On the origin of bilaterality : insights from the study of black corals (Cnidaria : Antipatharia)

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I am a Darwinian, and for us, life is the victory after a fight.

Arruda Furtado to Louis C. Miall

29 April 1881

To my grandmother Alzira

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CONTENTS

RÉSUMÉ **1**

PREFACE **7**

1 ANTIPATHARIA **15**

1.1 ANTIPATHARIA BELONGS TO THE PLYLUM CNIDARIA AND TO THE CLASS ANTHOZOA 19

1.2 PHYLOGENY AND GENERAL MORPHOLOGY OF ANTIPATHARIANS WITH FOCUS ON OUR MODEL SPECIES. 24

1.2.1 Taxonomy and phylogeny *24*

1.2.2 Description of Antipathes caribbeana *26*

1.2.3 Description of Plumapathes pennacea *29*

1.3 ANTIPATHARIAN DIVERSITY AND MORPHOLOGY 32

1.4 SYMMETRY IN THE TENTACLE DISPOSITION AND IN THE INTERNAL MORPHOLOGY OF CARIBBEAN SPECIES OF BLACK CORALS 50

1.4.1 Tentacle apparatus organization *50*

1.4.2 Symmetry linked to the internal organization *53*

1.5 ECOLOGY AND SOME ASPECTS OF ANTIPATHARIAN BIOLOGY 59

1.6 MISSION FOR COLLECTION OF BLACK CORALS 61

1.6.1 Collection *62*

1.6.2 Fixation *63*

2 MESENTERIC FORMATION AND SYMMETRY IN ANTHOZOA **67**

2.1 ELEMENTS OF SYMMETRY IN THE ADULT ANTHOZOAN POLYP 71

2.2 SYMMETRY AND MESENTERIC DEVELOPMENT ACROSS ANTHOZOAN CLASSES 80

2.2.1 Hexacorallia *81*

2.2.2 Ceriantharia *130*

2.2.3 Octocorallia *137*

2.2.4 Final considerations for the homology of mesenteric development across Anthozoa *142*

2.3 SYMMETRY AND THE ORIGIN OF BILATERALITY IN METAZOA 145

2.3.1 Metazoan phylogeny *145*

2.3.2 Anthozoan phylogeny *147*

2.3.3 The anthozoan position within Cnidaria and the evolution of bilaterality *151*

2.3.4 Opinions on the origin of bilaterality *155*

3 MORPHOLOGICAL CHARACTERIZATION OF *A. CARIBBEANA* POLYP BILATERAL ORGANIZATION AND MOLECULAR INSIGHTS ON THE ORIGIN OF ITS BILATERALITY 157

3.1 DETAILED ANATOMY OF *ANTIPATHES CARIBBEANA* 159

3.1.1 Musculature of the polyp 160

3.1.2 Detailed anatomy of the oral cone 170

3.1.3 Detailed anatomy of the tentacles 191

3.1.4 First description of hermaphroditism in an antipatharian polyp 195

3.2 DEVELOPMENT OF AN *IN SITU* HYBRIDIZATION PROTOCOL FOR ANTIPATHARIAN SPECIES 199

3.3 PAPER: BLACK CORALS (ANTIPATHARIA, ANTHOZOA) INSIGHTS ON PUTATIVE HOMOLOGY OF THE POLYP SECONDARY AXIS ACROSS ANTHOZOA (CNIDARIA) 201

4 MORPHOLOGICAL DIFFERENCE IN TENTACLE SYMMETRY IN TWO ANTIPATHARIAN SPECIES 208

4.1 EXPERIMENTAL PROCEDURE 211

4.2 RESULTS AND DISCUSSION 213

4.3 OBSERVATION OF ASEXUAL REPRODUCTION 219

5 GENERAL CONCLUSIONS 224

6 REFERENCES 230

7 SUPPLEMENTARY DOCUMENTS 254

RÉSUMÉ

L'origine des symétries et des polarités est l'un des thèmes centraux de l'évolution animale. Classiquement considérée comme une innovation propre aux animaux à symétrie bilatérale (Bilateria), la bilatéralité est en fait très largement répandue chez les cnidaires, groupe-frère des bilateria, principalement au sein de la classe des anthozoaires. En effet, chez ces organismes, à l'axe principal dit « oral-aboral » se superpose un axe secondaire perpendiculaire, dit « axe directeur » conduisant à une organisation bilatérale (Hyman 1940 ; Berking 2007). Celle-ci résulte principalement de l'organisation anatomique particulière des mésentères (ou cloisonnement de la colonne du polype) ainsi que de la présence d'une unique gouttière ciliée (ou siphonoglyphe) dans le pharynx.

L'homologie de l'axe secondaire des cnidaires avec l'axe dorso-ventral des Bilateria est une question qui suscite de nombreux débats et demeure largement ouverte. Sur la base d'arguments moléculaires certains travaux postulent que la bilatéralité est antérieure à la divergence cnidaires/ bilateria (Finnerty *et al*. 2004, Matus *et al*. 2006) et donc est un caractère commun homologue dans ces deux clades, alors que d'autres chercheurs mettent en avant l'hypothèse d'une convergence sur la base d'arguments anatomiques et phylogénétiques (Manuel 2009).

Les bases moléculaires de la bilatéralité des anthozoaires ont été principalement étudiées chez l'anémone *Nematostella vectensis* (ordre des actiniaires) (Rentzsch *et al*. 2006 ; Matus *et al*. 2006 ; Leclère *et al*. 2014) et, à moindre titre, chez

les coraux, en particulier du genre *Acropora* sp. (ordre des scléractiniaires) (Hayward *et al*. 2002, 2015). S'il est aujourd'hui admis que la voie *Wnt/b-catenin* est impliquée dans le développement du domaine oral chez les cnidaires (Kusserow *et al*. 2005 ; Kumburegama *et al*. 2011 ; Rottinger *et al*. 2012), le patterning de l'axe directeur chez *Nematostella* seraît contrôlé par la voie BMP. De nombreux facteurs de transcriptions ou des protéines sécrétées de cette voie sont exprimés asymétriquement le long de l'axe directeur. Ainsi, BMP2/4 et son antagoniste *Chordin* (homologues respectifs des gènes *decapentaplegic – dpp* - et *short gastrulation – sog* – de drosophile) sont conservés chez les cnidaires. Cependant des différences notoires apparaissent chez *Nematostella*. A la différence des Bilateria, *NvBMP2/4* et *NvBMP5-8* sont co-exprimés avec la BMP-*binding protein NvChordin*, d'abord autour du blastopore lors du développement précoce puis, plus tardivement, leur pattern est restreint au même coté de l'embryon, et donc polarisé dans des territoires partiellement chevauchant de l'ectoderme (Rentzsch *et al*. 2006 ; Matus *et al*. 2006). Cependant, l'étude de la forme active (protéine) du régulateur intracellulaire pSmad1/5/8 a permis de révéler que la voie de signalisation est activée sur le coté opposé (Leclère *et al*. 2014 ; Genikhovich *et al*. 2015). Ces résultats divergent fortement des bilateria pour lesquels on observe une expression de *chordin* loin du site principal d'expression des BMPs puis un rôle de cette molécule dans la concentration des BMPs au niveau de leur site de production. D'autres part, des différences notables dans l'autorégulation des BMPs existent entre cnidaires (régulation négative) et bilateria (régulation positive). De ce fait, les mêmes acteurs de la voie BMP paraissent être utilisés de façon différente (ou plus précisément dans différentes positions) au sein d'un réseau similaire de régulation (Saina *et al*. 2009). Pour l'heure, ces travaux sont principalement centrés sur quelques modèles et la conservation de tels mécanismes à l'échelle des cnidaires reste à démontrer. L'expression des gènes *Hox* se révèle être, chez les cnidaires, d'importance pour l'étude de l'axe secondaire. En effet, à la différence des bilateria chez qui les gènes *Hox* sont impliqués dans le patterning de l'axe antéro-postérieur, ils sont, chez *Nematostella*, différentiellement exprimés dans l'axe directeur et leur expression est contrôlée par la voie BMP (Finnerty 2004 ; Ryan *et al*. 2007 ; Leclère *et al*. 2014 ; Genikhovich *et al*. 2015). L'ensemble de ces approches moléculaires révèlent ainsi des similitudes importantes entre le patterning de l'axe dorso-ventral des bilateria et celui de l'axe secondaire des cnidaires.

A contrario, l'organisation anatomiques symétrique du polype des anthozoaires ne peut en aucun cas être corrélée aux structures dorso-ventralisées des bilateria et les caractères anatomiques qui engendrent les asymétries ne sont clairement pas homologues.

Au sein même des cnidaires, l'origine de la bilatéralité est également une question en suspend. A l'échelle des médusozoaires (hydrozoaires, scyphozoaires et cubozoaires), les organismes (polypes ou méduses) présentent une symétrie majoritairement radiaire. Seules les anthozoaires sont, en général, bilatéraux. La question du caractère dérivé ou plésiomorphe de la bilatéralité dans le clade des cnidaires demeure complexe d'autant que peu de taxons ont été étudiés en détail tant sur le plan de la morphologie que suivant une approche évo-dévo. Notre travail s'est donc focalisé sur un groupe peu étudié d'anthozoaires coloniaux : les coraux noirs ou antipathaires. Ces organismes ont été choisis d'une part en raison de leur diversité anatomique : des espèces bilatérales côtoient des espèces phylogénétiquement proches chez qui la radialité est plus nette, et aussi en raison de leur position phylogénétique : ce sont des hexacoralliaires, groupe-frère des scléractiniaires (coraux « durs ») et voisins des actiniaires (anémone de mer). Deux espèces ont été principalement étudiées : *Antipathes caribbeana*, à symétrie bilatérale et *Plumapathes pennacea* a symétrie radiaire qui différent, entre autre, par l'organisation de leurs tentacules.

Dans un premier temps, et afin d'analyser l'évolution des symétries à l'échelle des anthozoaires, l'anatomie fine des antipathaires a été étudiée. Pour une grande part méconnue, la morphologie des ces taxons a entièrement été réanalysée et étayée sur la base d'une littérature relativement ancienne (fin XIX^{ème} et début XX^{ème} siècles) et peu abondante. Une approche synthétique de l'organisation anatomique des antipathaires est proposée dans les chapitres 1 et 2. Ce travail était indispensable afin, d'une part de faire un point sur l'anatomie comparée de l'ensemble des anthozoaires et, d'autre part de vérifier sur les antipathaires bon nombre d'éléments que la littérature ancienne avait évoqué, suggéré ou affirmé avec des outils et approches de l'époque et sur un nombre limité d'espèces.

Dans un second temps, et afin de s'inscrire dans la problématique de cette thèse, c'est-à-dire l'évolution des symétries à différentes échelles taxonomiques (antipathaires, anthozoaires, cnidaires), une approche moléculaire a été entreprise. Elle s'est naturellement heurtée à de nombreuses mises au point techniques dans la mesure

où aucun travail de ce type n'avait été préalablement entrepris. L'une des deux espèces (*P. pennacea)* a été résolument récalcitrante à de nombreuses investigations, en particulier les hybridations *in situ*, ce qui a limité nos comparaisons. Ce travail s'est bien évidemment appuyé et a bénéficié des travaux sur l'un des cnidaires-modèles en évo-dévo et en biologie du développement : l'anémone de mer *Nematostella vectensis*. Les comparaisons avec cet organisme ont été fortement limitées pour deux raisons essentielles. Premièrement du fait que le développement précoce des antipathaires soit méconnu sinon inconnu. Bien que ces animaux soient principalement gonochoriques aucunes données n'existent sur le développement embryonnaire et les observations des larves planulas sont sporadiques. Deuxièmement, et pour les raisons qui précèdent, les comparaisons avec *Nematostella*, chez qui les observations publiées s'appuient principalement sur le développement précoce étaient difficiles. De ce fait, les résultats obtenus se fondent sur l'adulte d'antipathaires et l'analyse comparée a été réalisée avec les données d'autres anthozoaires : *Nematostella* (embryon ou jeune polype) et *Acropora* (embryons ou larves en cours de fixation). Bien entendu, ce point a limité la portée de nos résultats mais a permis de démontrer que bon nombre de gènes impliqués dans les symétries et polarités précoces voient leur expression polarisée conservée chez l'adulte d'antipathaire. Afin d'asseoir cette anatomie moléculaire comparée il a fallu polariser l'ensemble des animaux étudiés indépendamment des données moléculaires ce qui, sur des embryons en développement ou sur des adultes à organisation a priori radiaire, est un véritable « challenge ». Enfin, la méconnaissance de l'anatomie fine des antipathaires nous a conduit à y pallier en développant en parallèle de nos investigations génétiques des travaux d'anatomie comparée qui s'appuient sur de l'histologie ou de l'immunocytochimie. L'ensemble de ces approches est présenté dans le chapitre 3. Ces différents résultats permettent de conclure à la probable conservation des acteurs, et sans doute des processus, mis en jeu dans la mise en place de l'axe secondaire et dans le maintien de sa polarité, mais aussi à l'homologie probable de la symétrie bilatérale au sein des hexacoralliaires et, sans doute à l'échelle des anthozoaires. Ces résultats font l'objet d'un article dont un premier manuscrit est joint au chapitre 3 et qui est en voie de finalisation.

Enfin, une approche de transcriptomique comparée a été initiée dans le but de comparer deux espèces d'antipathaires voisines : l'une à symétrie bilatérale (*A. caribbeana*), l'autre à organisation radiaire (*P. pennacea*). La longue mise en œuvre des protocoles expérimentaux, l'obtention tardive des données et les difficultés de

validation de cette approche comparée par hybridation *in situ*, ont conduit à une exploitation très (trop) partielle de ces jeux de données. Toutefois, nous proposons au chapitre 4 des éléments de discussion concernant cet aspect de notre travail. L'un des points intéressants est la probable asymétrie « gauche / droite » que l'on peut supposer exister chez l'espèce à symétrie bilatérale.

En conclusion, ce travail participe à lever le voile sur un groupe de cnidaires coloniaux méconnus et dont l'anatomie, qui paraît « simple » à première vue, est de fait relativement complexe et diversifiée. Il paraît important de souligner que loin d'être anecdotique, l'organisation bilatérale des cnidaires est un élément de symétrie tout aussi répandue que l'organisation radiaire qui sert trop souvent à décrire le groupe. On peut même supposer que la bilatéralité est un caractère plésiomorphe au moins au sein des anthozoaires. A l'échelle des cnidaires la question reste ouverte et du coup l'homologie avec la symétrie bilatérale des Bilateria est difficile à résoudre. Toutefois, cette homologie paraît peu probable en raison d'aspects anatomiques fondamentaux qui séparent les deux groupes. En revanche, les données moléculaires semblent conforter l'idée d'une conservation des mécanismes de polarisation dans des contextes cellulaires pourtant bien différents.

PREFACE

Cnidarians, the sister group of Bilateria, have conventionally been described as a phylum with a radial symmetry. However, numerous cases of bilateral organization of morphoanatomical structures have been documented for more than a century in the anthozoan class (Cnidaria: Anthozoa). As will be detailed later in this work, the existence of bilaterality implies the existence of a secondary body axis in addition to the main oral-aboral axis of the anthozoans. This secondary body axis, so called "*directive axis*", that runs perpendicular to the oral-aboral axis, define a plane of bilateral symmetry (Layden *et al*. 2016).

Among cnidarians, radial symmetry prevails along medusozoan development and adult stages, but alternates or is replaced by a bilateral organization during the ontogenesis or adult stages of the polyps of Anthozoa.

In a context of cylindrical body organization and of radial symmetry, the repetitive segmentation of the cylindrical body in a plan perpendicular to the main axis of the cylinder makes the morphological switch between radiality and bilaterality relatively easy. The formation of a single element that breaks the radial symmetry makes the body automatically bilateral.

The BMP signalling pathway is the pathway responsible for patterning the dorso-ventral axis in Bilateria. This pathway was found to be differentially expressed during development in the secondary (directive) axis of the sea anemone *Nematostella vectensis* (Finnerty *et al*. 2004; Matus *et al*. 2006a; Matus *et al*. 2006b; Ryan *et al*. 2007) and of the scleractinian coral *Acropora millepora* (Hayward *et al*. 2002; Hayward *et al*. 2015; Okubo *et al*. 2016). Those findings, together with the sister group relationship between Cnidaria and Bilateria (Collins 1998, Kim *et al.* 1999, Medina *et al.* 2001; Ryan *et al*. 2013; Moroz *et al*. 2014; Whelan *et al*. 2015; Pisani *et al*. 2015) lead some authors to admit that the last common ancestor between bilaterians and cnidarians had a bilateral symmetry (Finnerty *et al*. 2004; Finnerty 2005; Matus *et al*. 2006a) and thus that the bilateral symmetries found between Cnidaria and Bilateria are homologous.

However, in the absence of a clear investigation of the nature of bilaterality present in the different anthozoan orders, it is important to understand if bilaterality is a derived trait of some anthozoans (e.g. autapomorphies of respectively octocorallians and hexacorallians) or if it comes out as a plesiomorphic trait. We need to understand if the gene expression patterns found in *Nematostella* and that gave rise to the "homology between the secondary body axis of Anthozoans and that of Bilaterians" hypothesis (Finnerty 2005; Matus *et al*. 2006a) are present in other groups and how do they relate to the visible bilaterality of those groups. In Bilataria numerous studies strongly suggest that the Bilateria last common ancestor had its dorso-ventral axis (secondary axis) patterned by the BMP-pathway (Arendt and Nübler-Jung 1994; De Robertis 2008; Genikhonovich *et al*. 2015) and, exception for vertebrates, its polarity is conserved (Holley *et al*. 1995; Arendt and Nübler-Jung 1999). The studies in *Nematostella vectensis* and in *Acropora millepora* are not yet sufficient to reconstruct the situation for the anthozoan last common ancestor and thus to correctly infer the situation of the common ancestor of cnidarians and bilaterians, as Manuel (2009) argued the bilaterality in Anthozoa and Bilateria may not be comparable.

Preface

In Anthozoa two sub-classes are fundamental to understand the origin of the bilateral symmetry in the order: the Octocorallia and the Hexacorallia. All Octocorallia orders present a bilateral symmetry (see chapter 2). Bilaterality seems then the plesiomorphic state of this sub-class. Hexacorallians however present both radial and bilateral symmetry. In this sub-class, main examples of asymmetrical expression of the BMP-pathway have been reported (*Nematostella* – Actiniaria order and *Acropora* – Scleractinia order). Therefore, we need to question in this subclass if the respective bilaterality is homologous (i.e. the ancestor state of hexacorallians) or if it is a derived trait in different orders.

In opposition to Bilateria (*i.e.* in this clade, *Hox* genes are expressed along the primary axis), *Hox* genes have recently been shown to be differentially expressed along the secondary axis of *Nematostella vectensis* (Finnerty *et al*. 2004; Matus *et al*. 2006a; Ryan *et al*. 2007; Leclère and Rentzsch 2014; Arendt *et al*. 2015).

Here we propose a study focusing on black corals, the order Antipatharia, as these organisms possess an extreme diversity of body forms for an anthozoan and as their bilaterality contrasts in relation to their colonial organization. Another reason to study this order is that it has been strongly understudied in relation to scleractinians or actiniarians and thus many aspects of their development are totally ignored. The asymmetric expression of the BMP-pathway in *Nematostella* relates to the bilaterality of the adult polyp. However the adult polyp of *Acropora* has a radial symmetry (see chapter 3) that is characteristic of scleractinians polyps whereas the BMP-pathway is probably conserved. The adult polyp bilateral morphology and the phylogenetic position of antipatharians as sister group to scleractinians are two major arguments to study this group. A detailed presentation of Antipatharia biology and anatomy will follow in **chapter 1**.

The morphological diversity of antipatharians is astonishing and must conquer the attention of any naturalist. However, the studies on the antipatharian morphology have so far been completely descriptive and no understanding how this diversity arises exists. It is obviously completely unrealistic to try to understand all the variety displayed. The two antipatharian species studied here: *Antipathes caribbeana* and *Plumapathes pennacea* present different symmetries of tentacle disposition on the polyp. *Plumapathes pennacea* presents a radial symmetry (most common symmetry of tentacles in Antipatharia) while *Antipathes caribbeana* presents a bilateral symmetry in the tentacle disposition.

The **first question** is: how does the morphological bilateral organization of antipatharians polyps relate to the organization of the polyps of the other anthozoan orders? A special focus will be given to the comparison with the bilateral organization of the sea anemone model *Nematostella vectensis* (Cnidaria: Anthozoa: Actiniaria). For this, a literature review will be conducted in order to compare: i) the morphological organization in terms of symmetry; ii) the modality development of the different anthozoan groups. This will allow to address a **second question**: is the anatomical bilaterality derived or pleisiomorphic in Anthozoa. All these themes are developed in **chapter 2**.

The **third question** is: are the secondary axis of *Nematostella* (Actiniaria) and Antipatharia homologous? To address this question the detailed anatomy of the *Antipathes caribbeana* polyp has been studied in order to establish very precisely its anatomical organization. Then we used a molecular approach: a characterization of the expression of the BMP-pathway and the *Hox* genes patterns to compare the expression of these developmental genes that are differentially expressed along the secondary axis of *Nematostella*. This comparison of gene expression patterns will allow us to understand if the results obtained in *Nematostella* could be generalized to another group of anthozoans that have a bilateral adult symmetry. Then we can discuss the hypothesis that bilaterality may have originated in the common ancestor between Anthozoa and Bilateria. These questions will be addressed in **chapter 3**.

The **fourth question** is: How did the bilateral organization of the tentacles of *Antipathes caribbeana* arise? With a RNA-seq comparative analysis between two antipatharian species with different tentacle symmetries we aim to highlight the molecular origin of these two types of organization and together with the results of chapter 3 partially understand the molecular basis for the polyps orientation and disposition in a colony. This will be addressed in **chapter 4**.

To address these questions many technical problems related to antipatharians needed to be overcomed, some examples are: the impossibility to maintain living colonies in the lab; little knowledge available on the reproduction of this group of animals and their anatomy; larval development unknown and so additional difficulty to compare them with other animals in terms of development; single opportunity to collect and fix them; laborious development of protocols that work and are both reliable and reproductible for these species.

1 ANTIPATHARIA

Antipatharia is an order of colonial and marine animals, belonging to Cnidaria, Eumetazoa. In classical taxonomy, this clade belongs to: Hexacorallia (subclass), Anthozoa (class), Cnidaria (phylum). They receive two common names: "black corals" or "thorny corals" because of their black skeleton (ranging to golden brown) (Wagner 2011) that is covered by skeletal spines. The term "Antipatharia" comes from the Greek words "*anti*" and "*pathos*", meaning "against disease" or "evil" (Castorena and Metaca 1979; Kenyon 1984; Romero 1997). In the red sea area they have been used to cure eye diseases and as an aphrodisiac (Castorena and Metaca 1979). In Indonesia, bracelets made of black coral were considered to increase virility and cure rheumatism (Grigg 1984; Tsounis *et al.* 2010). In Hawaii islands the skeleton powder was used with other ingredients to cure sores and lung diseases (Kaaiakamanu and Akina 1922; Nagata 1971). In Chinese traditional medicine they are said to prevent bleeding, relieve pain and spot convulsions (Bai *et al.* 2011). Nowadays, despite an occasional use in Chinese traditional medicine, the most common use of black coral is as a material to manufacture jewels and art objects in diverse parts of the world (Wagner 2011; Grigg 1975; Castorena and Metaca 1979). Black corals (the whole order) are protected by international treaties (*e.g.* CITES), restricting their exploitation and exportation/importation (Opresko and Sánchez 2005). Given the limited gene flow among black coral populations over long distances, as inferred by molecular markers (Miller 1997, 1998), as well as the low growth rates of black corals (Newton and Bak 1979; Oakley 1988), their long longevity (Roark *et al.* 2009) seems to be the key factor

for population maintenance and so overexploitation of black corals could easily lead to local population extinction.

In 1725, L. Marsigli published the first recognized illustration of an antipatharian specimen, a branch covered by spines collected in the coast of Marseille in the Mediterranean Sea (Opresko and Baron-Szabo 2001). Initially considered as a plant by him, as it was the fate for other corals at that time, it was only Linnaeus, in 1758, with the publication of the "Systema naturae", that definitively positioned corals as animals, ending an intense academic debate of the first decades of the 1700s. Bernard de Jussieu (**figure 1.1**), member of botanists family De Jussieu, which gives name to the Jussieu Campus of the University Pierre et Marie Curie (where this PhD thesis took place), contributed to place corals as animals in 1742. He remarks that those possessed an "animal nature" and adopts the French name of "polype" (polyp) to designate those animals (d'Orbigny 1837).

Figure 1.1. Bernard de Jussieu (Lyon 1699 – Paris 1777), member of the famous naturalist family De Jussieu recognized that corals had an "animal nature". Unknown artist – public domain.

The order "Antipatharia" comprises 7 families, divided into 43 genera and approximately 235 species (Cairns 2007; Daly *et al.* 2007; Bo 2008). This subdivision of antipatharian species is mainly based on major characteristics of the polyp (number of mesenteries), in colony form, spine form and disposition in the skeleton (for a recent taxonomic overview see Mariza Bo's PhD thesis: "Taxonomy and ecology of Antipatharians", 2008). The order is rather homogeneous (Opresko 2001) and characterized by the following:

- Exclusively colonial organisms;
- Spiny, proteinaceous skeleton (synapormorphy in Hexacorallia);
- Polyps with six unbranched, non-retractable tentacles (synapormorphy in Hexacorallia);
- 6 **primary mesenteries** and 0, 4 or 6 **secondary mesenteries**[*]

(Opresko 2001; Opresko & Baron-Szabo 2001; Daly *et al.* 2003; Opresko & Sanchez 2005; Daly *et al.* 2007; Bo 2008; Wagner 2011).

The antipatharian order comprises some of the oldest living animals, including some of the oldest continuously growing living beings, with a living *Leiopathe*s colony being dated as 4 265 years old (Roark *et al*. 2009). Black corals occur worldwide in all oceans from tropical to polar regions (Molodtsova 2006; Wagner 2011; Clark and Bowden 2015), from 2 meters of depth for tropical wire corals (Brook 1889; Davis and Cohen 1968; Parrish and Baco 2007; Bo 2008) to abyssal depths of more than 8600 meters for species living in the western pacific (Pasternak 1977; Molodtsova *et al.*

[*] The terms primary and secondary mesenteries are used in the Antipatharian taxonomy without any regards of identity or developmental significance between the primary and secondary mesenteries. The term primary refers to the mesenteries presented in the species that have the lowest number of mesenteries (6) and the term secondary to the remaining mesenteries presented in the species that present a total of 10 or 12 mesenteries.

2008; Parrish *et al*. 2015). However, most species (over 75%) inhabit at depths of more than 50 meters (Cairns 2007) and therefore are inaccessible by SCUBA diving. This accounts to the fact that many aspects of antipatharian biology and ecology are strongly unexplored. In fact most of the shallow water species exist in tropical waters, and due to their global distribution (*i.e.* tropical regions of the globe), are far away from the main academic centers and have then also been poorly studied. Despite being understudied, black corals are abundant and dominant components of the sessile invertebrate fauna at depths of over 50 meters (Genin *et al.* 1986; Opresko and Genin 1990; Chave and Malahoff 1998; Grigg 2001; Rogers *et al.* 2007; Bo *et al.* 2009a; Clark and Bowden 2015).

The phylogenetic placement of Antipatharia will be discussed in chapter 2. For understanding purposes, however, a brief description of the phylum (Cnidaria) and class (Anthozoa) they belong to will follow.

1.1 Antipatharia belongs to the plylum Cnidaria and to the class Anthozoa

Cnidaria

The phylum Cnidaria is a highly diverse group of animals that comprises two major clades, one being Anthozoa and the other Medusozoa. Anthozoa, comprising corals and the sea anemones, including the laboratory model *Nematostella vectensis*, and other not so famous animals like sea fans and zoanthids. Medusozoa include jellyfishes (Scyphozoa), the laboratory model *Hydra* and siphonophores (Hydrozoa), stalked jellyfishes (Staurozoa) and box-jellies (Cubozoa). A partial representation of the diversity of this phylum can be found in **figure 1.2**.

There are around 11 000 extant species of cnidarians (Appeltans *et al.* 2012). Part of their diversity results of their ability to form colonies by asexual reproduction, allowing them to attain sizes and forms that would not be reachable by a solitary organism. The colony organization is also a factor of morphological diversity (**figure 1.2 b, e, f**). Another fact that account for this cnidarian diversity is that many species of cnidarians show a dimorphic life cycle that includes two completely different body forms: a **polypoid body form** – fixed, generally benthic (**figure 1.2 c, d, e, g**) – and a **medusoid body forms** – planktonic and sexual (**figure 1.2a**) (Hyman 1940; Kaestner 1967).

Cnidarians are multicellular organisms and present "true" tissues. They possess two primary (germ) layers: **ectoderm** (or epiderm) and **endoderm**[†] (or gastroderm); possessing a middle layer, primarily ectodermally derived acellular matrix (**mesoglea**) or partly cellular (**mesenchyme**). They are generally qualified as epithelial animals. Classically, cnidarians share radial symmetry with their primary body axis being the

[†] These terms were invented by T. H. Huxley in 1849 to designate the two "*membranes*" layers of medusae (Hyman 1940). They were then afterwards used in Bilateria development to designate the germ layers.

oral-aboral axis. Other distinctive features of this phylum are the presence of tentacles in both polypoid and medusoid forms and the presence of stinging or adhesives cells: the cnidocyte, which contains one giant secretory organelle or ***cnida*** (plural *cnidae*) being a synapormorphy of this phylum. In contrast to Bilateria their nervous system is not centralized (but generally present a nerve net) and they lack discrete circulatory, respiratory or excretory organs. Mostly marine animals, some species inhabit fresh water environments such as *Hydra* (**figure 1.2 d**).

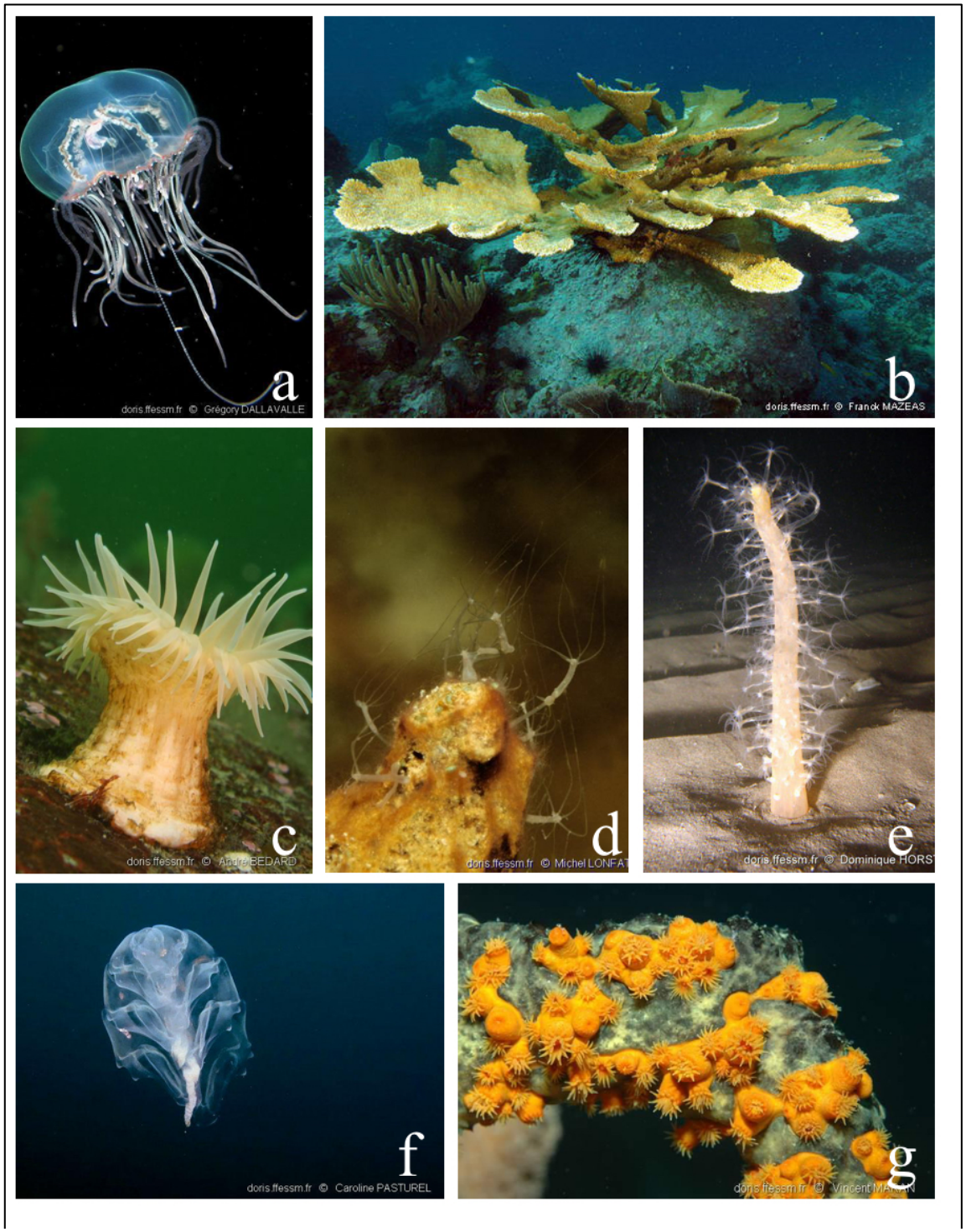

Figure 1.2. Partial representation of the cnidarian diversity: **a** – Medusa of the Hydrozoa class *Olindias phosphorica* (Delle Chiaje, 1841); **b** – Scleractinian coral *Acropora palmata* (Lamarck, 1816), a colonial species; **c** – Sea anemone, the actiniarian *Hormathia nodosa* (Fabricius, 1780); **d** – Polyps of *Hydra sp.* (Linnaeus, 1758 (genre)). *Hydra* has been a laboratory model for Cnidaria (Hydrozoa) in the past decades; **e** – Polyp colony here represented by the octocoral *Veretillum cynomorium* (Pallas, 1766), a sea pen; **f** - *Hippopodius hippopus* (Forskål, 1776) belongs to siphonophores, an order of exclusively pelagic colonial hydrozoides; **g** – Zoanthid polyps of the species *Parazoanthus swiftii* (Duchassaing and Michelotti, 1860). Photos from DORIS (http://doris.ffessm.fr), in order by Grégory Dallavalle, Franck Mazeas, André Bedard, Michel Lonfat, Dominique Horst, Caroline Pastorel and Vincent Maran.

Anthozoa

With more than 6000 described species, anthozoans account for more than 60% of the known cnidarians (Appeltans *et al.* 2012), they possess an extreme diversity of forms, from solitary anemones to 2 meters high black coral colonies and scleractinian corals that form impressive coral reefs, anthozoans play a major role in their ecosystems.

