia). Journal of Molluscan Studies 35(2-3): 82-85.

Guilmour, T.H.J. 1967. The defensive adaptations of *Lima hians* (Mollusca, Bivalvia). Journal of the Marine Biological Association of the United Kingdom 47: 209-221.

Guilmour, T.H.J. 1978. Ciliation and function of the food-collecting and waste-rejecting organs of lophophorates. Canadian Journal of Zoology 56(10): 2142-2155.

Gutsell, J.S. 1931. Natural history of the bay scallop. Bulletin of the Bureau of Fisheries (United States Bureau of Fisheries) 46: 569-632.

Haszprunar, G. 1985. The fine structure of the abdominal sense organs of Pteriomorpha (Mollusca, Bivalvia). Journal of Molluscan Studies 51: 315-319.

Hodgson, A.N. and Fielden, L.J. 1984. The structure and distribution of peripheral ciliated receptors in the bivalves molluscs *Donax serra* and *D. sordidus*. Journal of Molluscan Studies 50 (2): 104-112.

Jabbour-Zahab, R.; Chagot, D.; Blanc, F. and Grizel, H. 1992. Mantle histology, histochemistry and ultrastructure of the pearl oyster *Pinctada margaritifera* (L.). Aquatic Living Resources 5: 287-298.

Künz, E. and Haszprunar, G. 2001. Comparative ultrastructure of cephalic tentacles of primitive gastropod clades: Patellogastropoda, Neritaemorphi, and Vetigastropoda. Zoologischer Anzeiger 140(2): 137-165.

Lützen, J. and Nielsen, C. 2005. Galeommatid bivalves from Phuket, Thailand. Zoological Journal of the Linnean Society 144: 261-308.

Moir, A.J.G. 1977. Ultrastructural studies on the ciliated receptors of the long tentacles of the Giant Scallop, *Placopecten magellanicus* (Gmelin). Cell and Tissue Research 184: 367-380.

Morton, B. 1973. The biology and functional morphology of *Galeomma* (Paralepida) *takii* (Bivalvia: Leptonacea). Journal of Zoology 169: 133-150.

Morton, B. 2008. The evolution of eyes in the Bivalvia: new insights. American Malacological Bulletin 26(1-2): 35-45.

Moueza, M. and Frenkiel, L. 1974. Contribution a l'etude des structures palleales des Tellinacea. Morphologie et structure du manteaux de *Donax trunculus* L. Journal of Molluscan Studies 41(1): 1-19.

Narchi, W. 1972. Comparative study of the functional morphology of *Anomalocardia brasiliana* (Gmelin, 1791) and *Tivela mactroides* (Born, 1778) (Bivalvia, Veneridae). Bulletin of Marine Science 22(3): 643-670.

Nielsen, C. and Riisgard, H.U. 1998. Tentacle structure and filter-feeding in *Crisia eburnea* and other cyclostomatous bryozoans, with a review of upstream-collecting mechanisms. Marine Ecology Progress Series 168: 163-186.

Owen, G. and McCrae, J.M. 1979. Sensory cell/gland cell complexes associated with the pallial tentacles of the bivalve *Lima hians* (Gmelin), with a note on specialized cilia on the pallial curtains. Philosophical Transactions of the Royal Society of London B 287/1017: 45-62.

Richardson, C.A.; Runham, N.W. and Crisp, D.J. 1981. A histological and ultrastructural study of the cells of the mantle edge of a marine bivalve, *Cerastoderma edule*. Tissue & Cell 13: 715-730.

Passos, F.D. and Domaneschi, O. 2004. Biologia e anatomia funcional de *Donax gemmula* Morrison (Bivalvia, Donacidae) do litoral de São Paulo, Brasil. Revista Brasileira de Zoologia 21(4): 1017-1032.

Passos, F.D.; Domaneschi, O. and Sartori, A.F. 2005. Biology and functional morphology of the pallial organs of the Antarctic bivalve *Mysella charcoti* (Lamy, 1906) (Galeommatoidea: Lasaeidae). Polar Biology 28: 372-380.

Saeedi, H.; Costello M.J. and Cosel R. 2013. First report of anterior pallial tentacles in *Solen dactylus* (Bivalvia: Solenidae) from the Northern Persian Gulf, Iran. PLoS One 8(5): e63487.

Sartori, A.F.; Printrakoon, C.; Mikkelsen, P. and Bieler, R. 2008. Siphonal structure in the Veneridae (Bivalvia: Heterodonta) with an assessment of its phylogenetic application and a review of venerids of the Gulf of Thailand. The Raffles Bulletin of Zoology 18: 103-125.

Serb, J.M. 2008. Toward developing models to study the disease, ecology, and evolution of the eye in Mollusca. American Malacological Bulletin 26(1/2): 3-18.

Serb, J.M. and Eernisse, D.J. 2008. Charting evolution's trajectory: using molluscan eye diversity to understand parallel and convergent evolution. Evolution Education Outreach 1: 439-447.

Shimizu, H. and Namikawa, H. 2009. The body plan of the cnidarian medusa: distinct differences in positional origins of polyp tentacles and medusa tentacles. Evolution & Development 11(6): 619-621.

Stephens, P.J. 1978. The sensitivity and control of the scallop mantle edge. Journal of Experimental Biology 75: 203-221.

Tëmkin, I. 2006. Morphological perspective on the classification and evolution of Recent Pterioidea (Mollusca: Bivalvia). Zoological Journal of the Linnean Society 148: 253-312.

Viana, M.G. and Rocha-Barreira, C.A. 2007. The sensorial structures of *Spondylus americanus* Hermann, 1781 (Mollusca: Bivalvia, Spondylidae). Brazilian Archives of Biology and Technology 50(5): 815-819.

Waller, T.R. 1976. The behavior and tentacle morphology of pteriomorphian bivalves: a motion-picture study. Bulletin of the American Malacological Union for 1975: 7-13.

Waller, T. R. 2006. Phylogeny of families in the Pectinoidea (Mollusca: Bivalvia): importance of the fossil record. Zoological Journal of the Linnean Society 148: 313–342.

Wertz, A.; Rössler, W.; Obermayer, M. and Bickmeyer, U. 2006. Functional neuroanatomy of the rhinophore of *Aplysia punctata*. Frontiers in Zoology 3: 6.

Wilkens, L.A. 2006. Neurobiology and behaviour of the scallop. In: Shumway, S.E. amd Parsons, G.J. (Eds). Scallops: Biology, Ecology and Aquaculture. Amsterdam, Elsevier.

Wollesen, T.; Loesel, R. and Wanninger, A. 2009. Pygmy squids and giant brains: Mapping the complex cephalopod CNS by phalloidin staining of vibratome sections and whole-mount preparations. Journal of Neuroscience Methods 179: 63-67.

Yonge, C.M. 1957. Mantle fusion in the Lamellibranchia. Pubblicazioni della Stazione Zoologica di Napoli 29: 151-171.

Yonge, C.M. 1983. Symmetries and the role of the mantle margins in the bivalve Mollusca. Malacological Review 16: 1-10.

Young, J.Z. 1971. The anatomy of the nervous system of *Octopus vulgaris*. Oxford, Clarendon Press.

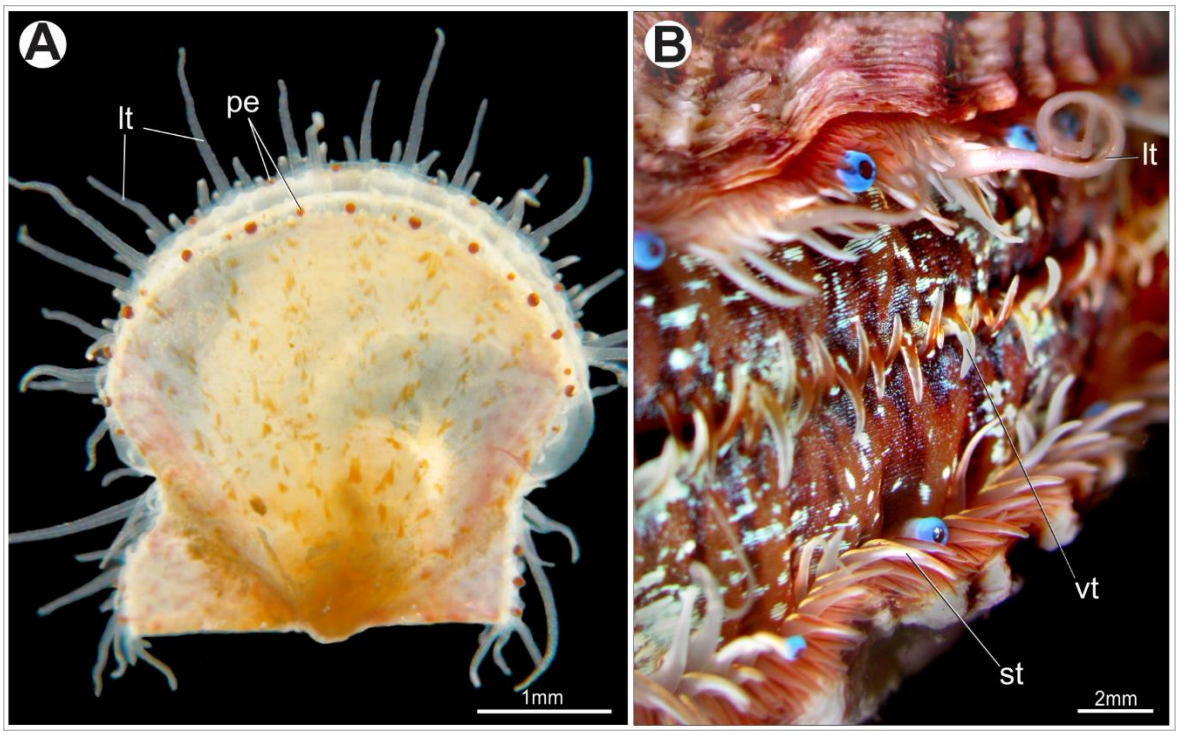

Figure1. *Nodipecten nodosus*. **A.** Postmetamorphic juvenile scallop with extended tentacles from the middle pallial fold. **B.** Detail of adult mantle margin displaying different tentacular organs. Abbreviations: *lt*, long tentacle; *pe*, pallial eye; *st*, short tentacle; *vt*, velar tentacle.

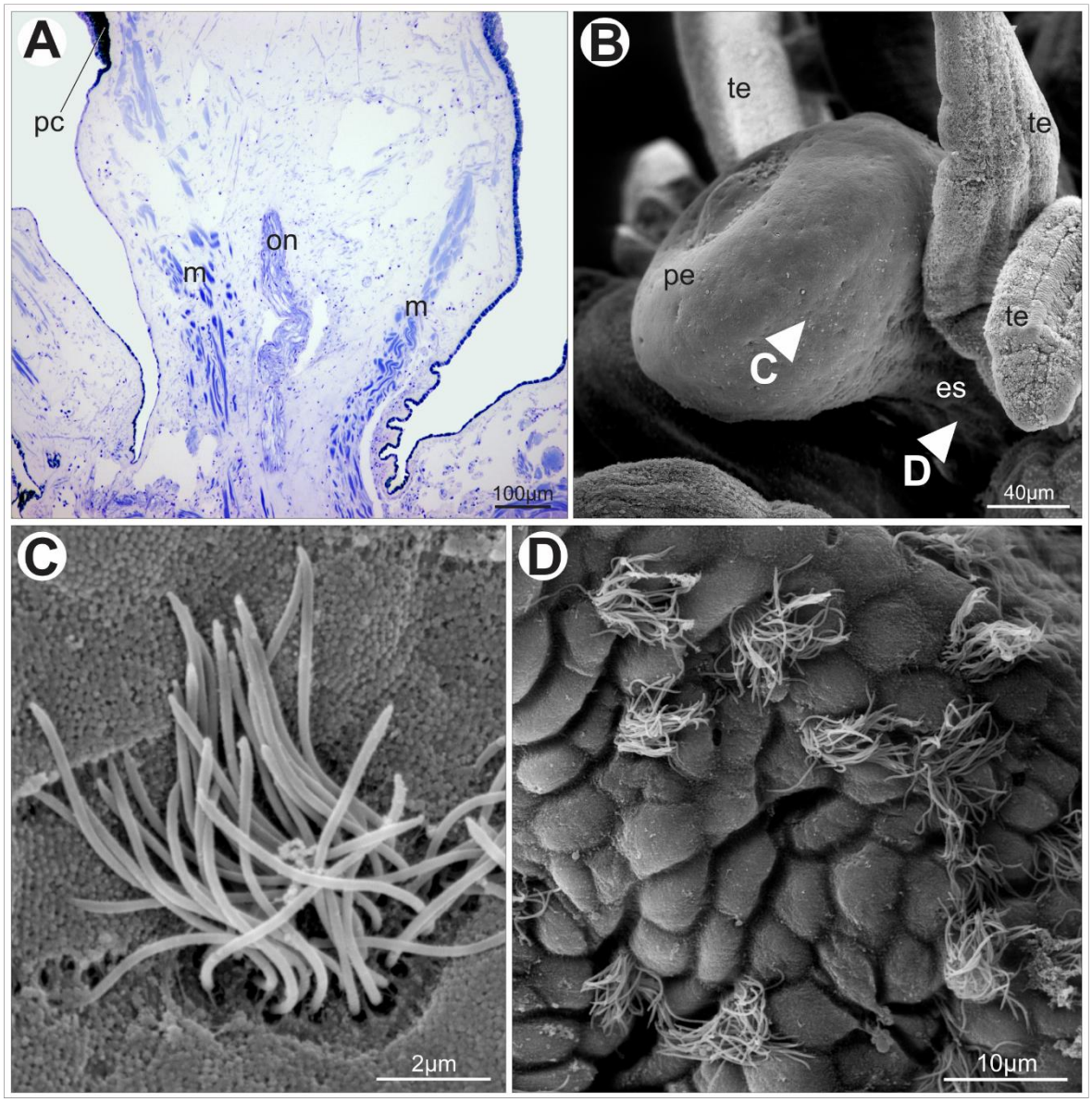

Figure 2. Eye-bearing tentacles from *N. nodosus*. **A.** Longitudinal section through the eyestalk showing internal muscular and nervous components. TB. **B.** General view of the pallial eye as revealed by scanning electron microscopy. **C.** Detail of a ciliary tuft present on the distal eyestalk epithelium of B. **D.** Detail of sparse ciliary tufts on the eyestalk of B. Abbreviations: *es*, eyestalk; *m*, muscle bundles; *on*, optic nerve; *pc*, pigmented epithelial cells; *pe*, pallial eye; *te*, tentacle.

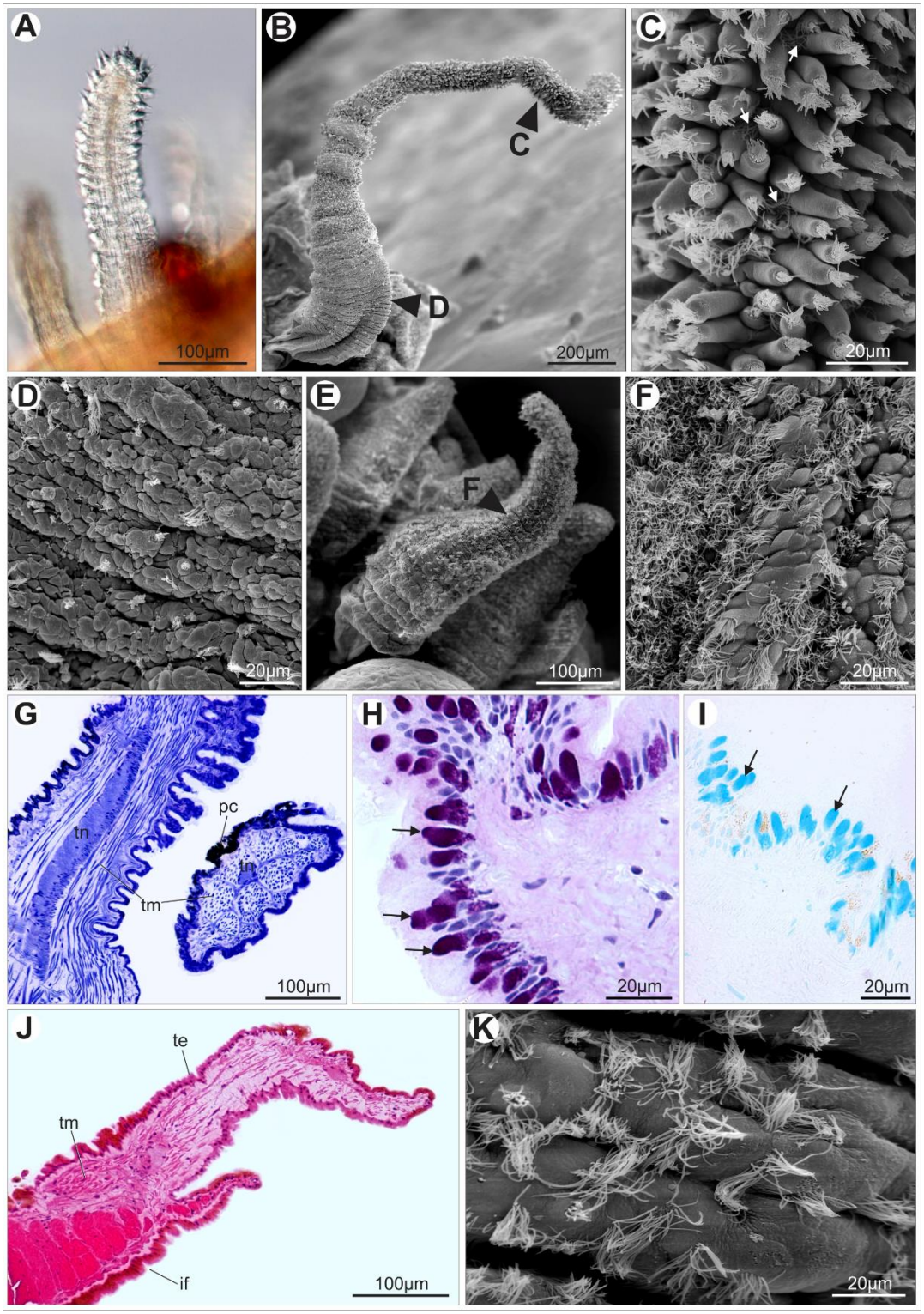

Figure 3. Tentacles from the middle mantle fold (A-I) and inner fold (J-K) of *N. nodosus*. **A.** General view of a long tentacle of a live specimen. **B.** Long tentacle morphology as revealed by scanning electron microscopy. **C.** Detail of the distal tentacular region of B, with cilia located on the tip of papillary projections and few ciliary tufts interspersed between the projections (arrows). **D.** Detail of the proximal tentacular region of B, with sparse ciliary tufts. **E.** Short tentacle morphology. **F.** Detail of the tentacular surface of E, with dense distribution of ciliary tufts. **G.** Longitudinal (left) and transverse (right) sections of short tentacles showing internal components, such as peripheral muscles and a central nerve. TB. **H.** Secretory cells from the tentacular epithelium with PAS-positive content (arrow). **I.** Same secretory cells from H showing affinity also for Alcian blue (acid mucopolysaccharide content; arrow). **J.** General histology of a velar tentacle. HE. **K.** Detail of the velar tentacle surface with sparse ciliary tufts. Abbreviations: *if*, inner mantle fold; *pc*, pigmented epithelial cells; *te*, tentacle epithelium; *tm*, tentacle muscle; *tn*, tentacle nerve.