The Anthozoa class can be defined as a polyp cnidarian with at least 6 mesenteries dividing their cylindrical body. The polyp is a synapormorphy of cnidarians: it is tube-shaped and possesses a basal (pedal) disc, a body column, and an oral disc bearing the mouth and tentacles (**figure 1.3**). This organization of the polyp leaves it with a polarity between the oral and the aboral end, from this result the first body axis of the polyp, the oral-aboral axis with the oral and the aboral poles (**figure 1.3**).

Anthozoans possess, as other cnidarians, an epithelial organization formed by two well-developed layers that correspond to the adult ectoderm and endoderm. An acellular matrix is intercalated as a middle layer, primarily ectodermally derived, the mesoglea.

The repetitive compartments (mesenteries) of the anthozoan polyps divide the body between the body wall and the pharynx wall, in a radial fashion (**figure 1.3**). From a general cylindrical body shape, anthozoan polyps internal anatomy becomes during development quickly radially or bilaterally symmetric due to the mesenteric arrangement. An external feature, the tentacle disposition in the oral disc gives insight of the body radial symmetry. The mesenteries usually possess longitudinal muscles (retractor muscles) that are disposed in a longitudinal fashion in relation to the oral-aboral body orientation. The disposition of these muscles is characteristic to each group and can give a bilateral symmetry to the polyp (see chapter 2 for details).

The bilateral symmetry found in anthozoan polyps contrasts with the radial symmetry found in Medusozoa polyps and medusae. The anthozoan polyp possesses, in contrast to the other Cnidarian polyps, a **pharynx**. It allows the communication between the mouth and the gastric cavity and is usually flattened along the secondary axis of the polyp as a slit. It is a tube originated from invaginated ectoderm (**figure 1.3**). Classically there is one or two **siphonoglyphs** (*i.e.* corner of the pharynx modified as a

ciliated groove) in the extremities of the slit but some groups, as scleractinians (but probably also all antipatharians), don't present any. Anthozoans possess tentacles, more or less retractable, surrounding the mouth and that allow the capture of preys (Pax 1987; Kaestner 1967; Brusca and Brusca 2003).

All anthozoans are marine animals and can be solitary or colonial and are in most cases attached to the substrate. Anthozoa is a monophyletic group that comprises the subclasses **Hexacorallia** and **Octocorallia**. The position of the **Ceriantharia** order, usually considered as hexacorallians is disputed, as they can be an independent subclass (see chapter 2). Therefore, the monophyly of Hexacorallia is disputed (Simion PhD thesis 2014; Zapata *et al*. 2015).

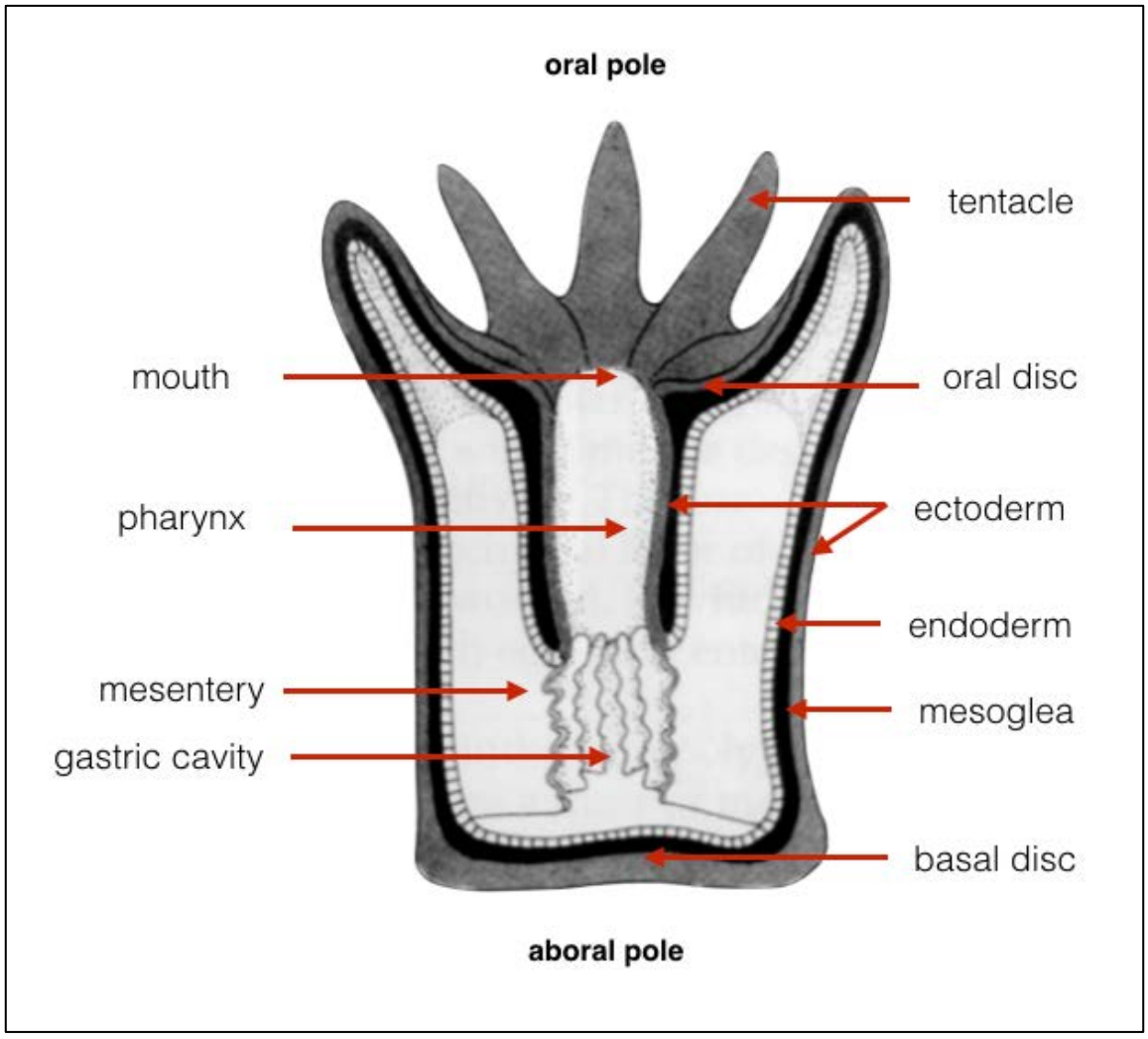

Figure 1.3. Schematic representation of the main characteristics of the anthozoan polyp. Adapted and corrected from Brusca & Brusca (2003).

1.2 Phylogeny and general morphology of antipatharians with focus on our model species.

1.2.1 Taxonomy and phylogeny

Antipathes caribbeana Opresko, 1996 and *Plumapathes pennacea* (Pallas, 1766) are two black coral species from Caribbean shallow waters (Opresko and Sánchez 2005), belonging to the Antipatharia order. They present several morphological differences between them (*e.g.* branching pattern of the colony and polyp size) and belong to different antipatharian families. One of their main morphological differences – their different external symmetry in tentacle disposition – is the reason why they were chosen as study species in this work. This aspect will be discussed in **section 1.4** of this chapter. However their exact position in the antipatharian phylogeny is hard to precise.

Antipathes caribbeana belongs to Antipathidae (Ehrenberg, 1834) family while *Plumapathes pennacea* belongs to the Myriopathidae (Opresko, 2001) family. The subdivision of antipatharian species in different families is mainly based on major characteristics of the polyp (number of mesenteries), in colony form and in spine form and disposition.

The evolutionary relationships between the antipatharian families have never been accessed by means of morphological cladistics analyses, and no serious studies tried to understand the evolutionary relations until molecular phylogenies with representatives of several families became available (Bo *et al*. 2012; Brugler *et al.* 2013).

The fact that molecular phylogenies have only been recently done in order to understand the evolutionary relations between the families and species of the antipatharian order account once again to the lack of studies on this group, not due to the lack of good quality publications by those that studies this group in recent years, good publications have been published in taxonomy and ecology (*e.g.* of authors: Opresko; Bo; Wagner; Grigg), but due to the small community of scientists presently studying this group of animals.

In 2013, Brugler *et al*. published the only molecular phylogenies including all the antipatharian families. In **figure 1.4** there is one of their phylogenetic reconstructions (Maximum likelihood method, details in Brugler *et al*. 2013), done with nucleotide mitochondrial sequences of *cox3* and *cox1* mitochondrial genes concatenated. The reconstruction was rooted with Actiniaria as outgroup (in black in the figure). Each species of the phylogeny is coloured in order to underline their taxonomic family. From this phylogenetic reconstruction it is evident that families are polyphyletic, especially Antipathidae (violet) and Aphanipathidae (green). The results showed that the morphological traits chosen to distinguish between families might not all be of valid use. The genera level also showed polyphyletic results in some cases, especially the *Stichopathes* species that branch in different clades of the tree. It is then problematic to address the question of whether two antipatharian species are more or less closely related.

Despite the difficulties accessing questions of phylogeny in Antipatharia, *Antipathes caribbeana* and *Plumapathes pennacea* belong to different families, and these families, Antipathidae and Myriopathidae, come out in different clades in Brugler *et al*. analysis. In **figure 1.4** the position of *Antipathes caribbeana* is marked with a **violet arrow** and the position of *Plumapathes pennacea* is marked with a **light blue arrow**. From these molecular results we can notice that the two species are quite distant in the evolutive history of black corals.

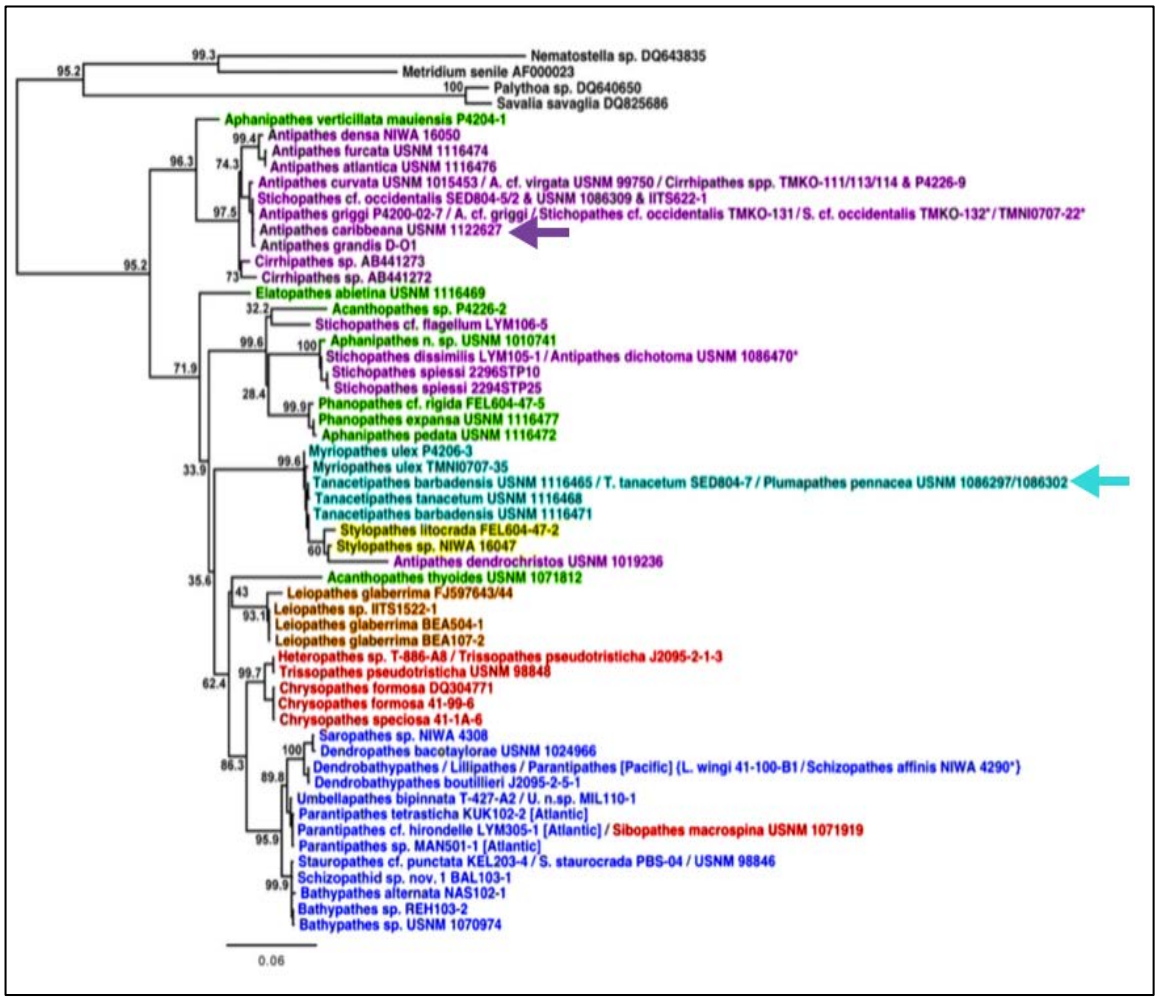

Figure 1.4. Adapted ML-based phylogenetic reconstruction of the evolutionary relations between antipatharian families with the cox3-cox1 mitochondrial genes nucleotide alignment, rooted to the Actiniaria (sea anemones) from Brugler *et al*. 2013. The colours and antipatharian families are as follow: Antipathidae: **violet**; Aphanipathidae: **green**; Myriopathidae: **light blue**; Stylopathidae: **yellow**; Leiopathidae: **orange**; Cladopathidae: **red** and Schizopathidae: **dark blue**. Bootstrap values are placed on the main branches.

1.2.2 Description of *Antipathes caribbeana*

Antipathes caribbeana described by Opresko (1996) is found especially on vertical wall faces at reef edges in areas of strong currents, at depths of 30 to about 100 meters (Opresko and Sánchez 2005) and known from the north of Colombia to the Bahamas islands (Opresko and Sánchez 2005). This species has been important commercially, the main branches of large colonies are suitable for jewellery (Opresko and Sánchez 2005).

Antipathes caribbeana belongs to the Antipathidae (Ehrenberg, 1834) family. Polyps with 10 mesenteries characterize this family of antipatharians: 6 primary mesenteries and 4 secondary mesenteries. Polyps of this family are not elongated, having a diameter of 1 to 3 millimetres (Bo 2008). Antipathidae is probably the most heterogeneous family of antipatharians and may be in need of a taxonomic revision (Bo 2008). In this family, the colony shape or external morphology do not allow to distinguish between genus (Bo 2008). Specific features like the shape of spines or the branching of the colony *caribbeana* need to be checked for in order to well identify the species.

Antipathes caribbeana is a species that forms bushy colonies (**figure 1.5 and figure 1.6**), branched to the 10th order or more – meaning that there may be 10 or more branching processes between a terminal branch and the main colony branch – and that measure one meter or more in height (Opresko 1996; Opresko and Sánchez 2005). Branches are linear, elongated, with narrow distal branch angles (30-45°) (**figure 1.6b and figure 1.7**). Smallest branchlets are straight or slightly curved, 0.15-0.30 mm in diameter (excluding spines) and 10 cm or more in length without becoming branched (Opresko 1996; Opresko and Sánchez 2005). Spines of the skelet are conical or sub-cylindrical in appearance; flared out distally and proximally at the junction with axis; they are covered with small cone-shaped or knob-like tubercles over apical one-half to three quarters of surface – **figure 1.6** (Opresko 1996; Opresko and Sánchez 2005). The polypar (in the polyp side of the branch) spines measure between 0.08 and 0.16 mm tall; the abpolypar (in the branch side without polyps) spines measure between 0.06 and 0.12 mm. Narrow conical secondary spines sometimes present on branches are 0.4 mm or more in diameter (Opresko 1996; Opresko and Sánchez 2005). Polyps of this species are 1.0 mm in transverse diameter with an interpolypar space of 0.3 to 0.5 mm; arranged on smallest branchlets in a single series with 6-10 polyps per centimetre. Polyps on larger branches and stem are less regularly distributed, sometimes occurring on all sides of the axis. Living colonies are brownish in colour (**figure 1.5**) but diverse morphotypes of colour might exist (Opresko – personal communication). Tentacles are partially translucent and present a white colour (Opresko and Sánchez 2005; personal observation).

Figure 1.5. Colony of *Antipathes caribbeana* in Guadeloupian waters at a depth of sensibly 30 meters. This colony has a height of more than one meter and presents a brownish colour. Photo by Alain Goyeau.

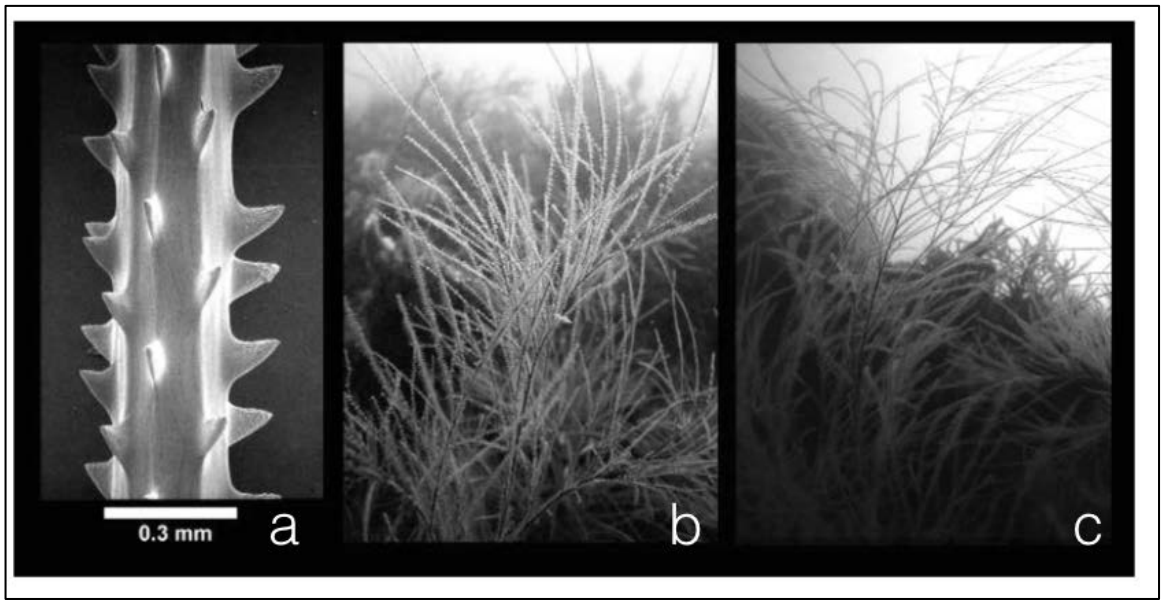

Figure 1.6. *Antipathes caribbeana*: **a** – Scanning electron microscope image of a part of the skelet (USNM 94379); **b** – branching in a colony (*in situ* photo); **c** – photo of a living colony. Figures taken from Opresko and Sánchez (2005).

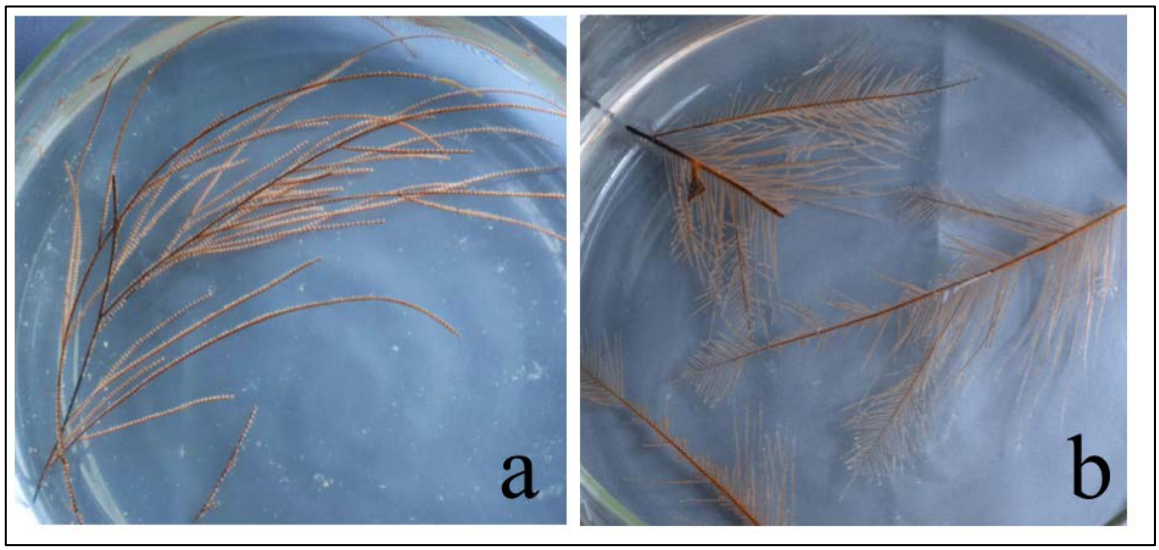

Figure 1.7. Photos of colony parts of *Antipathes caribbeana* (a) and *Plumapathes pennacea* (b) after collection (taken without scale). The differences in the branching pattern of both species are visible in this photos: **a** - Branches are linear, elongated, with narrow distal branch angles (30-45°) in *Antipathes caribbeana*; **b** - Branching in *Plumapathes pennacea* tends to be in one plane, stem and branches with two anterolateral rows of simple filiform pinnules up to 6 cm long (usually 2-4cm long). Pinnules in opposite rows arranged alternately with members of each row spaced 1-3 mm apart. Photos by Michaël Manuel.

1.2.3 Description of *Plumapathes pennacea*

Found at depths of 20 meters or more, *Plumapathes pennacea* is known from the north coast of Brazil to the coast of the Bahamas islands (Opresko and Sánchez 2005). This species is important commercially because the main branches of large colonies are suitable for jewellery (Opresko and Sánchez 2005).

Plumapathes pennacea belongs to the Myriopathidae family (Opresko, 2001). Opresko states that this family is established on the basis of the size and morphology of the polyps, tentacles and spines, and on the general pinnulation of the corallum. Myriopathidae is closely related to Antipathidae, but differs from that family in that the polyps are smaller (usually 0.5-1 mm), and the tentacles shorter with round tips, whereas in *Antipathes sensu stricto* the tentacles appear to narrow gradually to a very fine tip.

Plumapathes pennacea consist on large, sparsely to densely branched, pinnulated colonies (**figure 1.8 and figure 1.9d**), 0.5 m or more in height. Branching is irregular but tending to be in one plane (**figure 1.7b and 1.9c**), lower order branches one-half as long as stem or longer. The stem and branches have two anterolateral rows of simple filiform pinnules up to 6 cm long (usually 2-4cm long) (**figure 1.9b and figure 1.10**). Pinnules in opposite rows arranged alternately with members of each row spaced 1-3 mm apart (**figure 1.9b and figure 1.10**). Spines smooth to very faintly papillose, subcylindrical, acute; distally inclined (**figure 1.9a**); up to 0.35 mm tall on polypar side of the axis and up to 0.15 mm tall on abpolypar side of axis. Spines on pinnules arranged in 7-10 longitudinal rows with 35-70 spines per centimetre in each row.

Polyps measure between 0.45 and 0.8 mm in transverse diameter and are arranged in a single series with 10 to 13 polyps per centimetre. Colour of living colonies varies from greyish to orange-brown (**figure 1.8**) (Opresko and Sánchez 2005).

Figure 1.8. Colony of *Plumapathes pennacea* in Guadeloupian waters at a depth of 30 meters. This colony measures between 0.5 and 1 meter and presents an orange to brown colour. In the image it's visible the pinnulated aspect of this antipatharian. Photo by Alain Goyeau.

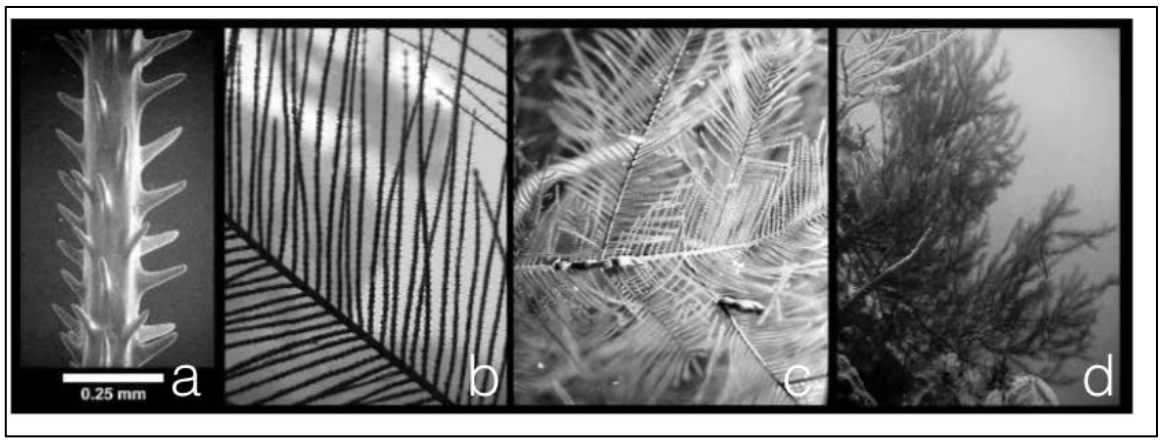

Figure 1.9. *Plumapathes pennacea*: **a** – Scanning electron microscope image of a part of the skelet (USNM 92976); **b** – branches have two anterolateral rows of simple filiform pinnules up to 6 cm long (*in situ* photo); **c** – photo of several main branches with pinnatulated branching; **d** – photo of a colony. Figures taken from Opresko and Sánchez (2005); colony photos taken at Cat Island, Bahamas at a depth of 25 meters.

Figure 1.10. Close-up photo of the branches of *Plumapathes pennacea*, allowing the visualization of their pinnulated branching. Photo taken in the Flower Garden Banks National Marine Sanctuary located in the Texas (United States of America) coast. This photo was taken using a Remotely Operated Vehicle (ROV) by the FGBNMS/National Undersea Research Center at the University of North Carolina Wilmington.

1.3 Antipatharian diversity and morphology

With around only 250 described species (Appeltans *et al.* 2012), Antipatharia presents an extraordinary morphological variation. From the most extremely different colony forms, passing to different ways of polyp disposition, skelet differences, color of colonies or differences in polyp anatomy.

Here there is a brief review of these variations as they are tightly linked to developmental differences and to ecology of the species. Thought these developmental differences have not yet been studied. Also the diversity of polyp disposition on the colony and its morphology will be approached.

Colony forms

Antipatharia presents a wide range of colony morphologies that form arborescent-like colonies being then considered to be phytomorphic (Doumenc and van Praët 1987). As other cnidarians, the antipatharian morphological unit is the polyp. Unlike their closely related scleractinians, antipatharian corals do not build reef structures and thus are considered ahermatypic (coral species that do not form coral reefs). They also don't possess any mineralized structures such as spicules or sclerites.

Colonies are attached to the substrate through a basal plate, with the exception of some species within the genera *Bathypathes* and *Schizopathes* that have a modified hook-like holdfast for support in soft sediments and mud (Pax 1918, Grigg and Opresko 1977; Pasternak 1977; Grasshoff 1981; Opresko 1997; Opesko 2002).

The Antipatharia present a huge variety of branching forms, from unbranched colonies to complex branching patterns. The general aspect of colonies can be grouped in five types:

- **Fan shape**: colonies that have their branching mainly in one plane and that present their branches close together tend to obtain a fan-like shape; example – *Trissopanthes pseudotristicha* **(**Opresko, 2003) in **figure 1.11a**;
- **Bush shape**: several antipatharian species display this form, big branches with many planes of branching contribute to obtain this colony form; example – *Antipathella subpinnata* (Ellis and Solander, 1786) in **figure 1.11b**;
- **Bottle-brush shape**: antipatharian species that branch in several planes around a main branch tend to present this colony shape; example – *Cupressopathes sp.* in **figure 1.11c**.
- **Feather shape**: colonies that have their branching mainly in one plane around a main branch end up with a feather-like shape; example – *Bathypathes sp.* in **figure 1.11d**.
- **Wire-like shape**: colonies that do not branch end with a wire-like shape, this colony shape corresponds to the *Cirripathes* and *Stichopathes* species; example: *Stichopathes lutkeni* (Brook, 1889) in **figure 1.11e**.

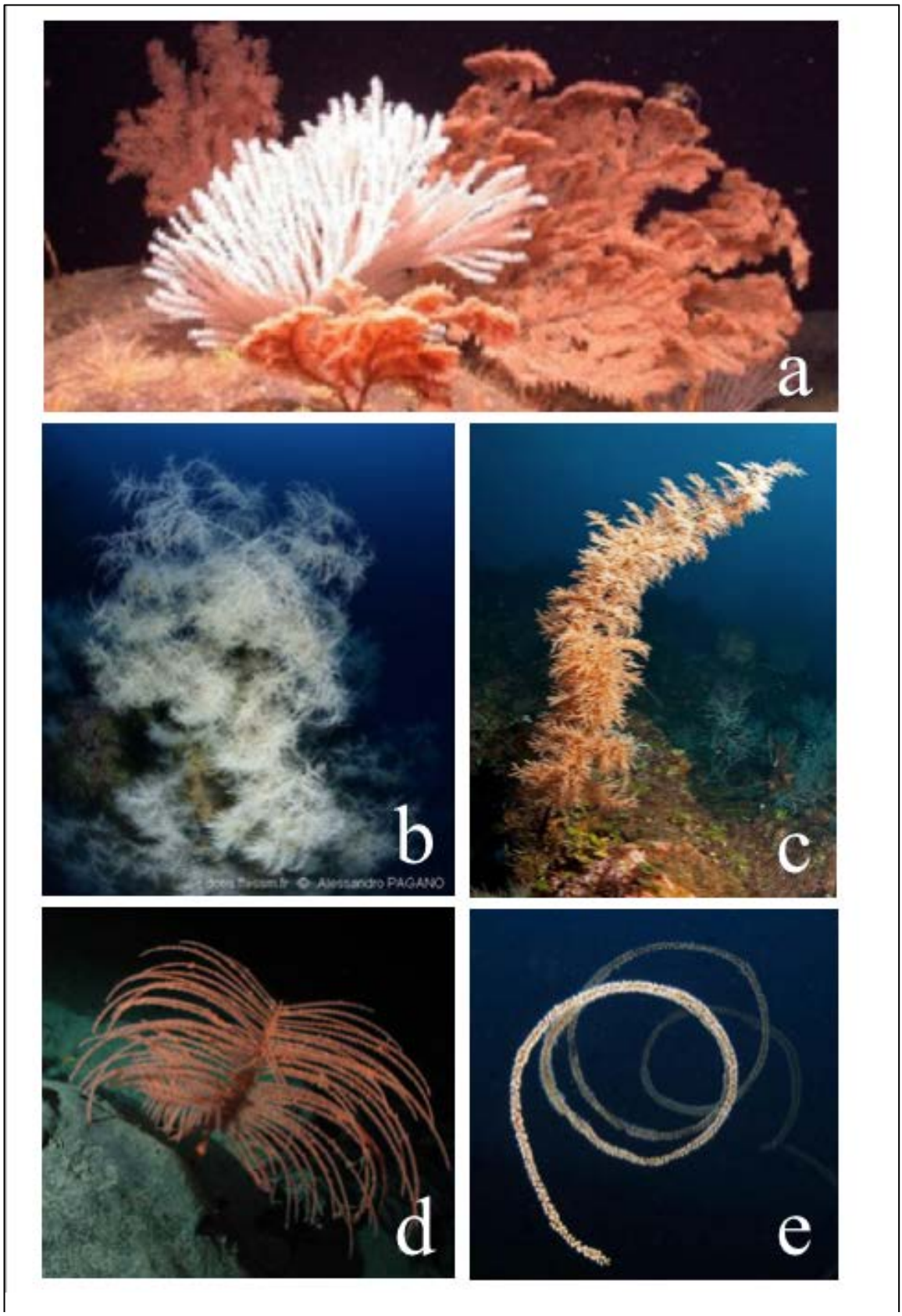

Figure 1.11. Antipatharian species present a huge variety of colony forms: **a** – **fan shape**: *Trissopanthes pseudotristicha* **(**Opresko, 2003) is a deep sea antipatharian, photo taken at 2 669m by NOAA/ MBARI; **b** – **bush shape**: *Antipathella subpinnata* (Ellis and Solander, 1786), a Mediterranean and Atlantic species that can attain 2 meters of high, photo of a specimen in the Sicilian coast (Italy) at 65 meters of depth, from DORIS (http://doris.ffessm.fr) by Alessandro Pagano; **c** – **bottle-brush shape**: *Cupressopathes sp.* from Indonesia; photo by Massimo Boyer; **d** – **feather-like shape**: *Bathypathes sp.* are deep sea

antipatharians, photo taken at 2 367 meters by NOAA/ MBARI; e – **wire-like shape**: *Stichopathes lutkeni* (Brook, 1889) is an unbranching colony, photo from DORIS (http://doris.ffessm.fr) by Patrick Giraudeau, taken at 37 meters of depth in Guadeloupe.

In **figure 1.12**, consisting of drawings of colony shapes and branching patterns from Pax (1918) after the original drawings of different authors, we can observe with more detail the variations on branching. This diversity of branching types can be expected to be tightly related to differences in the expression of genes and must then be taken into account when comparing species with different branching patterns.

In the representation of the distal part of a colony of *Antipathes dofleini* (Pax, 1915) – **figure 1.12a** – the branching takes place with angles of around 30º in this species as happens in most antipatharian species.

Branching can take different patterns in the same species, possibly depending on the environment. In **figure 1.12b and 1.12c** we can see this intraspecific variability, with different growing forms of *Antipathes abies* (Linnaeus, 1758): "*paniculata*" variant in **b** and "*normal*" variant in **c**.

Some species, as *Leiopathes grimaldi* (Roule, 1902) in **figure 1.12d**, present a dichotomously branched colony, meaning that each branch divides in two at each branch tip.

Branching can occur in a more regular or in a more irregular way, for example *Stauropathes punctata* (Roule, 1905) – in the left of **figure 1.12e** – presents its branches arranged in two membered whorls while *Antipathes abies* (Linnaeus, 1758) – in the right of **figure 1.12e** – presents a disperse branching.

The colony forms of species that do branch can be observed in **figure 1.12f**: bush shape with pseudodichotomous branching; **figure 1.12g** with representatives of feather-like, fan-like and bottle-brush shapes and **figure 1.12h** with a bush-like colony.

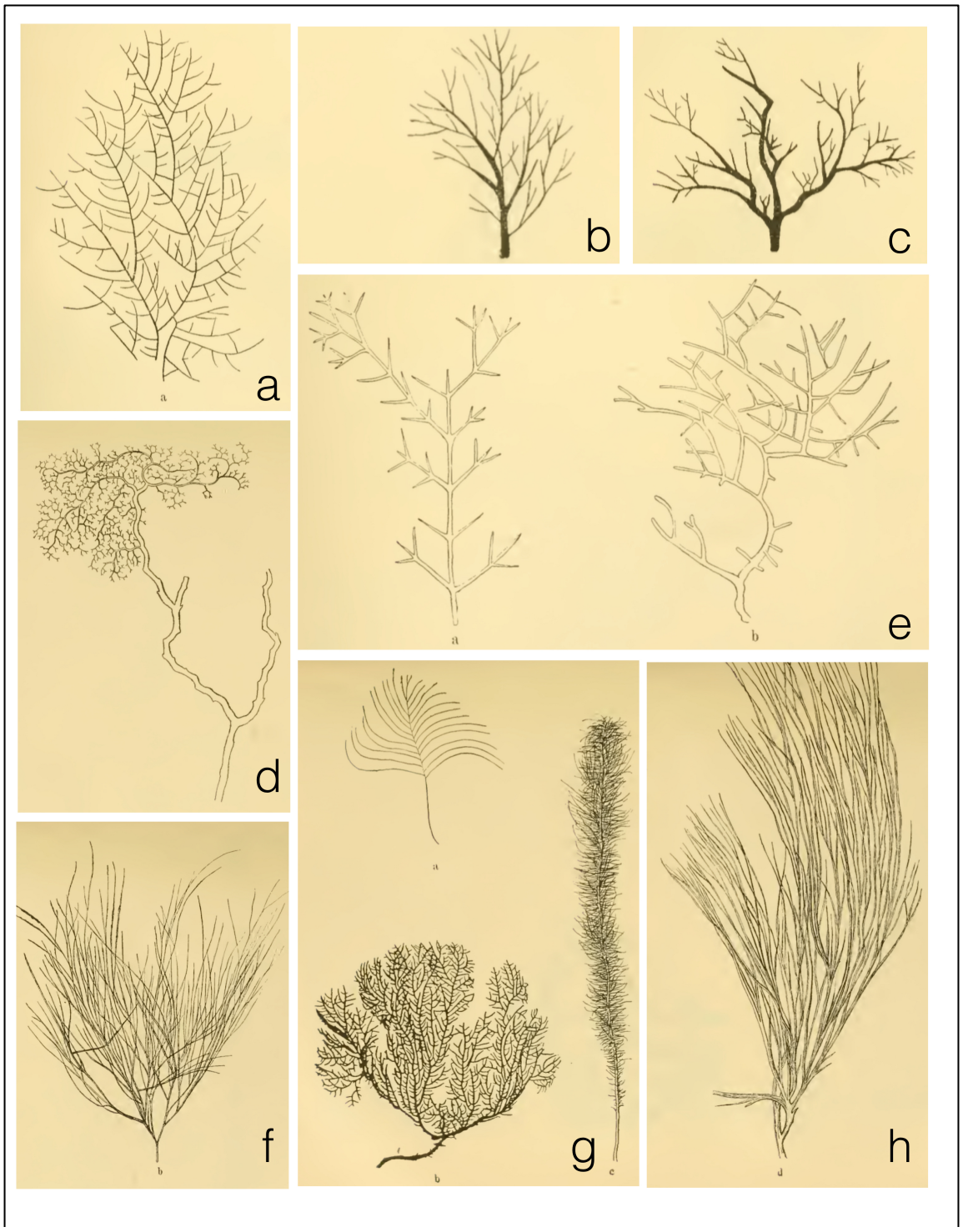

Figure 1.12. Antipatharian species present different colony forms, different branching patterns and may present intraspecific variability**: a** – distal part of a colony of *Antipathes dofleini* (Pax, 1915) (figure: Pax 1918 after Pax 1915); **b** and **c** – intraspecific variability: different growing forms of *Antipathes abies* (Linnaeus, 1758): "*paniculata*" variant in **b** and "*normal*" variant in **c** (figure: Pax 1918 after Cooper 1909); **d –** dichotomously branched colony of *Leiopathes grimaldi* (Roule, 1902) (figure: Pax 1918 after Roule 1905); **e** – *Stauropathes punctata* (Roule, 1905) – in the left of the image – presents its branches arranged in two membered whorls (figure: Pax 1918 after Roule 1905), *Antipathes abies* (Linnaeus, 1758) presents a disperse branching (figure: Pax 1918 after Cooper, 1909); **f** – pseudodichotomously branched colony of *Antipathes furcata* (Gray, 1857) (figure: Pax 1918 after Schultze 1902); g – different colony shapes in different antipatharian genra: up-left figure: *Bathypathes alternata* (Brook, 1889) has a feather-like shape (figure: Pax 1918 after Brook 1889); down-left figure: *Acathopathes hancocki* (Cooper, 1909) has a fan shaped colony (figure: Pax 1918 after Cooper 1902); right figure: *Stylopathes tenuispina*

(Silberfeld, 1909) has a bottlebrush colony shape (figure: Pax 1918 after Silberfeld 1909); **h** – *Antipathes densa* has a bush-like colony shape (figure: Pax 1918 after Silberfeld 1909).

Colour

Antipatharians, despite being called black corals (due to the colour of the skelet), display a wide variety of colours. Some species as *Cirripathes spiralis* (Blainville, 1834) can display different colour morphotypes, as yellow and green (**figure 1.13a and 1.13b**). Other colours displayed by antipatharians include: rose (**figure 1.13c**), white (**figure 1.13d**); orange/brown (**figure 1.13e**) and red (**figure 1.13f**). No studies have focused on explaining the different pigmentation of antipatharian species or the different colour of morphotypes of the same species.

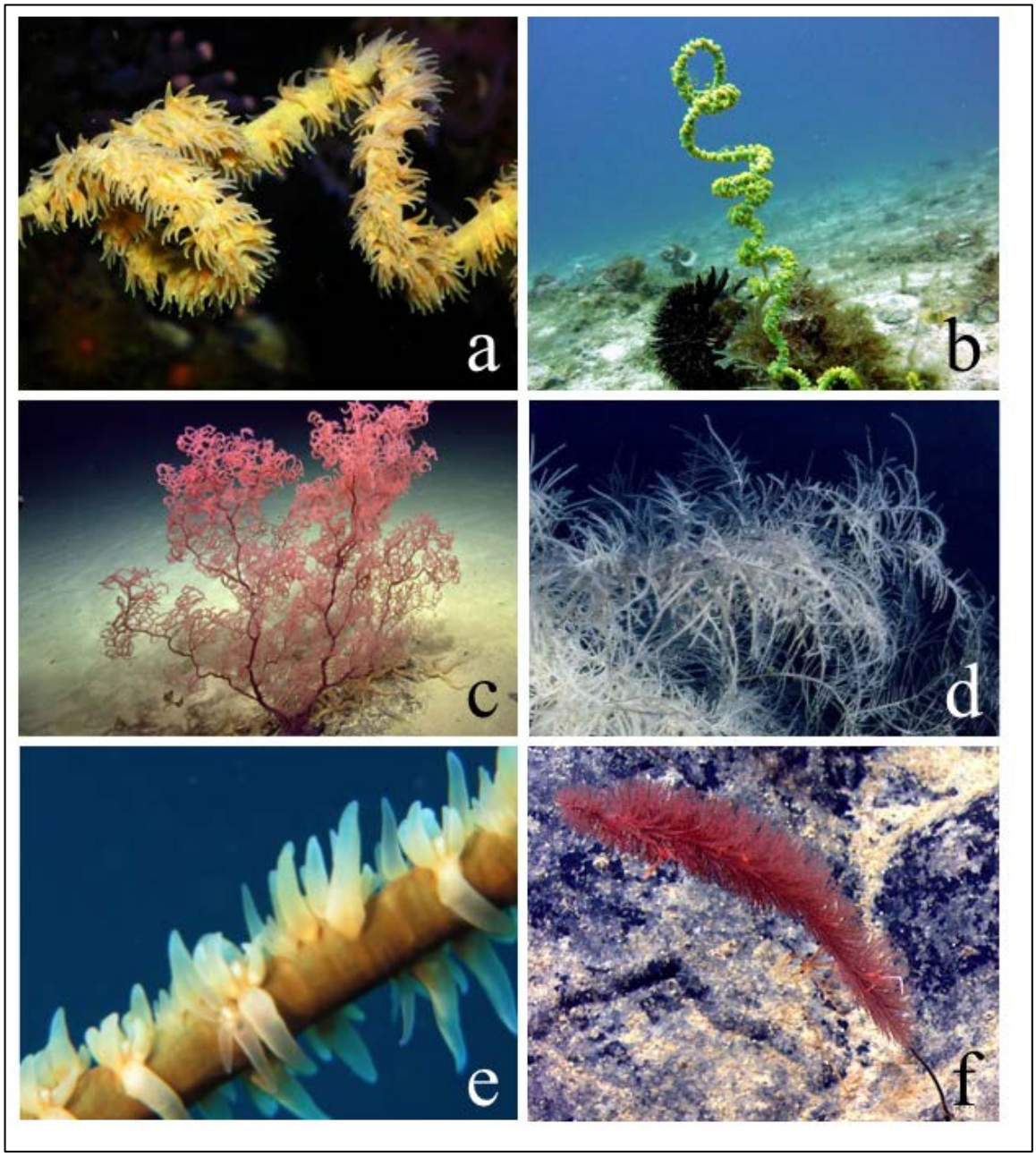

Figure 1.13. Antipatharian species present different colorations: **a** and **b** – *Cirripathes spiralis* (Blainville, 1834) presents different color morphotypes, here represented are **yellow (a)** and **green (b)**; **c** – *Leiopathes sp.*, Leiopathidae species present different colours, this individual presents a **rose** tonality; **d** – *Antipathella subpinnata* (Elis and Solander, 1786) presents a **white** color, forming authentic white forests in the Mediterranean sea; **e** – *Stichopathes lutneki* (Brook, 1889) presents colors ranging from **brown** to orange; **f** – *Parantipathes sp.* specimen displaying a **red** color. Photos by: a – Jaco Schoeman; b – Sylvie LB; c and f – Ocean exploration trust; d and e – DORIS (http://doris.ffessm.fr) by Alessandro Pagano and Dominique Marion.

Polyp external anatomy

The antipatharian polyp possesses the major traits of the organization of other anthozoan polyps. Looking at part of a branch of *Plumapathes pennacea* in **figure 1.16a** there are 6 polyps in the image, all of them are aligned and organized in a single row in one side of the skeletal axis. Each polyp has 6 tentacles around the oral cone with, in its centre, the mouth (**figure 1.16b**). In relation to its disposition in the skeletal axis the polyp has two "sides", a proximal pole in the side of the foot of the colony and a distal pole in the side of the colony apex. Then, both the colony and each polyp in the colony are polarized. The tentacles are distinguished as sagittal tentacles (the two tentacles situated along at opposite sides of flattered mouth) and two pairs of lateral tentacles that can be distinguished as proximal and distal pairs.

By transparency of the tissues its visible both in **figure 1.16a** and **figure 1.16b** the skelet axis with the spines that pierces the polyp and the coenenchyme. In some cases the spines can pass trough the coenenchyme and then be directly exposed to the environment.

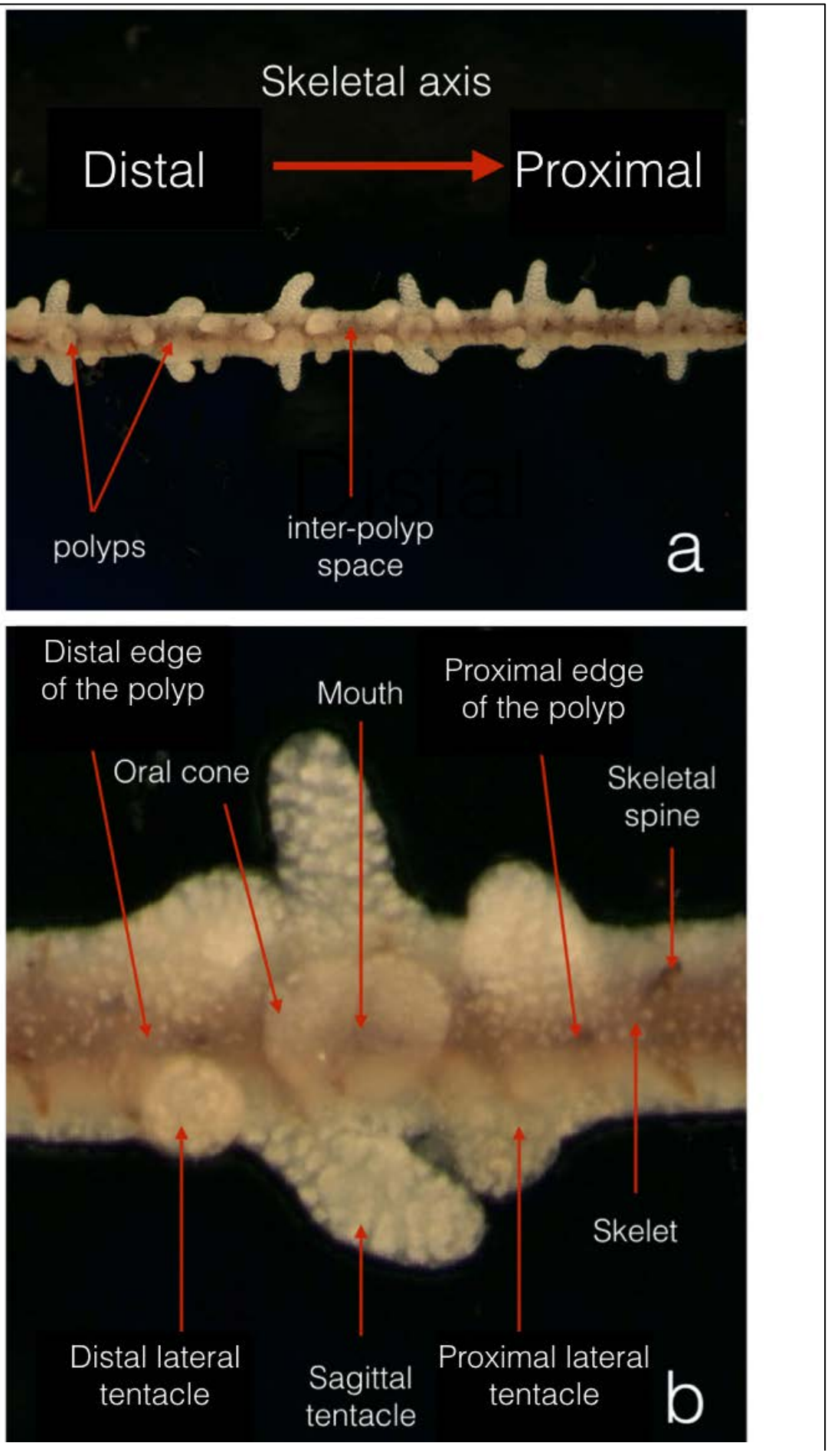
Skeletal axis
Distal
Proximal
polyps
inter-polyp space
a
Distal edge of the polyp
Mouth
Proximal edge of the polyp
Oral cone
Skeletal spine
Skelet
Distal lateral tentacle
Sagittal tentacle
Proximal lateral tentacle
b

Figure 1.16. – External morphology of *Plumapathes pennacea*: a – branch part with 6 polyps, the polyps are disposed in the same side of the branch and all possess 6 tentacles around the mouth, the skelet is visible by transparency of the tissues; b – close-up view of a polyp of *Plumapathes pennacea*, visible are the mouth in a central position in the centre of the oral cone around which are disposed 6 tentacles, 2 sagittal ones in each side of the flattered mouth and 2 pairs of lateral ones, a proximal lateral pair in the proximal edge of the polyp and a distal lateral pair in the distal edge of the polyp, also visible by transparency are the skelet and respective sketelat spines.

Coenenchyme and the limit between polyps

The **coenenchyme**‡ in antipatharians has been described by Brook (1889) as the basal portions of the polyps together with their connections with adjoining polyps. Its relative importance is described as varying deeply between genera.

In *Cirripathes sp*., Brook (1889) describes that the coenenchyme forms a sheath around the skelet in which the polyps are imbedded. In this genus the interzooidal areas (areas between polyps) are divided by means of mesogleal septa into a number of canals, mostly transverse to the skeletal axis and that allowed the communication between polyps (in this species the polyps are located all around the branches).

In *Leiopathes sp*., a species where polyps are disposed in a single row in one side of the branch, Brook (1889) described that the bases of the polyps fused forming a hollow tube in which the skelet is formed.

The three layers of the polyps are present in the coenenchyme. Externally there is a layer of ectoderm, followed internally by a layer of mesoglea and finally a layer of endoderm (Brook 1889; von Koch 1889).

‡ Terminology used at the anthozoan level to express the cellular environment secreted by adult polyps in which new polyps arise. The term is confusing because it also designates a separated layer (separated from the polyp), sometimes transitional, which separates two adjacent polyps. Also, in sea-pens (Pennatulacea) the large central polyp is composed mostly of coenenchyme from which very small polyps arise.

At the point opposite to the polyps, in the side without polyps, a slender longitudinal (according to the skelet axis) mesogleal septum occurs. This longitudinal septum continues and surrounds the skelet separating it from the endoderm of the polyp (**figure 1.14**). Between the mesoglea and the skelet there is a tissue, called by Brook (1889) as "*axis epithelium*" and by von Koch (1889) as "*achsenepithel*", German for "axis epithelium" and corresponds to the skeletal ectoderm, meaning that there is an ectodermal layer separating the polyp from the colony skelet (**figure 1.17**).

The skeletal sheath (tissues recovering the skelet) also includes a layer of endoderm continuous with that of the polyps, which forms the median portion of the floor of their gastric cavity (Brook 1889) (**figure 1.14**).

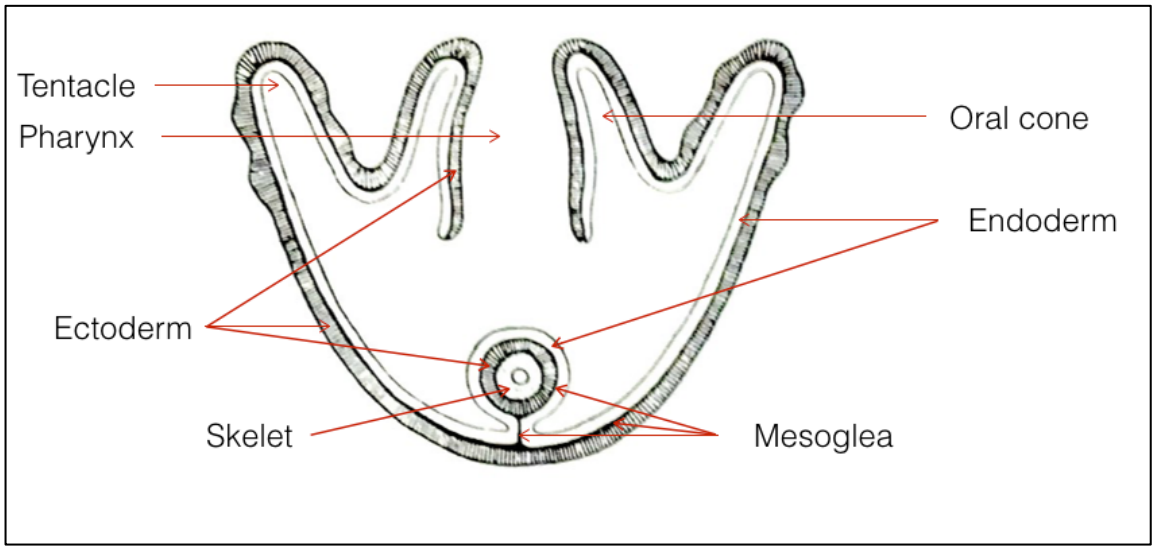

Figure 1.17. – Schematic representation of a transverse section (in relation to the colony) at a polyp level. The ectoderm corresponds to all externally exposed tissues of the polyp, including as in other anthozoans the pharynx. The antipatharian skelet is involved and secreted by a special type of ectoderm, the "*axis epithelium*" (Brook 1889). The endoderm is the internal tissue of antipatharians and both ectoderm and ectoderm are separated at all points by a sheet of mesoglea. Particularly there is a sheet of mesoglea separating two endoderm parts in the part of the branch bearing no polyps, corresponding to the mesogleal septum that runs longitudinally in relation to the skeletal axis.

In some species such as *Pteropathes fragilis* (Brook, 1889), the **coenenchyme** is confined to the back of each branch, the polyps on the polyp bearing surface being so closely packed together that there is no room for "*interzooidal*" tissue (Brook 1889). In other cases where the polyps are more isolated, the connections between the polyps (on the polyp bearing part of the branch) contain prolongations of their gastric cavity

(Brook 1889). Mesogleal septa usually occur, which incompletely separate the individuals from one another (von Koch 1889; Brook 1889, van Pesch 1914), see **figure 1.18**. In some species the mesogleal septa that separate polyps may either be absent or not visible in external observation, however in other species such as *Stichopathes aff. dissimilis* (Roule 1902) the septa dividing the polyps is evident and well marked in an external morphological observation (Molodtsova 2006).

The skelet of antipatharian species show no evident sign the mesogleal septa that may occur between polyps or of the coenenchyme that can occur between polyps of certain species. **This may mean that the ectoderm responsible for the skelet synthesis is not directly dependent of each particular polyp**. In **figures 1.6a and 1.9a** we can see that in *Antipathes caribbeana* and in *Plumapathes pennacea* no signs of polyp disposition are evident in the skelet.

In conclusion, a colony of *Antipathes caribbeana* (or *Plumapathes pennacea*) is formed by a series of polyps assembled longitudinally on a continuous skeletal element. In this species the coenenchyme is probably extremely reduced.

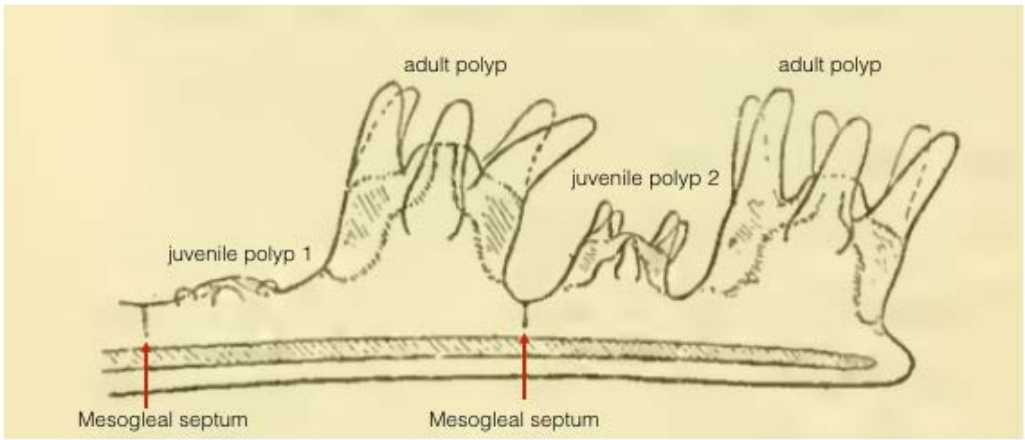

Figure 1.18. Schematic representation adapted from von Koch (1889) of a branch tip containing two adult polyps and two juvenile polyps. The juvenile polyps present different developmental states with juvenile polyp still emerging to the surface and with juvenile polyp 2 already emerged and completely formed. Von Koch observed mesogleal septa between the pairs of adult and juvenile polyps, showing that either the formation of septa between polyps takes place after the polyp reaches a certain development or that the juvenile polyps are directly dependent of the adult polyps (budding is discussed in detail in chapter 2).

Polyp disposition in the antipatharian colony

The polyps are disposed in different ways along the colony of antipatharian species. The antipatharian adult polyp himself is small, with 2 millimetres or less of sagittal diameter (Bo 2008). This maximal size is found in the polyps of the Leiopathidae family (Bo 2008). The smallest adult polyp is that of *Antipathes hirta* (Gray, 1857) with 0.5 millimetres of diameter (Echeverría 2002). The polyps can be disposed in different ways in the colonies. Different modalities of polyp disposition are: i) in a single row in one side of the branch in *Antipathes caribbeana*, *Plumapathes pennacea* and *Stichopathes sp*. (**figure 1.15a**), ii) in several rows in one side of the branch of *Pseudocirrhipathes mapia (*Bo *et al*. 2009b) (**figure 1.15b**) and iii) all around the colony axis in *Cirripathes* species, less densely (**figure 1.15c**) or more densely (**figure 1.15d**).

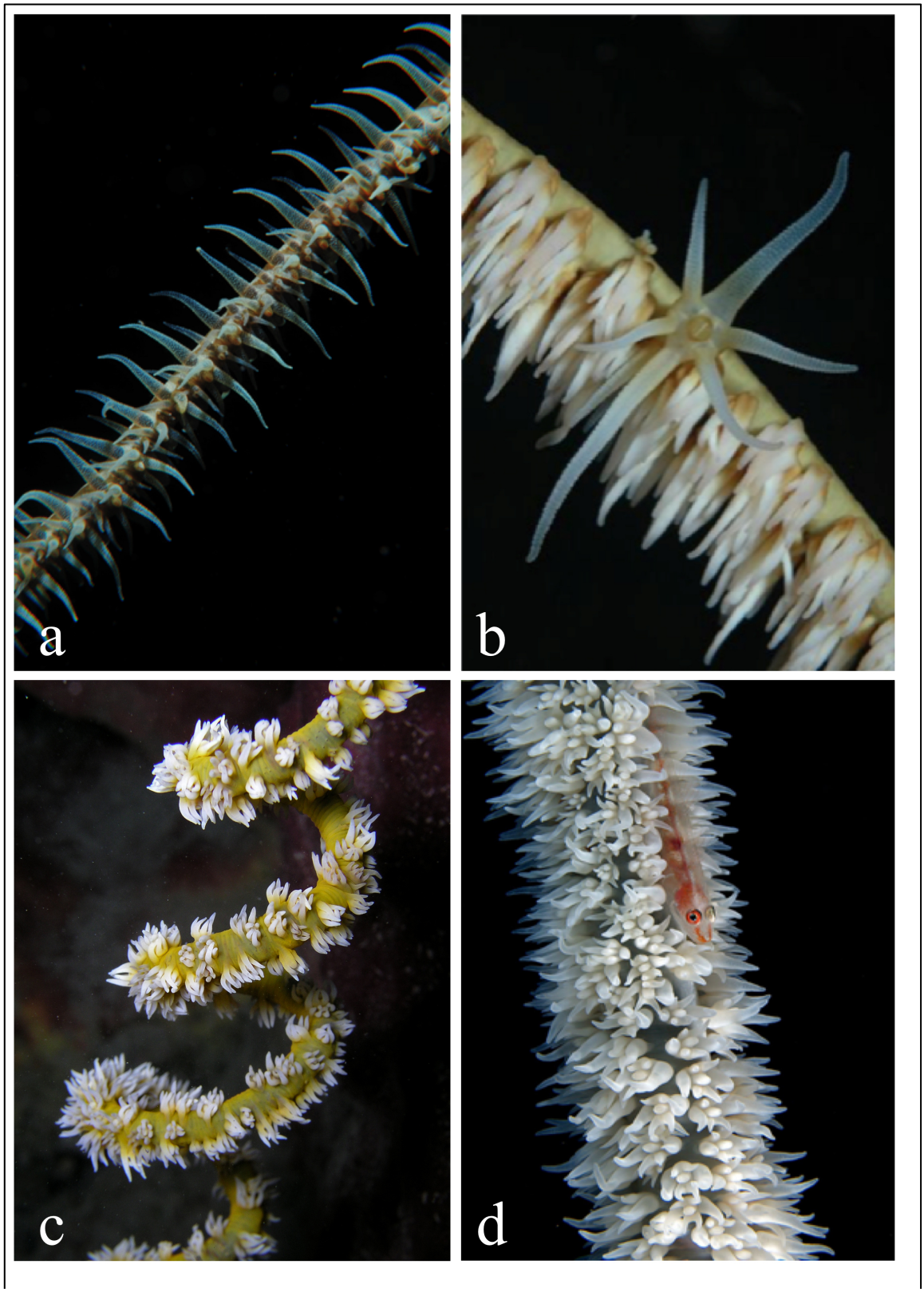

Figure 1.15. Variety of polyp distribution in antipatharian species: **a** – single row in one side of the branch in *Stichopathes sp*. (photo taken in Guadeloupian waters by Alain Goyeau); **b** - in several rows in one side of the branch of *Pseudocirrhipathes mapia* (Bo and Bavestrello, 2009), it is visible a polyp with the tentacles completely expanded in contrast with the other polyps (photo taken in Bunaken, Indonesia at 30 meters of depth, by M. Boyer); **c** and **d**– *Cirrhipathes* species have polyps all around the colony axis, the polyp density can vary (compare **c** and **d**), in **d** is visible the presence of a whip coral goby

(*Bryaninops yongei* - Davis and Cohen, 1969), this species live in association with wire corals (photos in c and d by Nick Hobgood taken in East-Timor coast).

Polyp internal anatomy

Antipatharians are diblastic eumetazoans, possessing endoderm and ectoderm and lacking mesoderm. **Mesoglea** is the intermediate layer situated between the ectoderm and the endoderm. It is generally thinner than in the majority of anthozoans. Usually the mesoglea lacks cells, but sometimes, astonishingly, as in the case of *Parantipathes larix*, it is partially cellularized (Pax 1987).

The internal anatomy of the antipatharian polyp is in a general way as the one of any other anthozoan polyp and can be appreciated in **figure 1.19**:

- The polyp possess a mouth that serves as the opening of the polyp to the surrounding environment;
- The mouth is situated in the edge of a pharynx that connects in the other end to the gastric cavity, this pharynx is ectodermic as in other anthozoans and is usually flattered in a right angle to the skeletal axis (details in chapter 2);
- The gastric cavity is surrounded by endoderm;
- The mouth is surrounded by the oral disk that is at the summit of the oral cone;
- The polyp is divided by mesenteries in radial non-communicating chambers, creating an internal organization and enlarging the area for changes with the medium;
- Chambers extend inside the tentacles, which are therefore hollow in the middle.
- Polyps possess 6 complete primary mesenteries, all of them complete, meaning that they go from the body all until the pharynx, and 0 to 6 secondary mesenteries
- Only two of the mesenteries bear the reproductive organs, the mesenteries that are aligned in the skeletal axis (see chapter 2).

- The skelet is secreted and surrounded by ectoderm;
- Gonads are present in both distal and proximal sides of the polyp;
- The formation of buds is visible as small protuberances in the colony[§].

However, the detailed morphology and cellular anatomy the antipatharian polyp will be discussed in **chapter 3** as detailed morphology was accessed by histology and immunohistochemistry in order to understand well their internal organization.

Elements of symmetry as the mesenteries and the associated retractor muscles, or the presence or absence of ciliated grooves in the pharynx will be exhaustively discussed in **chapter 2**.

[§] Details about bud formation can be found in chapter 2, it is important however to note that the formation of buds is directly dependent on adult polyps and that this fact is not depicted in the figure.

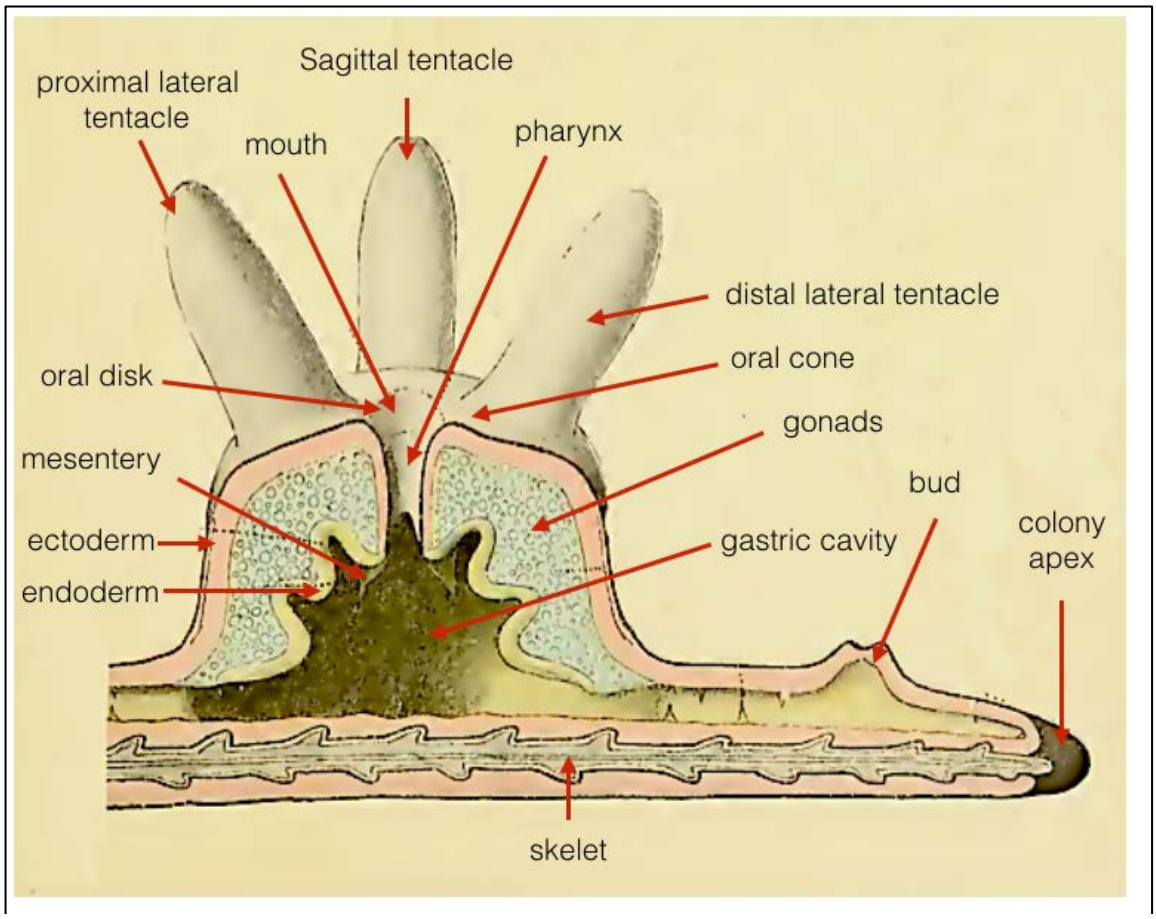

Figure 1.19. Morphology of the antipatharian polyp – schematic representation adapted from Delage and Hérouard (1901), terminology adapted from the French language.