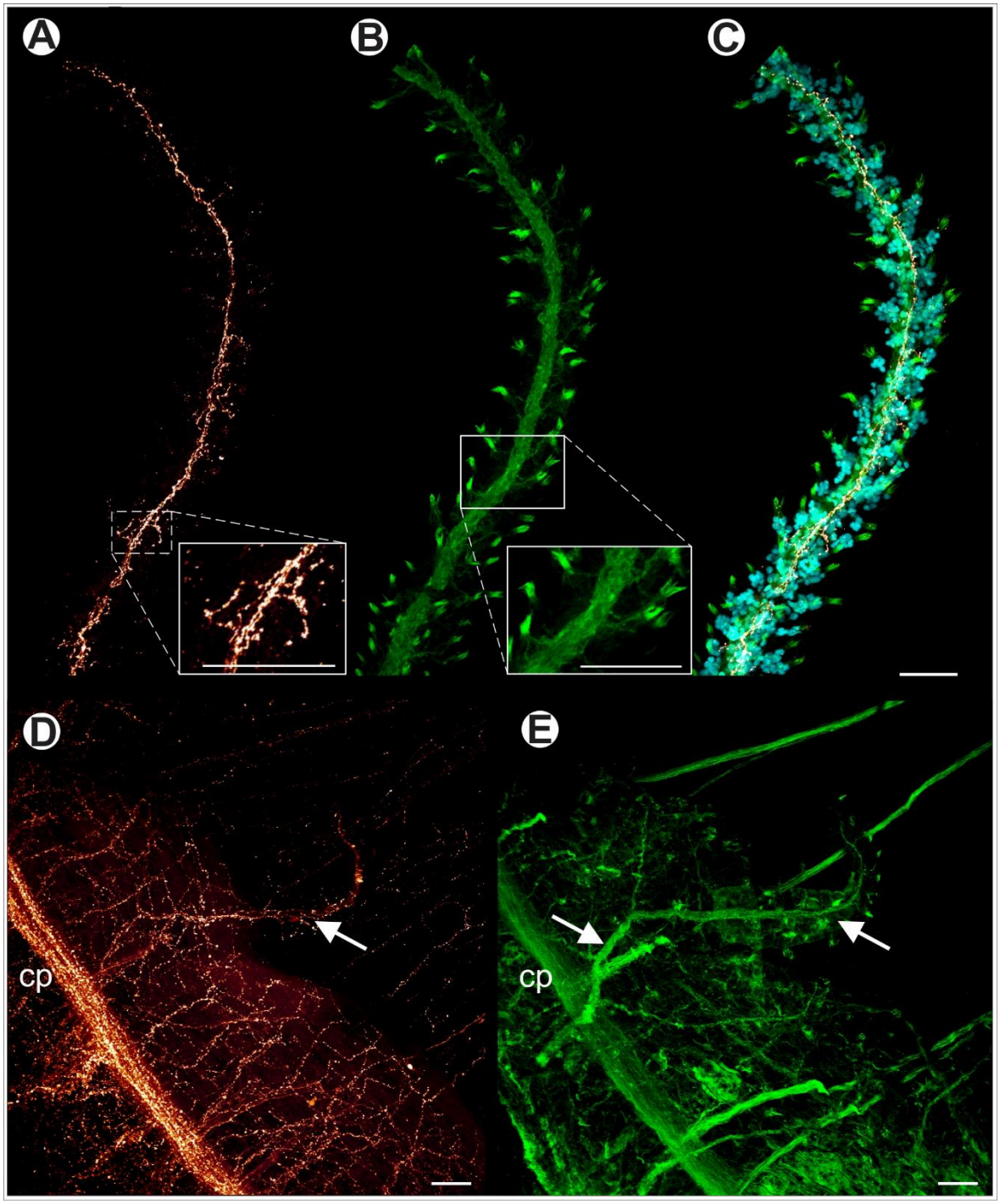

Figure 4. Nervous organization in tentacles from the middle mantle fold (A-C) and inner fold (D-E) of *N. nodosus*, as revealed by immunocytochemistry combined with confocal microscopy. **A.** Serotonergic immunoreactivity in the tentacular nerve; neuronal projections towards epithelial cells are shown in detail in the inset. **B.** Immunoreactivity to α-tubulin in the tentacular nerve; neuronal projections towards epithelial cells are shown in detail in the inset. **C.** Overlap of serotonin, α-tubulin and nuclei immunoreactivity in the tentacle. **D.** Serotonergic immunoreactivity in the velar tentacle nerve (arrow), which originates in the circum-pallial nerve passes through the inner mantle fold. **E.** Immunoreactivity to α-tubulin in the velar tentacle nerve (arrow). Abbreviation: *cp*, circum-pallial nerve. Scale bars: 30 μm.

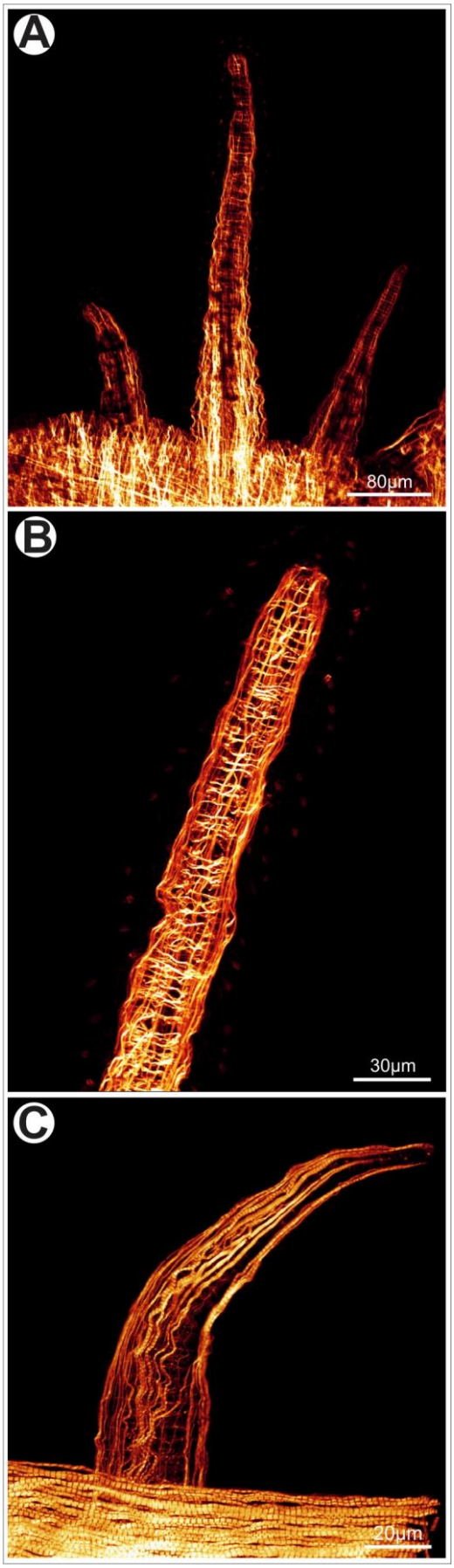

Figure 5. Muscular organization of tentacles from the middle mantle fold (A-B) and inner fold (C) of *N. nodosus*, as revealed by phalloidin staining combined with confocal microscopy. **A.** General muscle organization in short and long tentacles. **B.** Detail of a long tentacle musculature, showing non-striated longitudinal and transverse fibers. **C.** Detail of a velar tentacle musculature with striated longitudinal and transverse muscles.

CHAPTER 3

DEVELOPMENT OF THE PALLIAL EYE IN *NODIPECTEN NODOSUS* (MOLLUSCA: BIVALVIA): INSIGHTS INTO EARLY VISUAL PERFORMANCE IN SCALLOPS

CAPÍTULO 3

DESENVOLVIMENTO E ANATOMIA DOS OLHOS PALIAIS EM *NODIPECTEN NODOSUS* (BIVALVIA: PECTINIDAE)

RESUMO

Os olhos paliais dos pectinídeos são os sistemas visuais mais estudados dentre os moluscos bivalves. Apesar de avanços recentes no entendimento da função e evolução de tais estruturas, pouca atenção foi dedicada ao desenvolvimento ocular e sua performance visual inicial. O presente estudo investigou a anatomia e desenvolvimento dos olhos paliais da vieira *Nodipecten nodosus* (L. 1758) por meio de técnicas integradas de microscopia (*i.e.*, microscopias de luz, eletrônica e confocal). Após a metamorfose, vieiras juvenis desenvolvem pequenas papilas que rapidamente se diferenciam em diminutos órgãos oculares na prega mediana do manto. O epitélio distal gradualmente torna-se pigmentado, com exceção da córnea situada na extremidade central do olho. Internamente, a vesícula óptica compreende células indiferenciadas na região distal, enquanto placas refletoras são secretadas na base do sistema ocular, próximas às células pigmentadas. Em meio às células indiferenciadas, a retina proximal é a primeira a ser formada, seguida pela retina distal e, por último, pela lente. Olhos em indivíduos adultos são caracterizados pela ampla distribuição de pigmento no epitélio e lente com formato cônico acima da dupla camada de retina, que se encontra levemente curvada. Enquanto os olhos paliais em animais adultos são um complexo sistema visual baseado em um mecanismo de espelho para formação da imagem na retina, olhos em desenvolvimento sugerem um simples nível de fotopercepção direcional, sem visão espacial. Além disso, a morfologia da lente e córnea dos adultos de *N. nodosus* diferem significativamente de outras espécies filogeneticamente próximas, reforçando hipóteses prévias sobre variação ocular e evolução convergente no grupo.

Palavras-chave: bivalves, juvenil, morfologia funcional, sistema óptico, vieira.

O manuscrito a seguir contendo as informações detalhadas será submetido ao periódico internacional *Zoomorphology*.

CHAPTER 3

DEVELOPMENT OF THE PALLIAL EYE IN *NODIPECTEN NODOSUS* (MOLLUSCA: BIVALVIA): INSIGHTS INTO EARLY VISUAL PERFORMANCE IN SCALLOPS

ABSTRACT

Scallop pallial eyes have been the most studied optical system in bivalve molluscs. Even though recent advances have been achieved in understanding scallop eye's function and evolution, little attention has been focused on eye development and early visual performance. Here, the anatomy and development of pallial eyes were investigated in the scallop *Nodipecten nodosus* (Linnaeus, 1758) by means of integrative microscopy techniques (*i.e.*, light, electron and confocal microscopy techniques). After metamorphosis, juvenile scallops bear small papillae that rapidly transform into minute ocular organs on the middle mantle fold. The distal epithelium gradually becomes pigmented, except for the cornea formed at the distal center of the eye. Internally, the optic vesicle comprises undifferentiated cells at the distal region, while mirror plates are secreted at the base of the eye, next to pigmented cells. Within the undifferentiated cell mass, the proximal retina is the first to be formed, followed by the distal retina and then by the lens. Adult eyes are characterized by large pigment distribution in the epithelium, and conical lens above a slightly curved double retina. Whereas the pallial eyes from adult scallops are a complex visual system based on a mirror mechanism to form a focused image on the retina, early eye condition suggests a simple degree of directional photoreception, with no spatial vision. In addition, morphology of lens and cornea from adult *N. nodosus* significantly differs from other closely related scallops, reinforcing previous assumptions on eye variation and convergent evolution in the group.

Keywords: bivalves, functional morphology, juvenile, optical system, scallop

INTRODUCTION

In the diverse phylum Mollusca, eye morphology ranges from simple photoreceptor cells to complex ocular organs able to perform a variety of visual tasks (Serb, 2008). Consequently, molluscan eyes stimulated a myriad of scientific investigations, and provided suitable models to studies on photoreceptor structures evolution, visual ecology, and functional morphology (Salvini-Plawen, 2008; Serb and Eernisse, 2008). Cephalopod eyes are among the well-studied optic models in molluscs, including the pin-hole eye type of nautiloids and the camera-type eyes of coleoids (squids, cuttlefishes and octopuses), the latter type exhibiting a complex structure that strongly resembles vertebrate eyes, thus representing a striking example of convergent trajectory in eye evolution (Muntz and Raj, 1984; Young, 1971; Hanlon and Shashar, 2003). Most gastropods commonly display lens-bearing eyes located at the tip or base of cephalic tentacles (Zieger and Meyer-Rochow, 2008). While cephalic eyes prevail in gastropods and cephalopods, serially repeated photosensitive organs are located in the shell of chitons and on the mantle edge of bivalves (Serb and Eernisse, 2008). In the Polyplacophora, simple photoreceptors are located in aesthetes, embedded in the shell tegumentum, where they are likely associated to light-response behavior (Fitzgerald, 1975; Boyle, 1972). Nevertheless, complex ocelli with lens and retina may also be present in members of the family Chitonidae, forming a sophisticated optical system (Moseley, 1884; Speiser *et al.*, 2011a).

The Bivalvia is the second most diverse molluscan class, occurring in marine and freshwater environments, and with enormous variation in lifestyle, form and function (Giribet *et al.*, 2008). Bivalves comprise molluscs with a laterally compressed body enclosed by two valves, head reduction, and sensorial structures mainly distributed along the mantle margin (Stasek and McWilliams, 1973; Yonge, 1983). In comparison to other molluscan groups, bivalves exhibit the greatest diversity in photoreceptor organs (Serb, 2008). Besides light-sensitive nerves and larval cephalic eyespots, light perception is also performed by simple photoreceptors or even by complex organs located on one of the three pallial folds, hence the name "pallial eyes" (Morton, 2008). Bivalve eyes vary from simple pigmented pits in *Barbatia virescens* (Morton, 1987) to pigmented cups with lens in *Cerastoderma edule* (Barber and Land, 1967), eyespots in *Tridacna maxima* (Wilkens, 1986), compound eyes in *Arca noae* (Waller, 1980; Nilsson, 1994), and complex eyes in *Ctenoides floridanus* (Morton, 2000a), and *Laternula truncata* (Adal and Morton, 1973). In addition, the elaborate pallial eyes of the Pectinidae, distributed along the middle mantle

fold, are the most familiar example of eye complexity in bivalves (Morton, 2008; Serb and Errnisse, 2008). Consequently, they have been extensively studied regarding their anatomy, physiology and role in behavior (*e.g.*, Hayami, 1991; Hamilton and Koch, 1996; Morton, 2000b; Wilkens, 2006; Speiser *et al.*, 2011b).

The pallial eyes of Pectinidae were described for the first time by Poli (1795), and numerous subsequent descriptions were produced in attempt to understand general eye morphology (*e.g.*, Krohn, 1840; Hensen, 1865; Patten, 1886). Dakin (1910) was the first author to review previous knowledge of scallop eyes, providing fine details on the arrangement and anatomy of each ocular component. Later, more efforts were put forward to clarify scallop eye anatomy (Dakin, 1928; Ciocco, 1998), and more recently comparative studies have provided vital insights into the evolution and function of these organs (Speiser and Johnsen, 2008; Malkowsky and Jochum, 2014). Additionally, recent molecular approaches based on transcriptome and opsin genes have contributed to our understanding of the visual ecology in Pectinidae (Pairett and Serb, 2013; Serb *et al.*, 2013). Even though substantial knowledge of scallop eyes has been accumulated for about two centuries, the development of such visual organs remains obscure. Drew (1906) noticed that eye development had not been carefully investigated at his time, and Küpfer (1916) offered the first insights into this issue. Nevertheless, eye formation has been overlooked so far for pectinids and other bivalve groups. Therefore, to gain insight into the developmental sequence of the eye and early visual performance in scallops, the present study investigated eye development in postmetamorphic stages of *Nodipecten nodosus* (Linnaeus, 1758).

MATERIAL AND METHODS

Specimens of *Nodipecten nodosus* were obtained in the scallop farm *Institute of Eco-Development from Baía de Ilha Grande* (IED-BIG), Rio de Janeiro, Brazil. Juvenile individuals (*i.e.*, around a few weeks after metamorphosis, with a maximum length of 4 mm) were removed from artificial tanks, and adult mature individuals (about 8 cm in length) were collected from artificial lantern baskets. Prior to fixation, animals were anesthetized for 2 hours by gradual addition of drops of 7.5% $MgCl_2$. Observations of live animals were carried out under a Zeiss Stemi 2000-C stereomicroscope, images being captured using a Zeiss AxioCam MRc. Digital microscopy images were further processed

using Photoshop CS3 (Adobe Systems, USA) to adjust contrast and brightness, and complementary drawings were created using Corel Draw X5 (Corel Corporation, Canada).

Histology. Specimens were fixed for 3 hours at 4°C in a modified Karnovsky solution (2% paraformaldehyde + 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer at pH 7.4 and 1000 mosm adjusted with sucrose). Juveniles were decalcified for 12 hours at room temperature in a 3% ascorbic acid, while adult specimens were dissected after anesthesia to remove pallial margin fragments containing the eyes. Then, specimens were dehydrated in a graded ethanol and embedded in glycol-methacrylate resin (Leica Historesin Kit). Serial 3 μm sections were obtained using a Leica RM2255 microtome and stained with hematoxylin and eosin (HE) or toluidine blue and acid fuchsin (TB). Digital images were captured using a Nikon eclipse 80i microscope equipped with a Nikon DS-Ri1 camera.

Scanning and Transmission Electron Microscopy. For Scanning Electron Microscopy (SEM), juvenile and adult samples were previously fixed in modified Karnovsky solution. Post-fixation was performed for 30 minutes in 1% OsO_4 in buffer solution (sodium cacodylate buffer at pH 7.4), followed by 15 minutes in 1% tannic acid in buffer solution, and more 15 minutes in new solution of 1% OsO_4 at 4°C. Then, specimens were decalcified as previously described for histological procedures and dehydrated in graded ethanol series. Samples were critical point dried using CO_2 as a transitional fluid in a Balzers CPD 030, mounted on stubs, coated with gold in a Balzers SCD 050 sputter coater, and observed in a Zeiss DSM 940. For Transmission Electron Microscopy (TEM), only adult pallial eyes were post-fixed for 1 hour in 1% OsO_4 in buffer solution. Larval samples were embedded in Epoxi resin; ultrathin sections (50-70 nm) were cut using a Leica Ultracut UCT microtome, mounted on copper slot-grids, contrasted with uranyl acetate and lead citrate, and analyzed using a Zeiss EM 900 electron microscope.

Immunocytochemistry, Confocal Laser Scanning Microscopy and 3D Reconstruction. Juvenile specimens were fixed in 4% paraformaldehyde in 0.1 M phosphate buffered (PB) for 1 hour, followed by four rinses in buffer solution. Individuals were dissected to remove small fragments of the mantle margin containing the eyes. For neuronal staining, samples were incubated in 6% normal goat serum in PBT (block-PBT) overnight at room temperature. Subsequently, primary antibodies, *e.g.*, anti-serotonin raised in rabbit or anti-FMRFamide raised in rabbit, were applied at a concentration of 1:400 in block-PBT for 24 hours. Then, specimens were rinsed several times in block-PBT prior to application of a secondary fluorochrome-conjugated antibody (goat anti-rabbit

Alexa Fluor 488, Molecular Probes) in block-PBT at a concentration of 1:200 for 24 hours in the dark. Nuclei were stained by adding a 1 µl drop of 4', 6-diamidino-2-phenylindole (DAPI) (Invitrogen, 3 µg mL^{-1}) in conjunction with secondary antibody incubation. Then, samples were washed three times in PBS for about 30 minutes and mounted in Fluoromount G (Southern-Biotech, Birmingham, Alabama) on standard microscope slides. Analysis and image acquisition were performed on a Leica TCS SP5 II confocal laser scanning microscope equipped with the software Leica Application Suite Advanced Fluorescence (LAS AF), Version 2.6.0 (Leica Microsystems, Wetzlar, Germany). Confocal image stacks were recorded with 0.3 µm step size along the z-axis and digitally merged as maximum intensity projections. 3D reconstructions were created from selected image stacks using the software Imaris Version 4.1 (Bitplane, Switzerland).

RESULTS

Few weeks after metamorphosis of *Nodipecten nodosus*, numerous pallial eyes are found scattered along the entire middle mantle fold on both sides of the animal (Fig. 1A). At the juvenile stage, they consist of short projections not exceeding 100 µm, being intensively pigmented at the distal portion, but unpigmented at the eyestalk (Fig. 1B). A transparent cornea is already conspicuous at this stage (Fig. 1B). The ocular epithelium surface is quite similar throughout the length of the eye, bearing rare tufts of cilia, which are absent on the cornea (Fig. 1C). At the final stages of juvenile development, the eye displays the lens, retina, reflector layer and pigmented layer (responsible for the blue color of the eyes) ordered in a sequential arrangement (Fig. 1D). As will be seen, however, these structures do not develop simultaneously.

Juvenile eyes in early differentiation have a short stalk supporting an enlarged distal region (Fig. 2A). Their cornea contains columnar cells, while the surrounding epithelium gradually becomes pigmented, few pigmented cells forming a narrow band around the eye (Fig. 2A). Bellow the cornea, the optic vesicle, *i.e.* the inner cavity where the optical components develop, exhibits a cluster of undifferentiated cells, apparently with no defined structures, and the retinal matrix (Fig. 2A).

During ocular development in juveniles, more pigmented cells are added to the epithelium, enlarging the bluish band (Fig. 2B-D). In addition, both cornea and pigmented epithelium have their cells elongated, the columnar pattern becoming more conspicuous (Fig. 2B-D). The cellular mass is gradually organized in layers, the proximal retina being

apparently the first to differentiate, followed by the distal retina (Fig. 2B-D). At the base of the optic vesicle, numerous minute crystals (*i.e.*, refractional plates) form the mirror layer (or *argentea*), beneath the retina and above the developing pigmented cells (Fig. 2B, C, D). The cellular lens is the last component to be formed, comprising small cells pressed against the cornea (Fig. 2C, D) and enclosed basally by the double retina (Fig. 2C, D).

Even though the developing eyes are still in differentiation, innervation is already present. Whereas FMRFamide neuropeptide is weakly expressed inside pallial eyes (Fig. 3A), serotonin displays strong immunoreactivity (Fig. 3B). The circum-pallial nerve is responsible for emitting neuronal projections through each eye (Fig. 3B). The optic nerve goes through the eye and profoundly branches at the region of the proximal retina, exhibiting intense signal over the photoreceptor cells (Fig. 3C). Also, a small branch leaves the optic nerve at the optic vesicle level and innervates the distal retina (Fig. 3D).