The antipatharian skelet

The skeleton is secreted by axial epithelial tissues of the polyps in concentric layers around a central hollow core, the central canal (Daly *et al*. 2007) (**figure 1.14a**). The skelet is a result of the deposition of concentric layers (Pax 1918 after von Koch 1886), while in **figure 1.14b** we can see that the central canals from different branches are not connected (Pax 1918). Spines, like the spines of a rose, recover the antipatharian skelet. They are present in all antipatharian species and are as the spines of a rose probably important to the antipatharian defence from predators. There is a huge diversity of antipatharian spine forms. As an example there is, in a single species, *Stichopathes variabilis* (van Pesch, 1914), a variability of spine forms as can be observed in **figure 1.14c**. The spines can be arranged in several ways depending on the

species or in the age of the branch. However, spines are usually inclined towards the distal part of the colony, meaning that the free edge of the spine is pointing in the distal direction of the colony (Opresko 2001, 2002, 2003, 2004 and personal communication).

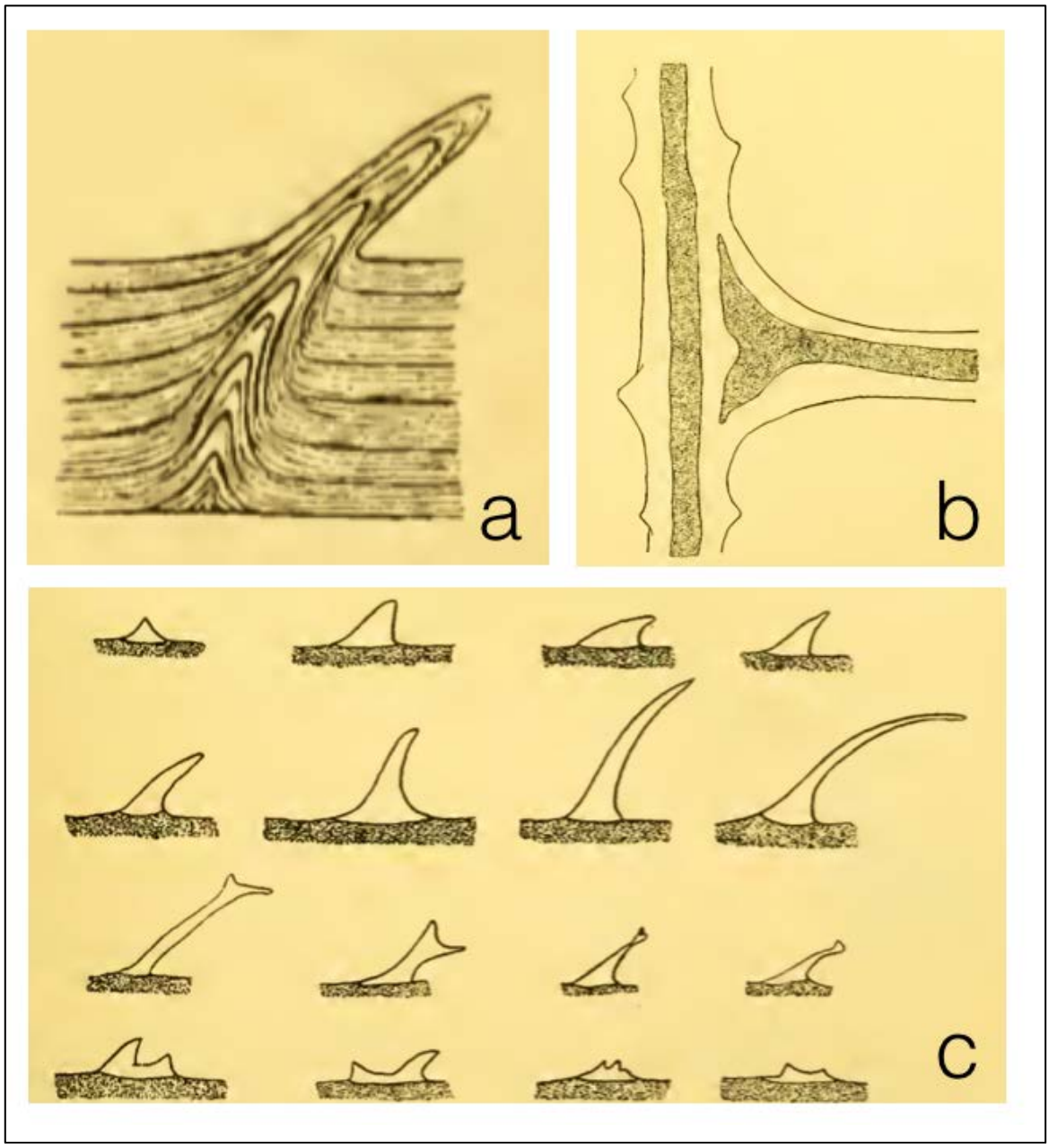

Figure 1.14. Skelet morphology in antipatharians: **a** – the skelet is a result of the disposition of concentric layers (Pax 1918, after von Koch1886) **b** – Skelet organization of an antipatharian specie by Pax (1918) showing that the skelet has an hollow core – the central canal – in its centre, but that the canals from different branches do not connect; **c** – antipatharian species possess a huge variety of spine morphologies. Here is the representation of the big variety found in *Stichopathes variabilis* by van Pesch (1914).

1.4 Symmetry in the tentacle disposition and in the internal morphology of Caribbean species of black corals

1.4.1 Tentacle apparatus organization

1.4.1.1 Bi-radial symmetry in the tentacle disposition of *Plumapathes pennacea*.

The tentacle disposition of the polyps of *Plumapathes pennacea* (**figure 1.20)** has a bi-radial symmetry, meaning that there are **two symmetry plans** for their disposition. This bi-radial symmetry comes mainly from two factors: the first being that the sagittal tentacles are inserted in a lateral position in relation to the colony, while the lateral tentacles are inserted in the polyp bearing side of the colony; the second is that the polyp is longitudinally elongated along the skelet axis (having their diameter longer in this skelet axis), making the polyp ovoid and not a perfect circle (this features are visible in **figure 1.20**). Amongst antipatharians this different level of insertion of the sagittal tentacles is usual as for most species they are inserted at a lower level than the lateral tentacles (van Pesch 1914).

1.4.1.2 Bilateral symmetry in the tentacle disposition of *Antipathes caribbeana* and *Stichopathes sp.*.

Antipathes caribbeana presents the proximal lateral pair of tentacles bigger than the distal pair of tentacles (**figure 1.20 a1 and a2)**. This different tentacle size makes that there is **a single plane of symmetry** for the tentacle disposition of *Antipathes caribbeana*, making its disposition bilaterally symmetrical (**figure 1.20 a2)**. It is important to note that this disposition of tentacles in relation to the polyp of *Antipathes caribbeana* is something common to all the branches of the colony and that all polyps of the same branch are oriented in this same way.

The polyps of *Stichopathes sp.* also present their proximal lateral pair of tentacles bigger than the distal lateral pair (**figure 1.20c1 and c2**) and have then also a single plane of symmetry for their tentacle disposition. These polyps present then also a disposition of tentacles around the oral cone of a polyp that is bilaterally symmetrical (**figure 1.20c2)**.

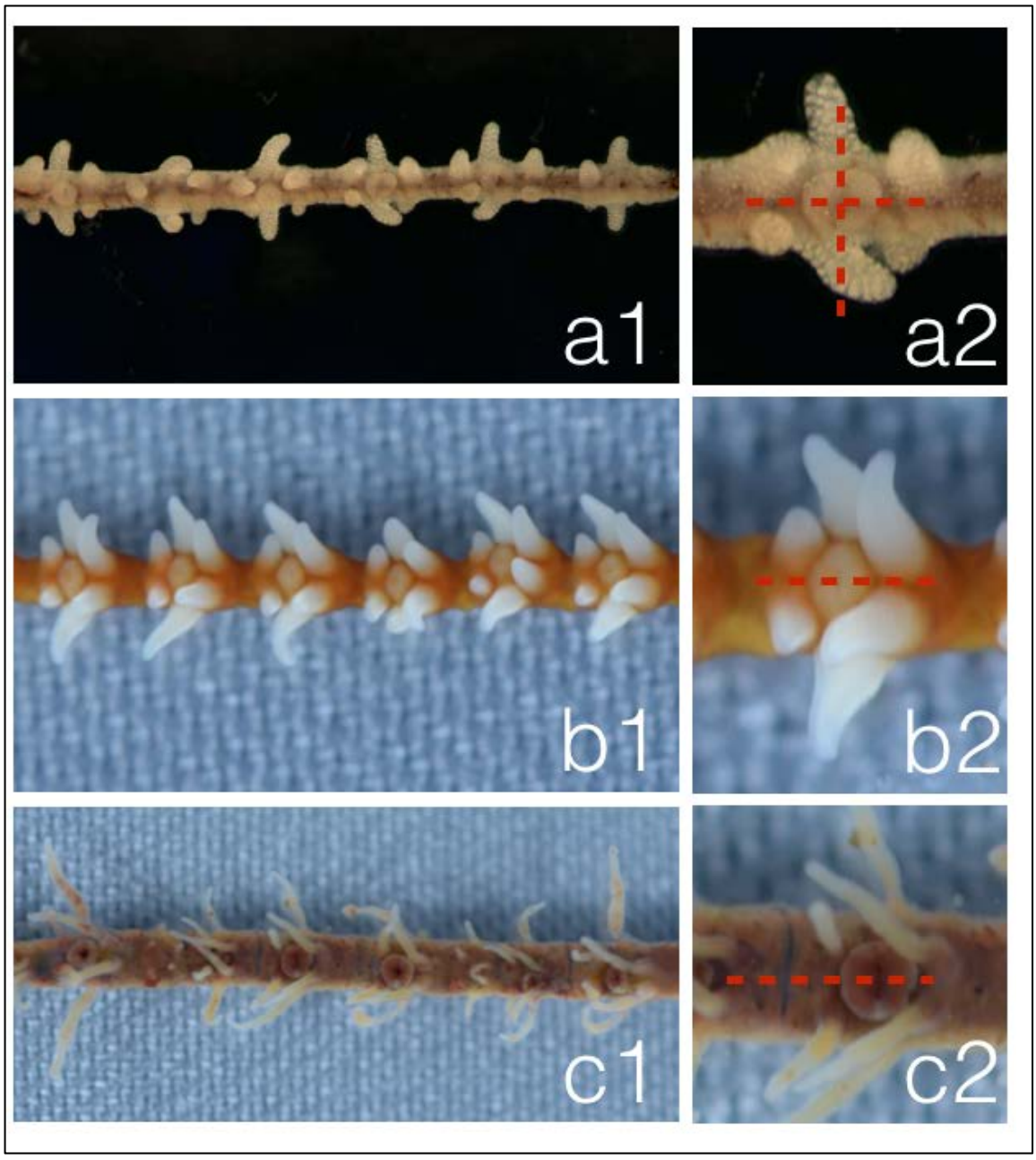

Figure 1.20. The antipatharian species *Plumapathes pennacea*, *Antipathes caribbeana* and *Stichopathes sp.* from Caribbean waters present different types of symmetry in their tentacle disposition: **a1** and **a2** – *Plumapathes pennacea* presents a bi-radial symmetry in their tentacle organization; **b1** and **b2** – *Antipathes caribbeana* present a single plane of symmetry for their tentacle organization because of the longer tentacle size of their proximal lateral pair in relation to the distal lateral pair; **c1** and **c2** – *Stichopathes sp.* also presents a single plane of symmetry for their tentacles as in this species the proximal lateral pair is also longer than the distal lateral pair. As in precedent figures the branches and polyps are oriented with their distal sides on the left and with their proximal sides on the right. Original photos from b1/b2 and c1/c2 by Michaël Manuel.

1.4.2 Symmetry linked to the internal organization

1.4.2.1 The antipatharian polyps have their internal symmetry perpendicular to the skeletal axis

The polyps of Antipatharia, however distributed they may be in the colony axis, present their main plane of **internal** symmetry perpendicular to the colony (or skeletal) axis as can be observed in **figure 1.21** (Schultze 1896). The flattered pharynx is represented in the figure in a central position with the mesenteries going from the pharynx to the body wall, disposed in a bilateral way (Pax 1918). The polyp is then in its internal anatomy bilaterally symmetric in relation to the antipatharian **directive axis**, the axis along which the pharynx is flattered. In the **figure 1.21** only the primary mesenteries are represented. Due to the elongated pharynx and the organization of the mesenteries, an isolated polyp presents a main plane of symmetry, which corresponds to two axes: the oral-aboral axis (not shown) and the directive axis.

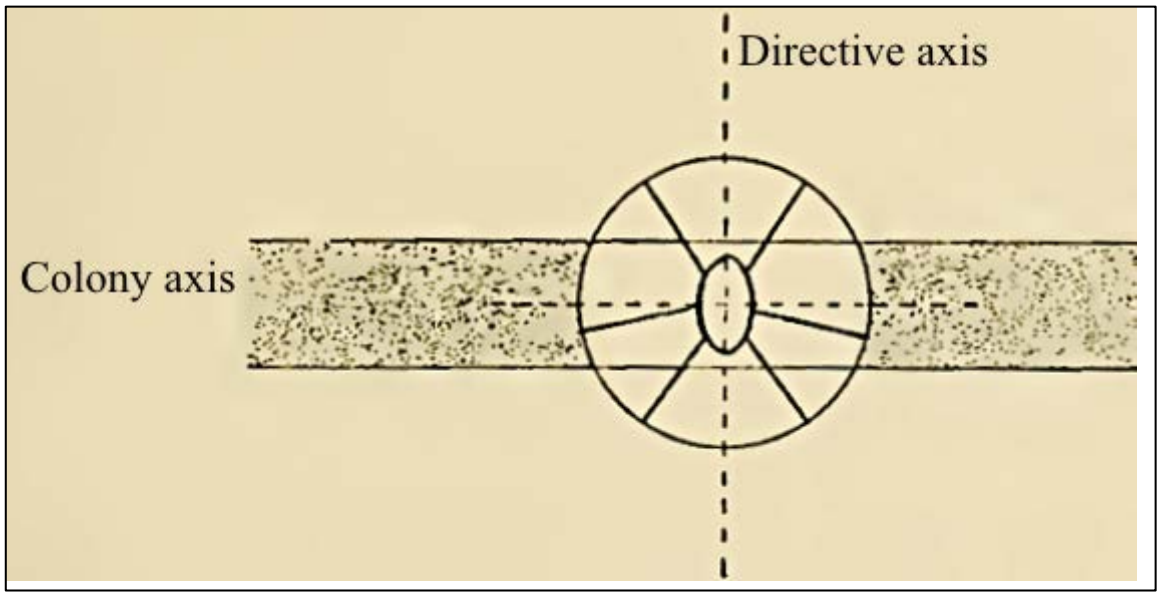

Figure 1.21. Representation of the antipatharian polyp disposition in relation to the colony axis from Pax (1918). In the figure by Pax (1918) it's visible that the antipatharian polyp is bilaterally organized in relation to the secondary axis that is perpendicular to the colony (or skeletal) axis: the flattered pharynx is represented in a central position of the polyp with mesenteries that go from the pharynx to the body wall and that are disposed in a bilateral way.

The disposition of the retractor muscles associated (see definition in chapter 2) to the mesenteries is also bilaterally symmetric in relation to the directive axis. Van Pesch (1914) described two modalities of retractor muscle disposition in the mesenteries of antipatharian species. These modalities can be observed in **figure 1.22** and are in both cases bilaterally symmetric in relation to the secondary axis (the axis along witch the pharynx is flattered.

A full review of the mesenteric arrangement and retractor muscle distribution is found in chapter 2. The point in this chapter is to understand that in terms of internal anatomy antipatharian polyps are bilaterally arranged along the directive axis, being the symmetry plane of the polyps along this directive axis.

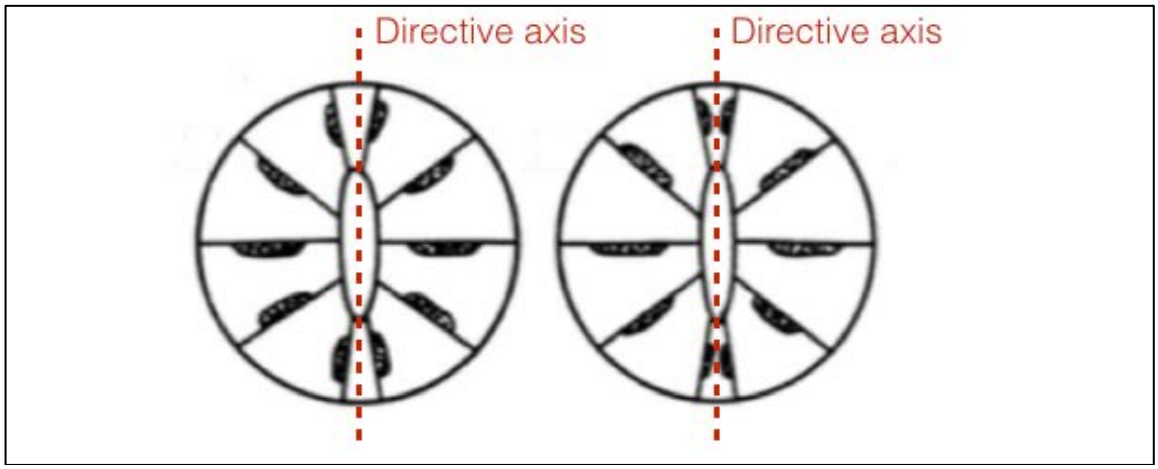

Figure 1.22. Schematic representation of the retractor muscles associated to antipatharian mesenteries by van Pesch (1914). Two types of distribution exist and both are symmetric in relation to the directive axis that corresponds to the axis along which the pharynx (in the central position) is flattered. **The directive and oral-aboral axis define a bilateral plane of symmetry**.

There is an asymmetry between the "*left*" (distal) and "*right*" (proximal) sides of each *Antipathes caribbeana* polyps

In *Plumapathes pennacea* the directive axis is a symmetry axis for both the internal anatomy as well as for the tentacle disposition. The polyp is then completely symmetric in relation to the directive axis (**figure 1.23a and 1.23b**).

However, in *Antipathes caribbeana* and *Stichopathes sp.* the only symmetry plane for the tentacle disposition runs along the skeletal axis (**as seen in figures 1.17b2 and 1.17c2**). The skeletal axis and the directive axis make a 90º angle, meaning that the difference in tentacle size in *Antipathes caribbeana* and *Stichopathes sp.* correspond to an asymmetry in relation to the symmetry plane that passes trough the directive axis of the polyps. For *Antipathes caribbeana* we can see this asymmetry by drawing (discontinuous red line) in each polyp the directive axis – **figure 1.23b1 and 1.23b2**. It is then visible that there is an asymmetry in tentacle size between the "*left*" side of the polyp – distal side in relation to the colony base – and the "*right*" side of the polyp – proximal side in relation to the colony base.

In conclusion, when we superpose the internal anatomy of *Antipathes caribbeana* (its symmetry along the main symmetry plane of antipatharian polyps) with its external anatomy we have that its tentacle organization is the result of a polyp asymmetry.

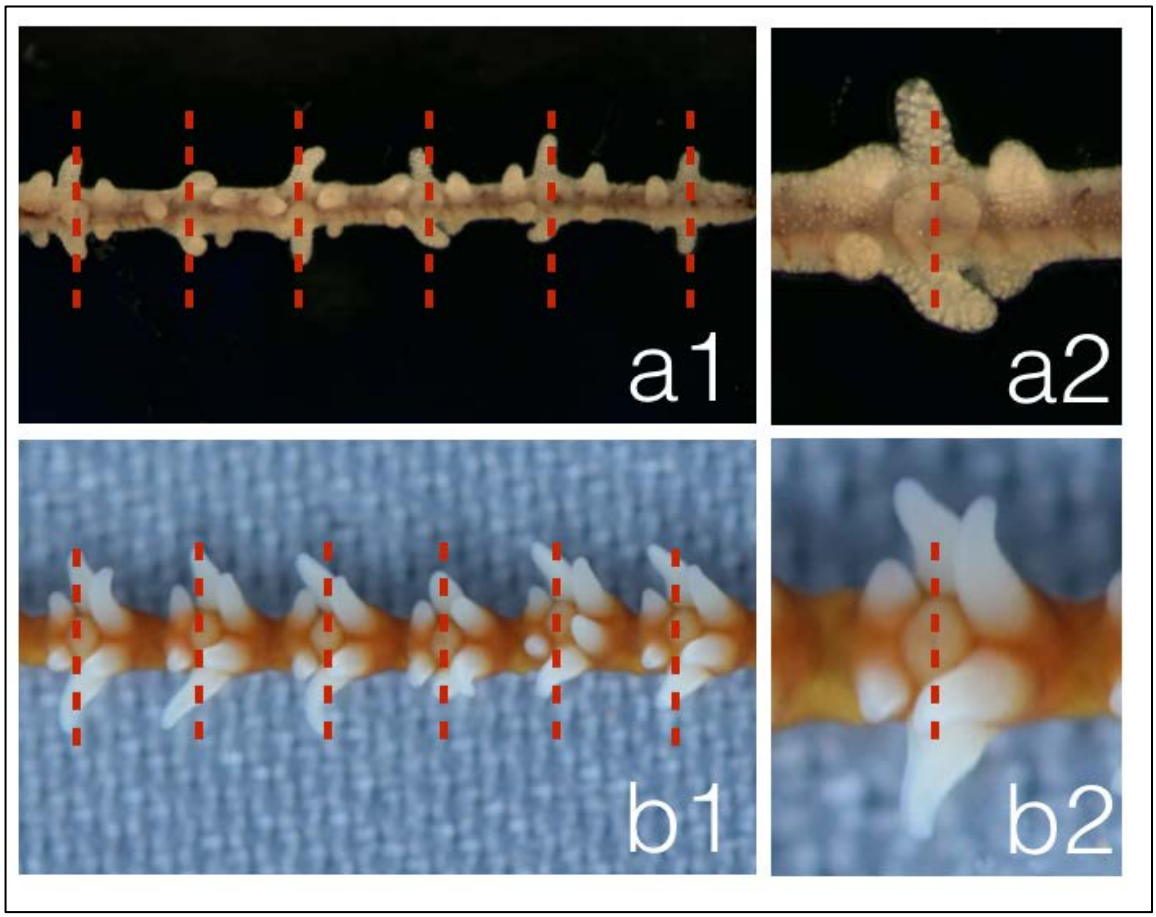

Figure 1.23. The antipatharian species *Plumapathes pennacea* and *Antipathes caribbeana* from Caribbean waters present different types of symmetry in their tentacle disposition: **a1** and **a2** – *Plumapathes pennacea* tentacles are symmetry in relation to their directive axis (red discontinuous line); **b1** and **b2** – *Antipathes caribbeana* tentacles are asymmetric in relation to their directive axis (red discontinuous line). As in precedent figures the branches and polyps are oriented with their distal sides on the left and with their proximal sides on the right. Original photos from b1/b2 by Michaël Manuel.

1.4.2.2 Tentacle asymmetries documented in the Antipatharia literature

Silberfeld has described an example of asymmetry between the size of the proximal lateral pair and size of the distal lateral pair of tentacles in his work from 1909 "*Japanische Antipatharien*". The author refers that the distal pair of tentacles of *Stichopathes filiformis* (Gray, 1868) is smaller that the proximal lateral pair. His representation of the polyps, where this difference is visible is found in **figure 1.24a**.

Van Pesch (1914) described the same asymmetry in *Stichopathes gracilis* (Gray, 1857), his representation is found in **figure 1.24b**. Van Pesch noted the polyp proximal lateral tentacles are "*bigger and more built than the distal pair*", having the proximal pair projected and covering the mouth.

Finally van Pesch has also found the same asymmetry in the polyps of *Antipathes dichotoma* (Pallas, 1776), he noted also that the polyps are distally inclined in this species: "*the polyps are inserted in a single row, there is great difference between the proximal and the distal pair of lateral tentacles, the proximal ones are inserted on a strikingly high level so that the entire polyp is exceedingly distally inclined, including the oral cone*". Pesch measured the tentacles, finding that the proximal pair has 0.3 mm against the 0.175 mm of the distal pair. The size difference between polyps and the distally inclined oral cone of this species can be appreciated in his representation (from Pesch 1914) in **figure 1.24c**.

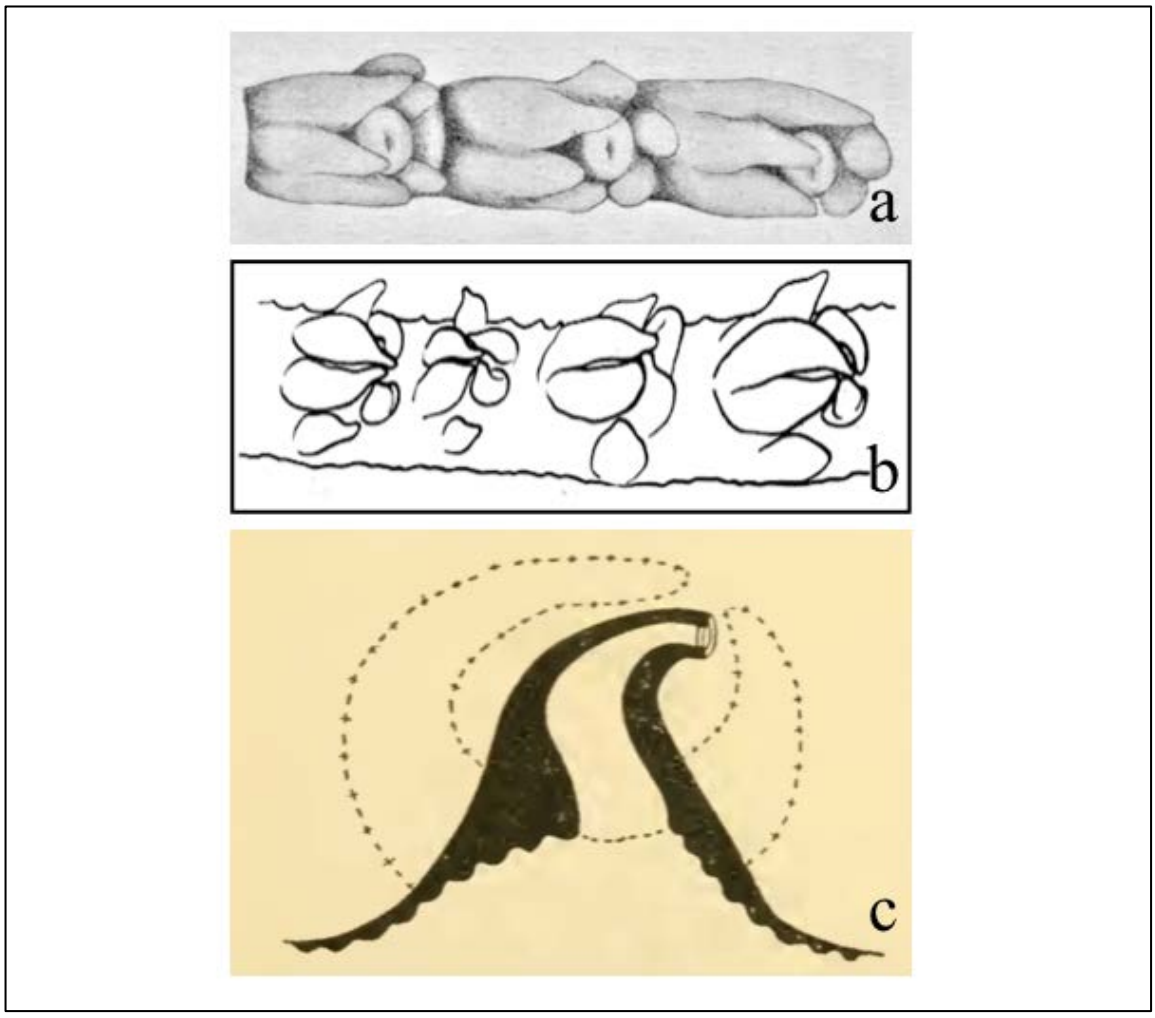

Figure 1.24. Some authors have documented asymmetries in size between the two lateral pairs of tentacles in antipatharian species: **a** – Silberfeld (1909) described that the proximal and distal pairs of lateral tentacles had different sizes in *Stichopathes filiformis* (Gray, 1868); **b** – van Pesch (1914) described the same difference in tentacle size for *Stichopathes gracilis* (Gray, 1857), with their proximal lateral pair of tentacles longer that the distal lateral pair, the four polyps of *Stichopathes gracilis* in the branch present the proximal lateral tentacles covering the mouth; **c** – van Pesch (1914) represented this same difference in a representation of one polyp of *Antipathes dichotoma* (Pallas, 1776), notorious is also the representation of the oral cone distally curved. As in precedent figures the branches and polyps are oriented with their proximal sides on the left and with their distal sides on the right.

1.5 Ecology and some aspects of antipatharian biology

Black corals are a characteristic component of seamount suspension-feeding fauna and they often host abundant associated fauna (Molodtsova and Budaeva 2007). In recent years some studies brought light in many aspects of their ecology.

Distribution

Antipatharians are marine organisms, which have never been found in brackish waters, some species however are adapted to low or medium salinities (Pax *et al*. 1987). They do not tolerate exposition to air and that is the reason why they are not found in infralittoral zones (Bo 2008).

The bathymetric spectrum of black corals is extremely wide, with a depth extension going from 2m (the tropical whip corals) to 8000 meters (the south-pacific species *Bathypathes patula*) (Pax *et al*. 1987). However this group is generally found from 100 to 1000 meters and is therefore considered part of the deep-sea coral group.

Antipatharians have as an order a wide range of demographic distribution. They are particularly abundant in the tropical and subtropical regions. However some species can be found in polar regions (as the Artic *Stauropathes arctica* or the Antarctic *Bathypathes bifida*) (Pax *et al*. 1987) as well as in temperate waters such as the Mediterranean Sea (Dantan 1921).

As for the biogeographical distribution of individual species very little is known, as studies on that matter are very scarce. Many species are only known from their type locality and have for that reason a very limited known range (Roberts and Hawkins 1999; Opresko 2001; Opresko 2006; Molodtsova 2005).

Feeding behaviour

Antipatharians are **suspension feeders**, having their colonies oriented in agreement with the water currents (Warner 1977). This is presumably also important for the polyp disposition inside the colony organization. Laboratory experiments by Grigg showed that the polyps were able to capture amphipods, copepods and chaetognaths (Grigg, 1965) and that the capture occurred through the use of both tentacles and cilia of ectodermic cells, moving the mucous film produced by the analysed species (Lewis 1978). M. Bo in her observations in antipatharians from Indonesian waters observed the production of such films as a defensive response but not as a predating mechanism (Bo 2008).

Golbert and Taylor (1989a and 1989b) indicated three trophic strategies in *Antipathella aperta*: predation, suspension feeding by mucous films (nets) created in part by the discharge of spirocyst microfibrils (see chapter 3 for definition), and filter feeding performed with the pharyngeal surface. It has been reported that several polyps may cooperate for the capture of bigger preys (Wagner 1981) with 2 or 3 polyps cooperating to trap large polychaetes. The study of the gut content of polyps of *Cirripathes sp*., demonstrated that large polyps do show a micro-predatory activity on small zooplankton.

1.6 Mission for collection of Black corals

In order to collect samples of black corals a collection mission of Black Corals was performed in the French Caribbean island of Guadeloupe. The mission took place between the 3rd and the 16th of April 2014. In addition to myself, Doctor Muriel Jager and Professor Éric Quéinnec also took place in the mission where they had an active role. It involved a before mission planning especially due to the need of bringing laboratory material and reagents from France to Guadeloupe in order to improvise a laboratory in Guadeloupe ("improvised laboratory" – **figure 1.25a**) at the University of the French West Indies and Guiana (UAG, Guadeloupe). We thank Olivier Gros for providing lab facilities. In a mission to a location like Guadeloupe everything needs to be rechecked, no single reagent or material may be missing has it can pose serious problems to a mission of this magnitude. A preliminary mission took place in 2013 for the sampling of cnidarian diversity (by Manuel, M., Simion, P. and Jager, M.).

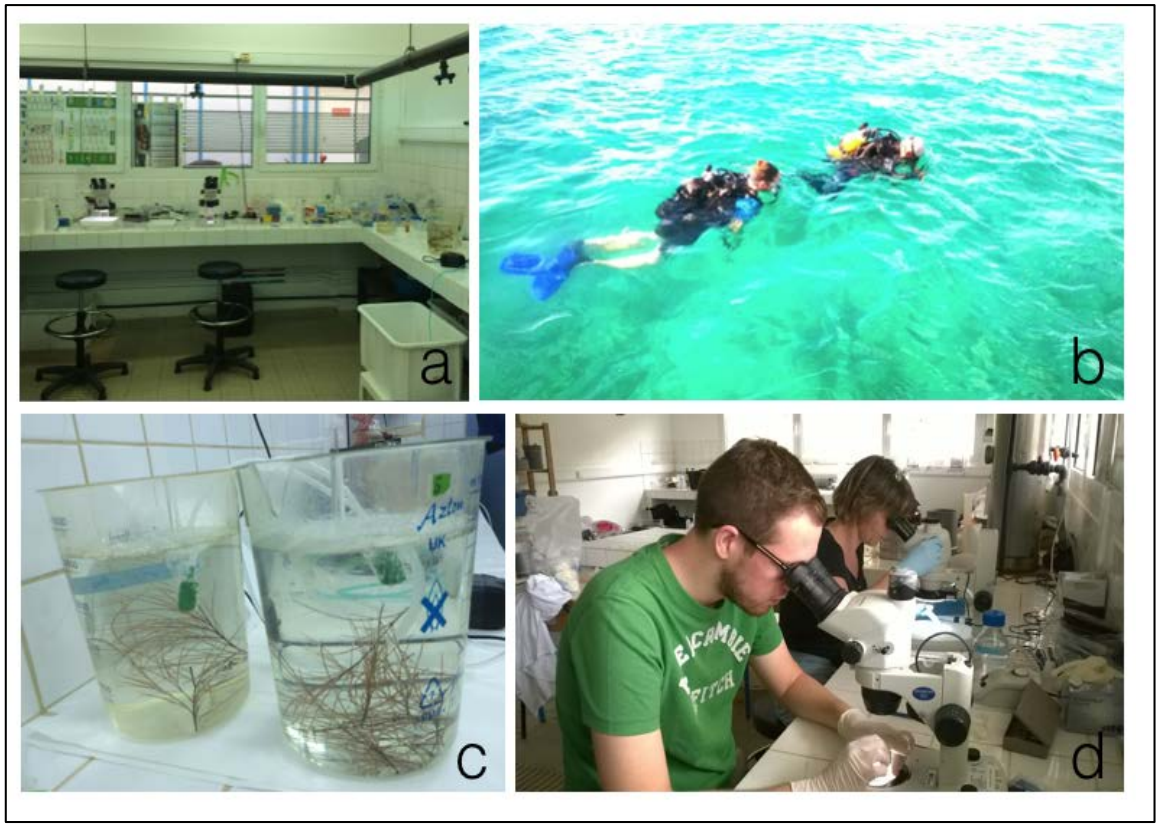

Figure 1.25. Guadeloupe mission for collection of antipatharian species: a – improvised laboratory; b – specimens collection took place by SCUBA diving; c – colony branches were kept in filtered oxygenated sea water before fixation; d – dissection of specimens.

1.6.1 Collection

Both *Antipathes caribbeana* and *Plumapathes pennacea* inhabit in the Caribbean region (white square in **figure 1.26a**). The collection of samples was performed off the coast of the Guadeloupe island (white square in **figure 1.26b**). It took place on the north of the "Grand-Terre", the main part of the island (white square in **figure 1.26c**).

More precisely the collection of the samples took place off the coast of the Port Louis village, in the rock arches that can be found in the "Pointe plate" coast zone (white square in figure **1.26d**) at 20 meters of depth. The precise coordinates of the sampling zone are 16º 27.220 N 61º 32.129 W.

Collection was performed by SCUBA diving (**figure 1.26b**). Diving took place in three different days of the mission, including the first one to explore the zone and discuss with local divers about the precise location of Black Coral colonies.

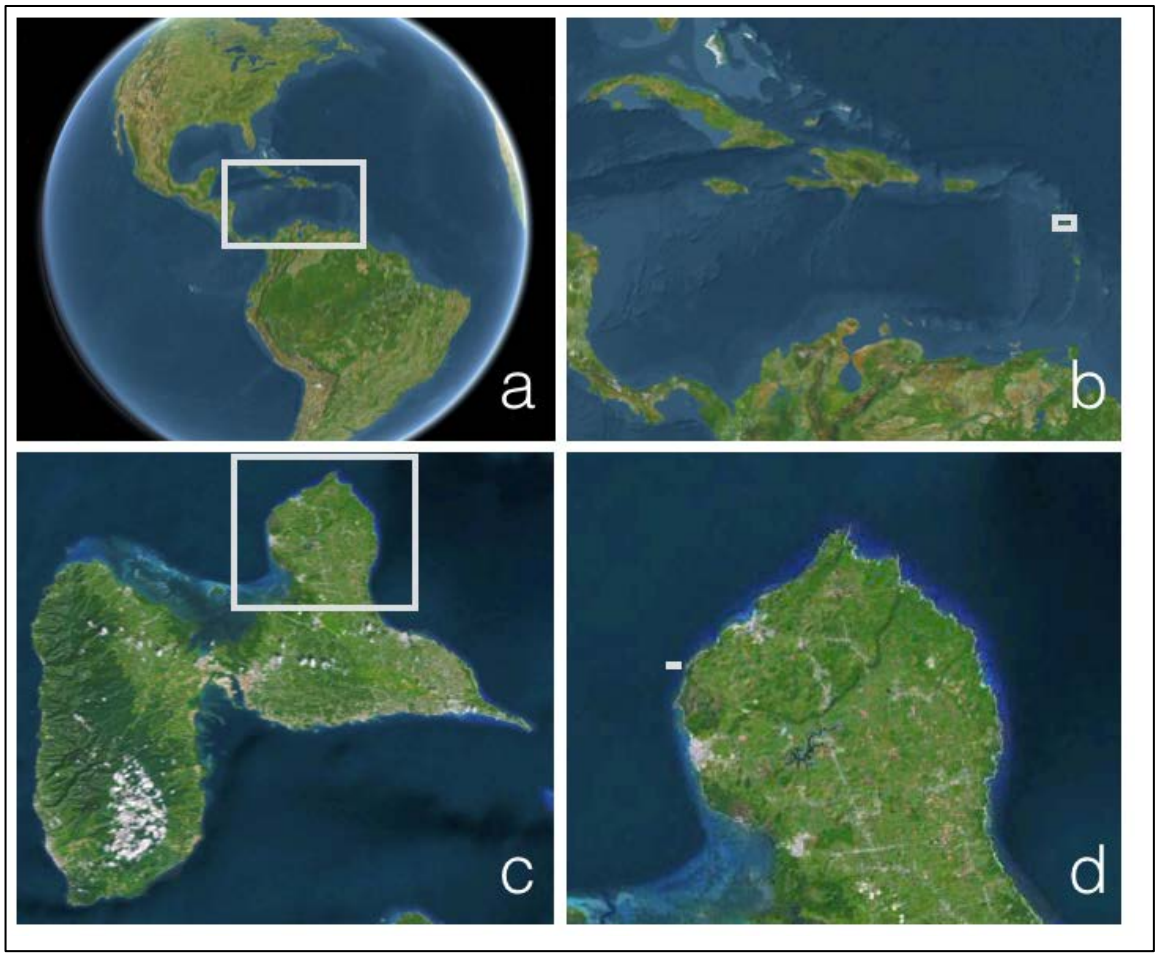

Figure 1.26. Collecting point of *Antipathes caribbeana* and *Plumapathes pennacea*: **a** – Both species inhabit in the Caribbean Sea; **b** – the collection of samples was performed off the coast of the island of Guadeloupe; **c** – collection took place on the north part of the "Grand-Terre" part of the island (right side of the island); **d** – precise location of collection, off the coast near the village of Port Louis.

Once collected the samples were maintained in filtered seawater in the most cool laboratory space we had available in Guadeloupe (no precise measures of temperature were possible, but the water was estimated to be around 22º C, which is close to the temperature of the sea water off the coast of Guadeloupe in April (at 20 meters of depth). The water was renewed regularly, at least each morning and afternoon and the containers were oxygenated by a mechanical air pump (**figure 1.15c**).

Ecological impact of the study

Antipathes caribbeana and *Plumapathes pennacea* present a big density of small polyps per centimetre of colony. Due to the polyp density and to their big colony size it is possible to collect hundreds of polyps by collecting only a small quantity of colony branches. It was then possible to conduct this study without compromising a single antipatharian colony.

The fact that we do not need to compromise a colony in order to conduct studies in these species is particularly important due to the extremely slow growth rate of most antipatharian species. In the case of *Plumapathes pennacea* its particularly important as the colony from which the samples were collected was the only colony that we observed in our diving location.

1.6.2 Fixation

The samples have been fixed with the main objective of performing *In Situ* Hybridizations (ISH) in both species but also the preparation of cDNA. As this has never been done with antipatharians different fixation methods were performed in order to maximize the chances of having a good fixation on which to perform the experiments.

For ISH the samples have been fixed by immersion in fixative with a volume of 20 times or more of volume in relation to the sample. A series of samples for ISH

have been fixed with a solution of 4% Paraformaldehyde another series with a solution of Formaldehyde 3.7%/Glutaraldehyde 0.2%. Both series were progressively dehydrated in ethanol and stocked in methanol once they arrived to Paris. A small part of the samples fixed with 4% Paraformaldehyde were no dehydrated in order to allow the performance of staining with phalloidin that can not be performed in dehydrated samples. The samples that were not dehydrated were kept in in PBS-tween (PBT) solution that was changed in Paris by a PSB azide solution.

The samples fixed in Paraformaldehyde were also prepared with the objective of allowing the performance of Immunohistochemistry.

For the preparation of cDNA, samples have been fixed in a commercial solution of RNAlater in order to preserve the sample RNA from which the cDNA can be synthetized.

The protocols of fixation, histology, IHC, ISH and phalloidin staining can be found in chapter 3.

Transcriptomes

The transcriptomes of both species were available in the lab previously to this mission. They were obtained in the scope of Paul Simion PhD thesis.

2 MESENTERIC FORMATION AND SYMMETRY IN ANTHOZOA

Animals present a diversity of "body plans" giving rise to marvellous forms, including an asymmetric organization for most Desmosponges, the bilaterality of vertebrates and the Ctenophores with their radial symmetries of different orders in the same organism. This marvellous diversity of eumetazoan forms allowed animal life to inhabit the most diverse environments and can be partially observed in **figure 2.1**, this diversity goes along with a famous quote from Charles Darwin in his famous book "On the Origin of Species by Means of Natural Selection, or the Preservation of Favoured Races in the Struggle for Life": "*Thus, from the war of nature, from famine and death, the most exalted object which we are capable of conceiving, namely, the production of the higher animals, directly follows. There is grandeur in this view of life, with its several powers, having been originally breathed into a few forms or into one; and that, whilst this planet has gone cycling on according to the fixed law of gravity, from so simple a beginning endless forms most beautiful and most wonderful have been, and are being, evolved*" (Darwin 1859 – page 490, 1st edition). This Darwin quote beautifully relates the diversification of body plans in animals from a single ancestor by means of natural selection, giving us in 1859 the pillar explanation for the diversity of the animal forms that surround us and how they come to be.

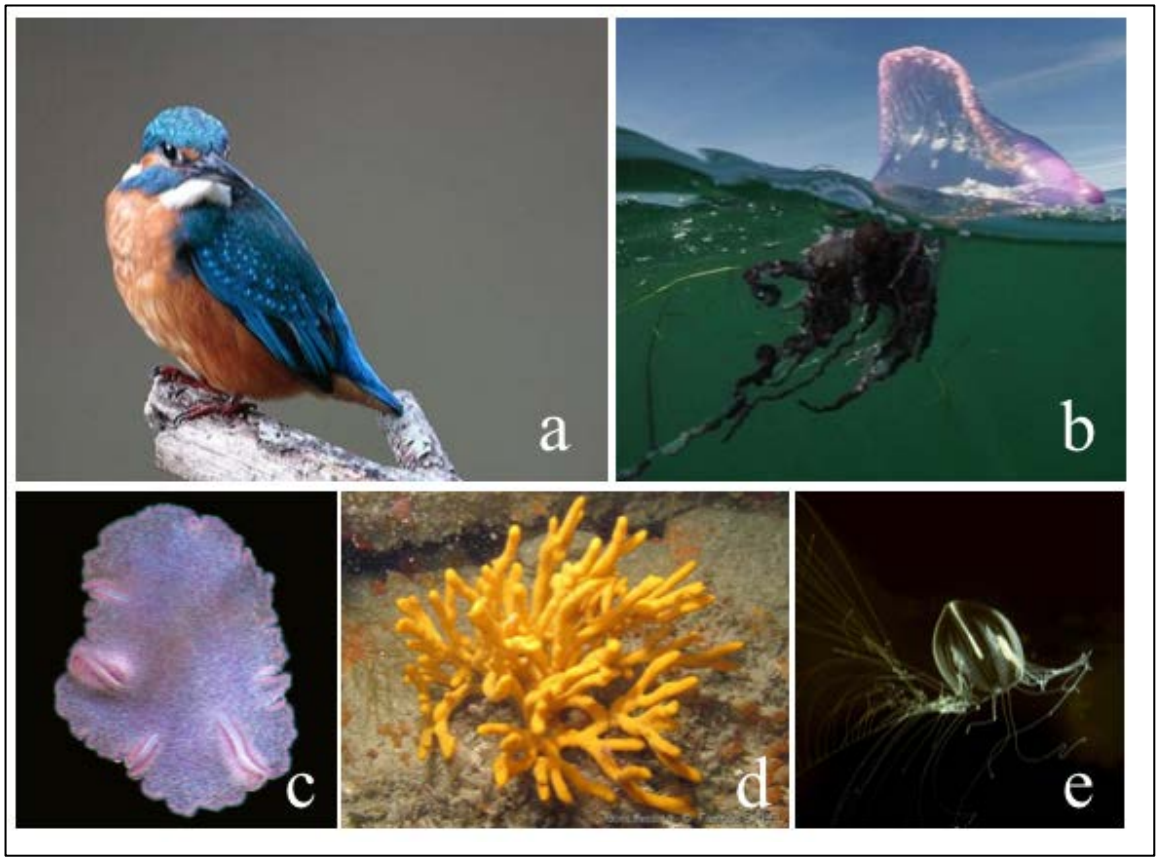

Figure 2.1. Partial representation of the diversity of body forms of Eumetazoa: **a** – Bilateria: *Alcedo atthis* (Linnaeus, 1758) the Common Kingfisher; **b** – Cnidaria: *Physalia physalis* (Linnaeus, 1758) the Portuguese man-of-war is a colonial hydrozoan; **c** – Placozoa: *Trichoplax adhaerens* (Schultze, 1883) is the only named species, although cryptic species may exist (Voigt *et al.* 2004; Pearse and Voigt 2007; Eitel *et al.* 2013); **d** – Porifera: *Homaxinella subdola* (Bowerbank, 1866) is a desmosponge with the characteristic asymmetric body plan of this group and a vibrant color; **e** - Ctenophora: *Pleurobrachia pileus* (Müller, 1776). Images **a**, **b** and **d** from DORIS (http://doris.ffessm.fr) by Maud Graillot-Denaix, Michel Barrabes and François Sichel respectively; image **c** by Ana Signorovitch and image **e** by Muriel Jager and Alexandre Alié.

The German word *Bauplan* (plural *Baupläne*) expresses the essence of animal architecture (Brusca and Brusca 2003) and can in English is usually used "*Bauplan*" that can be roughly translated as "body plan". As Brusca and Brusca (2003) put it: "*It captures in a single word the essence of structural range and architectural limits as well as functional aspects (...)*" and relates to the fact that for a given phyla we can relate it's diversity around a general "plane" of organization. This is due to the fact that even if we have this amazing diversity of forms there are "constrains", or better-putted "limits" in evolution because in the end there is a need of function, as organisms still need to breath, feed and reproduce. Richard Dawkins reflected this in his book the "River Out of Eden" as: "*But nobody, and I mean nobody, thinks that evolution has ever*

been jumpy enough to invent a whole new bauplan in one step." (Dawkins 1995). The extreme diversity of extant eumetazoans does not imply many different body plans, modern textbooks (*e.g.* Brusca and Brusca 2003) recognize around thirty phyla, but for example the clade Bilateria comprise the biggest number of phyla (Brusca and Brusca 2003) and present a relatively homogeneous type of bilateral symmetry and the differences between phyla of this clade can be compared because of their common body plan. We see then than symmetry and body organization are strongly correlated and it is thus important to define what is a symmetry plan of an organism: plane that divides the body in two completely equal body parts. In Bilateria, for example, the plane formed by two orthogonal axes, the antero-posterior axis and the dorso-ventral axis is the only that divide the body in two similar halves. Having defined this body symmetry or plane of organization, then asymmetries in relation to this plane can be studied (*e.g.* heart position in humans).

One fascinating group in terms of body organization and symmetries is Anthozoa, their polyps share several features with other Cnidarian polyps but have a different type of symmetry and of mesenteric formation (see this chapter section 2.1 for definition), and those two features are those that more deeply shape their internal anatomy and their development and in consequence imply a different body organization in relation to other cnidarian polyps.

Taxonomists for anthozoans have used body symmetry and the number of mesenteric cavities since Ehrenberg in 1834 used them to define the limits for Anthozoa, Plyactinia and Octactinia (Grebel'nyi 1981). Haeckel (1886) went in the same path for dividing Octocorallia, Hexacorallia and Tetracorallia (Grebel'nyi 1981). It was only later that Hertwig O. and Hertwig R., in 1879, suggested that for the classification of anthozoans the orientation of the organs (as mesenteries or muscles) and their sequence of development were more important than their number (Grebel'nyi 1981).

As the most common regard for the symmetry of cnidarians, the one that zoology students have in the beginning of their academic formation, many biologists naively have, also for anthozoans, the radial symmetry as a pillar characteristic for this group of animals. With the n-radial symmetries found in the medusa and polyp forms of Medusozoa, and in the external morphology of anthozoan polyps, one must be fascinated by finding a bilateral symmetry occurring in the internal organization of all

anthozoan classes (Van Pesch 1914; Chevalier 1987; Doumenc and Van Praët 1987; Tiffon 1987; Herberts 1987) and in some cases also in their external anatomy (Dawydoff 1928).

Just as Hertwig O. and Hertwig R. (1879) said, the orientation and development of internal organs, like mesenteries, is more important than their final number in the adult for the classification of anthozoans. The approach of comparing anthozoans based only on their adult mesenteric arrangement can induce major errors, especially when dealing with symmetry that is a character strongly correlated to development.

In this chapter, the description of the mesenteric formation of the different anthozoans groups will be presented with regards about the symmetry, this description will be useful for comparison among groups and also to understand how bilaterality came to be in this group. There is a special focus in antipatharian development and symmetry as this group has been for long time understudied and as a review of the literature can give insights that have not yet been properly discussed in literature. These insights on development and symmetry can now also be discussed taking into account the present knowledge about the phylogenetic relationship between anthozoan groups and the cnidarian position in the metazoan tree.

In this chapter it's presented a de novo synthesis of our knowledge about the anthozoan global polyp organization. Then an overview of comparative aspects is proposed.

2.1 Elements of symmetry in the adult anthozoan polyp

In order to understand the organization and development of the different anthozoan groups we firstly need to understand the main elements that compose their organization.

The anthozoan polyp **main body axis is the oral-aboral axis** that defines which part of the animal is open to the environment (feeding and respiration purposes), the mouth, and for fixing to the substrate, the foot (**figure 2.2a**).

The mouth is situated in the center of the oral disk. This oral disk presents tentacles that are radially arranged around the mouth. This oral disk seems radially arranged because of the tentacles position but looking carefully, many species present an elongated of ovoid mouth that divides the oral cone in two sides. This opening reflects the flattered pharynx of most anthozoans that correspond to a secondary axis of these animals. This pharynx of the anthozoan polyp is unique as other cnidarian polyps present a gastric cavity but not a pharynx with an ectodermal layer (Kaestner 1967, Brusca and Brusca 2003).

The anthozoan polyps are divided internally by 6 or more mesenteries (van Pesch 1914; Tixier-Durivault 1987; Brusca and Brusca 2003). The mesenteries (**figure 2.3)** are radial partitions of the gastrovascular cavity consisting of two layers of endoderm separated by a sheet of mesoglea, dividing the gastro-vascular cavity from the body wall in direction of the pharynx. On one side of the mesoglea lies a developed longitudinal layer of muscle. The position of this developed muscle, the **retractor muscle**, is consistent in all mesenteries of the same relative position and is crucial for the relative orientation of the polyp.

The polyps of Cubozoa and Hydrozoa don't possess mesenteries or pharynx and their gastric cavity is radially uniform (Kaestner 1967; Brusca and Brusca 2003). The Scyphozoa polyps present mesenteries, but always in the number of 4 and do not present a pharynx (Kaestner 1967; Brusca and Brusca 2003). Scyphozoa mesenteries simply subdivide the gastric cavity and thus their homology to the anthozoan mesenteries is not evident.

The anthozoan mesenteries possess mesoderm derived longitudinal muscles (retractor muscles) that are oriented in the oral-aboral axis and that are asymmetrically

disposed in relation to the mesoglea of each mesentery and are thus polarized in relation to each mesentery.

The disposition of the mesenteries and respective retractor muscles are the major element of symmetry of the anthozoan polyps, another important one is that in the pharynx of Anthozoans there may be present one or two ciliated grooves. These groves are called **siphonoglyphs**. These structures are especially ciliated and are responsible for directional circulation of water in the gastric cavity. The term "siphonoglyph" was invented by Hickson to denote the ventral groove of alcyonarians (Octocorallia) (Bourne 1919).

The adult octocorallians present a single symmetry plane and then a bilateral symmetry. The primary body axis (oral-aboral axis) is orthogonal to the secondary axis (directive axis). This can be observed in a cut done at the pharynx level transversely to the oral-aboral axis of the polyp as represented in **figure 2.2b** (cut level represented by the red dotted double arrow in **figure 2.2a**). The presence of a ciliated groove in one side of the pharynx, the siphonoglyph, and the disposition of retractor muscles in a single side of the mesenteries of this polyps only allow one plane of symmetry (**figure 2.2b**). Being that in this secondary axis both poles are different we can distinguish them as (**figure 2.2b**): pole bearing the siphonoglyph (red side of the double arrow) and for which the retractor muscles are directed (in same size as the siphonoglyph in relation to the mesoglea of the respective mesentery) and the pole without siphonoglyph (yellow side of the double arrow). Both sides receive names in the original legend by Tixier-Derivault (1987), the yellow side being called **asulcal** (without siphonoglyph) and the red side being called **sulcal** (with a siphonoglyph). This single plane of symmetry of the octocorallian polyp with two orthogonal axes leaves us with a true bilateral symmetry for the whole organism. The pharynx and associated endoderm (mesenteries) are the polarized.

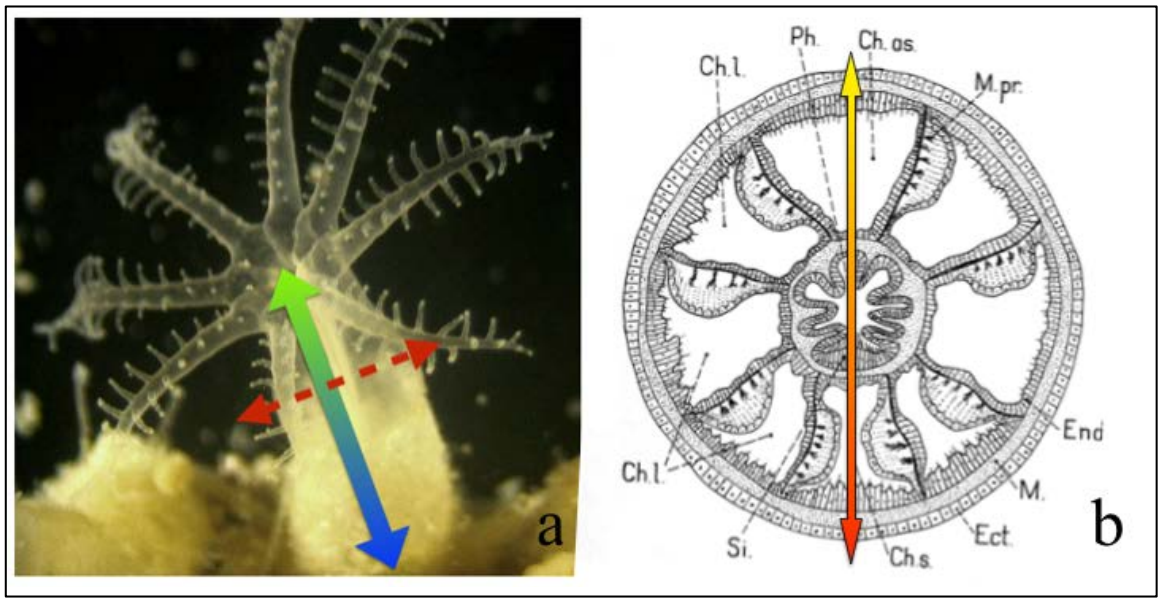

Figure 2.2. Representation of the anatomy of an octocorallian polyp: **a** – Polyp of the species *Cryptophyton goddardi*, an octocorallian. The full double arrow represent the main body axis of the polyp, namely the oral-aboral axis. In green the oral pole and in blue the aboral pole. The red double arrow represents the plane of the transversal cut that corresponds to figure b; **b** – schematic representation of a transversal cut to the oral-aboral axis of an octocorallian at the level of the pharynx: the disposition of the siphonoglyph in the pharynx, the repetition of the mesenteries, and the position of the retractor muscles associated to each mesentery imply a single plane of symmetry represented by the full double arrow passing by the middle of the siphonoglyph. Only this plane gives a mirror image of the polyp. Red end of the arrow represent the sulcal pole and yellow end the assulcal pole. Legend of the figure: Ch. as – assulcal chamber; Ch. l – lateral chamber; Ch. s. – sulcal chamber; end – endoderm; Ect: ectoderm; M – mesoglea; M. pr – retractor muscle; Ph – pharynx; Si – Siphonoglyph. Photo in **a** by Jeff Goddard (http://pl.reeflex.net/tiere/8059_Cryptophyton_goddardi.htm) and schematic representation on **b** by Andrée Tixier-Durivault (1987).

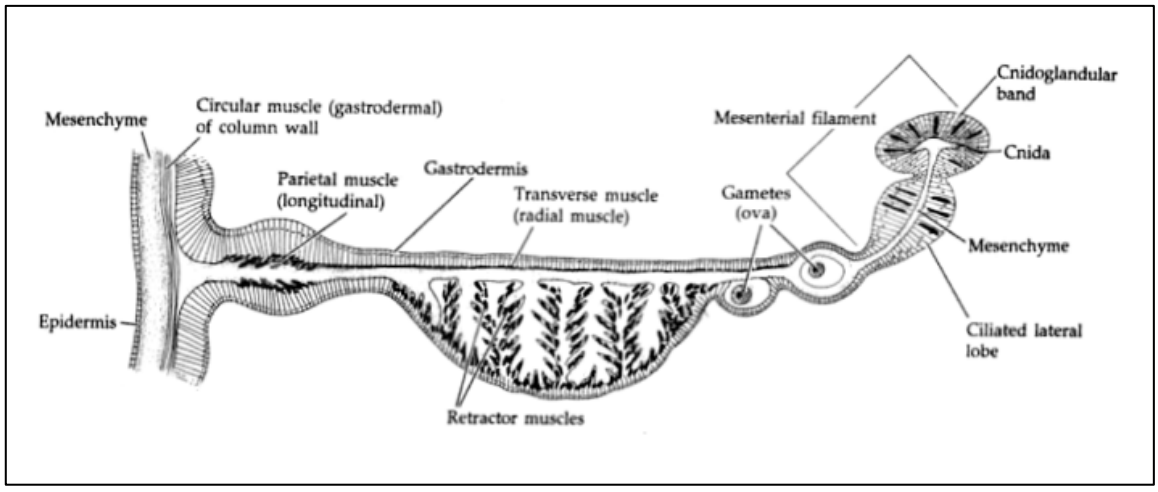

Figure 2.3. Schematic representation of one actiniarian mesentery. It is important to note the presence of muscle in both sides of the mesentery but that only in one side the muscle are enlarged and organized as a retractor muscle. Illustration by Nancy Haver, in the zoology textbook "Invertebrates" 2nd edition 2003 by Brusca & Brusca.

Most cnidarians present a true radial symmetry as is the case for the hydrozoan medusa (**figure 2.4a**). The number of planes of symmetry is constrained by the radial disposition, repetition and number of organs (such as tentacles, statocysts and gonads), so that in fact most cnidarians have a limited (usually pair) number of planes of symmetry (**radii**) in relation to which the structure of the body is truly symmetrical (**n-radial**), in the case represented on the **figure 2.4b** there is a **quadriradial symmetry**.

Adult anthozoans are, as cnidarians, usually regarded as primarily radially symmetrical, having their body parts symmetrically arranged around a central, longitudinal axis, the oral-aboral axis described. As an example of a radial symmetry of order two (bi-radial) we have some actiniarian (Anthozoa) adult polyps as represented in **figure 2.4b**: existence of two symmetry axis due to the elongated pharynx with two siphonoglyphs at opposite sides and to the radial disposition of the mesenteries and retractor muscles.

Cnidarians have then two different types of symmetry: **the n-radial symmetry (bi/tetra/hexa/octo-radiallity, etc)** (**figure 2.4**) and the **bilateral symmetry** (**figure 2.2**).

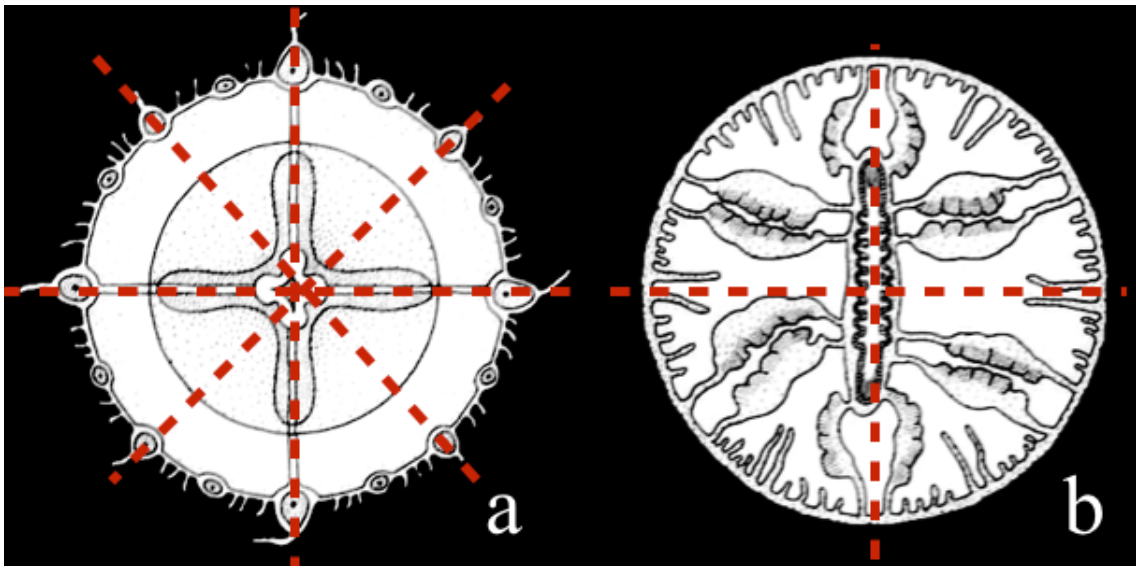

Figure 2.4. Schematic representation of radial symmetries of different order in cnidarians: **a** – hydrozoan medusa with four planes of symmetry, **b** – actiniarian polyp with two planes of symmetry generated by two opposite siphonoglyphs. Original illustrations are by Nancy Haver, in the zoology textbook "Invertebrates" 2nd edition (2003) by Brusca & Brusca.

As seen, the secondary axis of anthozoans with bilateral symmetry has two poles. The terminology for these two poles has been referred as **Dorsal** and **Ventral** by most authors (Hertwig 1882; Duerden 1905; Pax 1918; Hyman 1940; Tuzet 1961; Tiffon 1987; Chevalier 1987; Doumenc and Praët 1987; Ditlev and Ditlev 2003; Kise and Reimer 2016). Some others preferred a distinction in **anterior** and **posterior poles** (Brook 1889; van Beneden 1897). Finally the poles can be named as **sulcus side** and **sulculus side**, referring to the presence and position of different siphonoglyphs of Actiniaria (Haddon and Shackleton 1889; Bourne 1919; Maggenti *et al*. 2008) in each extremity of the flattered pharynx or even as **sulcus** side and **asulcus** side for the presence or absence of a siphonoglyph (Hyman 1940; Tixier-Durivault 1987) as in **figure 2.3b**. All these nomenclatures are problematic for different reasons. The **sulcus/sulculus nomenclature** is problematic because it implies a presence of two siphonoglyphs and some classes have only one or none. It is also problematic because it is based in a single class of anthozoans (Actiniaria) that sometimes present two slightly differentiated or undifferentiated siphonoglyphs. It is also problematic by the fact that homology between siphonoglyphs of different classes has not been always well established (see this chapter section 2.2.2 - Ceriantharia). The **sulcus/assulcus terminology** falls by the same reasons as the sulcus/sulculus terminology and so both these terminologies are hardly applicable for anthozoans. The **anterior/posterior nomenclature** should not be used as it suggests a homology between the secondary axis of anthozoans and the primary body axis of Bilateria (antero/posterior axis). Finally the **dorsal/ventral nomenclature** is also problematic as it implies a homology between the secondary axis of Bilateria and the secondary axis of Anthozoa and then a conserved polarity. However as no other nomenclature has been proposed that fits our need for a distinction between both poles that can be discussed in terms of several morphological features and not only on the presence or type of siphonoglyphs, from now on this chapter I'll make use of **the Dorsal/Ventral terminology that has also been the dominant one in textbooks**.

The terminology for dorsal and ventral comes from Hertwig R. in his "*Report on the Actiniaria Dredged by H.M.S. Challenger*" in 1882. In his text he describes that in the Zoanthidae family of Zoantharia the couples of mesenteries, with the exception of the **directives mesenteries** (mesenteries in both ends of the flattered pharynx, pair III and IV in figure 2.5), consist of a macro and a micro-mesentery and so that a dorsal and

a ventral zone of mesenteries must be distinguished. (Hertwig R. 1882). A **macro-mesentery** being a mesentery that reaches the pharynx and a **micro-mesentery** one that does not. A couple of mesenteries correspond to mesenteries that are disposed together without any other mesentery between them and bearing the retractor muscles pointing towards each other (**Figure 2.5:** in red the mesenteric couple consisting of a macro and a micro-mesentery). Historically it is important to note that, quickly, this terminology was used in Actiniaria as both groups present a flattered pharynx along the secondary axis with mesenteries developing in a bilateral way (in pairs – figure 2.5) in both sides of the pharynx. The first mesenteries to form on each couple correspond in Actiniaria to the first ones in Zoantharia (macro-mesenteries). Then, the Dorsal side corresponds to the side of the first appearing mesenteries of the lateral couples (for the couples that appear until the 12 mesenteries stage in both Actiniaria and Zoantharia) that can be macro-mesenteries (in Zoantharia). The ventral side corresponds to the side of the latter developing mesenteries of the lateral mesenteric couples (for the couples that appear until the 12 mesenteries stage in both Actiniaria and Zoantharia), that can be micro-mesenteries (in Zoantharia) and to the side where the siphonoglyph is always present in both groups.

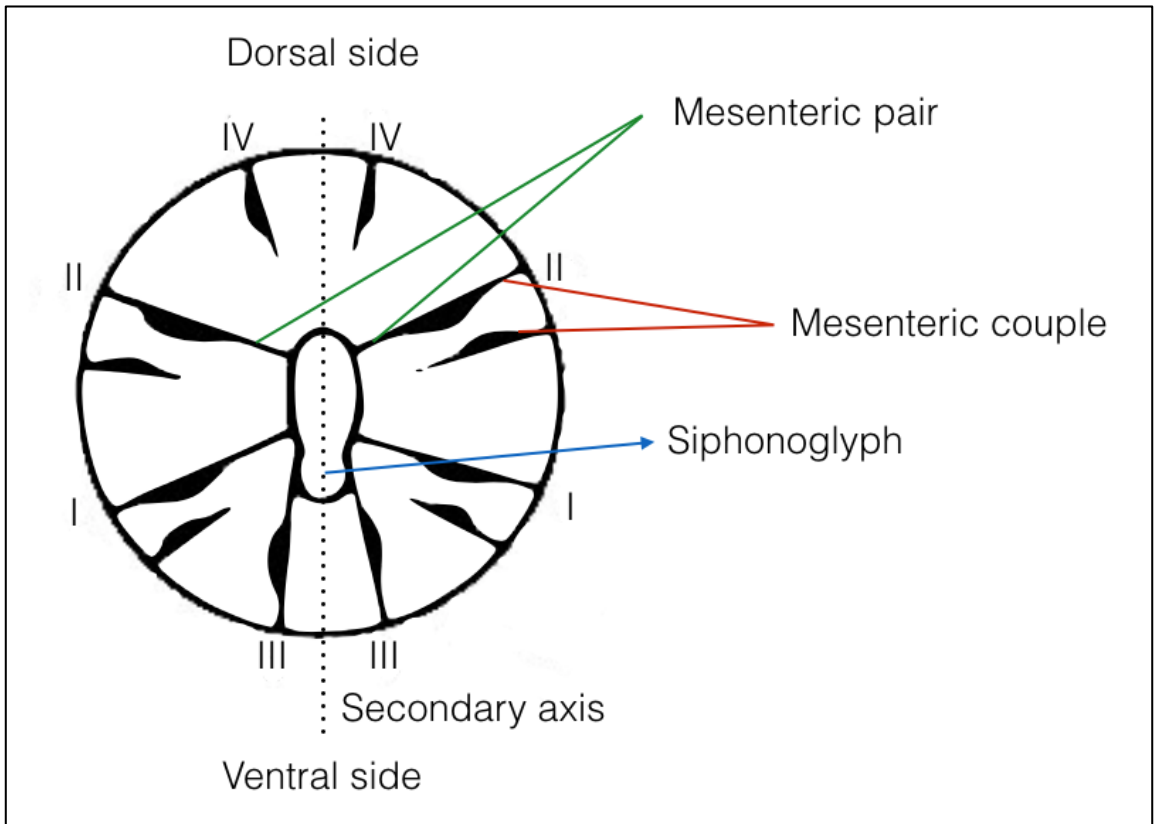

Figure 2.5. Schematic representation of a cut at the level of the pharynx in a zoanthid. The mesenteries develop in a bilateral fashion, with the disposition of pairs of mesenteries (green lines) along the secondary axis (doted line), mesenteries with the same roman number correspond to the same pair. The order of the numbers corresponds to the chronology of mesenteric development (of mesenteric pairs); a mesenteric couple corresponds to adjacent mesenteries without any mesentery between them and that present the retractor muscles in the space formed between the couple (red lines). The couples are formed by macro- (reaching the pharynx) and micro-mesenteries (do not reach the pharynx). Siphonoglyph (blue arrow) is present in the side of the micro-mesenteries. This arrangement of organs gave rise to the dorso-ventral terminology by Hertwig 1882. Original figure from: Chisholm, H**.** (1911) from *The Encyclopedia Britannica*.