In adults, the mantle margin is greatly enlarged, displaying numerous pallial eyes that reach 1 mm in diameter and almost 3 mm in length, being also more intensely pigmented (Fig. 4A). Externally, the transparent cornea is a conspicuous dome at the distal center of the eye, surrounded by a blue-pigmented epithelium (Fig. 4B). The eyestalk is also pigmented, but varying from white to brown (Fig. 4A, B).

Adult eyes preserve the sequential arrangement previously described for juveniles, *i.e.*, with cornea, lens, retina, mirror layer, and pigmented layer (Fig. 5A). The cornea is formed by tall columnar cells displaying microvilli, centered nuclei, and no pigmentation (Fig. 5B, C). Similarly, the pigmented epithelium is formed by thousands of numerous columnar cells covered by long microvilli, but differently from the cornea, large pigment granules occupy the basal cellular portion, the nuclei located at a more distal position (Fig. 5D, E). The lens comprises numerous irregularly shaped cells; its distal curvature is slightly convex, while the basal curvature is strongly curved (Fig. 5F). Change in lens shape (from flattened to conical) and the flattening of the retina during development create a free space around the lens, known as the distal chamber (Fig. 5A, F). The double retina is relatively more flattened, only slightly curved, and formed by rod cells with a typical, very elongated morphology (Fig. 5G, H), clearly distinct from their juvenile counterparts. The proximal region of the rod cells from the proximal retina is embedded within the retinal matrix, while the distal retina is in direct contact with the proximal region of the lens (Fig. 5G, H). Bellow the retinal matrix, crystal plates are organized in sheets forming the mirror layer (Fig. 5J, K, L). At the base of the eye, the pigmented layer consists of irregular cells

containing large pigment granules (Fig. 5I). Both mirror and pigmented layers are relatively more compact at this stage (Fig. 5G, H, J).

DISCUSSION

Summary of N. nodosus eye development

Eye development of *N. nodosus* is summarized in figure 6. Although final eye structure and morphology are achieved only later in development, all optical components are formed during scallop juvenile stages. Innervation is established early, with intense serotonergic immunoreactivity in the optic nerve and retinal branches. The elongation of the pallial eye seems to be caused by longitudinal extension of the epithelium, combined with general growth and cell proliferation within the optic vesicle. Differentiation of the optic vesicle has apparently a proximal-distal orientation. Both mirror and pigmented layers are early detected at the base of the vesicle (Fig. 6A). Subsequently, the proximal retina is formed, followed by the distal retina, and then, by the lens (Fig. 6A-D). Afterwards, the retina becomes more flattened and less curved (Fig. 6E), and its cells develop the characteristic rod morphology. The flattening of the retina combined with the more spherical shape of adult lens creates a free space in the distal portion of the optic vesicle (Fig. 6E). The organization of the plates from the mirror layer seems to become more compact with time, while the pigmented layer gradually increases pigmentation.

Pallial eye development in scallops

The anatomical changes described herein for eye development in *N. nodosus* suggest gradual modifications in eye morphology by differentiation of internal components combined with general growth. Similar patterns were suggested by Küpfer (1916) based on the anatomy of juvenile scallop eyes of *Palliolum incomparabile*. In both species, the eye develops from small papilla, the optic vesicle is formed at its distal portion, and, subsequently, internal cells gradually differentiate into proximal and distal retina, and then into the lens. Butcher (1930), investigating eye formation and regeneration in adult specimens of *Argopecten gibbus*, assumed that pallial eyes are formed as available space is provided on the mantle margin, and that the potential to form more eyes seems to be gradually lost as the scallop becomes larger. Similar to developing juvenile eyes in *N. nodosus*, during formation of new eyes in adults of *A. gibbus*, the cellular mass differentiates into the double retina, and the lens gradually appears above it. Nevertheless,

in the case of eye formation in adults, the epithelium first loses pigmentation to form the cornea (Butcher, 1930), but in *N. nodusus* the epithelium is first unpigmented, pigmented cells gradually developing around the cornea. Such difference may be explained by the distinct condition of the pallial epithelium in adults (pigmented) and juveniles (unpigmented). Therefore, despite minor variances, the general process of eye formation in adult scallops closely resembles the early eye development observed in juveniles of *N. nodosus*. The results provided herein, combined with developmental observations from Küpfer (1916) and Butcher (1930), suggest a single common process of eye organogenesis in these animals, regardless of the postmetamorphic stage.

Pallial eye anatomy

N. nodosus eye morphology greatly resembles that of *Chlamys islandica* and *Palliolum incomparabile* (Malkowsky and Jochum, 2014). Even though the same sequential organization has been constantly documented for scallop eyes, divergences in shape and size have attracted great attention in anatomical investigations. Recently, morphological variation in scallop ocular components was appointed as crucial for understanding eye diversity and visual performance (Speiser and Johnsen, 2008; Malkowsky and Götze, 2014).

The pigmented epithelium around the optic vesicle is typically formed by prismatic, columnar cells, densely filled with dark pigment granules (Drew, 1906). Apart from the distal region, epithelial cells gradually become cuboidal, exhibiting fewer pigments. The intense blue pigmentation observed in *N. nodosus* is also present in eyes of *Argopecten irradians*, *Aequipecten tehuelchus* and *Pecten maximus* (Speiser and Johnsen, 2008; Ciocco, 1998). However, color patterns may vary, with brown eyes occurring in *Amusium balloti*, and black pigmentation in other scallops such as *C. hastata* and *Spondylus americanus* (Speiser and Johnsen, 2008).

The most distal epithelial cells are differentiated into the transparent cornea, which differs in height and extension among scallop species. A layer of nucleated, cuboidal cells was detected in *Amusium balloti*, *A. irradians*, *C. hastata*, *C. rubida*, and *Placopecten magellanicus* (Speiser and Johnsen, 2008). Small, cubic cells were also reported for *Aequipecten opercularis*, *P. maximus* (Malkowsky and Jochum, 2014), *Flexopecten flexuosus*, *F. glaber* (Malkowsky and Götze, 2014), and for the sessile scallop *Spondylus americanus* (Viena and Rocha-Barreira, 2007). In contrast, columnar cells were observed in *Crassadoma gigantea* (Speiser and Johnsen, 2008), *Patinopecten yessoensis* (Morton,

2000b) and *Chlamys* (Dakin, 1928). Columnar cells similar to the ones described herein for the cornea of *N. nodosus*, *i.e.*, increasing in height towards the center, occur in *C. islandica* and *Palliolum incomparabile* (Malkowsky and Jochum, 2014).

In scallop pallial eyes, the lens are located centrally, bellow the cornea. In *P. maximus*, the lens was described as bi-convex, with a flattened distal surface and a dome-shaped proximal surface by Dakin (1910). Land (1965) described the lens of the same species as exhibiting a curved distal surface associated with the correction of spherical aberration caused by light reflection in the mirror layer. Spherical shape with a hyperbolic front surface was reported for *Amusium balloti* and *Placopecten magellanicus* (Drew, 1906; Speiser and Johnsen, 2008). In contrast, relatively spherical distal surfaces are present in the elliptical lens of *Argopecten irradians*, *C. hastata*, *C. rubida*, *Crassadoma gigantea* and *Spondylus americanus* (Speiser and Johnsen, 2008), *Patinopecten yessoensis* (Morton, 2000), *Aequipecten tehuelchus* (Ciocco, 1998), *Flexopecten flexuosus* and *F. glaber* (Malkowsky and Götze, 2014). In *N. nodosus*, the lens exhibits a conical shape, similar to the curvilinear triangulate pattern observed in *C. islandica* and *Palliolum incomparabile*, (Malkowsky and Jochum, 2014).

In the light of recent comparative studies on pallial eye anatomy, a correlation between the variation in shape of both the lens and the cornea was identified by Malkowsky and Götze (2014). According to recent molecular phylogenies, *N. nodosus* would be closely related to *P. maximus* within the subfamily Pectninae (Waller, 2006; Puslednik and Serb 2008; Alejandrino *et al.*, 2011). Nevertheless, the typical eye morphology of this group, with flat cornea and elliptical lens, is not present in *N. nodosus*. The general morphology of *N. nodosus*'s eye is, however, quite similar to that observed in *C. islandica* and *P. incomparabile*, including a similar columnar cornea combined with a conical lens. Both these characters' states were considered apomorphic within Pectinidae, but evolving independently in both *C. islandica* and *P. incomparabile* (Malkowsky and Götze, 2014). Considering the phylogenetic distance among *N. nodosus* and the remaining scallop species with similar eye morphology, combined with the plesiomorphic states displayed by its closest relatives, it seems reasonable to suggest that eye morphology in *N. nodosus* could represent another case of convergence, although this should be tested in a cladistic framework. This hypothesis would further reinforce several lines of evidence highlighting recurrent convergent and parallel evolution of morphological features and life habits within the Pectinidae (Alejandrino *et al.*, 2011; Serb *et al.*, 2011; Malkowsky and Götze, 2014).

The mirror layer, or *tapetum* according to Dakin (1910), comprises several layers of minute square plates above the pigmented cells as demonstrated herein for *N. nodosus* and for *P. maximus* by Barber *et al.* (1967). These plates, identified as guanine crystals by Land (1995) are organized in a curved shape, acting as a concave mirror responsible for focusing the reflected image at the level of the distal retina. A fluid cavity has been recurrently described between the proximal retina and the mirror layer in preserved specimens of several scallop species (Dakin, 1910; Land, 1965; Speiser and Johnsen, 2008), including in the present investigation on *N. nodosus*. Nevertheless, no cavity is observed in sectioned eyes *in vivo*, corroborating the argument of Dakin (1910) that such a cavity actually is an artefact resulting from tissue shrinking during fixation.

Functional perspectives in eye development

The functional implications of pallial eye development in scallops have, to our knowledge, never been investigated, which is surprising given the extensive list of studies concerning evolutionary significance and roles of eyes in the Pectinidae (*e.g.*, Morton, 2000b; Speiser and Johnsen, 2008). The emergence of eyes after metamorphosis has long been known to occur (Küpf, 1916; Sastry, 1967), but how light information could be processed and used by early juvenile scallops, with newly adopted benthic habits, is still obscure. But as the eyes develop and ocular components are gradually modified, some changes in optical performance are expected to occur during postmetamorphic development.

According to Nilsson (2013), adult scallop pallial eyes may be classified into directional photoreceptors of low-resolution vision (class III). Such a category of eyes is unique among bivalves because image can be formed in the retina by a concave mirror system (Land, 1965). Besides this sophisticated optical system, different functions and responses attributed to the adult double retina might provide visual clues for habitat selection and alarm against predators (Nilsson, 1994; Hamilton and Koch, 1996; Speiser and Johnsen, 2008).

Nevertheless, such assumptions might not be applicable in the case of developing eyes, for their morphology significantly differs from completely formed organs, as demonstrated herein. The present anatomical investigation with *N. nodosus* suggests that early developing eyes would not provide image resolution, but would instead be restricted to directional photoreception, *i.e.*, class II according to Nilsson (2013). The double retina comprises the layer of photoreceptor cells, and in the case of *N. nodosus*, these cells

rapidly differentiate during the juvenile stage. Serotonergic innervation was detected in the retina of forming eyes, suggesting the presence of early nervous activity in functional photoreceptors. The lens, however, is the last component to be formed, with a small size and flattened shape. Considering this ocular configuration, even if the crystal plates at the base of the optic vesicle could act as a concave mirror, the resulting spherical aberration would not be corrected. In other words, the light would pass through the cornea and retina, and would be reflected by the mirror region, but the reflected rays would not be correctly converged in a common focus on the retina, as is the case of adult eyes (Jonasova and Kozmik, 2008; Land and Nilsson, 2012).

In summary, the results obtained herein for *N. nodosus* suggest that developing eyes (present at the early juvenile stage) are most probably not capable of more than a simple degree of light perception. Our data indicate a gradual improvement in photoreceptive performance during eye development in scallops, starting from a directional photoreceptor structure to an image-forming organ. However, further investigations are needed to answer if juvenile eye condition is somehow associated to juvenile life habits or behavioral responses.

Other questions remain, though. For example, are eye developmental sequences similar among scallop species? What is the molecular basis for eye formation? How does gene expression mold eye organogenesis and visual activity? Over more than a century of studies on scallop pallial eyes, understanding their evolution and function is still challenging, which should stimulate future studies on these enigmatic ocular structures.

ACKNOWLEDGEMENTS

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REFERENCES

Alejandrino A, Puslednik L, Serb JM. 2011. Convergent and parallel evolution in life habit of the scallops (Bivalvia: Pectinidae). BMC Evolutionary Biology 11, 164.

Adal MN, Morton B. 1973. The fine structure of the pallial eyes of *Laternula truncata* (Bivalvia: Anomalodesmata: Pandoracea). Journal of Zoology 170:533-556.

Barber VC, Land MF. 1967. The eye of the cockle, *Cardium edule*: anatomical and physiological investigations. Cellular and Molecular Life Sciences 23(8): 677-678.

Barber VC, Evans EM, Land MF. 1967. The fine structure of the eye of the mollusc *Pecten maximus*. Zeitschrift für Zellforschung 76: 295–312.

Boyle PR. 1972. The aesthetes of chitons. 1. Role in the light response of whole animals. Marine Behavior & Physiology 1:171–84.

Butcher EO. 1930. The formation, regeneration, and transplantation of eyes in *Pecten* (*Gibbus borealis*). Biological Bulletin 59(2): 154–164.

Ciocco NF. 1998. Anatomía de la "vieira tehuelche", *Aequipecten tehuelchus* (d'Orbigny, 1846) (=*Chlamys tehuelcha*). IV, Sistema nervioso y estructuras sensoriales (Bivalvia, Pectinidae). Revista de Biología Marina y Oceanografía 33(1): 25–42.

Dakin WJ. 1910. The eye of *Pecten*. The Quarterly Journal of Microscopical Science 55: 49–112. Plates 6-7.

DakinWJ. 1928. The eyes of *Pecten, Spondylus, Amussium* and allied Lamellibranchs, with a short discussion on their evolution. Proceedings of the Royal Society London Series B, Containing Papers of a Biological Character 103(725): 355–365.

Drew GA. 1906. The habits, anatomy, and embryology of the giant scallop. (*Pecten tenuicostatus*, Mighels) (pp. 1–71). Maine: Orono.

Fitzgerald WJ. 1975. Movement patterns and phototactic response of *Mopalia ciliata* and *Mopalia muscosa* in Marin County, California. Veliger 18: 37–39.

Giribet G. 2008. Bivalvia. In: Ponder W and Lindberg D (eds). Phylogeny and Evolution of the Mollusca. California, University of California Press. Pp 105–141.

Hamilton PV, Koch KM. 1996. Orientation toward natural and artificial grassbeds by swimming bay scallops, *Argopecten irradians* Lamarck, 1819. Journal of Experimental Marine Biology and Ecology 199: 79–88.

Hanlon RT, Shashar N. 2003. Aspects of the sensory ecology of cephalopods. In: Collin SP and Marshall NJ (eds). Sensory processing in aquatic environments. New York, Springer New York. Pp 266–282.

Hayami I. 1991. Living and fossil scallop shells as airfoils: an experimental study. Paleobiology 17: 1–18.

Hessen V. 1865. Über die Augen von *Pecten jacobaeus*. Zeitschrift für wissenschaftliche Zoologie p. 220.

*Jonasova K, Kozmik Z. 2008. Eye evolution: lens and cornea as an upgrade of animal visual system. Seminars in Cell& Developmental Biology 19: 71–81.

Krohn A. 1840. Über augenähnliche Organe bei *Pecten* und *Spondylus*. Müllers Archiv für Anatomie und Physiologie 381–386.

Küpfer M. 1916. Entwicklungsgeschichtliche und neuro-histologische Beiträge zur Kenntnis der Sehorgane am Mantelrande der Pecten-Arten: mit anschliessenden vergleichend-anatomischen Betrachtungen (pp. 1–312). Jena: Fischer.

Land MF. 1965. Image formation by a concave reflector in the eye of the scallop, *Pecten maximus*. The Journal of Physiology, 179, 138–153.

Land MF. 1966. Activity in the optic nerve in *Pecten maximus* in response to changes in light intensity, and to pattern and movement in the optical environment. Journal of Experimental Biology 45: 83–99.

Land MF, Nilsson DE. 2012. Animal eyes. Oxford, Oxford University Press.

Malkowsky Y, Götze MC. 2014. Impact of habitat and life trait on character evolution of pallial eyes in Pectinidae (Mollusca: Bivalvia). Organisms Diversity and Evolution 14: 173–185.

Malkowsky Y, Jochum A. 2014. Three-dimensional reconstructions of pallial eyes in Pectinidae (Mollusca: Bivalvia). Acta Zoologica (Stockholm) 0: 1–7.

Moseley HN. 1885. On the presence of eyes in the shells of certain Chitonidae, and on the structure of these organs. The Quarterly Journal of Microscopical Science 25: 37–60.

Morton B. 1987. The pallial photophores of *Barbatia virescens* (Bivalvia: Arcacea). Journal of Molluscan Studies 53:241-243.

Morton B. 2000a. The pallial eyes of *Ctenoides floridanus* (Bivalvia: Limoidea). Journal of Molluscan Studies, 66, 449–455.

Morton B. 2000b. The function of pallial eyes within the Pectinidae, with a description of those present in *Patinopecten yessoensis*. In E M Harper, J D Taylor, and J A Crame (Eds.), The Evolutionary Biology of the Bivalvia (pp. 247–255). London: Geological Society. Special Publications, 177.

Morton B. 2008. The evolution of eyes in the Bivalvia: New Insights. American Malacological Bulletin 26(1/2): 35–45.

Muntz WRA, Raj U. 1984. On the visual system of *Nautilus pompilius*. Journal of Experimental Biology 109: 253–263.

Nilsson DE. 1994. Eyes as optical alarm systems in fan worms and ark clams. Philosophical Transactions of the Royal Society of London B Biological Sciences 346: 195–212.

Nilsson DE. 2013. Eye evolution and its functional basis. Visual Neuroscience 30: 5–20.

Pairett AN, Serb JM. 2013. *De novo* assembly and characterization of two transcriptomes reveal multiple light-mediated functions in the scallop eye (Bivalvia: Pectinidae). PLoS ONE 8(7): e69852.

Patten W. 1887. Eyes of Molluscs and Arthropods. Journal of Morphology 1(1): 67–92. Plate 3.

Poli J. 1795. Testacea utriusque Siciliae eorumque historia et anatome tabulis aeneis illustrata. Parma. Pars II. *Spondylus gaederopus*, p. 107, *Pecten jacobaeus*, p. 153.

Puslednik L, Serb JM. 2008. Molecular phylogenetics of the Pectinidae (Mollusca: Bivalvia) and effect of increased taxon sampling and outgroup selection on tree topology. Molecular Phylogenetics and Evolution 48: 1178–1188.

Salvini-Plawen LV. 2008. Photoreception and the polyphyletic evolution of photoreceptors (with special reference to Mollusca). American Malacological Bulletin 26(1/2): 83–100.

Sastry AN. 1965. The development and external morphology of pelagic larval and postlarval stages of the bay scallop, *Aequipecten irradians concentricus* Say, reared in the laboratory. Bulletin of Marine Science 15(2): 417–435.

Serb JM. 2008. Toward developing models to study the disease, ecology, and evolution of the eye in Mollusca. American Malacological Bulletin 26(1/2): 3–18.

Serb JM, Eernisse DJ. 2008. Charting evolution's trajectory: using molluscan eye diversity to understand parallel and convergent evolution. Evolution Education Outreach 1: 439–447.

Serb JM, Alejandrino A, Otárola-Castillo E, Adams DC. 2011. Morphological convergence of shell shape in distantly related scallop species (Mollusca: Pectinidae). Zoological Journal of the Linnean Society 163(2): 571–584.

Serb JM, Porath-Krause AJ, Pairett AN. 2013. Uncovering a gene duplication of the photoreceptive protein, opsin, in scallops (Bivalvia: Pectinidae). Integrative and Comparative Biology 53: 68–77.

Speiser DI, Johnsen S. 2008. Comparative morphology of the concave mirror eyes of scallops (Pectinoidea). American Malacological Bulletin 26(1/2): 27–33.

Speiser DI, Eernisse DJ, Johnsen S. 2011a. A chiton uses aragonite lenses to form images. Current Biology 21: 665–670.

Speiser DI, Loew ER, Johnsen S. 2011b. Spectral sensitivity of the concave mirror eyes of scallops: potential influences of habitat, self-screening and longitudinal chromatic aberration. The Journal of Experimental Biology 214: 422–431.

Stasek CR, McWilliams WR. 1973. The comparative morphology and evolution of molluscan mantle edge. The Veliger 16:1–19.

Viana MG, Rocha-Barreira CA. 2007. The sensorial structures of *Spondylus americanus* Hermann, 1781 (Mollusca: Bivalvia, Spondylidae). Brazilian Archives of Biology and Technology 50(5): 815–819.

Waller TR. 1980. Scanning electron microscopy of shell and mantle in the order Arcoida (Mollusca: Bivalvia). Smithsonian Contributions to Zoology 313: 1–58.