Many terminologies have been used to differentiate the mesenteries in Anthozoa. Mesenteries can be called directive, pair, couple, primary, secondary, dorsal, ventral and other redundant terminologies that have been used in literature. Description of the terminology for mesenteries and other symmetry elements that is used in this chapter and currently by literature follows:

Directive mesenteries: Mesenteric pair that alone forms an endocoel, meaning that no mesenteries form between this mesenteries. They are always disposed in one or

both the poles of the secondary axis and can be distinguish as dorsal and ventral directive mesenteries in agreement with their position along the secondary axis;

Couple of mesenteries: Mesenteries (non directives) that are disposed together without any other mesentery between them and bearing the retractor muscles pointing towards each other;

Pair of mesenteries[**] **:** Until the 12 mesenteries phase of development, mesenteries form by pairs, with one forming on the left side of the secondary axis (pharynx) and one on the right side;

Primary mesenteries: considered to be those that form until the 12 mesenteries stage of Actiniaria, those that are bilaterally disposed during development.

Secondary mesenteries[††]**:** Considered as the mesenteries formed by pairs after the 8 mesenteries stage of Actiniaria;

Complete mesenteries / Macro-mesenteries: Mesentery that reaches the internal wall of the pharynx with which it fuses;

Incomplete mesenteries / Micro-mesenteries: Mesentery that do not reach the internal wall of the pharynx;

Retractor muscle: Muscular longitudinal fibber, formed by gastroderm cells, that run in the oral-aboral axis the interior of each mesentery.

Endocoel: Radial chamber between the two complete mesenteries of a mesenteric couple;

Exocoel: Radial chamber between two complete neighbour mesenteries that do not form a pair;

[**] According to French terminology: **Couple of mesenteries** in English literature refer to **pair of mesenteries** in French literature and Pair of mesenteries in English literature refers to couple of mesenteries in French literature.

[††] Secondary mesenteries can be seen in literature to refer to the mesenteries that form as couples after the 12 mesenteries stage.

Siphonoglyph[‡‡]: Ciliated groove in the pharynx with the function of introducing water in the gastrovascular cavity;

Sulcus: Ventral siphonoglyph (the siphonoglyph that is always present in Actiniaria);

Sulculus: Dorsal siphonoglyph (the secondary siphonoglyph in Actiniaria);

Sulcal: Sulcus side – with a siphonoglyph (Ventral side);

Asulcal: Side without a siphonoglyph in Octocorallia (Dorsal side) and in Actiniarians with only one siphonoglyph (Dorsal side);

Mesenteries can also be named by their relative side: as ventral (VD) or dorsal directives (DD), and as ventral lateral (VL) or dorsal lateral (DL) mesenteries.

Note that these terminologies has been mostly developed for the Actiniaria order and their usage can be controversial in some groups that present different types of mesenteric development.

A good example to show the limitations of the current terminology is the Octororallia group. Octocorallians have no sulculus, no secondary mesenteries, no mesenteric couples and so no endocoel. Nevertheless some other terminologies are easily applied to octocoralians and the homology between the ventral and dorsal sides of Octocorallia and Actiniaria groups has not been of dispute due to the disposition of the retractor muscles in the lateral mesenteries and the presence of a siphonoglyph (for details see 2.2.3 – Octocorallia, later in this chapter).

[‡‡] The term was invented by Hickson to denote the ventral groove of Alcyonarians (Octocorallia) (Bourne 1919).

2.2 Symmetry and mesenteric development across anthozoan classes

The first description of anthozoans goes as way back as Aristotle (384-322 b.C.). In his texts the author described two octocorals: Pneumōn – posteirously *Alcyonium palmatum* (Pallas, 1766) and Holothourion – posteriorly *Veretillum cynomorium* (Pallas, 1766) and two sea anemones (Actiniaria) (Acalēphē sklērē – posteriorly *Actinia equina* (Linnaeus, 1758) and Acalēphē edōdimos – posteriorly *Anemonia viridis* (Forskål, 1775) (Voultsiadou and Vafidis 2007).

Two **subclasses** of extant anthozoans are usually considered:

- **Octocorallia** with and eight-fold symmetry, which includes the orders: Alcyonacea (soft corals and gorgonians), Helioporacea (blue corals) and Pennatulacea (sea pens), **this orders have an eight-fold symmetry**;
- **Hexacorallia with a six-fold symmetry**, which includes the orders: Actiniaria (sea anemones), Antipatharia (black corals) Scleractinia (scleractinian corals), Corallimorpharia (corallimorpharians) and Ceriantharia (tube anemones) that due to their disputed position in the Anthozoan tree will be treated independently from the other classical hexacorallians.

In this part will follow a detailed description of the mesenteric development in Octocorallia and for each Hexacorallia classes. This focus on the mesenteric development comes from the fact that all anthozoans possess mesenteries, that the mesenteric development has strong consequences in their symmetry in their adult stage and that they can be compared between groups. Other body parts such as siphonoglyphs and tentacles also have an influence on the body symmetry but by their small number or absence the siphonoglyphs furnish less information to be compared between groups. Tentacles by their different arrangement between groups and sometimes due to their high number are not easy to compare or are not informative. However information on the siphonoglyphs and tentacles will be presented when relevant.

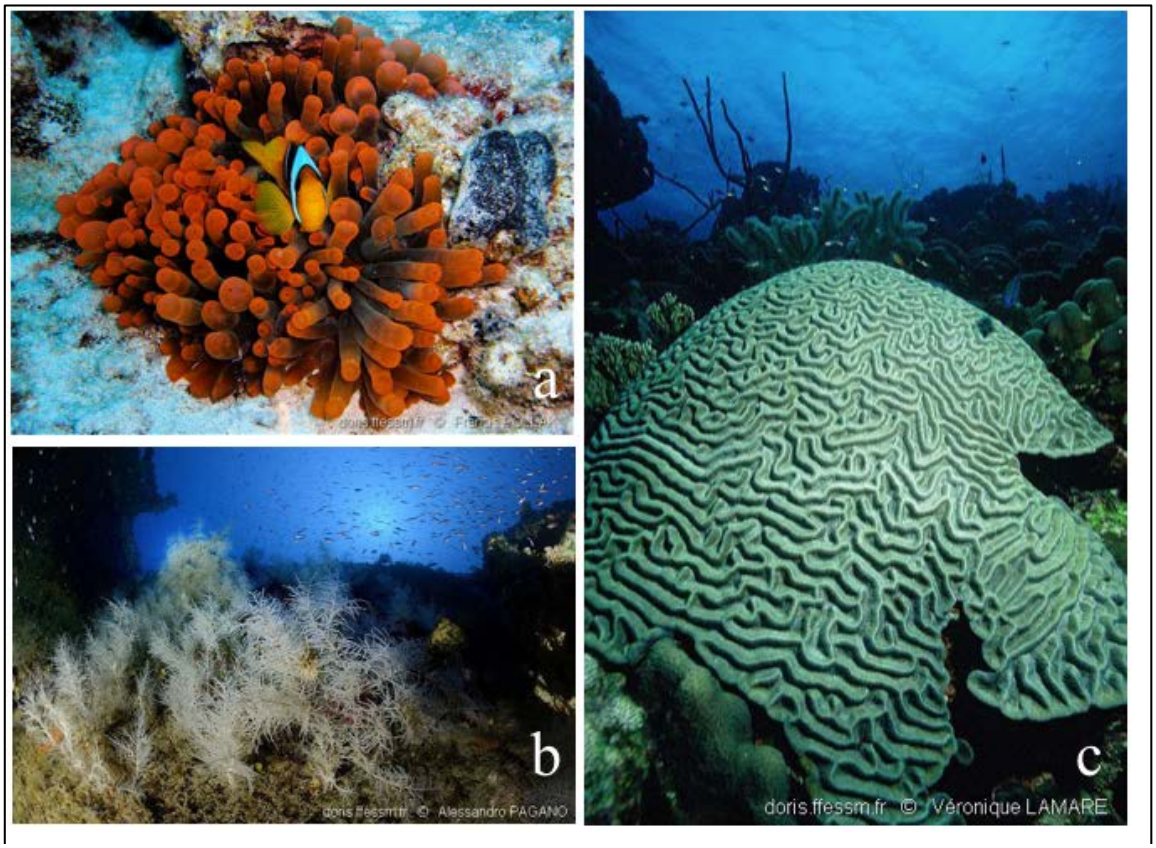

Figure 2.6. Anthozoans play a major role in ecosystems (*e.g.* the formation of coral reefs (Furla *et al.* 2005) and present an enormous diversity of forms, solitary or colonial: **a** - *Entacmaea quadricolor* (Leuckart, in Rüppell & Leuckart, 1828) is a solitary anemone that can reach 40 centimeters and has 13 species of clown fish associated, like the specimen of *Amphiprion bicinctus* (Rüppell, 1830) present on the picture; **b** – *Antipathella subpinnata* (Ellis & Solander, 1786) is a black coral that can reach 2 meters as a colony formed by polyps smaller than 1 millimeter; **c** – *Colpophyllia natans* (Houttuyn, 1772) is a Scleractinian from the Caribbean coral reefs. Images from DORIS (http://doris.ffessm.fr) by Francis Pollak, Alessandro Pageno and Véronique Lamare respectively.

2.2.1 Hexacorallia

Hexacorallians comprise more than 3.100 species (Appeltans *et al.* 2012) and are typically regarded, as their name anticipates, as 6n-radial organisms, meaning that they would have their body organs arranged in multiples of 6. In this chapter, we'll see that this is not always the case. Due to the presence of both bilateral and radial symmetries in their internal organization and to the different orders of mesenteric development, each order will be discussed separately, allowing to understand the prevalence of each symmetry in this sub-class, in each order and each type of mesenteric formation.

2.2.1.1 Actiniaria

Actiniarians (**sea anemones**) have been one the most studied orders of anthozoans in different fields, such as ecology and physiology. They also have been the most studied in terms of development. One reason for this is that they are the second order with most described species in **hexacorallians** with around 1100 described species (Appeltans *et al.* 2012). They inhabit all oceans, from tropical waters to the artic. In comparison, Scleractinia order has more described species but they produce a mineralized skeleton that makes their development harder to study and their distribution in the globe is more restricted. Actiniarians can be solitary or colonial (Brusca and Brusca 2003). The existence of solitary species is another reason why their development is more studied than orders that do not present solitary forms, as in solitary species the organism morphology is easier to approach. This accounts for the fact that the development of other anthozoan orders and especially their mesenteric arrangement has mostly been compared to the developmental phases of actiniarians, being the reason why their development is here described in first place.

Actiniarians present a huge diversity of body organization types, varying in the number of mesenteries or tentacles and their disposition (tentacles - **figure 2.7**; mesenteries - **figure 2.9** and **figure 2.11**;), possessing different symmetries (e.g. different symmetries in mesenteric arrangement in adult Actiniarians: **figure 2.9** – bilateral symmetry and **figure 2.11 –** bi-radial symmetry) between species or even in the same organism in different parts of the polyp (see **figure 2.13**).

Figure 2.7. Diversity in Actiniaria: The number of tentacles and their disposition vary greatly, from anemones presenting 12 tentacles as in **a** - *Halcampa chrysanthellum* (Peach *in* Johnston, 1847); dozens of tentacles arranged in a few rows as in **b** – *Bunodosoma biscayense* (Fisher, 1874) and **c** – *Diadumene lineata* (Verrill, 1869) or hundreds of tentacles disposed in several concentric rows as in **d** – *Stichodactyla helianthus* (Ellis, 1768). Images from DORIS (http://doris.ffessm.fr), respectively by Marc Cochu, Frédéric André, Vincent Maran, Véronique Lamare.

Formation of the first pair of mesenteries:

Actiniarian adult polyps mesenteric arrangement varies greatly but the initial steps of development are quite the same for all studied specimens. It starts with the development of a **first pair of mesenteries** – the **Ventro-lateral pair (VL)** – that grow from the outer wall (Hyman 1940 after Faurot 1895). Both the mesenteries of this first pair forms synchronously (**figure 2.8**) in the left and right side of the secondary axis and asymmetrically in relation to the secondary axis, resulting in two compartments of unequal size. The bigger corresponds to the one usually named "dorsal" in actiniarians, and the smaller to the "ventral" part (**figure 2.8**). This size difference results from the fact that the angle of this first pair of mesenteries orthogonal in relation to the flattered pharynx. From the development of this pair of mesenteries that is followed by the formation of the flattered pharynx (along which passes the secondary axis) we have in

this first stage of mesenteric development of the actiniarian polyp a clear **bilateral symmetry** as can be seen in the **figure 2.8**. This bilateral symmetry comes from the fact there is a single plane of symmetry for the polyp. This plane passes longitudinally from oral to aboral by the secondary axis (visible in **figure 2.8**). The ventro-lateral pair (VL) in Actiniaria will always have their retractor muscles pointing towards the ventral side (smaller compartment), being them also an element of symmetry. **All actiniarians pass by this bilateral phase of development**.

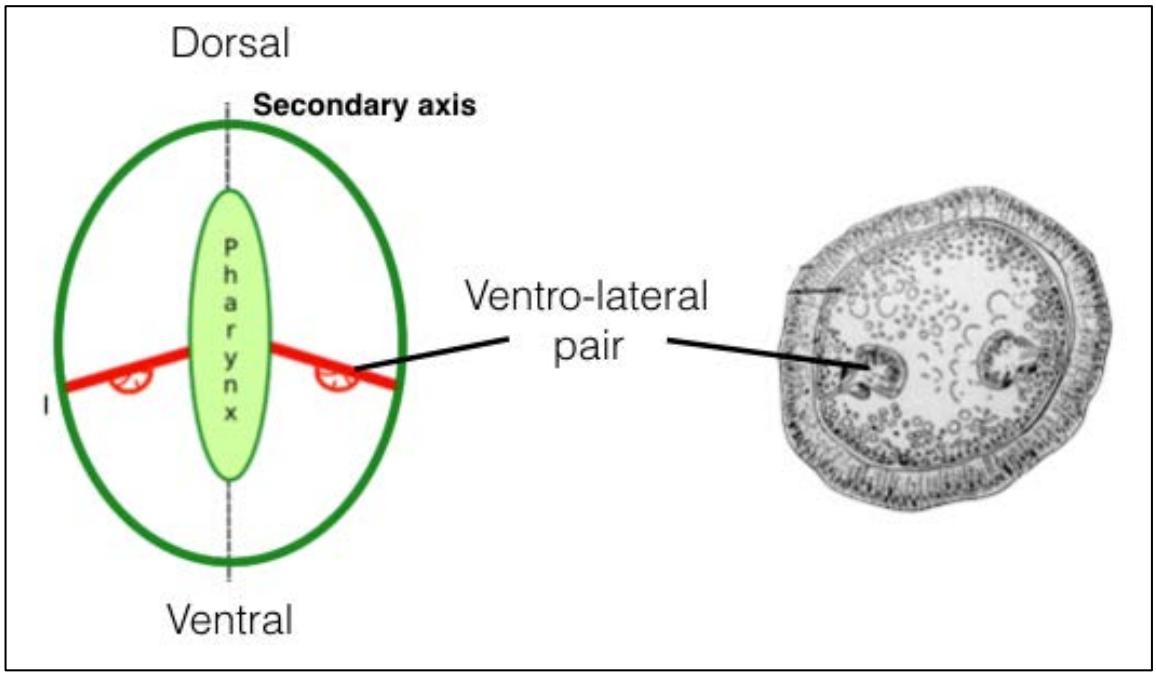

Figure 2.8. Early bilateral symmetry in Actiniaria (sea anemones). The first pair (**I**) of mesenteries to form (**ventro-lateral pair: VL**) divides the animal in two differentially-sized compartments, by the formation of a mesentery on each side of the flattered pharynx with an angle different to 90º in relation to the secondary axis. In the left there is a schematic representation where the position of the retractor muscles that will be associated to the first pair of mesenteries is represented in the ventral part of the mesenteries. In the right we can see the representation (from Hyman 1940 after Faurot 1895) of a cross section of *Adamsia sp* (Actiniaria) showing the precedence of the ventro-lateral pair over the others: note that at this stage the pharynx is not yet form and that the retractor muscles are not yet present, in the schematic representation they are represented for understanding purposes.

Development of the "Edwardsia stage"

This first pair (VL) is followed by the quick addition of three other pairs of mesenteries, resulting in the formation of what is classically called the "**Edwardsia stage**" (**figure 2.9**). This stage is named for the Edwardsiidae, sea anemones that retain this mesenteric conformation in their adult form. This addition of 3 mesenteric pairs (6 mesenteries) results that the Edwardsia stage of Actiniaria contains 4 mesenteric pairs (8 mesenteries) (**figure 2.9**).

The growth of this three last mesenteric pairs has been historically disputed and can follow different schemes (**figure 2.9**):

I - From McMurrich (1891) that studied the embryologic development of *Isoaulactinia stelloides* and Reitzel *et al.* (2009) in *Edwardsiella lineata*, the second pair to be formed is the **dorso-lateral pair (DL)** followed by the **ventral directive pair (VD)** and finally the **dorsal directive pair (DD)** (**figure 2.9a**).

II – A different order (**Figure 2.9b**) was described by Lacaze-Duthiers (1872) in *Actinia nigropunctata*, with the second pair to form is the **dorso-directive pair (DD)** followed by the **ventro-directive pair (VD)** and finally by the **dorso-lateral pair (DL)** (**figure 2.9b**).

In this "Edwardsia stage" the retractor muscles are oriented towards the ventral part of the polyp, exception made for the retractor muscles of the directive ventral pair (**figure 2.9**). A single plane of symmetry is still present. This plane of symmetry conserved at each stage of development of the 3 pairs of mesenteries added at this stage, regardless of their order of development. It is the same plane of symmetry that existed in the developmental phase with a single pair of mesenteries. It corresponds to the plane passing through the flattered pharynx and that divides each pair of mesenteries in two equal parts.

The "Edwardsia stage" can be defined as simply as the actiniarian stage of development comprising 4 mesenteric pairs (8 mesenteries). All actiniarians have at least this number of mesenteries; the disposition of the retractor muscles is constant between species. This accounts that all actiniarians pass by this bilateral phase of development.

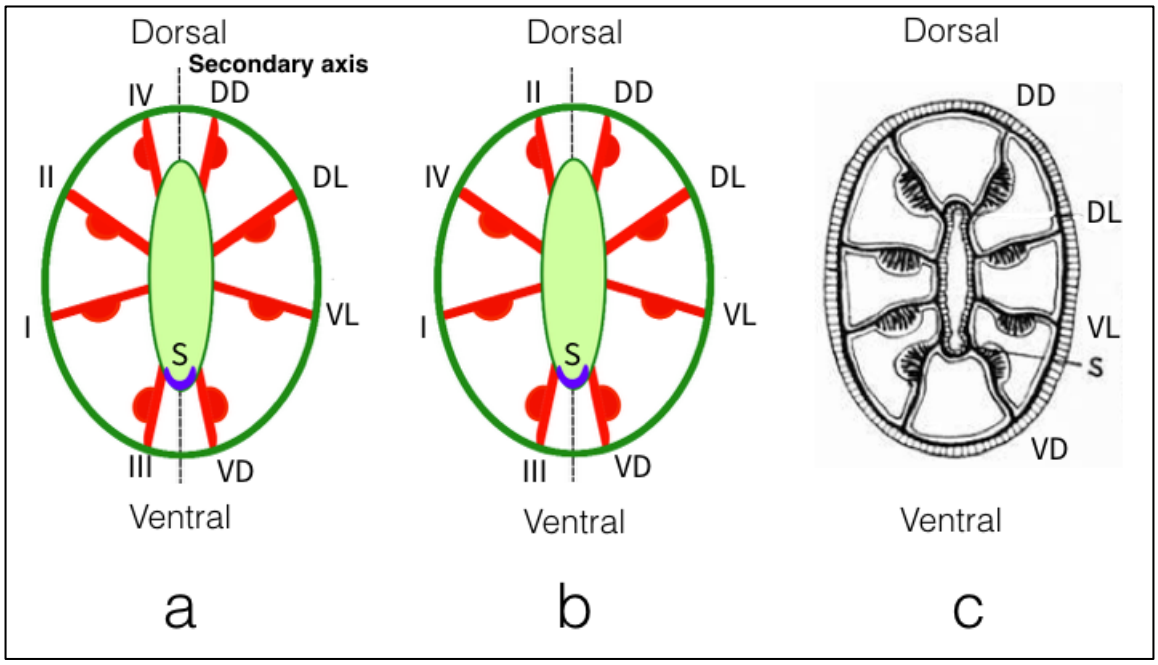

Figure 2.9. Edwardsia stage of development in Actiniaria corresponds to the phase with 4 pairs of mesenteries (M=8) dividing the polyp. **a** and **b**: different orders of mesenteric development in Actiniarians have been described and are shown by roman numerals: **a** – **I** – McMurrich (1891) and Reitzel *et al*. (2009); **b** – **II** –Lacaze-Duthiers (1872); c – Edwardia stage showing the disposition of the retractor muscles of *Edwardsia claparedii* (after A. Andrès 1881) from Chisholm, H**.** (1911). *The Encyclopedia Britannica*. Legend: **DD**: Dorsal-directive mesentery; **DL**: dorso-lateral mesentery; **VL**: Ventro-lateral mesentery; **VD**: Ventro-directive mesentery; **S**: siphonoglyph.

Nematostella vectensis polyp organization

The laboratory model species for Anthozoa *Nematostella vectensis* (Stephenson, 1935) presents this conformation of mesenteries corresponding to the "Edwardsia stage" in their adult form (**figure 2.10**) (Frank and Bleakney 1976). This species is then bilateral in their adult stage.

The exact order of mesenteric development of the 4 pairs of mesenteries of Nematostella is not yet completely clear. Authors agree that the first pair to form is as in other actiniarians the ventro-lateral pair (**VL**) (Leclère and Rentzsch 2014; Jahnel *et al*. 2014). Tarrant *et al*. (2015) reported in their paper on the directions and future perspectives that came from the third *Nematostella* research conference that Andy Aman (from Technau laboratory, University of Vienna, Austria) confirmed that the first pair of mesenteries to develop was the ventro-lateral pair but that the 3 other pairs (DD, VD and DL) **formed simultaneously** without a defined progression.

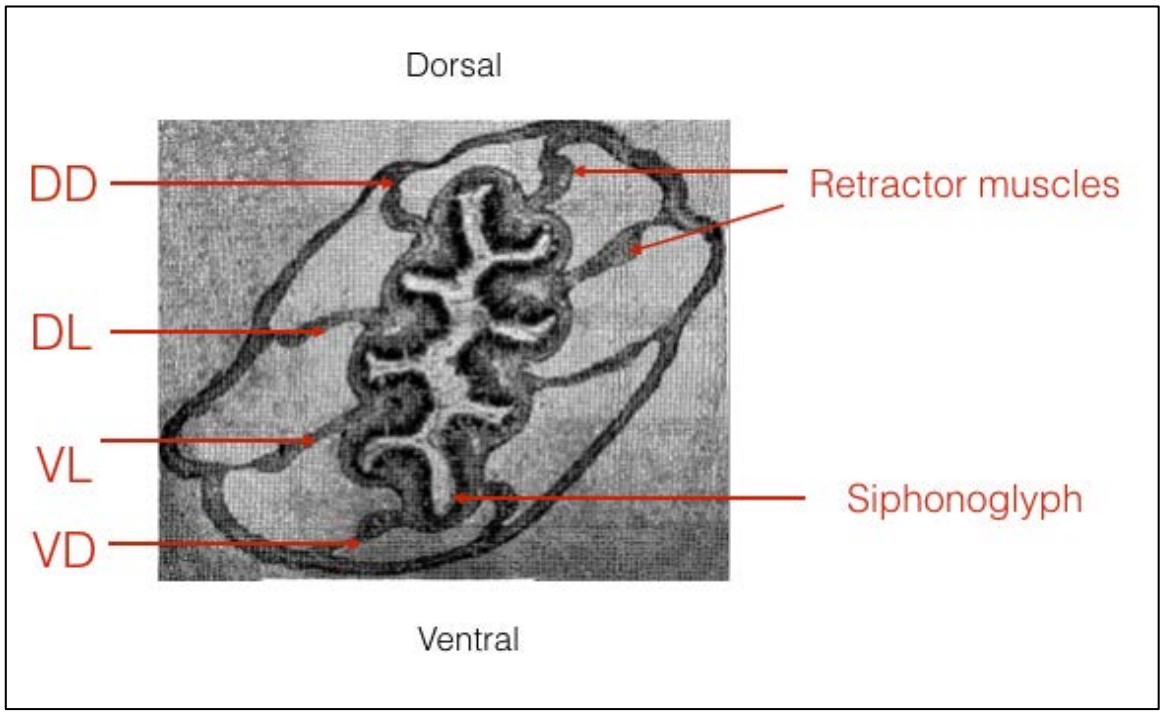

Figure 2.10. Original histological cross-section at the pharynx level of ***Nematostella vectensis*** in Frank and Bleakney (1976), **In the histological section we can see that the adult polyp of *Nematostella* possesses 4 pairs of mesenteries (8 Mesenteries) corresponding to the Edwardsia stage** pairs as seen in figure 2.9a) Legend: **DD**: Dorsal-directive mesentery; **DL**: dorso-lateral mesentery; **VL**: Ventro-lateral mesentery; **VD**: Ventro-directive mesentery; **S**: siphonoglyph.

Development of the "*Halcampoides stage*"

After this "Edwardsia stage", some actiniarians continue to add more mesenteries to their internal organization. The next step is the growth of two lateral mesenteric pairs, named **Secondary Ventro-lateral pair (SVL)** and **Secondary Dorso-lateral pair (SDL)** in **figure 2.11**. They appear each one ventrally to one existing lateral mesentery pair and have their retractor muscles pointing towards them. Both pairs form at around the same time and no big controversy exist between different authors. However McMurrich (1891) represented an order of formation for these pairs: the 5th pair to form in Actiniaria is the **SVL** pair, immediately ventrally to the VL pair and it's retractor muscle points towards **VL,** the 6th pair is the **SDL** that forms also immediately ventrally to the **DL** pair having its retractor muscle pointing towards **DL**. The mesenteries pairs **DL** and **SDL** form two couples of mesenteries and the same happens with the pairs **VL** and **SVL** that also form two mesenteric couples, the space between this couples is an endocoel.

Some anemones "stay" in this stage, with 6 mesenteric pairs (12 mesenteries): the **Halcampoides** for which the stage was named. With the growth of the 5th (**SVL**) and 6th pair (**SDL**) in the direction of the pharynx we reach a **bi-radial symmetry** (**figure 2.11a**). The "*Halcampoides stage*" can also be found in literature as "*Halcampella stage*" (McMurrich 1891) or as "*Halcampula* stage" (Grebel'nyi 1981).

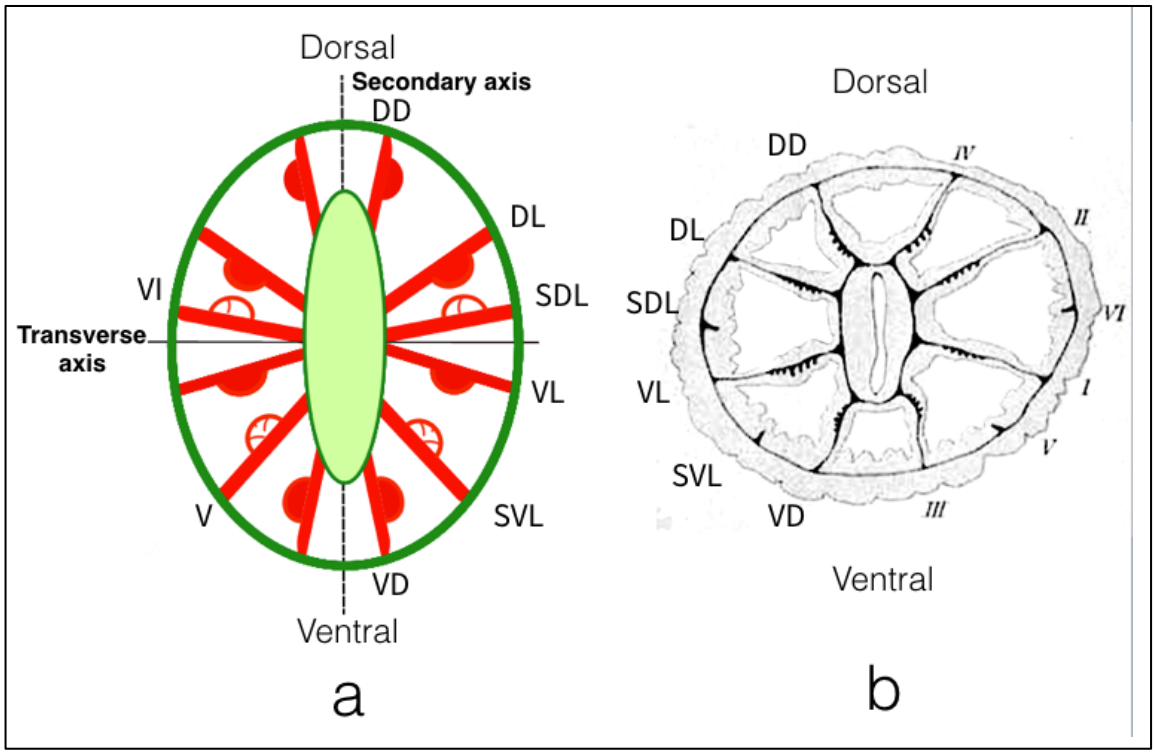

Figure 2.11. Halcampoides stage of development in Actiniaria corresponds to the phase when 6 pairs of mesenteries (M=12) divide the polyp. In relation to **figure 2.9** there is the addition of two secondary pairs that will couple with the dorso-lateral pair and with the Ventro-lateral pair: the Secondary ventro-lateral pair (SVL) and the secondary dorso-lateral pair (SDL). This new formed pairs are indicated with the roman numeral V and VI in roman numerals in relation to their order of development in **a** and **b**. In **a** the retractor muscles in the secondary mesenteries are represented differently in this pairs for better visualization. With the addition of this pairs the polyp becomes bi-radial as we can obtain two similar parts by dividing it by the **secondary axis** or by the **transverse axis**; image corresponds to the original image from McMurrich (1891) in **b** shows the development of the secondary mesenteric pairs SVL and SDL in *Isoaulactinia stelloides* (McMurrich, 1891). Legend: **DD**: Dorsal-directive mesentery; **DL**: dorso-lateral mesentery; **VL**: Ventro-lateral mesentery; **VD**: Ventro-directive mesentery; **SDL: secondary dorso-lateral mesentery**; **SVL: secondary ventro-lateral mesentery**.

From this point on, the addition of mesenteries happens in couples in the exocoel sections of the polyps. In **figure 2.12** we have 6 mesenteric couples (12 mesenteries) that have been developed after the 12 mesenteric stage, leaving the polyp with 24 mesenteries arranged in 12 couples (but only 12 mesenteries are paired). It is important to note that in polyps with this mesenteric conformation we have a **bi-radial symmetry** but that these polyps have passed during their development by **bilateral** phases.

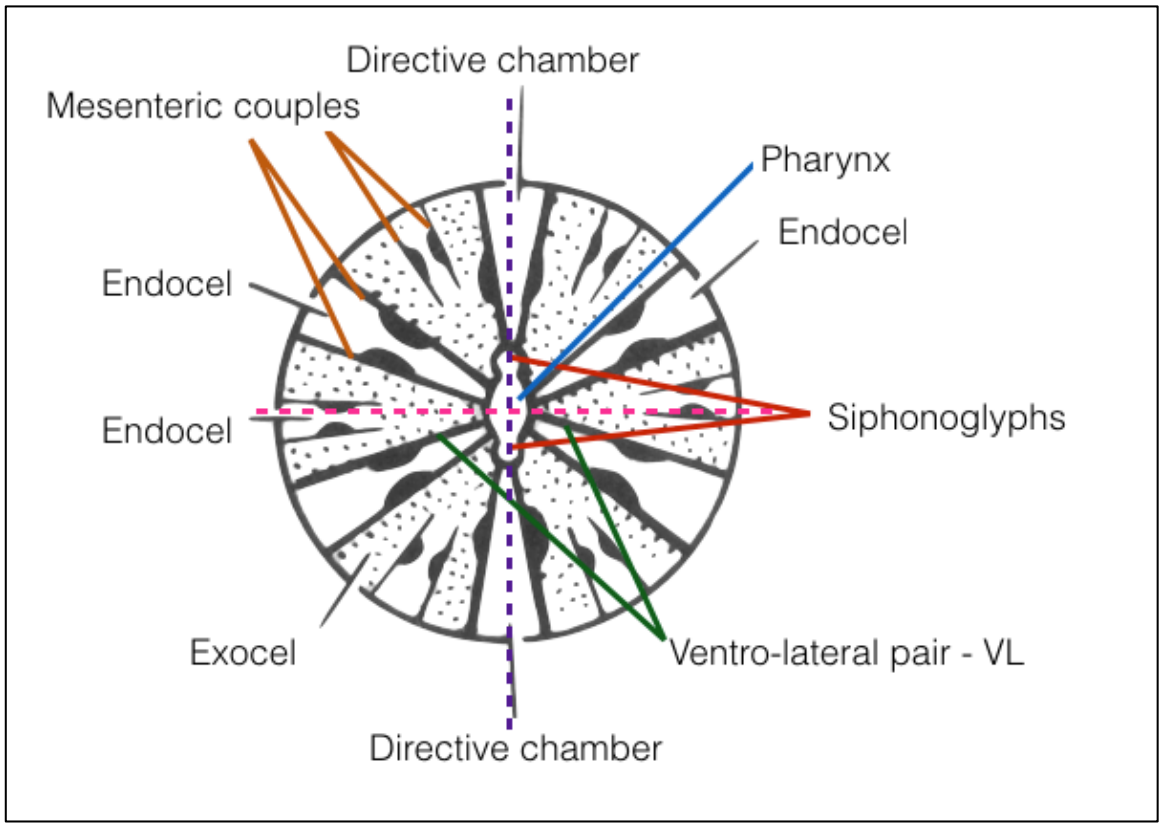

Figure 2.12. Advanced stage of development in Actiniaria with 24 mesenteries: in this polyp with two siphonoglyphs and a flattered pharynx there are two symmetry planes, one passing by the directive chambers and both siphonoglyphs of the flattered pharynx (violet intermittent line), the only symmetry plane in the first developmental stages, and another symmetry plane, usually called transverse plane (rose intermittent line) that is created by the addition of supplementary mesenteries. We can in this phase clearly see the presence of exocoel and endocoel zones between mesenteric couples. The ventro-lateral pair is indicated to facilitate the correspondence to previous developmental stages. Original figure from (1967).