Waller TR. 2006. Phylogeny of families in the Pectinoidea (Mollusca: Bivalvia): importance of the fossil record. In Bieler R (ed) *Bivalvia—a look at the Branches*. Zoological Journal of the Linnean Society 148: 313–342.

Wilkens LA. The visual system of the giant clam *Tridacna*: behavioral adaptation. Biological Bulletin 170: 393–408.

Wilkens LA. 2006. Neurobiology and behaviour of the scallop. In Shumway SE and Parsons GJ (eds). Scallops: Biology, Ecology, and Aquaculture. Elsevier, New York. Pp. 317–356.

Yonge CM. 1983. Symmetries and the role of the mantle margins in the bivalve Mollusca. Malacological Review 16: 1–10.

Young JZ. 1971. The anatomy of the nervous system of *Octopus vulgaris*. Oxford, Clarendon Press.

Zieger MV, Meyer-Rochow VB. 2008. Understanding the cephalic eyes of pulmonate gastropods: a review. American Malacological Bulletin 26(1/2): 47–66.

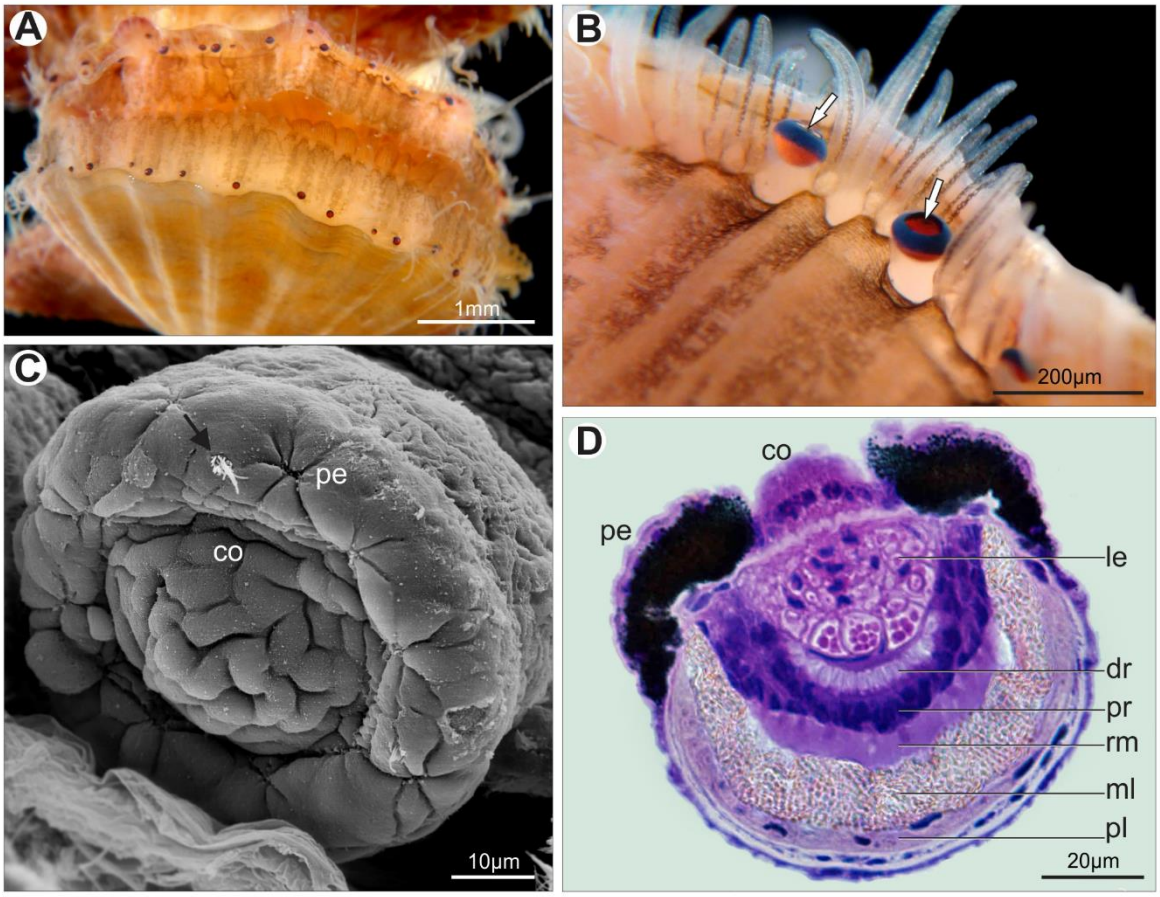

Figure 1. Pallial eyes in juvenile *N nodosus*. **A.** Ventral view of a juvenile specimen, showing pallial eyes scattered along both mantle margins. **B.** Detail of the middle pallial fold with eyes interspersed with tentacles; arrow points to the cornea. **C.** Scanning electron microscopy of a juvenile eye, showing a tuft of cilia (arrow) in the ocular epithelium. **D.** Section of the eye, showing all ocular components already present at this stage. TB. Abbreviations: *co*, cornea; *dr*, distal retina; *le*, lens; *ml*, mirror layer; *pe*, pigmented epithelium; *pl*, pigmented layer; *pr*, proximal retina; *rm*, retinal matrix.

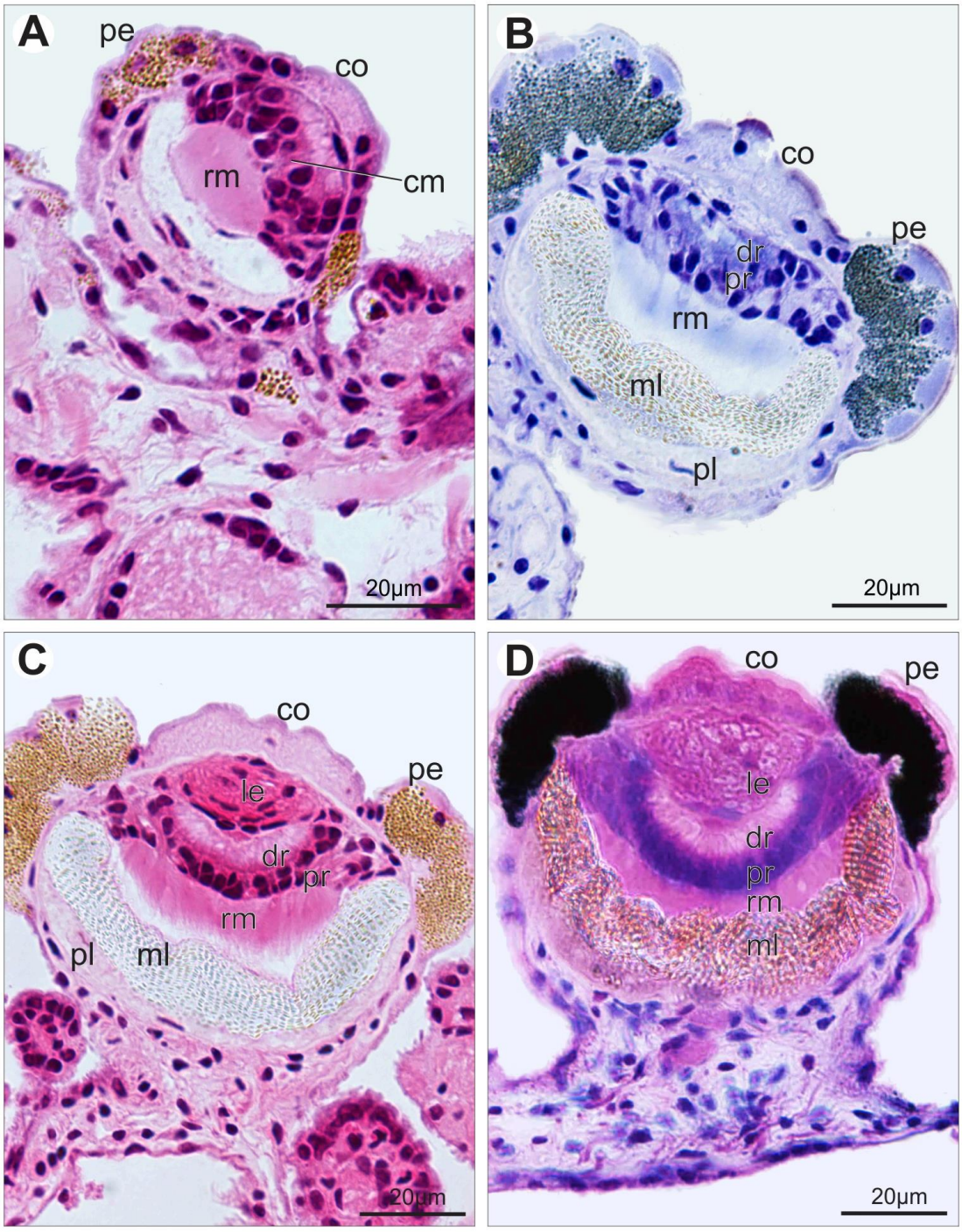

Figure 2. Median cross sections of developing pallial eyes in juvenile *N. nodosus*. **A.** Early stage with few pigmented cells in the epithelium, and optic vesicle with an undifferentiated cell mass in the distal portion, and retinal matrix at the center. HE. **B.** Intermediate stage with pigmented epithelium expanded, proximal and distal retinas beginning to differentiate, and mirror layer at the base of the optic vesicle. TB. **C.** Intermediate stage with developing lens, and distal and proximal retinas more differentiated and curved. HE. **D.** Late juvenile stage with optic vesicle completely filled by ocular components, and curved retina enclosing the base of the lens. TB. Abbreviations: *cm*, cell mass; *co*, cornea; *dr*, distal retina; *le*, lens; *ml*, mirror layer; *pe*, pigmented epithelium; *pl*, pigmented layer; *pr*, proximal retina; *rm*, retinal matrix.

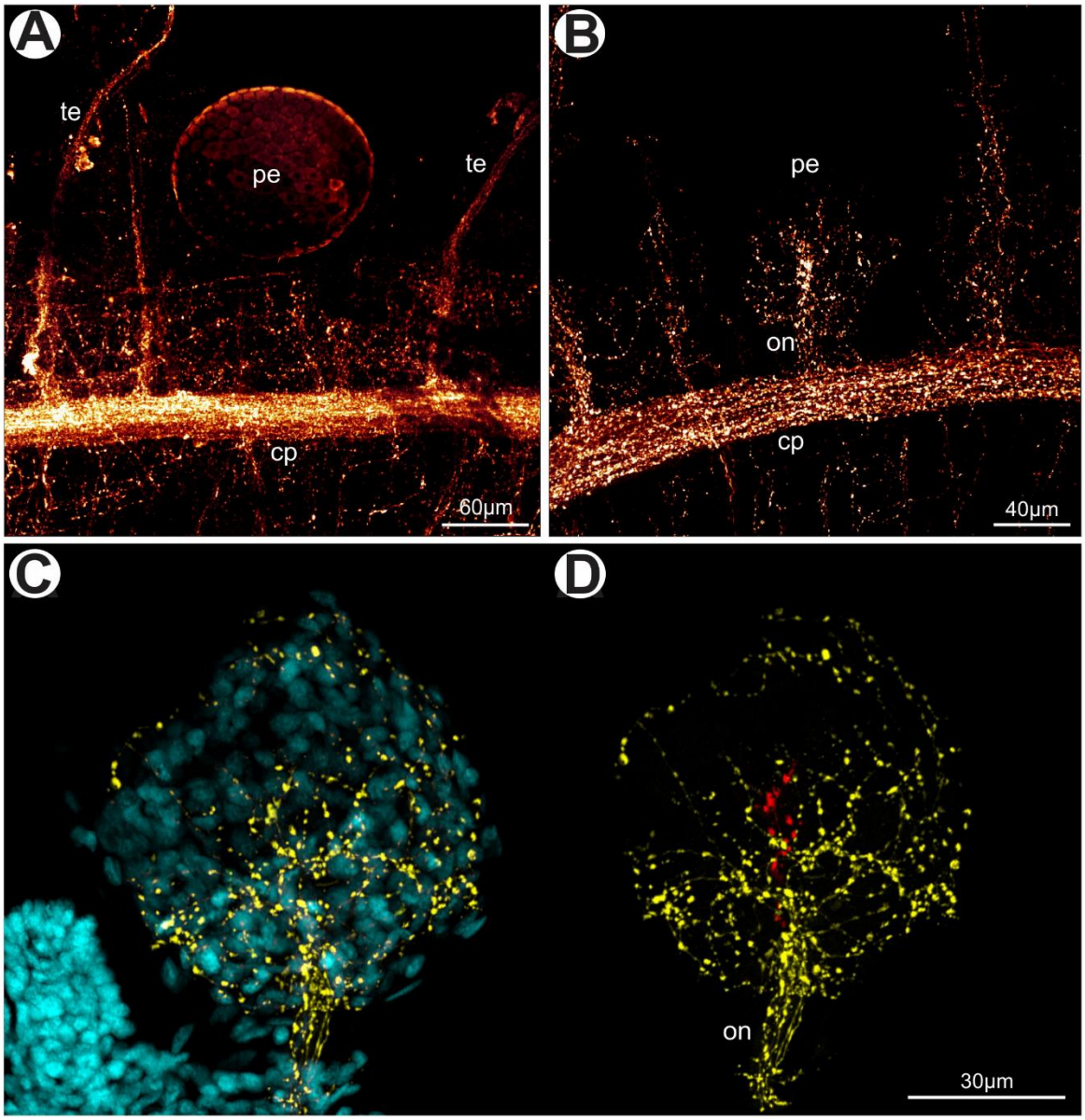

Figure 3. Neuronal marker immunoreactivity in developing pallial eyes of juvenile *N. nodosus*, as revealed by confocal microscopy. **A.** Expression of FMRFamide in the mantle margin, showing very weak signal within the eye. View from the base of the eye. **B.** Serotonergic immunoreactivity in the mantle margin, showing the circum-pallial nerve and its projection to the eye via optic nerve. **C.** Neuronal serotonergic projections in the retina (yellow), as observed by 3D reconstruction based on confocal image stacks. Nuclei stained in blue by DAPI. **D.** Same reconstruction exhibiting the innervation of the proximal retina (yellow), and a small branch innervating the distal retina (red). Abbreviations: *cp*, circum-pallial nerve; *on*, optic nerve; *pe*, pallial eye; *te*, tentacle.

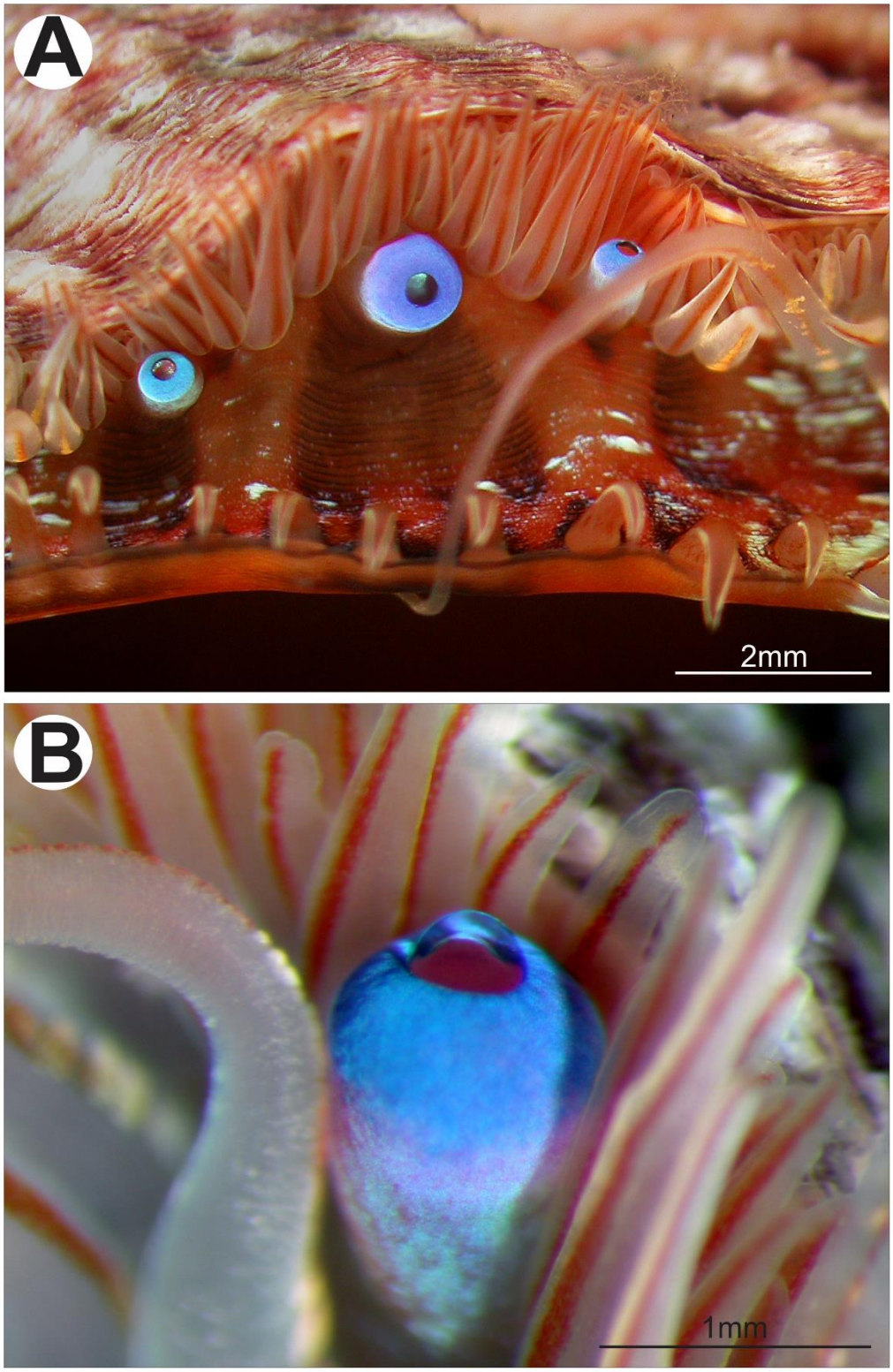

Figure 4. Pallial eyes of adult *N. nodosus*. **A.** Detail of the mantle margin with pallial eyes. **B.** External ocular morphology.

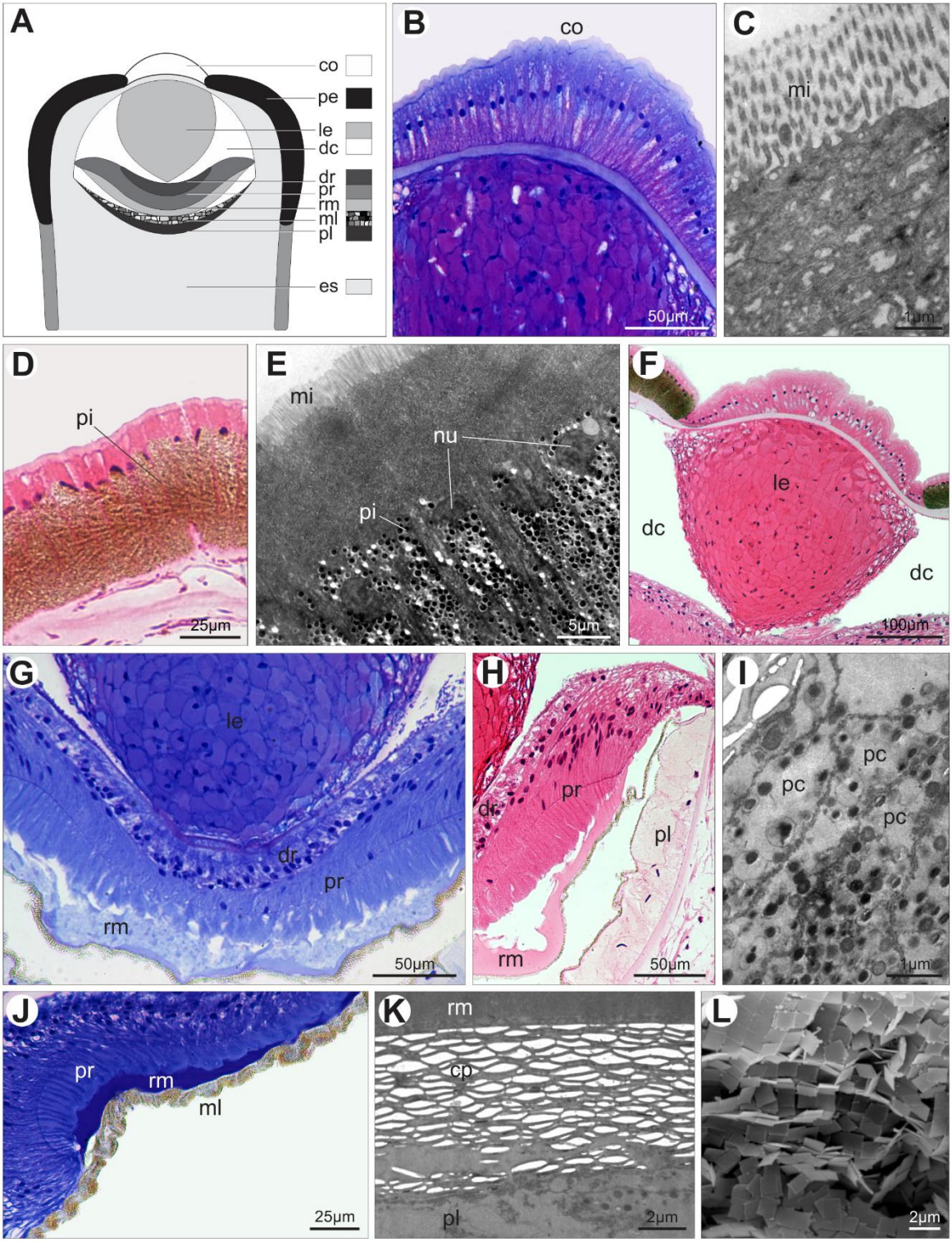

Figure 5. Pallial eye's ocular components of adult *N. nodosus*. **A.** General eye anatomy. **B.** Columnar, unpigmented cells forming the cornea. TB. **C.** Microvilli from cornea cells. TEM. **D.** Pigmented epithelium. HE. **E.** Detail from the pigmented epithelium, with columnar cells with basal pigment granules and microvilli at the apical surface. TEM. **F.** Lens. HE. **G.** Proximal and distal retina. TB. **H.** Histology of rods, with proximal cell portion embedded within the retinal matrix, and of the pigmented layer at the base of the optic vesicle. HE. **I.** Pigmented layer with irregular cells containing large pigment granules. TEM. **J.** Mirror layer located adjacent to the retinal matrix. TB. **K.** Layers of crystal plates forming the mirror layer. TEM. **L.** Detail of the square plates forming the mirror layer. SEM. Abbreviations: *co*, cornea; *cp*, crystal plates; *dc*, distal chamber; *dr*, distal retina; *es*, eyestalk; *le*, lens; *mi*, microvilli; *ml*, mirror layer; *nu*, nucleus; *pc*, pigmented cell; *pe*, pigmented epithelium; *pi*, pigment granules; *pl*, pigmented layer; *pr*, proximal retina; *rm*, retinal matrix.

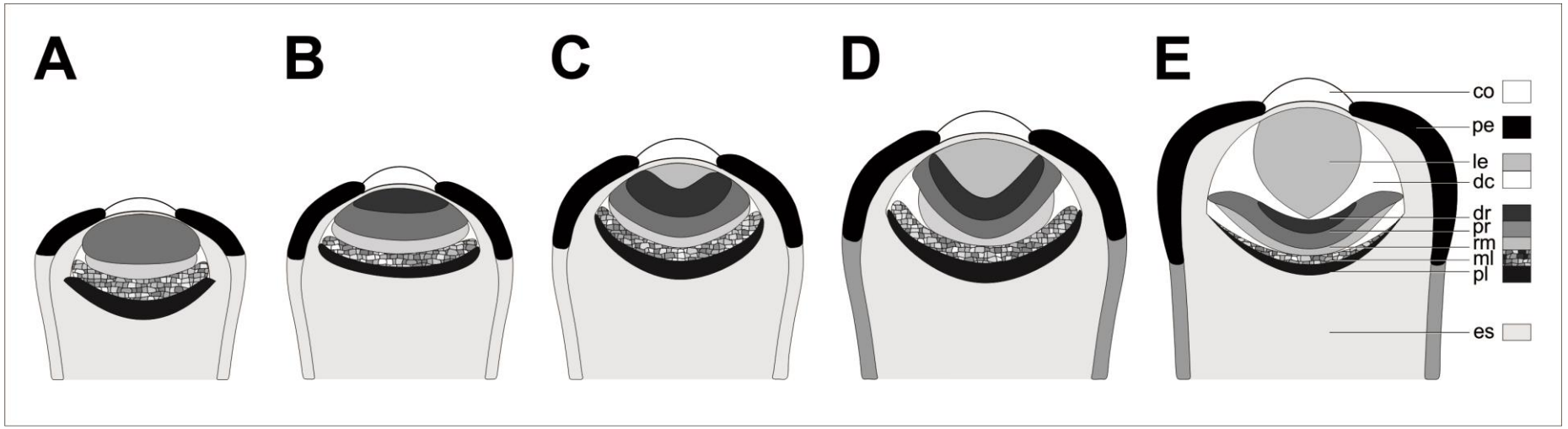

Figure 6. Schematic representation of pallial eye developmental sequence in *N. nodosus*. **A.** Early juvenile stage, including short pigmented epithelium, undifferentiated retina (cell mass), retinal matrix, and presence of mirror and pigmented layer at the base of the optic vesicle. **B.** Intermediate juvenile stage, with distal differentiation of the optic vesicle, with development of the distal retina. **C.** Intermediate juvenile stage, with a developing lens enclosed basally by the retina. **D.** Late juvenile stage, with eye growth and elongation of the pigmented epithelium. **E.** Adult stage, with reorganization of ocular components by change in lens shape and flattening of the retina, resulting in the formation of the distal chamber; mirror and pigmented layers are relatively more compact. Abbreviations: *co*, cornea; *dc*, distal chamber; *dr*, distal retina; *es*, eyestalk; *le*, lens; *ml*, mirror layer; *pe*, pigmented epithelium; *pl*, pigmented layer; *pr*, proximal retina; *rm*, retinal matrix.

CONSIDERAÇÕES FINAIS

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Os resultados obtidos com *Nodipecten nodosus* sustentam diferentes linhas de evidência que permitiram confrontar especulações prévias da literatura e propor um modelo generalizado para o desenvolvimento da margem palial. A anatomia larval da espécie, em conjunto com descrições da literatura (Creek, 1960; Elston, 1980; Waller, 1981; Cragg, 2006; Altnöder and Haszprunar, 2008), sugere a ausência de pregas como condição inicial da margem do manto. Ao longo do desenvolvimento, dois processos de evaginação são cruciais na formação das pregas paliais. O primeiro ocorre no estádio de pedivéliger, originando também o sulco do perióstraco, enquanto o segundo ocorre após a metamorfose, responsável pela origem da prega palial mediana. As informações coligidas corroboram hipóteses anteriores sobre a origem da prega mediana a partir da porção interna da prega interna do manto (Waller, 1980; Morton & Peharda, 2008). Estruturas associadas, como tentáculos e olhos paliais, são formadas apenas após a metamorfose, e compõem a complexa condição final da margem do manto em Pectinidae. Apesar de mudanças drásticas na morfologia pós-metamorfose, o presente estudo revela significativa importância do período larval para a constituição da margem palial. A emergência dos sistemas muscular e nervoso na borda do manto ocorre ainda na fase de pedivéliger, o que representa uma etapa essencial na organização da região, assim como o surgimento e distribuição de cílios no epitélio. Em suma, tanto modificações larvais quanto pós-metamórficas são essenciais para definição da condição de três pregas paliais. Desse modo, a investigação realizada com *N. nodosus* proporcionou o primeiro modelo geral para o desenvolvimento da margem palial em Pectinidae, além de prover profunda contribuição para o entendimento da morfogênese da região em Bivalvia. Consequentemente, o estudo detalhado da formação e anatomia de órgãos paliais associados, como tentáculos e olhos, também representa amplo avanço no conhecimento das especializações do manto.

Embora estruturas tentaculares sejam comuns na margem palial de bivalves, estudos detalhados de morfologia, função e desenvolvimento ainda são escassos para tais órgãos (Guilmour, 1967; Moir, 1977; Yonge, 1983; Tëmkin, 2006). O estudo com *N. nodosus* forneceu contribuições substanciais à caracterização do desenvolvimento e da anatomia de tentáculos paliais em Pectinidae, revelando, por exemplo, a organização comum em epitélio ciliado, musculatura periférica e inervação central. A ampla plasticidade tentacular do grupo é evidenciada por diferentes tipos tentaculares formados após a metamorfose, que diferem

em tipos ciliares sensoriais, atividade secretora e percepção visual. Os dados obtidos para a espécie de estudo sugerem também importantes similaridades com os demais pectinídeos, além de diferenças particulares com outras espécies de bivalves. Neste sentido, análises comparativas de caracteres tentaculares em Bivalvia ainda são muito escassas, o que evidencia a necessidade de mais estudos ontogenéticos e morfológicos para o grupo.

Enquanto diferentes abordagens e técnicas foram empregadas nos estudos de olhos paliais em Pectinidae (*e.g.*, Dakin, 1910; Ciocco, 1998; Morton, 2001; Speiser & Johnsen, 2008; Serb *et al.*, 2013; Malkowsky & Jochum, 2014), a origem e o desenvolvimento de tais órgãos foram considerados apenas em esparsas contribuições (Küpfer, 1916; Butcher, 1930). Em *N. nodosus*, a formação de olhos paliais se assemelha às descrições de regeneração e formação de olhos em outras espécies de pectinídeos (Küpfer, 1916; Butcher, 1930). Neste sentido, uma sequência de desenvolvimento geral foi proposta para descrever a origem e modificação dos componentes oculares na família. A diferenciação da cápsula óptica ocorre em sentido proximal-distal, gerando os componentes proximais (camada refletora e pigmentada), e um grupo celular distal que se diferencia em: retina proximal, retina distal e, por último, em lente. Enquanto os olhos paliais em vieiras adultas possuem um complexo sistema visual, baseado em um mecanismo refletor para formação de imagem (Land, 1965), a organização de olhos em formação sugere um simples nível de fotopercepção direcional, aparentemente incapaz de gerar visão espacial. Desse modo, as modificações da morfologia ocular ao longo do desenvolvimento parecem estar vinculadas ao desempenho óptico da estrutura, revelando pontos vitais na compreensão da morfogênese funcional desses órgãos. Em olhos completamente formados de *N. nodosus*, a morfologia da lente e córnea diverge significativamente de outros pectinídeos, corroborando hipóteses prévias sobre estruturas-chave na variação da morfologia ocular do grupo (Speiser & Johnsen, 2008; Malkowsky & Jochum, 2014). Finalmente, o estudo realizado deve servir como ponto de partida para novas linhas de investigação sobre olhos de bivalves, especialmente sobre mecanismos associados ao desenvolvimento ocular e performance visual em pectinídeos.

REFERÊNCIAS BIBLIOGRÁFICAS

Altnöder, A. & Haszprunar, G. 2008. Larval morphology of the brooding clam *Lasaea adansonii* (Gmelin, 1791) (Bivalvia, Heterodonta, Galeommatoidea). Journal of Morphology 269: 762–774.

Butcher, E. O. 1930. The formation, regeneration, and transplantation of eyes in *Pecten* (*Gibbus borealis*). Biological Bulletin 59(2): 154–164.

Ciocco, N. F. 1998. Anatomía de la vieira tehuelche, *Chlamys tehuelcha* (d´Orb). IV. Sistema nervioso y estructuras sensoriales (Bivalvia, Pectinidae). Revista de Biologia Marina y Oceanografía 33(1): 25–42.

Cragg, S. M. 2006. Development, physiology, behavior and ecology of scallop larvae, p. 45–122. In: Shumway, S. E. & Parsons, G. J. (Eds). Scallops: biology, ecology and aquaculture. Elsevier.

Creek, G. A. 1960. The development of the lamellibranch *Cardium edule* L. Proceedings of the Zoological Society of London 135(2): 243–260.

Dakin, W. J. 1910. The eye of *Pecten*. Quarterly Journal of Microscopical Science 55: 49–112.

Elston, R. 1980. Functional anatomy, histology and ultrastructure of the soft tissues of the larval American oyster, *Crassostrea virginica*. Proceeding of the. National Shellfish Association 70: 65–93.

Guilmour, T. H. J. 1967. The defensive adaptations of *Lima hians* (Mollusca, Bivalvia). Journal of the Marine Biological Association of the United Kingdom 47: 209–221.

Küpfer, M. 1916. Entwicklungsgeschichtliche und neuro-histologische Beiträge zur Kenntnis der Sehorgane am Mantelrande der *Pecten*-Arten: mit anschliessenden vergleichend-anatomischen Betrachtungen (pp. 1–312). Jena, Fischer.

Land, M. F. 1965. Image formation by a concave reflector in the eye of the scallop, *Pecten maximus*. The Journal of Physiology 179: 138–153.

Moir, A. J. G. 1977. Ultrastructural studies on the ciliated receptors of the long tentacles of the Giant Scallop, *Placopecten magellanicus* (Gmelin). Cell and Tissue Research 184: 367–380.

Malkowsky, Y. & Jochum, A. 2014. Three-dimensional reconstructions of pallial eyes in Pectinidae (Mollusca: Bivalvia). Acta Zoologica (Stockholm) 0: 1–7.

Morton, B. 2001. The function of pallial eyes within the Pectinidae, with a description of those present in *Patinopecten yessoensis*. Geological Society, London, Special Publications 177: 247–255.

Morton. B. & Peharda, M. 2008. The biology and functional morphology of *Arca noae* (Bivalvia: Arcidae) from the Adriatic Sea, Croatia, with a discussion on the evolution of the bivalve mantle margin. Acta Zoologica (Stockholm) 89: 19–28.

Serb, J. M.; Porath-Krause, A. J. & Pairett, A. N. 2013. Uncovering a gene duplication of the photoreceptive protein, opsin, in scallops (Bivalvia: Pectinidae). Integrative and Comparative Biology 53: 68–77.

Speiser, D. I. & Johnse,n S. 2008. Comparative morphology of the concave mirror eyes of scallops (Pectinoidea). American Malacological Bulletin 26: 27–35.

Tëmkin, I. 2006. Morphological perspective on the classification and evolution of Recent Pterioidea (Mollusca: Bivalvia). Zoological Journal of the Linnean Society 148: 253–312.

Waller, T. R. 1980. Scanning electron microscopy of shell and mantle in the order Arcoida (Mollusca: Bivalvia). Smithsonian Contributions to Zoology 313.

Waller, T. R. 1981. Functional morphology and development of veliger larvae of the European oyster, *Ostrea edulis* Linné. Smithsonian Contributions to Zoology 328.

Yonge, C. M. 1957. Mantle fusion in the Lamellibranchia. Pubblicazioni della Stazione Zoologica di Napoli, 29: 151–171.

Yonge, C. M. 1983. Symmetries and role of mantle margins in the bivalve Mollusca. Malacological Review 16: 1–10.

RESUMO

O atual conhecimento sobre a margem do manto em moluscos bivalves é extenso, incluindo informações sobre morfologia, função e diversidade. Bivalves da família Pectinidae, também conhecidos como vieiras, possuem complexa margem palial, organizada em três pregas, incluindo olhos e tentáculos. Questões acerca do desenvolvimento da margem do manto em bivalves continuam amplamente incompreendidas, assim como a relação entre características paliais ao longo dos diferentes estádios do ciclo de vida. Neste contexto, a presente investigação utilizou a espécie de vieira *Nodipecten nodosus* como modelo para compreensão da morfogênese da margem palial em Pectinidae, com ênfase na origem e diferenciação das pregas paliais e estruturas associadas. Para contemplar esses objetivos, espécimes em diferentes estádios de desenvolvimento larval e pós-metamórfico foram analisados por meio de técnicas integradas de microscopia (*i.e.*, histologia, microscopia eletrônica de varredura e transmissão, e imunocitoquímica aplicada à microscopia confocal). Inicialmente, a margem palial em larvas véliger de *N. nodosus* não é pregueada, porém, ao longo do desenvolvimento, dois processos de evaginação são determinantes na formação das pregas paliais. O primeiro ocorre no estádio de pedivéliger, originando as pregas externa e interna, bem como o sulco do perióstraco. O segundo ocorre após a metamorfose, sendo responsável pela origem da prega palial mediana a partir da porção interna da prega interna. Os sistemas muscular e nervoso da margem palial têm origem durante o período larval, tornando-se amplamente desenvolvidos posteriormente. Estruturas associadas, como tentáculos e olhos paliais, são formadas apenas após a metamorfose, e compõem a complexa condição final da margem do manto em Pectinidae. Os diferentes tipos tentaculares possuem desenvolvimento e anatomia similar, entretanto diferem quanto ao tamanho, tipo de musculatura, organização ciliar e presença de células glandulares. Os olhos paliais em formação diferenciam-se gradualmente em sentido proximal-distal, essas características morfológicas sugerindo um nível simples de fotopercepção direcional como condição inicial. Os dados aqui apresentados para *N. nodosus* permitiram propor um modelo geral para o desenvolvimento da margem palial em Pectinidae, além de contribuir para o entendimento da morfogênese dessa região em Bivalvia.

Palavras-chave: bivalves, desenvolvimento, larva, moluscos, prega palial.

ABSTRACT

Current knowledge of the bivalve mantle margin is extensive, covering several aspects of its morphology, function and diversity. Bivalves from the family Pectinidae, also known as scallops, bear three pallial folds at the mantle margin, including complex structures, such as tentacles and eyes. The development of the bivalve mantle margin is still poorly understood, the morphogenesis and functional anatomy of mantle margin features during developmental stages being enigmatic. The present investigation used the scallop *Nodipecten nodosus* (L. 1758) as a model species to understand mantle margin morphogenesis in the Pectinidae, with emphasis on the origin and differentiation of pallial folds and associated pallial structures. To achieve these goals, specimens from larval and postmetamorphic stages were thoroughly analyzed by means of integrative microscopy techniques (*i.e.*, histology, scanning and transmission electron microcopy, and immunocytochemistry combined with confocal microscopy). In veliger larvae of *N. nodosus*, the mantle margin is initially unfolded, two folding processes being crucial for pallial fold establishment during further development. The first one occurs by the pediveliger stage, forming the outer and inner folds, as well as the periostracal groove. The second folding process takes place after metamorphosis and is responsible for the formation of the middle pallial fold from the inner region of the inner mantle fold. The emergence of muscular and nervous systems in the mantle margin occurs early during development, at the larval stage. Associated pallial structures, including tentacles and eyes, develop only after metamorphosis, and contribute to the complex final condition of the mantle margin in Pectinidae. Although different tentacular types have similar development and anatomy, they differ in size, muscle type, ciliary organization, and gland cells distribution. Developing pallial eyes exhibit gradual differentiation in a proximal-distal direction, and their morphological features suggest a simple level of directional photoreception as the initial ocular condition in juveniles. The present investigation conducted with *N. nodosus* provided a general model to understand mantle margin development in the Pectinidae, as well as insights into the morphogenesis of this region in the Bivalvia.

Keywords: bivalves, development, larva, molluscs, pallial fold.

ANEXOS

ANEXO 1

Relação taxonômica dos principais grupos viventes contidos em Bivalvia, baseado em Bieler *et al.* (2010).

CLASSE BIVALVIA Linnaeus, 1758

Subclasse Protobranchia Pelseneer, 1889

Ordem Nuculida Dall, 1889

Ordem Solemyida Dall, 1889

Ordem Nuculanida Carter *et al.*, 2000

Subclasse Autobranchia Grobben, 1894

Superordem Pteriomorphia Beurlen, 1944

Ordem Mytilida Férussac, 1822

Ordem Arcida Gray, 1854

Ordem Pteriida Newell, 1965

Ordem Ostreida Férussac, 1822

Ordem Pectinida Gray, 1854

Ordem Limida Moore, 1952

Superordem Heteroconchia Hertwig, 1895

Clado Palaeoheterodonta Newell, 1965

Ordem Trigoniida Dall, 1889

Ordem Unionida Gray, 1854

Clado Heterodonta Neumayr, 1884

Ordem Lucinida Gray, 1854

Ordem Carditida Dall, 1889

Ordem Venerida Gray, 1854

Ordem Myida Stoliczka, 1870

Ordem Pholadomyida Newell, 1965 = Anomalodesmata Dall, 1889

ANEXO 2

SÍNTESE DO CONHECIMENTO SOBRE A DIVERSIDADE DE SISTEMAS VISUAIS EM MOLLUSCA, COM ÊNFASE EM BIVALVIA

SYNTHESIS OF THE KNOWLEDGE OF VISUAL SYSTEMS DIVERSITY IN MOLLUSCA, WITH EMPHASIS ON BIVALVIA

RESUMO

A diversidade de órgãos fotorreceptores encontrada em Metazoa é surpreendente. O estudo dos sistemas visuais animais vem crescendo nos últimos anos e contribuindo com avanços significativos, principalmente por meio da pesquisa baseada em diferentes táxons e pela incorporação de novas técnicas moleculares e de microscopia. A maior variedade de sistemas visuais ocorre no filo Mollusca, em especial na classe Bivalvia, sendo por isso animais considerados potenciais modelos para ampla gama de investigações no contexto da evolução da visão em Metazoa. Primeiramente, este artigo expõe as principais discussões atuais sobre homologias e convergências da fotopercepção animal. Em seguida, é apresentada uma revisão crítica do conhecimento sobre sistemas visuais em moluscos, destacando-se a diversidade estrutural e os aspectos centrais da organização ocular dos bivalves. Finalmente, são apontadas lacunas no conhecimento e novas perspectivas de estudos empregando moluscos, considerando o histórico de investigações no grupo e os atuais paradigmas de diversidade e evolução da visão animal.