Tentacles, due to their huge number, are sometimes harder to look at in terms of symmetry. However, in polyps of species with fewer tentacles they have also been compared in terms of symmetry, for example by Dawydoff in 1928. Dawydoff described (**figure 2.13**) two different sea anemones, compared in terms of tentacle symmetry at the "Halcampoides stage": *Cibrina sp.* in the left of the figure with a bilateral arrangement of the tentacles and *Peachia sp.* in the right with a radial disposition of the tentacles. It is interesting to see that there is no agreement between the symmetry of the internal organization of these anemones and their external symmetry. In both cases when the mesenteric pairs SVL and SDL reach the pharynx level, the anemone will present a radial symmetry for the disposition of mesenteries and retractor muscles. Looking at the pharynx level we see that in *Cibrina* we have a bi-radial symmetry and in *Peachia* a bilateral symmetry due to the presence of a single siphonoglyph. These symmetries on the internal organization contrast with the symmetries these anemones present for the tentacle disposition. In both cases the animal *in toto* is bilateral. We can here see that the presence of a single siphonoglyph can also break a radial symmetry giving a bilateral symmetry to an otherwise radial polyp – **figure 2.13b**).

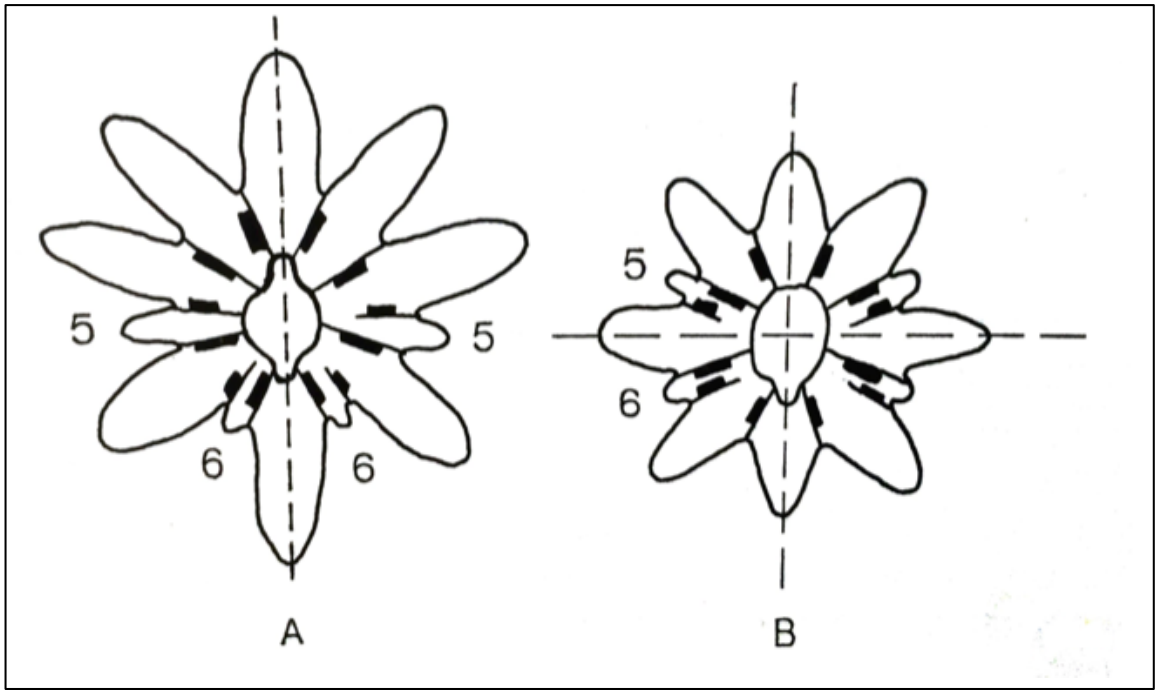

Figure 2.13. Tentacle symmetry in Actiniaria at the Halcampoides stage: A – Bilateral symmetry of the tentacle disposition in *Cribrina sp.;* B – Radial symmetry of the tentacle disposition in *Peschia sp.*; 5 and 6 refer to the SDL and SVL respectively. Image from Doumenc and van Praët (1987), after Dawydoff (1928).

2.2.1.2 Antipatharia

In terms of anatomy, the Antipatharia is one of the most understudied orders of hexacorallians and comprises around 250 described species (Appeltans *et al.* 2012). Usually considered as animals from higher depths they can be found in depths as shallow as 15 meters in tropical waters (*e.g.* Trinidad (Warner 1981); Bocas del Toro, Panama (Guzmán *et al*. 1999)). General information on antipatharian morphology and ecology can be found in chapter 1.

The development of antipatharian species remains mysterious. The larval stage of this order has only been observed for two species *Antipathella fiordensis* in laboratory conditions (Miller 1996, Kai *et al*. 2001). From the description of Miller we have that the ciliated planulae measured 200 µm in length and developed within 36 hours post-fertilization (Miller 1996). The larvae were weak swimmers, likely non-feeding larvae, negatively buoyant and lived for a maximum of 10 days, none of the larvae settled (Miller 1996). Kai *et al*. however described some more details: the embryos exhibited pseudospiral cleavage and became hollow ball-shaped blastulas. It Endoderm formed without invagination, by delamination from the ectoderm and before the formation of the oral pore. The embryos developed into bullet shaped planula larvae with an oral pore at the posterior end (Kai *et al*. 2001).

<u>The budding process in antipatharians</u>

Development of this species has only been studied seriously by Dantan in 1921 with his observation on budding in the species: *Parantipathes larix* (Esper, 1788), *Antipathella subpinnata* (Ellis and Solander, 1786) and *Leiopathes glaberrima* (Esper, 1788) from "*Le golfe de Naples*" – Mediterranean Sea. The budding process corresponds to a form of asexual reproduction with the formation of new polyps from pre-existing polyps of the colony and is the way of development of all colony polyps with the exception of the founding polyp that himself results from the settlement of a larvae that resulted from sexual reproduction.

Before Dantan, only the existence, place and form of the budding had been observed. Brook firstly in 1889 and van Pesch in 1914 described budding as an amorphous or round protuberance that usually appeared in between two adult polyps. Dantan observed this for *Leiopathes glaberrima* but not for *Antipathella subpinnata* or *Parantipathes larix* for which he observed several buds between two adult polyps.

Van Pesch described in 1914 that the sagittal tentacles are the firsts to be formed. This fact was then confirmed by Dantan observations, as in **figure 2.14** where we can see the presence the initial stage of formation of the sagittal tentacles preceding the formation of the lateral tentacles. In this figure we can already see that the antipatharian polyp secondary axis is perpendicular to the axis of the colony. This can be seen more clearly in **figure 2.18**.

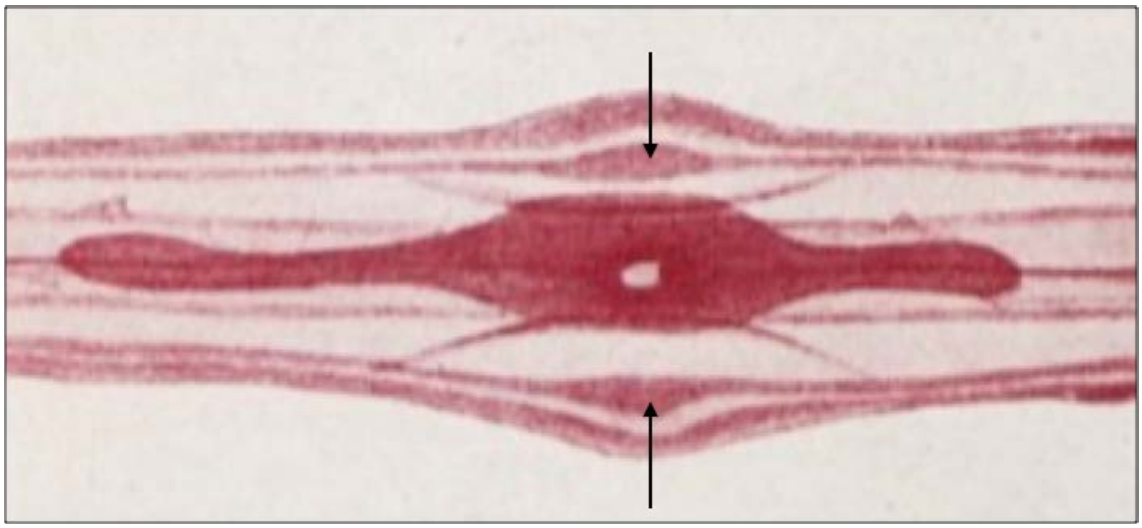

Figure 2.14. Representation of a bud of *Parantipathes larix* from Dantan 1921, presenting the formation of the sagittal tentacles (arrows) before the lateral ones.

However only two authors, van Pesch (1914) and Dantan (1921), looked more closely to budding. Both authors agree that the budding process does not start in the coenenchyme between polyps and that the beginning of the process is directly dependent of an adult polyp. However some main differences were related between authors. Van Pesch observed, in *Cirripathes contorta*, that the budding took place in the oral cone of adult polyps. He observed in a single polyp three “oral openings” (3 buds) orientated in the dorso-ventral axis plane between the sagittal tentacles of the adult polyp. Each of the three buds had it’s own pharynx and mesenteries. The main

difference in relation to the description of Dantan is that this author observed in *Parantipathes larix* the formation of a single bud at a time, forming in an adult polyp. This difference in budding formation between these species may be related to the different polyp disposition in the colony, being that in *Cirripathes* species polyps are disposed all around the branch of the colony, while in *Parantipathes* species they are disposed in a single row in the colony branch.

Dantan detailed in *Parantipathes larix,* that he fixed with Bouin-Hollande fixative, that the **buds** did not form in any point of the colony but that they **formed specifically in the extension of a lateral mesenteric chamber** (named "cloison latérale"). Then the formation of the bud directly relied to the adult. The consequence is that the adult polyps are always perfectly aligned and that their lateral mesenteries correspond perfectly. Dantan showed that the lateral mesenteries are perfectly correspond even when the adult polyps are not completely aligned (**figure 2.15**).

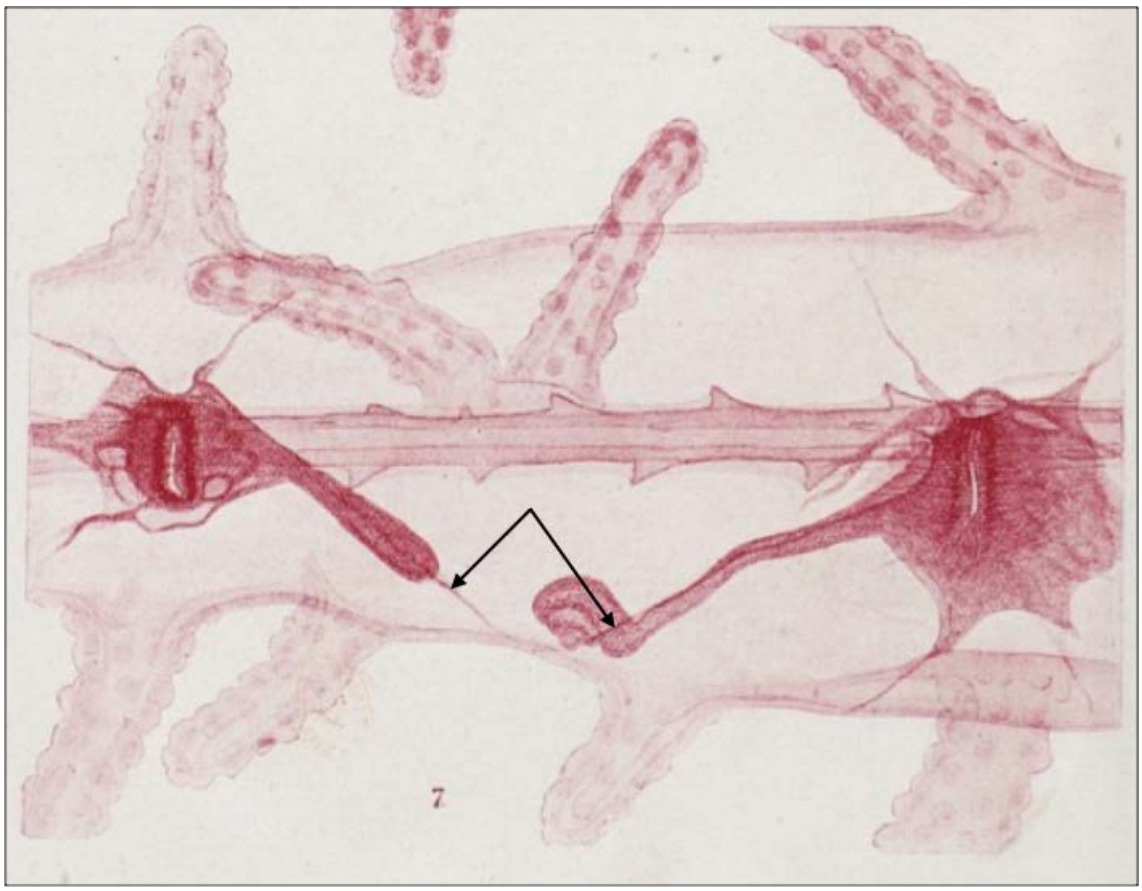

Figure 2.15. Dantan 1921 showed that the lateral mesenteries (arrows) of two adjacent adult polyps correspond perfectly even when the polyps are not aligned.

Dantan (1921) showed that these primary lateral mesenteries corresponded to the first pair of mesenteries to be formed together with the pharynx (flattered along the secondary axis) – **figure 2.16a** and **2.16b**. Note that this pair has a different nomenclature (**primary lateral pair**) than the first pair to develop in Actiniaria (the **ventro-lateral pair**). The homology of this pair will be discussed later in this chapter, as this discussion will take into account other elements of the antipatharian development and morphology.

After this primary pair there is the formation of the **dorso-directive mesenteric pair** (**DD**) and the **ventro-directive mesenteric pair** (**VD**) together with the mouth and simultaneously (**figure 2.16c**). No homology problems exist between these pairs and the **dorso-directive mesenteric pair** (**DD**) and the **ventro-directive mesenteric pair** (**VD**) of Actiniaria. They are, in both species, situated in the extremities of the flattered pharynx, both are pairs that alone form an **endocoel** and so correspond perfectly to the definition of **directive pair**.

Some species of antipatharians only possess this three mesenteric pairs (6 mesenteries) in their adult polyps. It is the case of species of the family Cladopathidae, including the *Cladopathes*, *Sibopathes*, *Heliopathes*, *Chrysopathes*, *Trissopathes* and *Hexapathes* (Bo 2008). The number of 6 mesenteries of these adult polyps corresponds to the smallest number of mesenteries found in an adult anthozoan polyp.

In **figure 2.16c** we can also see that the formation of mesenteries of a chamber precedes the formation of the corresponding tentacle. Dantan reinforced the idea that as in Actiniaria the first tentacles to form correspond to the directive chambers (directive endocoels). Dantan notes also that the endodermal tissue associated to the primary mesenteries never present the same size on both primary mesenteries, one being one third shorter than the other (**figures 2.16c/d/e**). From the orientation of the spines we can deduct that the distal one is the smaller (the spines of antipatharian skelet, apart from abnormalities point towards the distal part of the colony (Opresko 2001, 2002, 2003, 2004 and personal communication)). Finally there is the formation of the two so-called **lateral mesenteric pairs**, visible in **figure 2.16d** (adult polyp in the figure) and **2.16e**. One of this pairs form between the dorso-directive pair and the primary lateral pair and the other between the lateral primary pair and the ventro-directive pair, leaving the antipatharian adult polyp with 10 mesenteries: 6 primary ones and 4 secondary ones.

Most Antipatharian species have this mesenteric number (Opresko 2001, 2002, 2003, 2004; Bo 2008). All this development takes place without the existence of an oral opening or an oral cone.

In another species, *Leiopathes glaberrima*, Dantan described the addition of an extra pair of mesenteries immediately dorsally to the primary lateral pair, leaving the polyp of *Leiopathes* with 12 mesenteries. There are only 6 species belonging to the Leiopathidae family. This family is characterised by the presence of 12 mesenteries (Bo 2008).

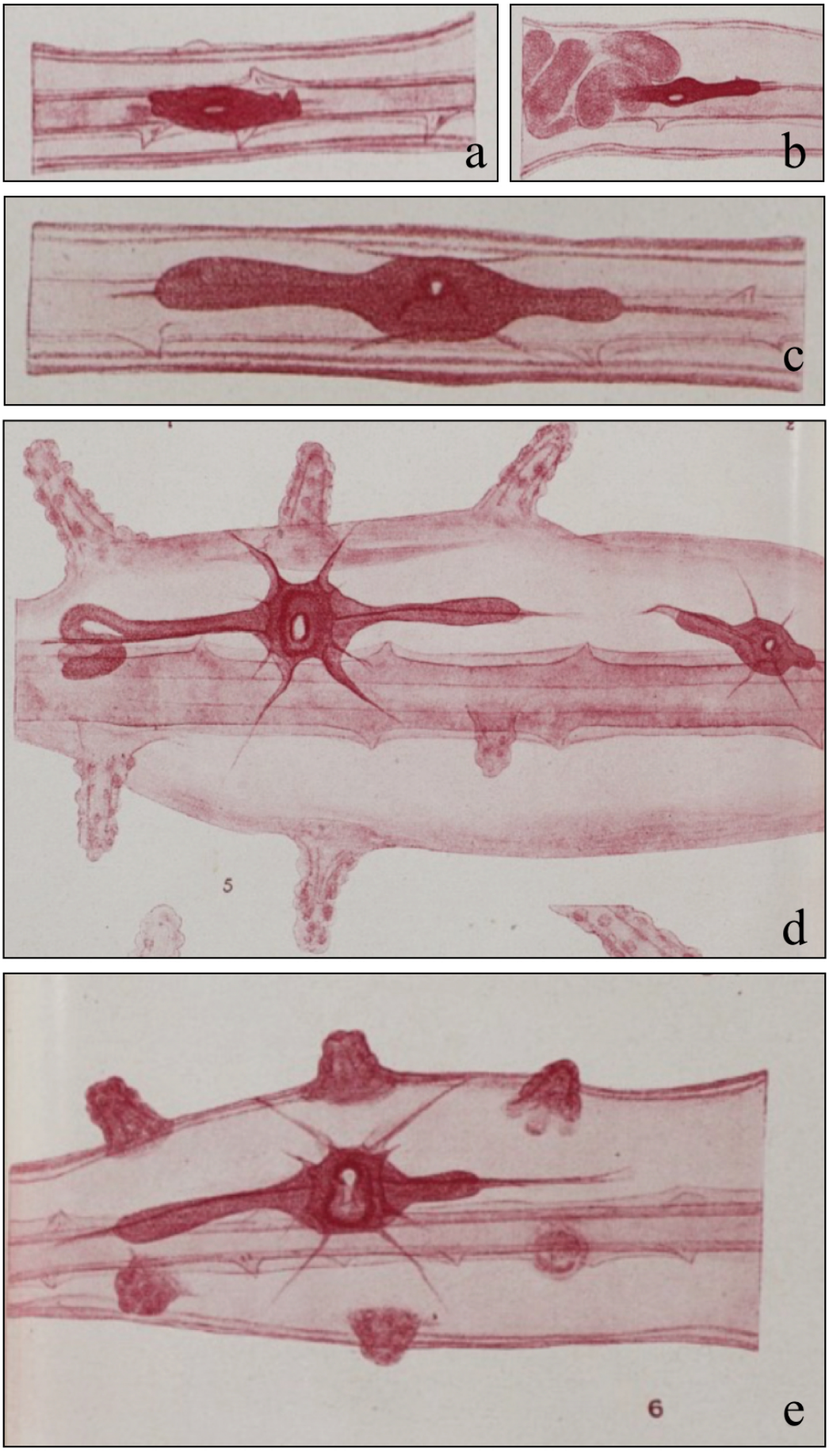
a
b
c
5
d
6
e

Figure 2.16. Figures from Dantan 1921 representing the budding process: **a** and **b** – young buds presenting only the primary lateral mesenteries, in the first the mouth is not yet open but it is visible by transparency and in the second a growth of the mesenteries and endodermic associated tissue is visible in the colony axis, or transverse axis of the polyp; **c** – young bud with six mesenteries, the primary lateral pair and the two directive pairs; **d**: adult polyp (left) and young bud (right), the bigger primary mesentery and corresponding endodermic tissue are located on the same side of the corresponding polyps – proximal side of the colony, the young bud presents 6 mesenteries and as the bud in on figure c; **e**: young polyp that already presents 6 tentacles and 10 mesenteries corresponding to the 6 previously represented and the 4 secondary lateral ones.

Dantan resumed in **figure 2.17** the order of apparition of the mesenteric pairs along the secondary axis of antipatharians:

a – Formation of the **primary lateral mesenteric pair**(I), with one mesentery forming in the left side and another on the right side of the secondary axis;

b – after a short break, development of the **directive mesenteries**, the **dorsal directive pair – DD** (II) and the **ventro-directive pair - VD** (III).

c – formation of the two pairs of lateral mesenteric pairs – called **secondary mesenteric pairs** in antipatharian literature: one dorsally in relation to the primary lateral pair (IV) and one ventrally (V). Most antipatharian species present this conformation in the adult polyps;

d – Formation of an extra pair of mesenteries (VI) in *Leiopathes glaberrima*, immediately dorsally to the primary mesenteric pair (I).

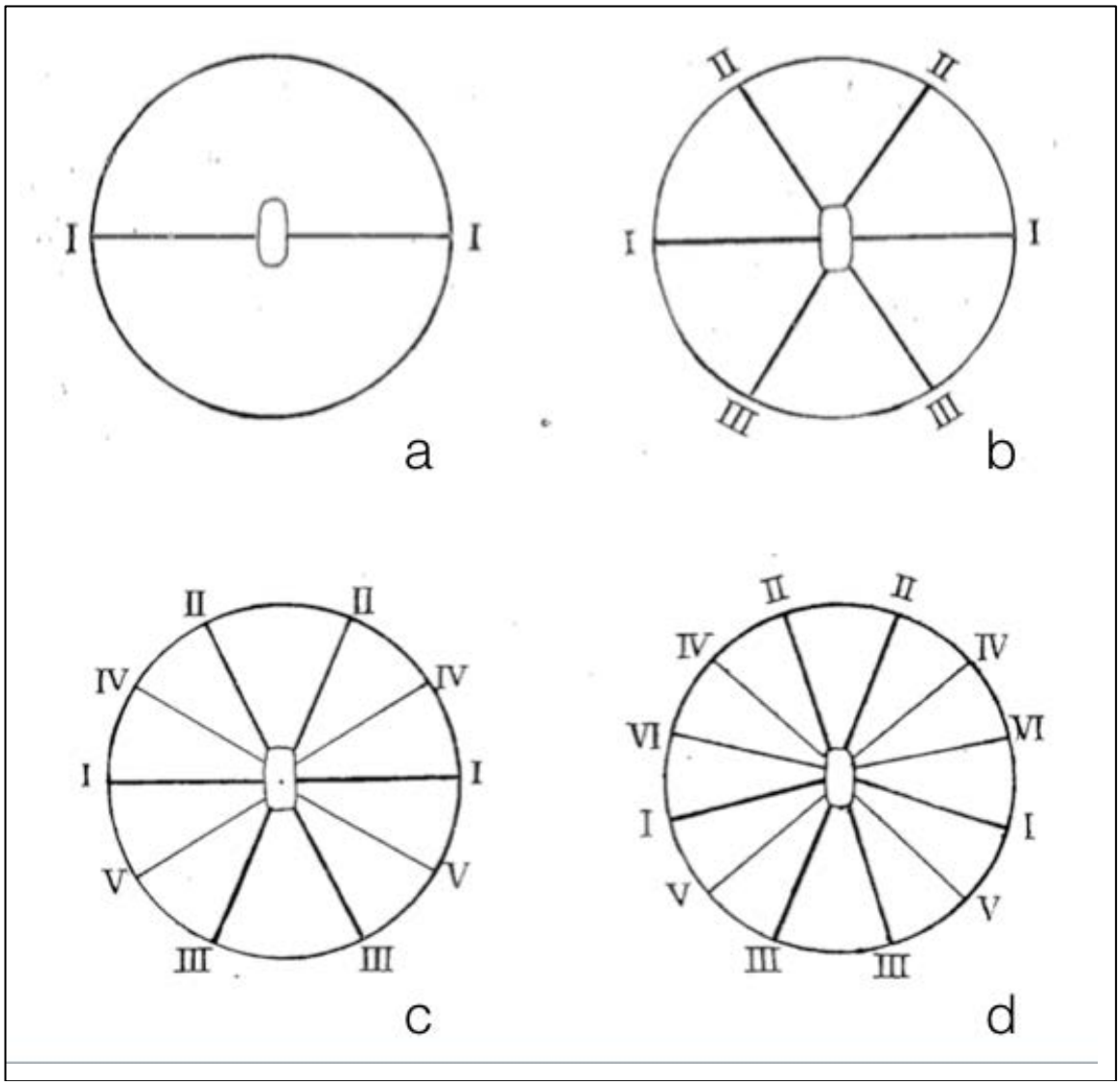

Figure 2.17. Order of mesenteric formation in Antipatharia as described by Dantan in 1921: **a** – first phase of mesenteric formation with the insertion of the first pair of mesenteries - I, the primary mesenteries, perpendicular to the secondary axis; **b** –second phase of mesenteric formation with formation of the directive mesenteries simultaneously, II – dorsal directives, III – ventral directives; adult stage of the Cladopathidae family **c** – third phase of mesenteric formation with formation of the lateral secondary mesenteries, IV – Dorsal secondary mesenteric pair, V – Ventral secondary mesenteric pair; adult stage of most antipatharian species **d** - fourth phase of mesenteric formation: formation of an extra pair of mesenteries in the dorsal side of *Leiopathes glaberrima* between the primary mesenteries (I) and the dorso-lateral pair (IV). Adapted from Dantan (1921).

Position of the retractor muscles in the mesenteries of Antipatharia: orientation of the secondary axis and the formation of the first mesenteric pair

All authors that studied the morphology of antipatharians (such as Brook 1889, Lacaze-Duthiers 1865, Dantan 1921, van Beneden 1897) failed to distinguish retractor muscles associated to mesenteries. The only author successful in doing so was van Pesch (1914) in the monograph he did about the antipatharians collected in the Siboga expedition. Van Pesch used Delafield-haematoxylin for part of the microtome preparations and haemalum for other part of the preparations, both gave good results but he prefers the first since it allowed a clearer and better differentiation.

Van Pesch described the retractor muscle distribution for 10 species of antipatharians (**figure 2.18**). He used the anterior/posterior nomenclature for the poles of the secondary axis and discovered that the retractor muscles associated to the primary lateral mesenteric pair (the one Dantan described as being the first pair to be formed) always presented their retractor muscles pointing towards the posterior (ventral) side of the polyp. This direction of the retractor muscles is the same as the first pair of mesenteries to form in Actiniaria, the ventro-lateral pair (VL).

A question remains about the angle of formation of this first pair: does it also divide the body of the polyp in two unequal chambers as it happens with the first pair formed in Actiniaria? Firstly we have to take into account that the formation of new polyps by budding presents a constrain as the primary pair of mesenteries is in continuity with the primary mesenteric pair of the mother polyp and that in many species the polyps are completely aligned in a single side of the branch of the colony. As the primary mesenteries to form in Antipatharia have the same orientation as the skelet/colony and in a certain way centre the polyp in the colony axis together with the other polyps, this first pair might have been constrained to a more central position than in Actiniaria. Nevertheless Pax (1918) based on Schultze (1896) described the angle of the primary mesenteric pair as not being perpendicular to the directive axis (**figure 2.19**), leaving the ventral side smaller than the dorsal side as in Actiniaria. This author calls the directive axis as "***Sagittalbene***", German for "**sagittal plane**", and the colony axis as "***Transversalebene***", German for "**transversal plane**". The orientation of the retractor muscles of the first mesenteric pair to form and the angle of its formation show

the homology between the primary lateral pair of Antipatharia and the VL pair of Actiniaria.

Dantan defends this homology arguing that the first pair to form in Antipatharia and Actiniaria is the same: "(*...) car les premières cloisons formées correspondent bien, chez les Antipathaires, au maître couple des Hexactiniaires. Ce sont elles qui, pour employer l'expression d'E. van Beneden, dominent toute la marche du développement: elles ont des relations étroites avec la formation de l'actinopharynx, elles portent les entéroïdes, c'est à leur intérieur que prennent naissance les produits sexuels, enfin ce sont elles qui dirigent le bourgeonnement*".

The first pair of mesenteries to form, the **Ventro-lateral pair** (**VL**) in both Antipatharia and Actiniaria divides the body of the polyp in a Dorsal and a Ventral sides (**figure 2.20**), it is important to note that in antipatharians, as it happends in Actiniaria, the pharynx is flattered along the secondary axis (**figure 2.26**). Therefore, as in Actiniaria, antipatharians present at this stage of development, with one pair of meseteries, a **bilateral symmetry**. The first stage of development is homologous in the two groups.

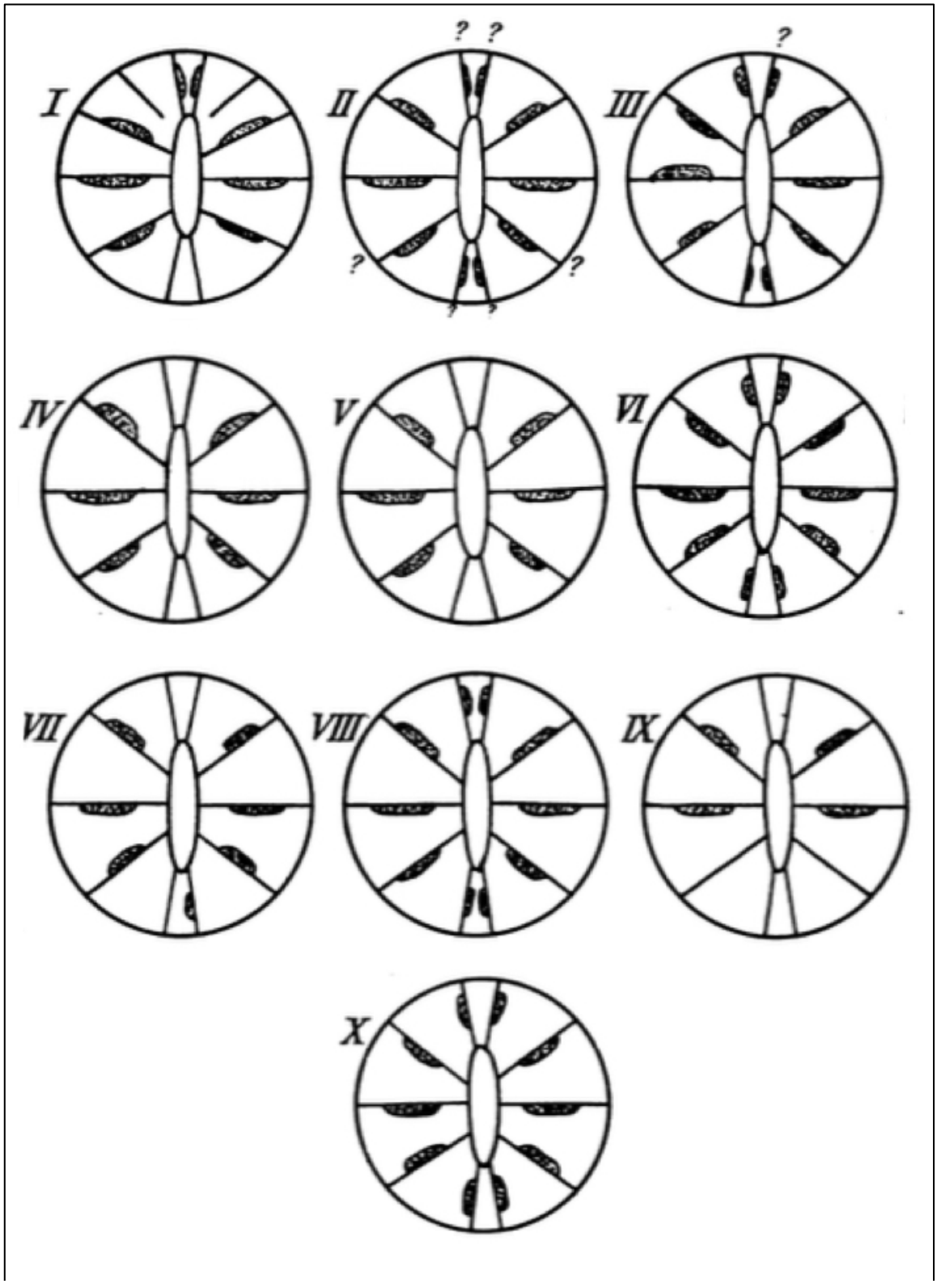

Figure 2.18. Schematic representations for the disposition of the longitudinal musculature in the mesenteries of several antipatharian species by Adrianus Jacobus van Pesch in it's work "The Antipatharia of the Siboga expedition": **I** - *Cirrhipathes contorta* (van Pesch, 1910); **II** - *Cirrhipathes anguina* (Dana, 1846); **III** - *Cirrhipathes musculosa* (van Pesch, 1910); **IV** - *Stichopathes variabilis* (van Pesch, 1914); **V** - *Stichopathes solorensis* (van Pesch, 1914); **VI** - *Stichopathes gracilis* (Gray, 1857); **VII** – *Stichopathes saccula* (van Pesch, 1914); **VIII** – *Stichopathes ceylonensis* (Thomson and Simpson, 1905); **IX** – *Antipathes plana* (Cooper, 1909); **X** – *Antipathes longibrachiata* (van Pesch, 1914). For each figure the upper part corresponds to the dorsal side and the lower part to the ventral side of the polyp.