Palavras-chaves: bivalves, convergências, fotorreceptores, moluscos, olhos.

ABSTRACT

The diversity of metazoan photoreceptor structures is astonishing. Studies on animal visual systems are increasingly growing in number and advancing our knowledge by incorporating various taxa and novel molecular and microscopy techniques. The phylum Mollusca exhibits huge diversity of ocular structures and Bivalvia is the most diverse class in this aspect. Besides morpho-functional variation, these animals represent potential models for different kinds of studies; therefore, investigations on molluscan eyes are vital within the context of evolution of vision in Metazoa. Firstly, this paper presents the main current

hypotheses about homologies and convergences of animal photoreception. Then, a critical review of the knowledge of molluscan visual systems is presented, with emphasis on the structural diversity exhibited by several of its representatives, as well as central aspects of ocular organization in bivalves. Finally, gaps in our knowledge and potential perspectives for future research are described based on the historical record of investigations for the group, and current paradigms for the diversity and evolution of animal vision are discussed.

Keywords: bivalves, convergences, eyes, mollusks, photoreceptors.

INTRODUÇÃO

Estruturas fotossensíveis estão presentes nas mais diversas espécies de seres vivos, desde fotorreceptores simples em bactérias e eucariontes unicelulares, até olhos complexos em muitos animais (Nilsson, 2009). Há pouca informação disponível sobre o surgimento de órgãos fotossensíveis em Metazoa, principalmente porque os registros mais antigos sobre estruturas oculares estão presentes apenas em fósseis de trilobitas do início do Cambriano, há cerca de 530 milhões de anos atrás (Land & Nilsson, 2012). Após este período, no início do Ordoviciano (488 maa), o registro fóssil já contém representantes da maioria dos filos de metazoários atualmente reconhecidos e, surpreendentemente, também revela ampla variedade de estruturas oculares (Nilsson, 2009; Land & Nilsson, 2012).

A compreensão da evolução e função adaptativa dos fotorreceptores é um tema complexo, amplamente estudado e com avanços recentes significativos a partir do emprego de diferentes abordagens (*e.g.*, Erclik *et al.*, 2009; Vopalensky & Kozmik, 2009; Gehring, 2012). Este artigo apresenta uma revisão crítica sobre o atual conhecimento relacionado à diversidade de estruturas fotorreceptoras em Mollusca, com ênfase nos moluscos bivalves. Para abordar o tema em um contexto mais integrado, uma síntese geral da fotopercepção em Metazoa é apresentada com o objetivo de pontuar as atuais discussões sobre homologias e convergências associadas à evolução da fotopercepção. Considerando tais perspectivas e também o histórico de estudos em Mollusca, são apontadas lacunas no conhecimento e potenciais campos de pesquisa relacionados à diversidade e evolução dos sistemas visuais no grupo.

FOTOPERCEPÇÃO

Apesar do emprego recorrente na literatura científica de termos como “visão” e “olho”, esses conceitos nem sempre são claramente definidos. O crescente avanço no estudo

da fotopercepção animal faz com que este seja um tema constantemente reavaliado. A proposta de classificação apresentada a seguir baseia-se no estado da arte da literatura sobre fotopercepção em Metazoa (Land & Nilsson, 2012; Nilsson, 2013), sendo particularmente útil na compreensão da diversidade ocular em Mollusca, como discutido mais adiante. Neste contexto, são consideradas quatro classes morfofuncionais associadas a comportamentos visualmente guiados:

I. *Fotopercepção não-direcional*. Células fotorreceptoras simples, geralmente isoladas, apenas identificam a presença, intensidade e ausência de luz no ambiente.

II. *Fotopercepção direcional*. Células fotorreceptoras são acompanhadas de células pigmentadas que atuam sombreando os fotorreceptores, de modo que a luz é detectada em algumas direções, mas não em outras. Deve-se ressaltar que qualquer resposta de fototaxia exibida por um animal é dependente de um sistema direcional de fotopercepção.

III. *Visão de baixa resolução*. Quando diferentes células fotorreceptoras encontram-se organizadas, por exemplo, em taças pigmentares, diferentes direções são simultaneamente percebidas. Este é o princípio da visão, onde a estrutura que contém os fotorreceptores é informada sobre a variação de luz no espaço de forma simultânea e comparada.

IV. *Visão de alta resolução*. A inclusão de diferentes componentes e estruturas ao sistema III está associada ao avanço da resolução óptica e, consequentemente, ao aumento da diversidade de morfologias oculares. O surgimento de lentes e outros mecanismos para obtenção de foco foram inovações-chave na evolução de comportamentos visualmente guiados mais específicos e complexos.

Em suma, apenas as classes III e IV são considerados visão em razão de sua acuidade espacial. Consequentemente, olhos são estruturas visuais incluídas nestas duas classes. Os tipos I e II são classes de fotopercepção mais simples que não correspondem a olhos propriamente ditos devido à ausência de uma morfologia conspícua e limitações ópticas (Nilsson, 2013). Os princípios gerais expostos acima correspondem a um modo de avaliar a diversidade de morfologias oculares presente entre os animais por meio da variação conjunta entre gradientes de complexidade dos elementos constituintes, suas propriedades ópticas e as respostas comportamentais associadas (Land & Nilsson, 2012).

Estruturas fotorreceptoras em Metazoa

As células fotorreceptoras animais podem apresentar dois tipos de modificação da membrana celular relacionadas à sensibilidade e resposta à luz (Eakim, 1965): rabdoma (Fig. 1A), conjunto de microvilosidades presente em diversos filos de animais protostômios, e

cílio (Fig. 1B), no qual há dobras na região da membrana que o envolve, presentes nos deuterostômios (Eakim, 1965; Nilsson, 2004; Fain *et al.*, 2010). Receptores rabdoméricos e ciliares também diferem entre si quanto às proteínas envolvidas na fotopercepção e quanto ao tipo de cascata de sinalização celular produzida (Arendt, 2003). Embora se observe uma aparente dicotomia entre deuterostômios e protostômios, a evolução das células receptoras ainda é tema de discussão, pois há muitas controvérsias e exceções (Fain *et al.*, 2010). Por exemplo, invertebrados protostômios como bivalves e artrópodes também apresentam receptores ciliares semelhantes aos de vertebrados, revelando casos de convergência evolutiva (Arendt, 2003). De forma consensual, a evolução da morfologia das células fotorreceptoras é considerada homoplástica, com inúmeros casos de convergência e evolução independente em distintos táxons animais (Salvini-Plawen, 2008).

A fotopercepção mais simples em Metazoa corresponde às classes I e II, presentes na maioria dos filos animais (Nilsson, 2009; Land & Nilsson, 2012). Entretanto, estruturas como manchas ocelares frequentemente variam quanto à classificação (II ou III) conforme critérios ópticos e comportamentais (Nilsson, 2013). Os sistemas oculares (classes III e IV) são classificados de acordo com os mecanismos de formação de imagem, podendo ser subdivididos em olhos compostos e simples. Os olhos compostos são formados pelo arranjo de milhares de unidades fotorreceptoras denominadas omatídeos, cuja organização estrutural e distribuição dos pigmentos estão classificadas em seis subtipos distintos (Land, 1992). Este tipo ocular é característico dos artrópodes, principalmente de representantes de Crustacea e Insecta, além de ocorrer também em outros grupos, como poliquetos e bivalves (Nilsson, 1994). Os olhos simples são formados por camadas de células fotorreceptoras associadas a células pigmentadas; entretanto, muitos outros elementos podem estar presentes (Land, 1992; Jonasova & Kozmik, 2008). Tanto olhos compostos quanto simples são chamados de complexos quando possuem maior acuidade e/ou sensibilidade visual. Olhos nesta condição ocorrem em apenas seis filos de metazoários: Cnidaria, Annelida, Mollusca, Onycophora, Arthropoda e Chordata (Land & Nilsson, 2012).

HOMOLOGIAS

Como descrito acima, olhos são encontrados em diversos filos e possuem uma variedade morfológica surpreendente. O desenvolvimento ocular é distinto entre os grandes grupos de animais e, apesar de haver semelhanças, em geral assume-se que não há qualquer homologia estrutural envolvida (Land & Nilsson, 2012). Mesmo dentro dos filos, como no caso de Mollusca, a evolução dos fotorreceptores e estruturas oculares é reconhecidamente

não-homóloga (Salvini-Plawen, 2008). Neste sentido, estudos detalhados de anatomia e ontogenia são fundamentais para compreensão de convergências evolutivas e possíveis homologias (Serb & Eernisse, 2008).

Apesar das evidências morfológicas apontarem para caminhos independentes e convergentes, nas últimas décadas dados da biologia molecular colocaram em questão a completa ausência de homologia entre tantas estruturas visuais. Pesquisas na área de regulação gênica de estruturas oculares revelaram que genes da família *Pax* estão intimamente relacionados à regulação de transcrições envolvidas na formação de estruturas visuais em metazoários (*e.g.*, Gehring, 2002; Nilsson, 2004; Fernald, 2006). Recentemente, diversos estudos foram realizados para avaliar a expressão desses genes e sua ação de regulação sobre transcrições em diferentes grupos animais (*e.g.*, Gehring, 2005, 2012; Vopalensky & Kozmik, 2009). A variedade *Pax6* aparece como iniciador do processo de formação de estruturas fotorreceptoras em Bilateria, atuando na transcrição das duas principais classes de opsinas que estão presentes no sistema de receptores rabdomérico e ciliar (Nilsson, 2009). As opsinas são proteínas trans-membranas de domínios característicos, essenciais na cascata proteica envolvida na despolarização ou hiperpolarização da membrana celular durante a transdução do sinal luminoso (Fain *et al.*, 2010). Além de iniciarem a cascata de eventos da fotopercepção, estudos apontam que esse grupo de proteínas se apresenta altamente conservado em Metazoa (Fain *et al.*, 2010). Por exemplo, a formação de olhos em medusas das classes Cubozoa e Hydrozoa está associada à expressão de genes da família *Pax* (também presente em outros grupos de Cnidaria), que, por apresentar variações nos domínios principais, recebeu o nome de *PaxB* (Piatigorsky, 2003). Estudos recentes sugerem ainda que o gene *PaxB* possa corresponder à condição ancestral, a qual, por meio de eventos de duplicação e diversificação, teria originado as variedades *Pax6* e *Pax 2/5/8* dos bilatérios (*e.g.*, Ruzickova *et al.*, 2009). Recentemente, a expressão de opsinas em Ctenophora também foi constatada, contribuindo para o conhecimento sobre a origem e conservação dessas proteínas (Schinitzler, 2012).

Em suma, as evidências acumuladas para diferentes grupos animais revelam que, apesar da origem embrionária distinta e das demais diferenças no desenvolvimento de órgãos fotorreceptores, há uma base molecular ancestral comum: os animais compartilham genes ortólogos reguladores de transcrição, fundamentais para formação de estruturas visuais (*e.g.*, Nilsson, 2004; Fernald, 2006; Ruzickova *et al.*, 2009; Gehring, 2012). Sendo assim, pode não haver homologia nos tecidos que originam os olhos, porém ela existe quando considerada a base molecular reguladora dessa formação. Como apontado por Piatigorsky

(2008), é fundamental se pensar em diferentes níveis de homologia quando se trata da evolução das estruturas visuais nos animais.

MOLLUSCA

O contexto evolutivo e morfológico dos sistemas visuais dos metazoários é fundamental para o embasamento das discussões sobre evolução e caracterização das estruturas fotorreceptoras de Mollusca. O filo corresponde a um dos mais diversificados grupos de metazoários, com cerca de 130.000 espécies descritas para a fauna atual e 70.000 para o registro fóssil (Haszprunar *et al.*, 2008). A diversidade de estruturas fotossensíveis também é surpreendentemente diversa, pois, além da fotopercepção direcional e não direcional (classes I e II), a visão no grupo pode ocorrer em taças ou cúpulas pigmentares (classes II e III) ou em olhos do tipo câmera (classes III e IV). A variedade de estudos sobre fotopercepção no filo inclui abordagens genéticas, experimentais, fisiológicas e anatômicas, permitindo o reconhecimento de padrões gerais bem como especificidades em cada classe.

Uma das sinapomorfias de Polyplacophora é justamente a presença de estruturas sensoriais características denominadas estetos, presentes em canais que atravessam as placas valvares (Todt *et al.*, 2008). Células fotorreceptoras presentes em tais canalículos percebem a luminosidade do meio externo, permitindo ao animal uma resposta de exposição ou fuga de ambientes mais iluminados (Moseley, 1884). Quítons do gênero *Mopalia* (Mopaliidae), por exemplo, apresentam fototaxia negativa, ou seja, movimentam-se na direção contrária à luz (Fitzgerald, 1975). A distribuição de ocelos pode ser regular ou irregular e, em alguns casos, com maior concentração nas valvas anteriores (Speiser *et al.*, 2011a). Pode haver também variedade nos tipos estruturais presentes, como ocelos intra e extra-pigmentares, que permitem a percepção de intensidade e direção da luz (Crozier, 1920). Em uma condição mais simples, células fotorreceptoras rabdoméricas podem estar associadas a células pigmentadas ao longo do canalículo, como observado em *Chiton olivaceus* (Chitonidae) (Fischer, 1978). Surpreendentemente, casos de visão em baixa resolução ocorrem no grupo a partir de estruturas diferenciadas. Por exemplos, estudos de morfologia e comportamento do quíton *Acanthopleura granulata* (Chitonidae) revelaram a presença de estetos cujos ocelos (Fig. 2A) contêm lentes compostas por aragonita, uma curiosa exceção entre os animais, os quais, em sua maioria, possuem lentes formadas por proteínas (Speiser *et al.*, 2011a). Investigações conduzidas com a espécie sugerem que a lente de aragonita seja responsável, em parte, pela capacidade de formação de imagem na retina, tanto na condição submersa como em exposição ao ar (Speiser *et al.*, 2011a). Apesar das descrições gerais e

dos estudos aprofundados com *A. granulata*, as estruturas fotorreceptoras dos poliplacóforos ainda foram pouco investigadas. Detalhes da anatomia e variação entre os diferentes grupos da classe são escassos, assim como estudos genéticos, ontogenéticos e fisiológicos.

Os gastrópodes, em sua maioria, apresentam olhos cefálicos localizados na base dos tentáculos. No caso dos gastrópodes pulmonados terrestres (Stylommatophora), os olhos são terminais, localizados no ápice dos tentáculos cefálicos (Mordan & Wade, 2008). Duas morfologias oculares podem estar presentes em Gastropoda: olhos fechados, que podem apresentar lentes, e olhos sem córnea, abertos para o meio (*open pit*) (Serb, 2008). De modo geral, tais condições morfológicas sugerem fotopercepção associada às classes II e III. Caramujos e lesmas terrestres, como o *scargot* (*Helix aspersa*, Helicidae), possuem estrutura ocular terminal com córnea, retina e lente celular (Fig. 2B) (Meisenhaimer, 1912). Gastrópodes marinhos do gênero *Littorina* (Littorinidae), comuns em costões rochosos, possuem organização similar, embora seus olhos ocorram na base dos tentáculos cefálicos e apresentem lente celular com diferentes índices refrativos (Seyer, 1992). Já em outros grupos, como no gênero *Haliotis* (Haliotidae), conhecidos como abalones, o olho possui uma abertura estreita para o meio, além de uma cavidade com retina e preenchimento gelatinoso homogêneo (Fig. 2C) (Cox, 1962). Componentes oculares com funções específicas já foram descritos para diversos gastrópodes, como retinas acessórias em Limacidae (lesmas terrestres), potencialmente envolvidas na percepção de infravermelho (Kataoka, 1975). É interessante notar que, além dos olhos cefálicos, estudos morfofisiológicos também foram conduzidos com ocelos localizados em papilas dorsais de lesmas marinhas da família Onchidiidae (Katagiri *et al.*, 2002).

As áreas mais estudas no contexto da fotopercepção em Gastropoda são a ultraestrutura e a fisiologia, principalmente com espécies de pulmonados terrestres e aquáticos, modelos comuns em investigações sobre olhos (*e.g.*, Bobkova *et al.*, 2004; Zieger & Meyer-Rochow, 2008). A regeneração ocular é outro tema muito explorado, havendo grande variedade de informações compiladas para diferentes espécies de gastrópodes (Tuchina & Meyer-Rochow, 2010). Detalhes da formação do órgão visual foram descritos para *Helix aspersa* (Eakin & Bradenburger, 1967), entretanto estudos mais recentes de morfogênese e expressão gênica ainda são escassos para o grupo (*e.g.*, O'Brien & Degnan, 2002).

Em Cephalopoda ocorrem dois padrões básicos de olhos cefálicos do tipo câmera. Na subclasse Nautiloidea, cujos representantes são os náutilos, os olhos possuem uma organização conhecida como *pinhole camera*, pois se trata de uma cavidade aberta para o

meio, sem lente nem córnea (Fig. 2D). Sendo assim, a retina está localizada ao fundo do olho e em contato direto com a água do mar (Muntz & Raj, 1984). Os olhos dos náutilos são relativamente grandes quando comparados aos demais cefalópodes e possuem capacidade de regular a pupila em resposta à intensidade de luz (Muntz & Raj, 1984). Estima-se que esse arranjo ocular permita percepção de movimento e resolução potencialmente alta, contudo a sensibilidade é muito reduzida devido à pequena abertura do olho, o que limita a captura de luz (Muntz & Raj, 1984; Muntz, 1999). Diferentes hipóteses sobre a evolução dos olhos associada a hábitos de vida em náutilos já foram levantadas, explorando principalmente a compatibilidade do sistema visual com visão a curta distância, movimentos lentos de natação e migração vertical (Muntz, 1999; Colicchia, 2006).

Na subclasse Coleoidea (grupo que abrange as lulas, sépias e polvos) ocorrem olhos do tipo câmera, cuja organização é a mais complexa dentre os invertebrados e de notável convergência com o modelo ocular de vertebrados (Fig. 2E) (Serb, 2008). Os olhos desses cefalópodes são classificados dentro da classe IV em razão de sua complexidade estrutural, óptica e vasto repertório de comportamentos visualmente guiados, de modo que este é o único grupo de Mollusca a receber tal classificação (Nilsson, 2013). Esse tipo ocular apresenta alta resolução, contendo um sistema de retina, lente, íris e córnea, porém, diferente dos receptores ciliares dos vertebrados, a organização da membrana nos coleoides é rabdomérica, ou seja, por microvilosidades (Young, 1971). Outro aspecto fundamental em que ambos diferem é a organização dos tecidos: a camada de células ganglionares está situada sob a retina nos coleoides, situação inversa a dos vertebrados (Young, 1971). Dentre os cefalópodes, o olho mais sofisticado pertence às lulas (ordem Teuthida), contudo há grande variação na forma, simetria e composição ocular, de modo que diferentes aspectos da evolução do sistema visual em Coleoidea vêm sendo investigados em conjunto com informações sobre hábito de vida pelágico, profundidade de ocorrência, metabolismo e captura de presas (Sweeney *et al.*, 2007; Serb, 2008). O sistema sensorial e nervoso de cefalópodes foi e ainda é o mais intensamente estudado em comparação com os demais grupos de moluscos. Neste sentido, é interessante ressaltar que informações mais detalhadas podem ser encontradas na literatura em extensos conjuntos de trabalhos sobre anatomia ocular, neurofisiologia, comportamento e ecologia visual de cefalópodes (*e.g.*, Hanlon & Messenger, 1996; Hanlon & Shashar, 2003; Nixon & Young, 2003; Sweeney *et al.*, 2007).

BIVALVIA

Os moluscos bivalves são animais de corpo mole protegido por duas valvas e que apresentam extensa diversidade de formas e hábitos de vida (Giribet, 2008). O adulto não possui cabeça e os diferentes tipos de órgãos sensoriais ocorrem principalmente concentrados no manto e em sua margem livre (Stasek & McWilliams, 1973; Yonge, 1983). Dentre os demais moluscos, os bivalves apresentam a maior diversidade de estruturas fotorreceptoras (Serb, 2008). Nessa classe, a percepção luminosa varia de acordo com a localização da estrutura receptora, a complexidade de elementos envolvidos e o mecanismo de fotopercepção, podendo inclusive haver olhos notadamente complexos (Serb & Earnisse, 2008). Segundo Morton (2008), a fotopercepção neste grupo pode ocorrer basicamente por três tipos: receptores simples do manto, ocelos cefálicos ou olhos paliais. O primeiro tipo corresponde a fotorreceptores simples, onde o próprio nervo palial contém células sensíveis à luminosidade (Kennedy, 1960). Essa condição sugere uma fotopercepção não-direcional vinculada à classe I. Respostas à sombra são típicas nesse caso, por exemplo, com consequente retração dos sifões. Esse tipo de mecanismo é observado em diversos bivalves, principalmente em habitantes de águas mais rasas e de substratos pouco profundos, como é o caso do escavador *Donax* (Donacidae), que responde a variações de luminosidade com ajustes de sua posição no sedimento (Ansell *et al.*, 1998).

Os ocelos cefálicos foram descritos para a fase larval de representantes de apenas algumas famílias da infraordem Pteriomorphia (*e.g.*, Cole, 1938; Hodgson & Burke, 1988; Carriker, 1990), agrupamento que inclui as ostras, vieiras e mexilhões, o que levou à hipótese de que tais órgãos teriam surgido apenas uma vez em Bivalvia (Morton, 2008). Situados na região cefálica da larva (anteriormente às brânquias), os ocelos cefálicos possuem forma de taça pigmentar uniforme, podendo inclusive haver uma lente amorfa (Waller, 1981; Hodgson & Burke, 1988). Tais estruturas ocorrem apenas na fase larval e sofrem degeneração ao longo do desenvolvimento (Carriker, 1990). Apesar de aspectos funcionais serem ainda especulativos, os estudos realizados até o momento permitem o reconhecimento de um padrão morfológico para esses ocelos cujas características sugerem uma fotopercepção direcional (classe II).

Os olhos paliais certamente compõem o tipo ocular que mais se destaca, tanto pela diversidade quanto pela plasticidade (Fig. 3). Tais estruturas ocorrem na margem livre do manto e assume-se que seu surgimento tenha ocorrido diversas vezes de forma independente em diferentes linhagens de bivalves ao longo da evolução do grupo (Morton, 2008). A hipótese da evolução homoplástica de estruturas oculares em Bivalvia, principalmente de

olhos paliais, é fortemente sustentada por diferentes dados morfológicos e ultraestruturais sobre evolução e diversidade de fotorreceptores na classe (Salvini-Plawen, 2008). Tal cenário é congruente com a evolução da fotopercepção em Mollusca e também entre os demais grupos de metazoários, cujo recrutamento e interação independente de genes regulatórios são responsáveis pela formação da diversidade observada de estruturas oculares, muitas vezes com notáveis convergências evolutivas (Jonasova & Kozmik, 2008; Piatigorsky, 2008).

A borda livre do manto apresenta geralmente três pregas distintas e, segundo Morton (2008), a classificação dos olhos paliais em bivalves pauta-se na localização nessas pregas, havendo, portanto, três categorias de olhos. No primeiro tipo ocular, os olhos são formados na face interna da prega externa, sendo a porção mais externa responsável pela secreção dos componentes da valva. O arranjo ocular pode ser de taça pigmentar ou de taça evertida, formando uma cúpula fotorreceptora (Morton & Peharda, 2008). É interessante notar que o sulco entre a prega mediana e a externa é responsável pela secreção do perióstraco, camada proteica mais externa da concha. Devido a sua localização, as estruturas visuais ficam permanentemente sob a camada do perióstraco, o que limita a percepção luminosa (Morton, 1995). Tais olhos paliais estão presentes em poucas famílias de Pteriomorphia (*i.e*., Pteriidae, Limopsidae, Anomiidae e Arcidae) (Morton, 2008). Para *Pteria brevialata* (Pteriidae), foram descritos detalhes da organização da margem do manto, incluindo as células fotossensíveis e células pigmentadas presentes na prega externa sob o perióstraco (Morton, 1995). Em espécies de Arcidae com ocorrência em águas rasas, além de olhos em forma de cúpula ou taça, podem ocorrer olhos peculiares denominados multifacetados, que correspondem a olhos compostos (Fig. 3D) (Morton & Peharda, 2008). Em *Barbatia virescens* e *Anadara notabilis* observam-se células fotossensíveis entremeadas às células pigmentadas formando pequenas cúpulas na porção anterior do manto (Morton, 1987; Nilsson, 1994). Já *Arca zebra*, *A. noe* e *B. cancellaria* apresentam centenas de olhos compostos multifacetados cuja organização em omatídeos se assemelha superficialmente aos olhos compostos dos artrópodes (Waller, 1980; Nilsson, 1994; Morton & Peharda, 2008). Os olhos multifacetados dos Arcidae são circulares, côncavos e organizados em dezenas de facetas com alta densidade de microvilosidades (Waller, 1980). Cada unidade formadora corresponde a um tubo afunilado, desprovido de lente e com células receptoras ao fundo cercadas por células pigmentadas (Nilsson, 1994). Tal arranjo indica uma fotopercepção direcional (classe II) associada à resposta de fechamento das valvas por detecção de sombra e movimentos (Nilsson, 1994, 2013).

A formação de olhos paliais nas pregas mediana e interna é apontada como uma das possíveis condições que favoreceram o surgimento de estruturas visuais mais complexas. A vantagem oferecida no desenvolvimento de estruturas oculares nessas pregas é a ausência da cobertura do perióstraco, condição que pode estar associada ao aumento da resolução e sensibilidade visual (Morton, 2008). A segunda categoria ocular corresponde aos olhos formados pelo lobo palial mediano, que estão presentes nas famílias Limidae, Pectinidae e Spondylidae (Dakin, 1928). Na base dos tentáculos de *Ctenoides floridanus* (Limidae), os olhos correspondem a invaginações da porção externa da prega mediana e possuem forma de taça pigmentar que envolve os fotorreceptores (Fig. 3F) (Morton, 2000a). Outro aspecto relevante é a presença de uma lente celular oposta à retina, contudo, o olho possui, curiosamente, conexão com o meio externo, sendo preenchido por uma matriz amorfa (Morton, 2000a). Com exceção dessas informações e daquelas acerca da fisiologia dos fotorreceptores de *Lima scabra* (Nasi, 1991), dados mais detalhados da anatomia, desenvolvimento e variabilidade ocular são ausentes para família.

Situação inversa ocorre com os olhos de vieiras (Pectinidae), possivelmente o grupo mais bem estudado dentre os Bivalvia quanto a aspectos visuais (Serb & Earnisse, 2008). Os olhos paliais deste grupo apresentam córnea, lente, retina dupla e diversos outros componentes (Fig. 3A) (Dakin, 1910). É interessante notar que esse tipo ocular complexo ocorre tanto em Pectinidae, família de bivalves com grande capacidade de mobilidade, inclusive de natação, como em Spondylidae, que reúne bivalves que apresentam uma das valvas cimentadas ao substrato e que, portanto, são sésseis (Dakin, 1928; Speiser & Johnsen, 2008). Devido à proximidade de parentesco entre ambas as famílias citadas, supõe-se que esses olhos paliais já estivessem presentes em seu ancestral comum (Morton, 2008).

Os olhos das vieiras vêm sendo extensamente estudados quanto a aspectos de anatomia e fisiologia desde o início do século 20 (*e.g.*, Dakin, 1910; Land, 1964; Barber *et al.*, 1967; Speiser & Johnsen, 2008). Localizados no ápice de pequenos pedúnculos, os olhos ocorrem entre centenas de tentáculos na prega mediana da margem palial (Dakin, 1910). A retina dupla destes olhos é formada por fotorreceptores em uma camada proximal, rabdomérica, e uma camada distal, ciliar (Barber *et al.*, 1967). Abaixo da retina dupla há uma camada côncava refletora, denominada argênteo, composta por cristais de guanina que refletem a luz como um espelho (Barber *et al.*, 1967; Wilkens 2006). A luz que entra pela abertura ocular passa pela córnea e lente, sendo refletida no fundo do olho pelo argênteo de modo que a imagem em baixa resolução resultante é formada na retina (Land, 1964, Nilsson, 2013). Além de diferirem quanto à morfologia das células fotorreceptoras, as camadas de

retina possuem diferentes sensibilidades ao espectro de luz, o que se soma ao conjunto de evidências que apontam para a hipótese da especialização dessas retinas para diferentes tarefas visuais (Speiser *et al.*, 2011b).

Duas vias distintas de fototransdução foram observadas em pectinídeos, ou seja, duas cascatas de eventos intracelulares podem ser desencadeadas pela percepção luminosa, porém análises de transcriptoma ocular em vieiras indicam a presença de uma terceira via associada ao ritmo circadiano e, possivelmente, a outras funções sensoriais (Pairett & Serb, 2013). Estudos pioneiros em genética de pectinídeos revelaram como genes que codificam fotoproteínas variam nesses animais, fornecendo dados moleculares para hipóteses sobre origem e diversificação da ecologia visual no grupo (Serb *et al.*, 2013).

Debates sobre função dos olhos em Pectinidae e Spondylidae são extensos, e muitas vezes incertos, principalmente devido à complexidade de fatores associados à evolução de tais órgãos (Morton, 2000b). Especula-se que o papel funcional dos olhos nestes animais esteja relacionado à resposta de fuga de predadores em espécies com capacidade de natação, embora haja controvérsias nessa hipótese devido à participação de quimiorreceptores em tais tarefas (Wilkens, 2006). Funções relacionadas à orientação do animal no ambiente e substrato também foram sugeridas a partir de experimentos comportamentais com *Argopecten irradians* (Hamilton & Koch, 1996). Dados de anatomia comparada também indicam variação ocular em função da mobilidade do organismo, embora o papel funcional desses olhos ainda esteja em ampla discussão (Speiser & Johnsen, 2008). Em suma, apesar das controvérsias, os dados de anatomia, fisiologia e comportamento sugerem uma condição ocular associada à fotopercepção de baixa-resolução (classe III).

Finalmente, a última categoria de olhos corresponde àqueles formados na prega palial interna (*i.e.*, famílias Myidae, Cardiidae, Tridacnidae, Laternulidae). A presença de um tufo ciliar associado ao órgão fotorreceptor parece comum neste caso, porém sua função é desconhecida (Morton, 2008). Na família Cardiidae, os olhos paliais mais estudados são os que ocorrem nos tentáculos dos sifões de *Cerastoderma edule*, cuja organização ocular é em forma de cúpula com células pigmentadas envolvendo os fotorreceptores (Fig. 3B) (Barber & Land, 1967). Os representantes do gênero *Tridacna* (Tridacnidae), os maiores bivalves existentes, possuem numerosos ocelos ao longo da margem hipertrofiada do manto, a qual abriga milhares de zooxantelas (*i.e.*, dinoflagelados fotossintetizantes endossimbiontes) em hemoceles paliais (Yonge, 1932). Morfologicamente os ocelos de *Tridacna* são muito similares aos de Cardiidae, contendo córnea, lente e retina; não obstante, há uma diferença fundamental associada à presença das zooxantelas localizadas no entorno dos componentes

visuais (Fig. 3E). Essa organização ocular parece se utilizar dos simbiontes como refletores de luz, aumentando a eficiência de percepção direcional da luz (Fankboner, 1981; Wilkens, 1986). Apesar de haver poucos dados ultraestruturais e fisiológicos sobre os ocelos de Tridacnidae, um vasto repertório de comportamentos visualmente guiados foi registrado em estudos experimentais, como retração do manto e sifão, produção de jatos de água e fechamento das valvas (Wilkens, 1986; Land 2003). De modo geral, o conjunto de informações comportamentais que se soma aos dados de anatomia sugere uma fotopercepção direcional (classe II) como condição desses bivalves.

Outro exemplo notável de organização estrutural está presente nos representantes de Laternulidae, cujos olhos são muito semelhantes e tão complexos quanto os de Pectinidae. Entretanto, a distância filogenética entre essas famílias sugere um alto grau de convergência (Morton, 2008). Os olhos de *Laternula truncata*, associados aos tentáculos dos sifões na prega interna do manto, possuem córnea, lente e duas camadas de retina (ambas com fotorreceptores rabdoméricos), além de um órgão sensorial ciliar acessório (Fig. 3C) (Adal & Morton, 1973; Morton, 1973). Todavia, não há mais informações além da anatomia geral desses olhos.

CONSIDERAÇÕES FINAIS

Ao longo das últimas décadas novas metodologias vêm sendo empregadas e integradas a diferentes áreas do conhecimento na tentativa de esclarecer questões sobre a evolução e diversidade dos sistemas visuais em animais. Dentre elas, destacam-se técnicas de biologia molecular, genética, ecologia comportamental e microscopia. Os debates sobre homologias e convergências ganham continuamente mais elementos à medida que novas informações são descobertas para diferentes grupos de animais, genes e proteínas. Consensos estão finalmente sendo definidos, principalmente quanto à variabilidade no recrutamento e expressão de genes regulatórios ortólogos que estão diretamente envolvidos na formação de uma infinidade de estruturas oculares. De modo geral, entretanto, a maior parte dos estudos sobre sistemas visuais é baseada em animais vertebrados, sendo estes os principais objetos de estudo na área. Para melhor compreender a evolução da visão em Metazoa, os invertebrados ganharam mais atenção nas últimas décadas, principalmente artrópodes, moluscos e, mais recentemente, cnidários.

O filo Mollusca é extensamente diverso, assim como a vasta gama de estruturas visuais já registradas para o grupo. Foram descritos olhos que diferem na origem, localização, morfologia, composição e mecanismos de fotopercepção. Dentre os moluscos,

os cefalópodes são o grupo mais bem estudado neste aspecto, principalmente em virtude da complexidade neurocomportamental. Ainda assim, outros grupos de moluscos se destacam nessa área, como os gastrópodes e bivalves e, ainda de forma incipiente, os poliplacóforos.

Em Bivalvia, a diversidade de formas e organizações oculares é notável. A fotopercepção no grupo é muito variada, atingindo surpreendentes níveis de especialização e convergência. Neste contexto, a borda do manto parece ser a região com maior plasticidade e variabilidade de sistemas oculares, abrigando tipos variados nas mais diferentes famílias de bivalves. Podem ocorrer olhos simples ou compostos, cuja combinação de componentes (*e.g.*, lente, córnea, retina, cílios, refletores e pigmentos) é muito variável. Embora descrições gerais de órgãos visuais sejam abundantes, essa classe de moluscos ainda carece de dados comparativos de anatomia e comportamento que correspondam à sua diversidade taxonômica. Diferentemente da situação já alcançada para outros representantes de Mollusca, informações morfológicas e ultraestruturais ainda são insuficientes para compreensão da diversidade e funcionamento de órgãos visuais para a maioria dos grupos. Deve-se ressaltar ainda que a plasticidade de estruturas fotorreceptoras dos bivalves torna esses animais excelentes modelos para investigações sobre diversificação da fotopercepção. Estudos moleculares voltados à pesquisa de fotorreceptores são escassos para o grupo, embora iniciativas recentes tenham contribuído significativamente no entendimento da maquinaria molecular a partir de dados de transcriptoma. Neste sentido, novas perspectivas na compreensão da evolução dos olhos em bivalves devem surgir a partir de estudos de expressão gênica e regulação da formação de olhos. De forma complementar, investigações ontogenéticas também são carentes e devem acrescentar substancial informação sobre o tema ao grupo. Em síntese, o uso integrado de diferentes técnicas e abordagens mostra-se vital na continuidade dos estudos sobre estruturas fotorreceptoras em Bivalvia.

LITERATURA CITADA

Adal, M. N. & Morton, B. 1973. The fine structure of the pallial eyes of *Laternula truncata* (Bivalvia: Anomalodesmata: Pandoracea). Journal of Zoology 170: 533–556.

Ansell, A. D.; Gunther, C. P. & Burrows, M. T. 1998. Partial emergence of the bivalve *Donax vittatus* in response to abrupt changes in light intensity and before spawning. Journal of the Marine Biological Association of the United Kingdom 78: 669–672.

Arendt, D. 2003. Evolution of eyes and photoreceptor cell types. International Journal of Developmental Biology 47: 563–571.

Barber, V. C.; Evans, E. M. & Land, M. F. 1967. The fine structure of the eye of the mollusk *Pecten maximus*. Zeitschritt fur Zellforschung 76: 295–312.

Barber, V. C. & Land, M. F. 1967. The eye of the cockle, *Cardium edule*: anatomical and physiological investigations. Cellular and Molecular Life Sciences 23(8): 677–678.

Bobkova, M. V.; Gál, J.; Zhukov, V. V.; Shepeleva, I. P.; Meyer-Rochow, V. B. 2004. Variations in the retinal designs of pulmonate snails (Mollusca, Gastropoda): squaring phylogenetic background and ecophysiological needs (I). Invertebrate Biology 123(2): 101–115.

Carriker, M. R. 1990. Functional significance of the pediveliger in bivalve development. In: Morton, B (Ed). The Bivalvia: Proceedings of a Memorial Symposium in honour of Sir Charles Maurice Yonge (1899-1986) at the 9th International Malacological Congress, Edinburgh, 1986. Hong Kong, Hong Kong University Press.

Cole, H. A. 1938. The fate of larval organs in the metamorphosis of *Ostrea edulis*. Journal of Marine Biological Association of the United Kingdom 22: 469–484.

Colicchia, G. 2006. Ancient cephalopod scavenges successfully with its pinhole eye. *Physics Education* 41: 15–17.

Cox, K. W. 1962. California abalones, family Haliotidae. Fish Bulletin 118.

Crozier, W. J. 1920. On the photic sensitivity of the chitons. The American Naturalist 54: 376–380.

Dakin, W. J. 1910. The eye of *Pecten*. Quarterly Journal of Microscopical Science 55: 49–112.

Dakin, W. J. 1928.The eyes of *Pecten*, *Spondylus*, *Amussium* and allied Lamellibranchs, with a short discussion on their evolution. Proceedings of the Royal Society of London B 103: 355–365.

Eakin, R. M. 1965. Evolution of photoreceptors. Cold Spring Harbor Symposia on Quantitative Biology 30: 363–370.

Eakin, R. M. & Bradenburger, J. L. 1967. Differentiation in the eye of a pulmonate snail *Helix aspersa*. Journal of Ultrastructure Research 18: 391–421.

Erclik ,T.; Hartenstein, V.; McInnes, R. R. & Lipshitz, H. D. 2009. Eye evolution at high resolution: The neuron as a unit of homology. Developmental Biology 332: 70–79.

Fain, G. L.; Hardie, R. & Laughlin, S. B. 2010. Phototransduction and evolution of photoreceptors. Current Biology 20: 114–124.

Fankboner, P. V. 1981. Siphonal eyes of giant clams and their relationship to adjacent zooxanthellae. Veliger 23: 245–249.

Fernald, R. D. 2006. Casting a genetic light on the evolution of the eyes. Science 313:1914-1918.

Fischer, F. P. 1078. Photoreceptor cell in chiton aesthetes. Spixiana 1: 209–213.

Fitzgerald, W. J. 1975. Movement patterns and phototactic response of *Mopalia ciliata* and *Mopalia muscosa* in Marin County, California. Veliger 18: 37–39

Gehring, W. J. 2005. New perspectives on eye development and the evolution of eyes and photoreceptors. Journal of Heredity 96(3): 171–184.

Gehring, W. J. 2012. The animal body plan, the prototypic body segment, and eye evolution. Evolution & Development 14: 34–46.

Giribet, G. 2008. Bivalvia. In: Ponder, W. & Lindberg, D (Ed). Phylogeny and Evolution of the Mollusca. California, University of California Press.

Hamilton, P. V. & Koch, K. M. 1996. Orientation toward natural and artificial grassbeds by swimming bay scallops, *Argopecten irradians* (Lamarck, 1819). Journal of Experimental Marine Biology and Ecology 199: 79–88.

Hanlon, R. T. & Messenger, J. B. 1996. Cephalopod Behaviour. Cambridge, Cambridge University Press.

Hanlon, R. T. & Shashar, N. 2003. Aspects of the sensory ecology of cephalopods. In: Collin, S. P. & Marshall, N. J. (Ed). Sensory processing in aquatic environments. New York, Springer New York.

Haszprunar, G.; Schander, C. & Halanych, K. M. 2008. Relationships of higher molluscan taxa. In: Ponder, W. & Lindberg, D. R. (Ed). Phylogeny and Evolution of the Mollusca. California, University of California Press.

Hodgson, C. A. & Burke, R. D. 1988. Development and larval morphology of the Spiny Oyster, *Chlamys hastata*. Biological Bulletin 174: 303–318.

Jonasova, K. & Kozmik, Z. 2008. Eye evolution: lens and cornea as un upgrade of animal visual system. Seminars in Cell & Developmental Biology 19: 71–81.

Katagiri, N.; Suzuki, T.; Shimatani, Y. & Katagiri, Y. 2002. Localization of retinal proteins in the stalk and dorsal eyes of the marine gastropod, *Onchidium*. Zoological Science 19(11): 1231–1240.

Kataoka, S. 1975. Fine structure of the retina of a slug, *Limax flavus* L. Vision research 15(6): 681–686.

Kennedy, D. 1960. Neural photoreception in a lamellibranch mollusc. Journal of General Physiology 44: 277–299.

Land, M. F. 1992. The evolution of eyes. Annual Review of Neurosciences 15: 1–9.

Land, M. F. 2003. The spatial resolution of the pinhole eyes of giant clams (*Tridacna maxima*). Proceedings of the Royal Society of London B Biological Sciences 270: 185–188.

Land, M. F. & Nilsson, D. 2012. Animal eyes. Oxford, Oxford University Press.

Meisenheimer, J. 1912. Die weinbergschnecke *Helix pomatia* L. Leipzig, Werner Klinkhardt.

Mordan, P. & Wade, C. 2008. Heterobranchia II: the Pulmonata. In: Ponder, W. & Lindberg, D. R. (Ed). Phylogeny and Evolution of the Mollusca. California, University of California Press.

Morton, B. 1973. The biology and functional morphology of *Laternula truncata* (Lamarck 1818) (Bivalvia: Anomalodesmata: Pandoracea). Biological Bulletin 145: 509–531.

Morton, B. 1987. The pallial photophores of *Barbatia virescens* (Bivalvia: Arcacea). Journal of Molluscan Studies 53: 241–243.

Morton, B. 1995. The biology and functional morphology of *Pteria brevialata* (Bivalvia: Pterioidea) associated with gorgonians in Hong Kong. Journal of Zoology 236: 223–241.

Morton, B. 2000a. The pallial eyes of *Ctenoides floridanus* (Bivalvia: Limidae). Journal of Molluscan Studies 66: 449–455.

Morton, B. 2000b. The function of pallial eyes within the Pectinidae, with a description of those present in *Patinopecten yessoensis*. In: Harper, E. M.; Taylor, J. D. & Crame, A. J. (Ed). The Evolutionary Biology of the Bivalvia. The Geological Society of London, Special Publications 177: 247–255.

Morton, B. 2008. The evolution of eyes in the Bivalvia: new insights. American Malacological Bulletin 26(1/2): 35–45.

Morton, B & Peharda, M. 2008. The biology and functional morphology of *Arca noae* (Bivalvia: Arcidae) from the Adriatic Sea, Croatia, with a discussion on the evolution of the bivalve mantle margin. Acta Zoologica 89: 19–28.

Moseley, H. N. 1884. On the presence of eyes and other sense-organs in the shells of the Chitonidae. Annals and Magazine of Natural History 5: 141–147.

Muntz, W. R. A & Raj, U. 1984. On the visual system of *Nautilus pompilius*. Journal of Experimental Biology 109: 253–263.

Muntz, W. R. A. 1999. Visual systems, behaviour, and environment in cephalopods. In: Archer, S. N.; Djamgoz, M. B. A.; Loew, E. R.; Partridgeand, J. C. & Vallerga, S. (Ed). Adaptive Mechanisms in the Ecology of Vision. Dordrecht, Kluwer Academic Publishers.

Nasi, E. 1991. Whole-cell clamp of dissociated photoreceptors from the eye of *Lima scabra*. Journal of General Physiology 97: 35–54.

Nilsson, D. 1994. Eyes as optical alarms systems in fan warms and ark clams. Philosophical Transactions of the Royal Society B 346: 195–212.

Nilsson, D. 2004. Eye evolution: a question of genetic promiscuity. Current Opinion in Neurobiology 14: 407–414.

Nilsson, D. 2009. The evolution of eyes and visually guided behaviour. Philosophical Transactions of the Royal Society B 364: 2833–2847.

Nilsson, D. 2013. Eye evolution and its functional basis. Visual Neuroscience 30: 5–20.

Nixon, M. & Young, J. Z. 2003. The brains and lives of cephalopods. Oxford, Oxford University Press.

O'Brien, E. K. & Degnan, B. M. 2002. Developmental expression of a class IV POU gene in the gastropod *Haliotis asinina* supports a conserved role in sensory cell development in bilaterians. Development Genes and Evolution 212: 394–398.

Pairett, A. N. & Serb, J. M. 2013. *De novo* assembly and characterization of two transcriptomes reveal multiple light-mediated functions in the scallop eye (Bivalvia: Pectinidae). PLoS ONE 8(7): e69852.

Piatigorsky, J. & Kozmik, Z. 2004. Cubozoan jellyfish: an Evo/Devo model for eyes and other sensory systems. International Journal of Developmental Biology 48: 719–729.

Piatigorsky, J. 2008. Evolution of mollusc lens crystallins: Glutathione S-transferase/S-crystaliins and aldehyde dehydrogenase/Ω-crystallins. American Malacological Bulletin 26(1/2): 73–81.

Ponder, W. F. & Lindberg, D. R. 2008. Phylogeny and Evolution of the Mollusca. California, University of California Press.

Ruzickova, J.; Piatigorsky, J. & Kozmik, Z. 2009. Eye-specific expression of an ancestral jellyfish Pax B gene interferes with Pax6 function despite its conserved Pax6/Pax2 characteristics. International Journal of Developmental Biology 53: 469–482.

Salvini-Plawen, L. 2008. Photoreception and the polyphyletic evolution of photoreceptors (with special reference to Mollusca). American Malacological Bulletin 26(1/2): 83–100.

Serb, J. M. 2008. Toward developing models to study the disease, ecology, and evolution of the eye in Mollusca. American Malacological Bulletin 26(1/2): 3–18.

Serb, J. M. & Eernisse, D. J. 2008. Charting evolution's trajectory: using molluscan eye diversity to understand parallel and convergent evolution. Evolution Education Outreach 1: 439–447.

Serb, J. M.; Porath-Krause, A. J. & Pairett, A. N. 2013. Uncovering a gene duplication of the photoreceptive protein, opsin, in scallops (Bivalvia: Pectinidae). Integrative and Comparative Biology 53: 68–77.

Seyer, J. 1992. Resolution and sensitivity in the eye of the winkle *Littorina littorea*. Journal of Experimental Biology 170: 57–69.

Speiser, D. I. & Johnsen, S. 2008. Comparative morphology of the concave mirror eyes of scallops (Pectinoidea). American Malacological Bulletin 26: 27–33.

Speiser, D. I.; Eernisse, D. J. & Johnsen, S. 2011a. A chiton uses aragonite lenses to form images. Current Biology 21: 665–670.

Speiser, D. I.; Loew, E. R. & Johnsen, S. 2011b. Spectral sensitivity of the concave mirror eyes of scallops: potential influences of habitat, self-screening and longitudinal chromatic aberration. The Journal of Experimental Biology 214: 422–431.

Stasek, C. R. & McWilliams, W. R. 1973. The comparative morphology and evolution of molluscan mantle edge. The Veliger 16:1-19.

Sweeney, A. M.; Haddock, S. H. D. & Johnsen, S. 2007. Comparative visual acuity of coleoid cephalopods. Integrative and Comparative Biology 47(6): 808–814.

Todt, C.; Okusu, A.; Schander, C. & Schwabe, E. 2008. Solenogastres, Caudofoveata, and Polyplacophora. In: Ponder, W. & Lindberg, D. R. (Ed). Phylogeny and Evolution of the Mollusca. California, University of California Press.

Tuchina, O. & Meyer-Rochow, V. B. 2010. Regeneration of the visual system in gastropods (Mollusca). Invertebrate Biology 129(1): 27–38.

Vopalensky, P. & Kozmik, Z. 2009. Eye evolution: common use and independent recruitment of genetic components. Philosophical Transactions of the Royal Society B 364: 2819–2832.

Waller, T. R. 1980. Scanning electron microscopy of shell and mantle in the order Arcoida (Mollusca: Bivalvia). Smithsonian Contributions to Zoology 313: 1–58.

Waller, T. R. 1981. Functional morphology and development of veliger larvae of the European oyster, *Ostrea edulis*. Smithsonian Contributions to Zoology 328: 1–70.

Wilkens, L. A. 1986. The visual system of the giant clam *Tridacna*: behavioral adaptations. Biological Bulletin 170: 393–408.

Wilkens, L. A. 2006. Neurobiology and behaviour of the scallop. In: Shumway, S. E. & Parsons, G. J. (Ed). Scallops: Biology, Ecology and Aquaculture. Amsterdam, Elsevier.

Yonge, C. M. 1936. Mode of life, feeding, digestion and symbiosis with zooxanthellae in the Tridacnidae. Scientific Reports of the Great Barrier Reef Expedition 1928-1929: 283–321.

Yonge, C. M. 1983. Symmetries and the role of the mantle margins in the bivalve Mollusca. Malacological Review 16: 1–10.

Young, J. Z. 1971. The anatomy of the nervous system of *Octopus vulgaris*. Oxford, Clarendon Press.

Zieger, M. V. & Meyer-Rochow, V. B. 2008. Understanding the cephalic eyes of pulmonate gastropods: a review. American Malacological Bulletin 26: 47–66.

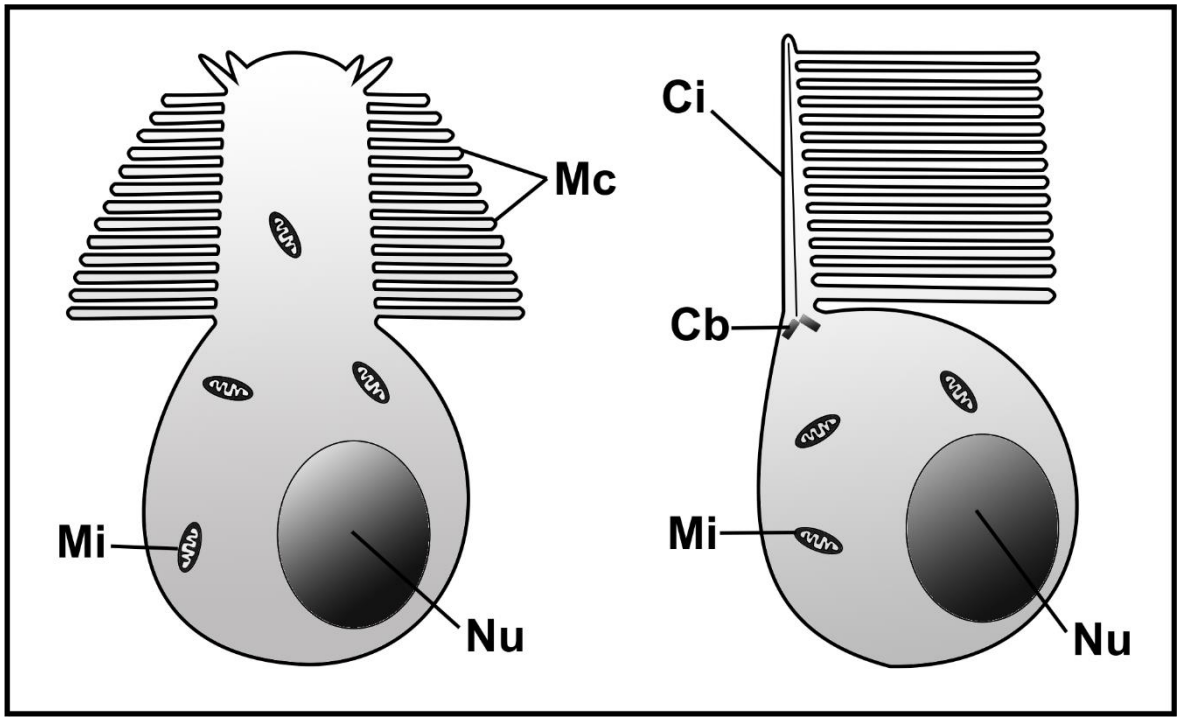

Figura 1. Representação esquemática dos dois tipos morfológicos de células fotorreceptoras animais. **A**. Célula fotorreceptora rabdomérica cujas proteínas fotossensíveis (opsinas) estão agrupadas na membrana celular organizada em microvilosidades (rabdomas). **B**. Célula fotorreceptora ciliar onde as opsinas encontram-se presentes em expansões da membrana celular do cílio modificado. *Cb*, corpúsculo basal; *Ci*, cílio; *Mc*, microvilosidades; *Mi*, mitocôndrias; *Nu*, núcleo.

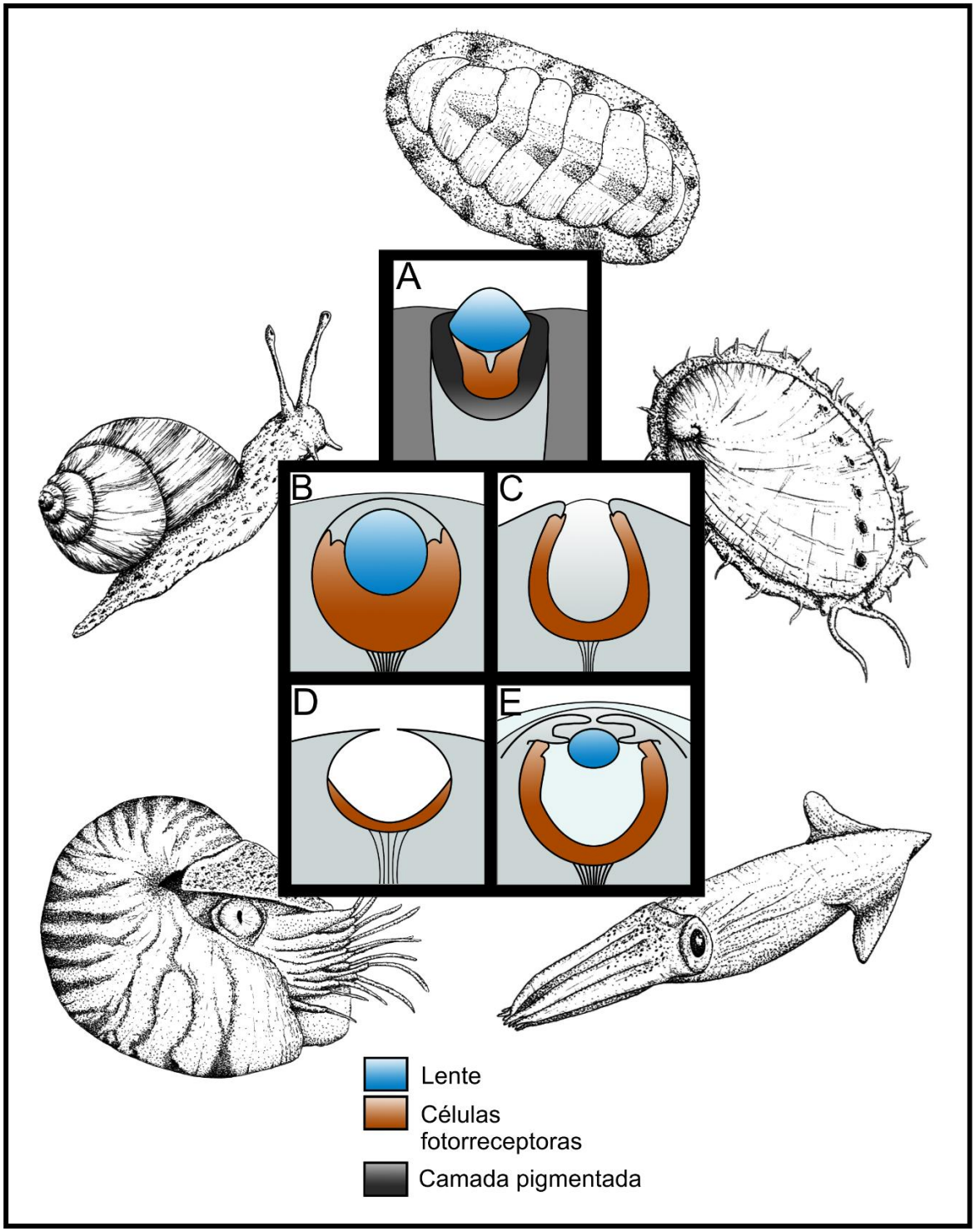

Figura 2. Diversidade de estruturas oculares em Mollusca. **A**. *Acanthopleura granulata* (Polyplacophora: Chitonidae). Quítons podem possuir milhares de estetos com ocelos desenvolvidos distribuídos ao longo das placas valvares. **B**. *Helix aspersa* (Gastropoda: Helicidae). Alguns gastrópodes terrestres apresentam olhos fechados no ápice dos tentáculos cefálicos. **C**. *Haliotis* sp. (Gastropoda: Haliotidae). Muitas espécies de gastrópodes marinhos possuem olhos abertos, preenchidos por uma matriz homogênea, e localizados na base dos tentáculos cefálicos. **D**. *Nautilus pompilius* (Cephalopoda: Nautilidae). Os náutilos possuem um par de olhos abertos do tipo câmera e com o interior em contato direto com o ambiente. **E**. *Doryteuthis plei* (Cephalopoda: Loliginidae). Lulas, sépias e polvos apresentam característico arranjo ocular fechado, do tipo câmera e com diversos componentes oculares.

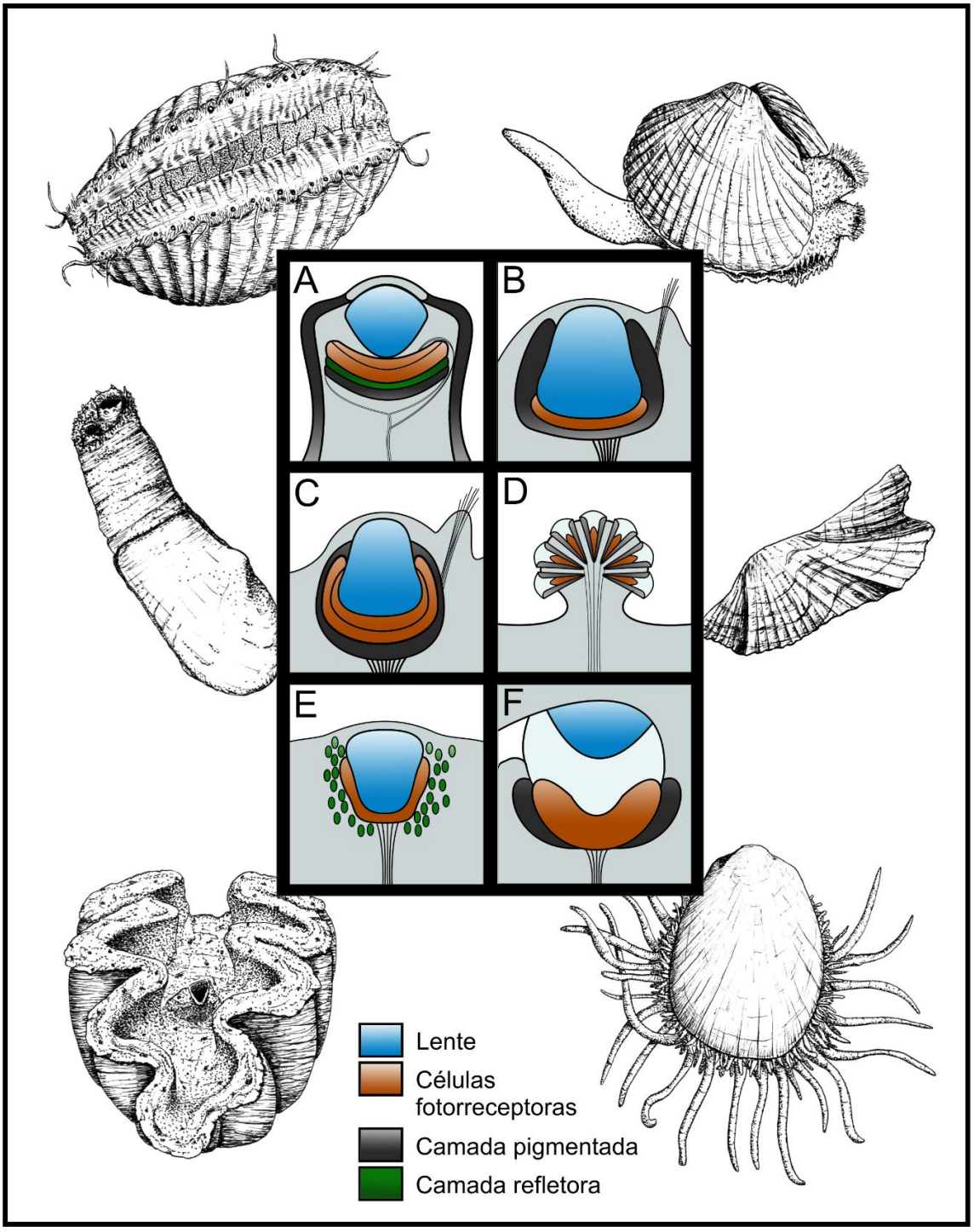

Figura 3. Representação de diferentes membros da classe Bivalvia com seus respectivos esquemas de olhos paliais em destaque. **A**. *Argopecten irradians* (Pectinidae), vieira que possui dezenas de olhos paliais espelhados na prega mediana do manto e distribuídos em ambos os lados do animal. **B.** *Cerastoderma edule* (Cardiidae), bivalve que apresenta pequenos ocelos na região da prega palial interna em tentáculos associados aos sifões e à abertura ventral. **C**. *Laternula truncata* (Laternulidae), bivalve com olhos complexos presentes em tentáculos ópticos dos sifões. **D**. *Arca zebra* (Arcidae), bivalve que possui vários olhos compostos distribuídos na prega palial externa. **E**. *Tridacna máxima* (Tridacnidae), bivalve com milhares de ocelos associados a zooxantelas presentes na prega interna hipertrofiada do manto. **F**. *Ctenoides floridanus* (Limidae), bivalve com olhos abertos para o ambiente e localizados na prega palial mediana.