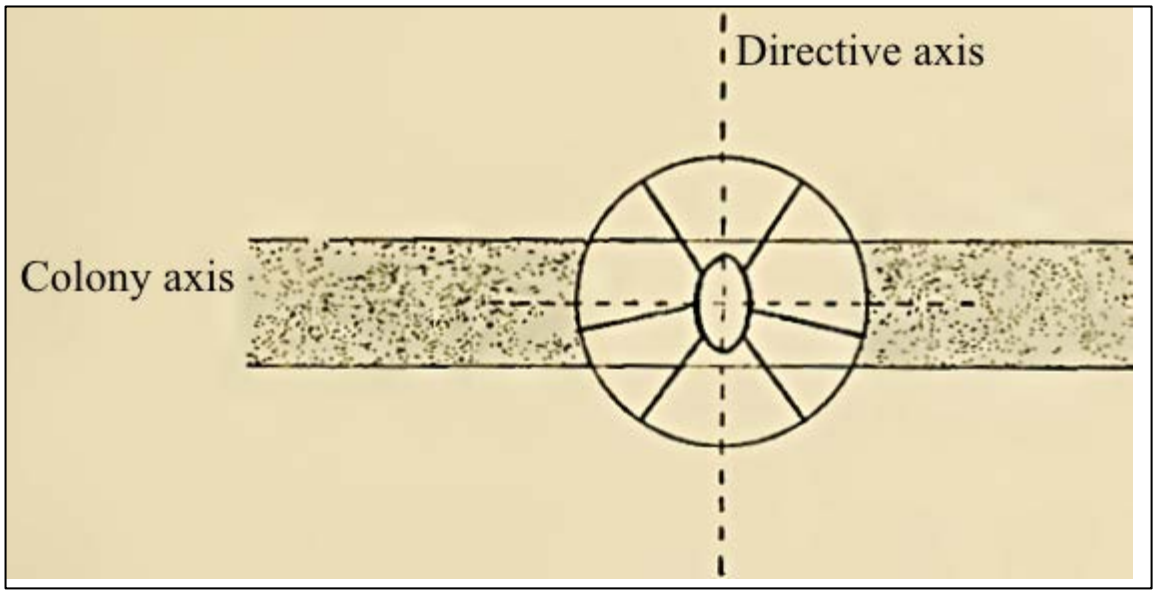

Figure 2.19. Representation of the antipatharian polyp disposition in relation to the colony axis from Pax (1918). In the figure, we see the two directive pairs disposed near the ends of the flattered pharynx and the Ventro-lateral pair, represented by Pax with an angle different from the angle formed between the colony axis and the directive axis, leaving two compartments with different sizes in the polyp.

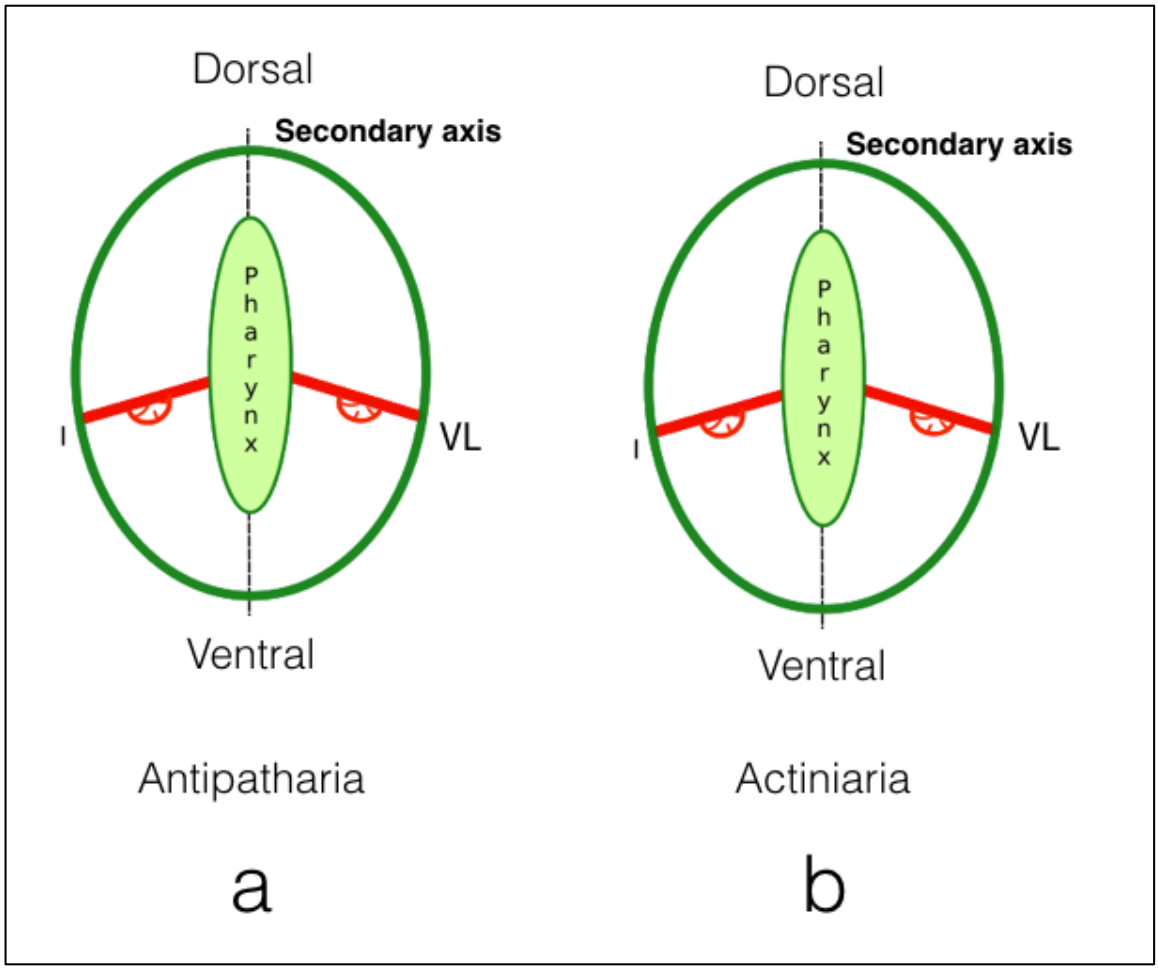

Figure 2.20. The first pair (**I**) of mesenteries to form (**ventro-lateral pair: VL**) is the first pair of mesenteries to form in both antipatharians (**a**) and actiniarians (**b**). In both groups the orientation of the retractor muscles is towards the ventral part of the polyp.

Van Pesch described two systems of retractor muscle distribution based on the disposition of mesenteries in antipatharians, one, **type A**, corresponds to the muscle disposition of the species (from **figure 2.18**): **VI** - *Stichopathes gracilis*; **VII** – *Stichopathes saccula* and **X** – *Antipathes longibrachiata*. This muscle system has been described by him as: "*the longitudinal muscle fibers are found on the anterior side of the posterior sagittal pair and of the posterior secondary pair, but on the posterior side of the other three pair of mesenteries*". The representation of this is on **figure 2.21a** and corresponds to the complete muscle system found for **VI** and **X**. This muscle system can be present in some species as an incomplete muscle system (*e.g.* absence of retractor muscles in the directive mesenteries in **VII**). The presence of a single retractor muscle in one of the ventral directive mesenteries of **VII** seems an artifact of observation as seems also to be the case for the description of the retractor muscle disposition in **III** - *Cirrhipathes musculosa* for which van Pesch admits that a problem on the orientation of the cuts obtained at the level of the pharynx can be in the origin of observation problems that lead to his description.

The second system of retractor muscle distribution on antipatharians described by van Pesch, which we'll call **type B and** corresponds to the muscle disposition of the species: **I** - *Cirrhipathes contorta*; **II** - *Cirrhipathes anguina*; **IV** - *Stichopathes variabilis*; **V** - *Stichopathes solorensis*; **VIII** – *Stichopathes ceylonensis* and **IX** – *Antipathes plana*. The muscle system for this group has been described by van Pesch as: "*the longitudinal muscle fibers are found on the anterior side of the anterior side of the anterior sagittal pair and of the anterior secondary pair, but on the posterior side of the other three pairs of mesenteries*". The representation of this is on **figure 2.21b** with and corresponds to the species **VIII** – *Stichopathes ceylonensis* that possesses a complete muscle system with all mesenteries bearing a retractor muscle associated, other species previously described as belonging to this muscle system present incomplete muscle systems as **I**, **IV**, **V** and **IX**.

Recalling that the anterior side corresponds to the dorsal side as the nomenclature in Actiniaria and that the posterior side corresponds to the ventral side an easier definition of the systems can be proposed:

- **Type A – figure 2.21a**. The primary mesenteric pair has its retractor muscle pointing towards the ventral side. **Both directive mesenteries pairs have the retractor muscles pointing in opposite directions** and

the secondary mesenteries of each pole respect the disposition of the retractor muscles of the directive pair of the corresponding pole;

- **Type B – figure 2-21b**. The primary mesenteric pair has their retractor muscles pointing towards the ventral side. **Both directive mesenteries pairs have the retractor muscles pointing towards each other** and the secondary mesenteries of each pole respect the disposition of the retractor muscles of the directive pair of the corresponding pole.

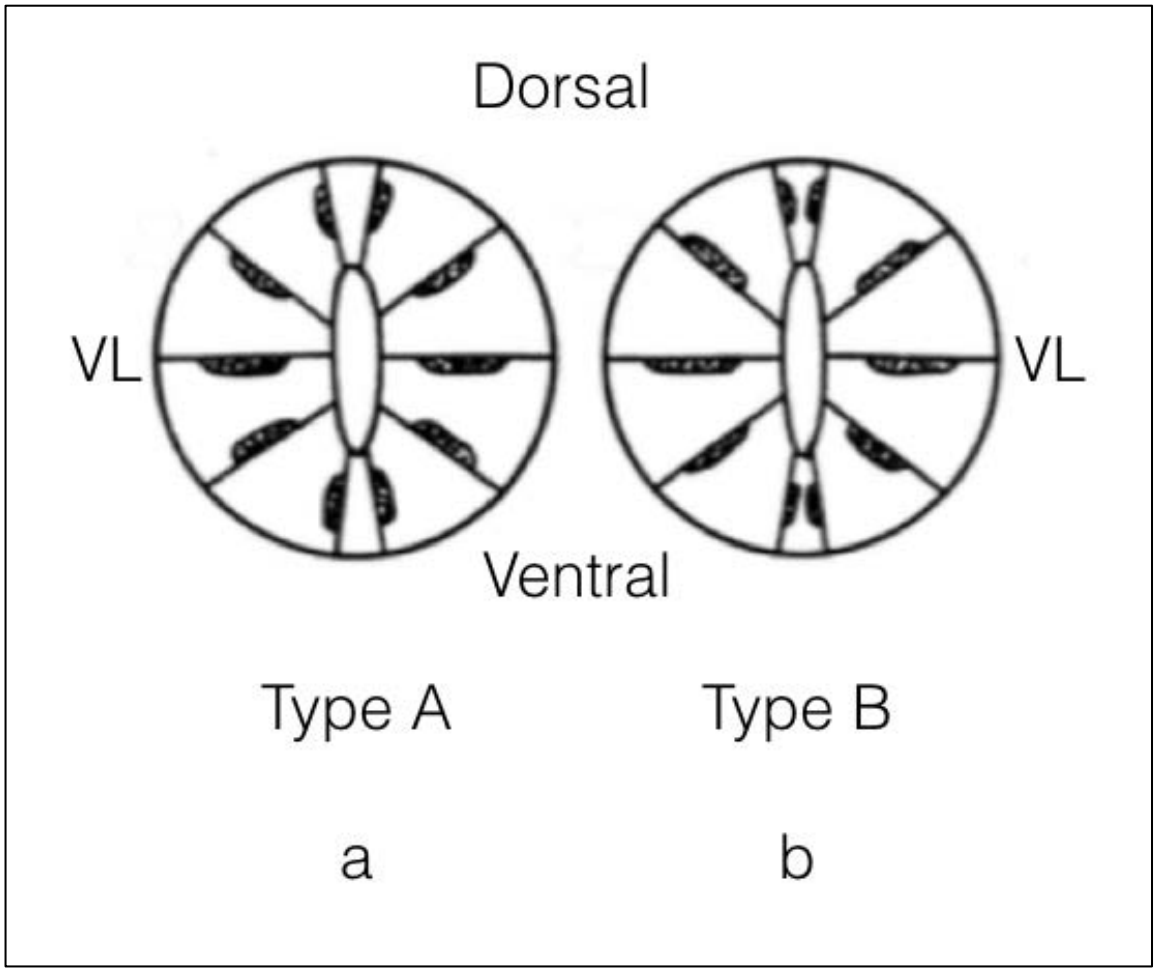

Figure 2.21. Retractor muscle distribution in antipatharian mesenteries by van Pesch (1914). Two types of distribution exist (upper part of the figures corresponds to the dorsal side while the lower part corresponds to the ventral side): **a** – type A: both directive mesenteries pairs have the retractor muscles pointing in opposite directions; **b** – type B: both directive mesenteries pairs have the retractor muscles pointing towards each other. The muscles of the ventro-lateral mesenteric pair (VL in the figure) are always directed towards the ventral side while the muscles in the secondary mesenteric pairs are in agreement with the directive pair of its own side (dorsal or ventral).

Development of the directive pairs

If we take into account the information from work of van Pesch about the retractor muscle distribution and also the work of Dantan about the order of mesenteric formation it corresponds to the stage of three mesenteric pairs to what happens in Actiniaria, namely to what happens in *Actinia nigropunctata* according to Lacaze-Duthiers (1872). As we can see in **figure 2.22**, in the **type A** of Antipatharia, the muscle disposition at the 3 mesenteric pair stage is exactly the same as in Actiniaria. For **type B** of Antipatharia the muscle disposition in the directive pairs is completely inversed in relation to type A of Antipatharia and to Actiniaria. Nevertheless the disposition of the retractor muscles of the primary pair to be formed (the ventro-lateral pair: **VL**) is always consistent as is its order of development also is. The organization for antipatharians at this stage of development clearly indicates a bilateral symmetry due to: i) mesenteric arrangement and ii) retractor muscles distribution.

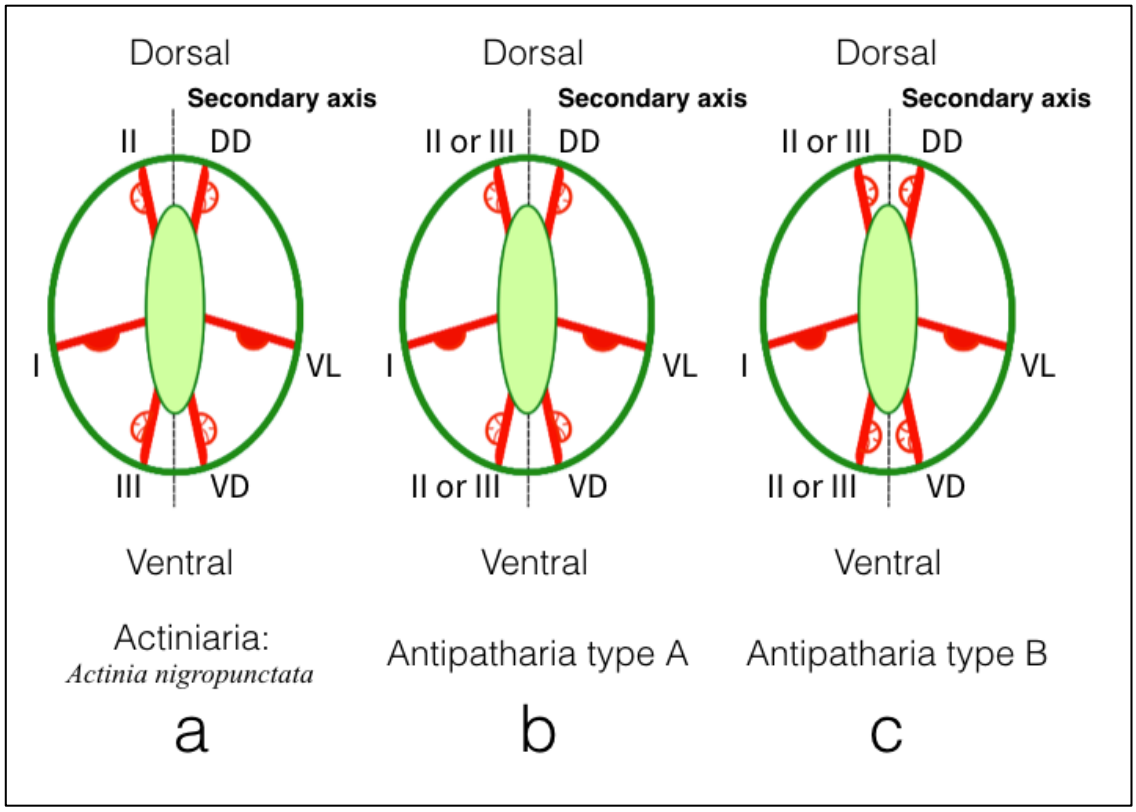

Figure 2.22. Schematic representation of the 3 mesenteric pairs developmental stage of **a** – actiniarian (Lacaze-Duthiers (1872); **b** – antipatharian type A; **c** – antipatharian type B.

Development of the 4th mesenteric pair

Antipatharians do not pass a clear "Edwardsia stage" (*i.e.* a developmental stage with 8 mesenteries) (Dantan 1921), because both 4th and 5th pair of mesenteries would form rather simultaneously and no clear order has been discriminated by authors. However from the so-called secondary pairs of antipatharians the only than can be compared with an "Edwardsian" pair of Actiniaria is the dorsal secondary pair, the one forming dorsally to the ventro-lateral pair. This is so because in Actiniaria the only mesenteric pair that exists in Edwardsia stage, other than the Ventro-lateral pair and both directive pairs forms dorsally to the Ventro-lateral pair. Dantan has represented in his figure (**see figure 2.17**) the pair that forms dorsally to the Ventro-lateral as the 4th pair, even if in its text he has not precise an order between the 4th and 5th pair.

In **figure 2.23** there is a schematic representation of the Edwardsia stage of Actiniaria (**a**) with all possible orders of mesenteric development and the representation for both types of Antipatharia, **b** and **c**. It is remarkable to see, that for Antipatharia type A, the disposition of the retractor muscles is exactly the same as in Actiniaria as happens in the previous phases of development. Antipatharia type B presents the same difference that it already showed in previous developmental phases, the inversion of retractor muscles in all but the Ventro-lateral pair (VL). In both antipatharian types at this stage of development a bilateral symmetry is present as the development of mesenteries is done in a bilateral way. From this we have that the so-called dorsal secondary pair of Antipatharia corresponds in fact to a **Dorso-lateral pair** (**DL**).

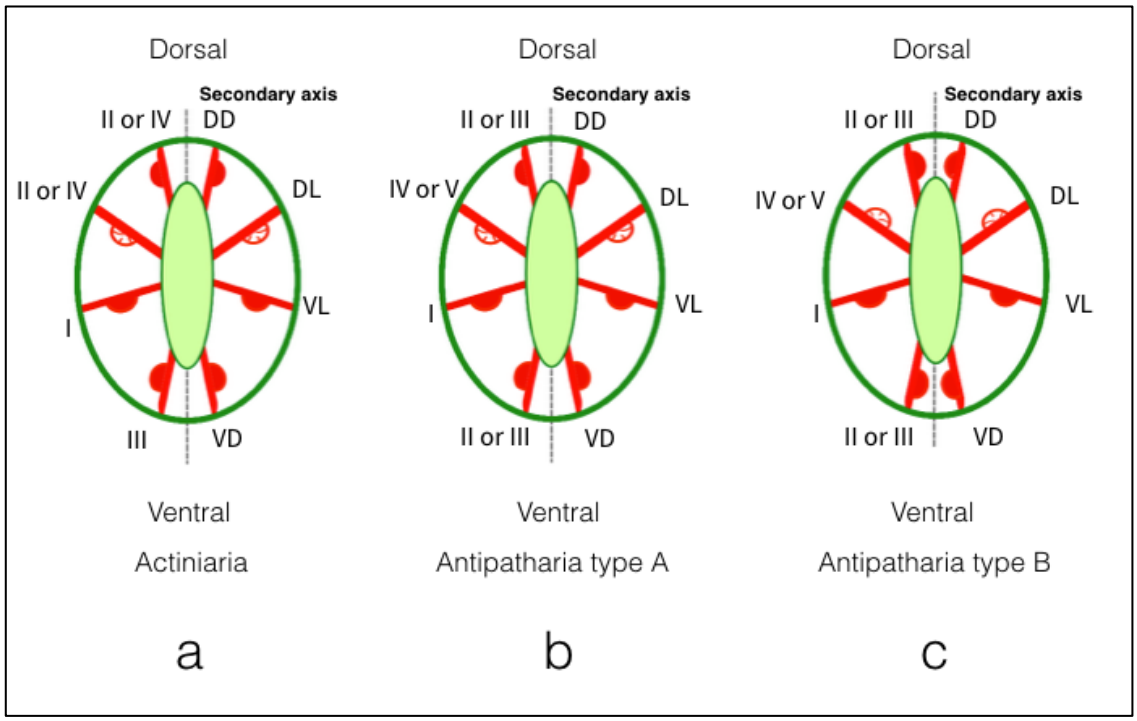

Figure 2.23. Schematic representation of the 4 mesenteric pairs phase for (**a**) Actiniaria – "Edwardsia stage"; (**b**) Antipatharia type A and (**c**) Antipatharia type B. The dorsal secondary pair will form immediately after the previous pairs, either before or accompanied by a 5th pair (not shown).

Development of the 5th mesenteric pair

In Actiniaria the 5th pair to form, according to McMurrich (1891), is the secondary ventro-lateral pair. Comparison can be made between Actiniaria and Antipatharia at 10 mesenteries stage (**figure 2.24**). The Type A of Antipatharia correspond perfectly once again to the 10 mesenteries developmental stage of Actiniaria. So perfectly, that in that this addition of a secondary pair of mesenteries in the ventral part of the animal forms the first lateral endocoels to be formed in both cases – between mesenteries of the Ventro-Lateral Pair (VL) and the Secondary Ventro-lateral pairs (SVL). **Type B** of antipatharians does not form an endocoel as in this antipatharian type the retractor muscles are inverted. **In both types polyps exhibit a bilateral symmetry**. **This case represents most adult polyps of antipatharians**. From the presented comparison comes that the so-called Secondary ventral pair of mesenteries in Antipatharia corresponds to the Secondary ventro-lateral pair of Actiniaria (SVL).

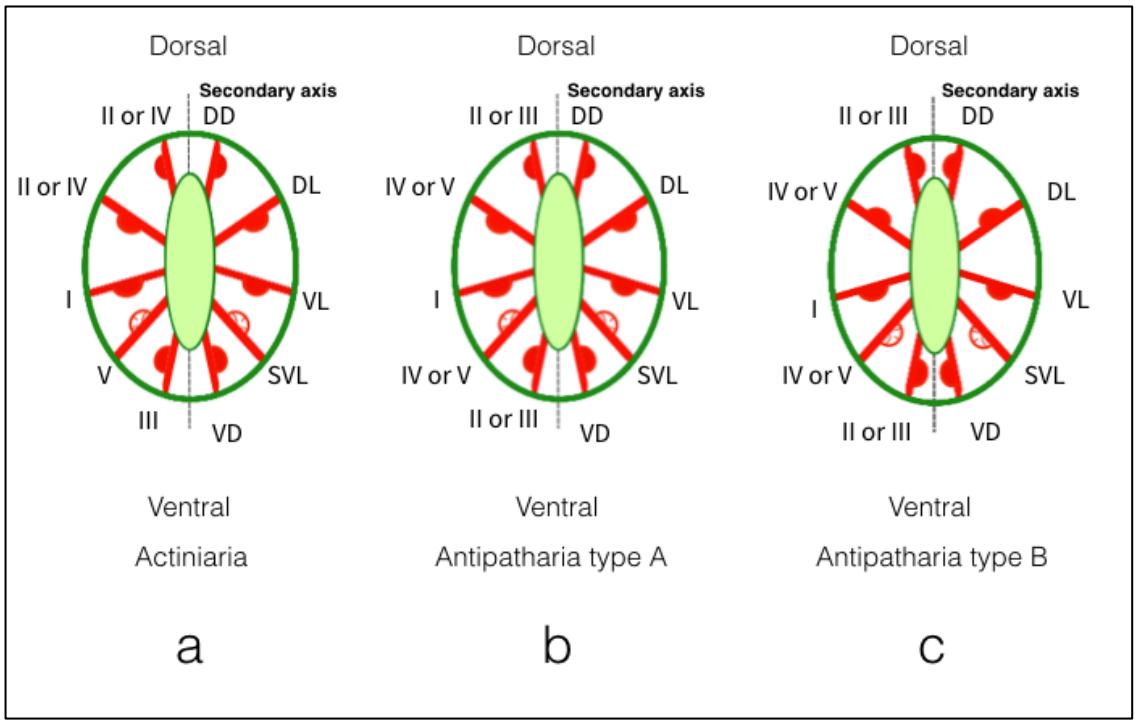

Figure 2.24. Schematic representation of the 5 mesenteric pairs phase for Actiniaria (**a**), Antipatharia type A (**b**) and Antipatharia type B (**c**).

Development of the 6th mesenteric pair

Only few species of antipatharians form a 6th pair of mesenteries. Van Pesch described this 6th pair in of *Cirrhipathes contorta* as mesenteries forming between the dorsal directive pair and the dorso-lateral pair and disproved of retractor muscles (**figure 2.25**).

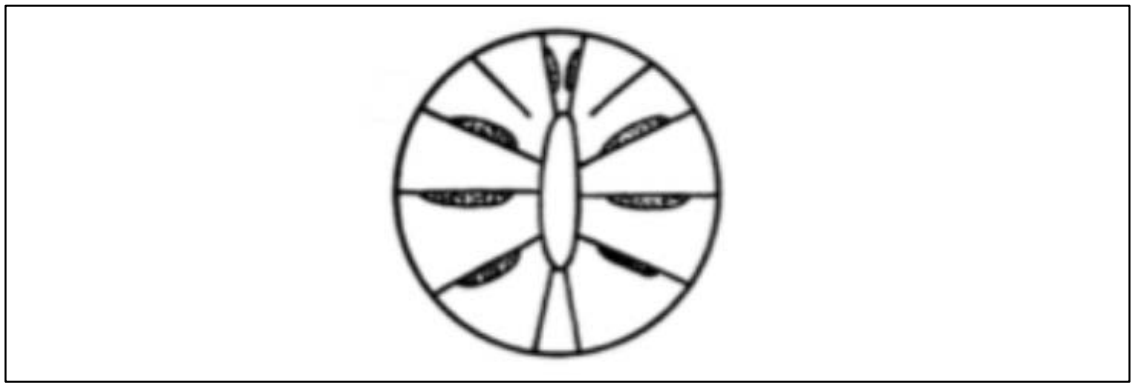

Figure 2.25. Schematic representation by Van Pesch of the mesenteries and associated retractor muscles in *Cirrhipathes contorta*, with 12 mesenteries, the supplementary pair is represented as not reaching the actinopharynx and between the dorso-lateral pair and the dorso-directive pair.

This additional 6th pair has not the same development as the others when present. This may reflect the fact that the dorsal part of the polyp is smaller than it would be for an Actiniarian polyp (relative size between dorsal and ventral compartment) due to the more central position of the Ventro-lateral pair (VL). This pair represents an extra pair in a dorsal side that has the same relative space that the ventral compartment. So, probably for space and functional constrains, this pair might have been lost for most antipatharian groups.

Dantan (**figure 2.17**) and van Beneden (1897) (**figure 2.26** – red arrows) described for *Leiopathes glaberrima*, the development of a 6th mesenteric pair between the ventro-lateral pair (VL) and the dorso-lateral pair (DL).

Another main difference exists between the description of van Pesch in *Cirrhipathes contorta* and van Beneden in *Leiopathes glaberrima*. In the first the 6th pair of mesenteries to form does not reach the pharynx, while in the later it does not reach the body wall. The absence of a retractor muscle together with this fact and the centred Ventro-lateral pair that leaves a dorsal compartment with less relative space than in Actiniaria may indicate that the 6th pair of mesenteries may have been lost in most antipatharians as it seems to be a vestigial organ.

As we do not know the retractor muscle disposition it is impossible to the organization of *Leiopathes glaberrima* as either a type A antipatharian or a Type B, We have then to consider both possibilities. For the 6th pair to form in Antipatharia we have various possibilities, two possibilities for the formation chamber (exocoel):

- Between the pair DD and DL – Type B antipatharian (van Pesch 1914).
- Between the pair VL and DL – Type A antipatharian or Type B antipatharian.

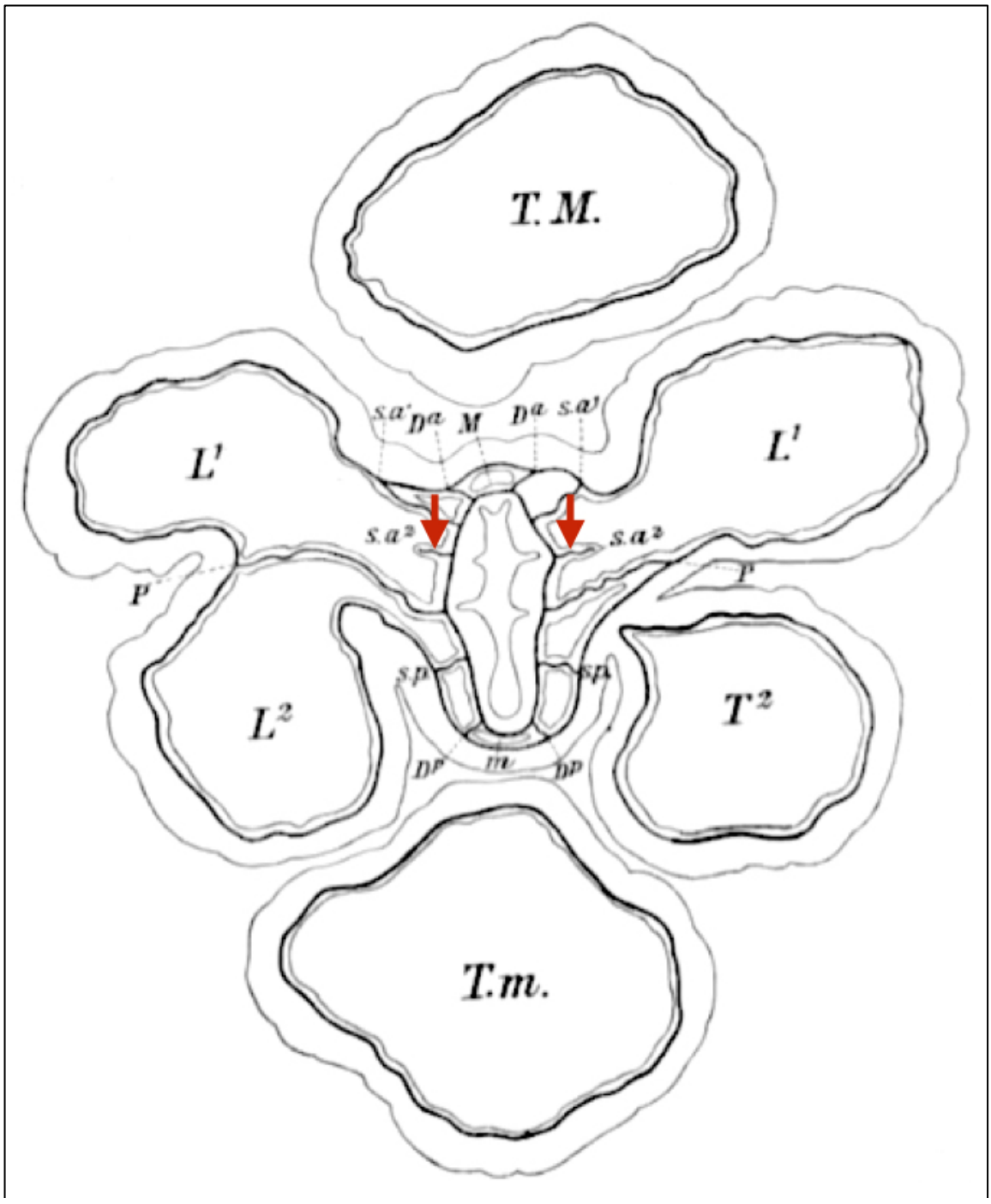

Figure 2.26: Representation from van Beneden (1897) showing a transversal cut of the polyp of *Leiopathes glaberrima*, represented are the six tentacles or corresponding mesenteric spaces around the oral cone and the 12 mesenteries present on this species. The 6th mesenteric pair (s.a^2 in the figure) forms between the primary mesenteric pair and the dorso-lateral pair. Legend of the figure is as van Beneden described in French: L^1 and L^2 – Lateral "*loges*"; T.M – *tentacule median anterieur*; T.m. – *tentacule median posterieur*; T^2 – *tentacule laterale posterieur*; M – *loge mediane anterieure*; m – *loge mediane posterieure*; P – *cloisons primaires*; D^a – *cloison primare anterieure*; D^p – *cloison primaire posterieure*; s.a.^{1} – *cloison assessoire anterieure*; s.p. – *cloisson assesoire posterieure*; s.a.^{2} – *cloisson assesoire anterieure supplementaire*.

The schematic representation of these possibilities can be found in **figure 2.27**, considering that the 6th pair of mesenteries to form in Antipatharia goes from the pharynx until the body wall, together with the representation of the "Halcampoides stage of development" of actiniarians. From this figure that represents the possible 12 mesenteric phase of development in antipatharians and actiniarians we can see than as we do not have or know the retractor muscle disposition that may be associated with the 6th pair of mesenteries of antipatharians the comparison to Actiniarians is harder to make. If *Leiopathes glaberrima* is a type A antipatharian the new formed mesenteries will form couples with the Dorso-lateral pair (**DL**) and so result in a Halcampoides stage, attaining a bi-radial symmetry (**figure 2.27b**).

For Antipatharia type B independently of the chamber where pair 6 will form no radial symmetry will come out of the possible 6th pair development, as the retractor muscles of the mesenteries formed dorsally to the VL pair of type B antipatharians are always disposed towards the ventral side.

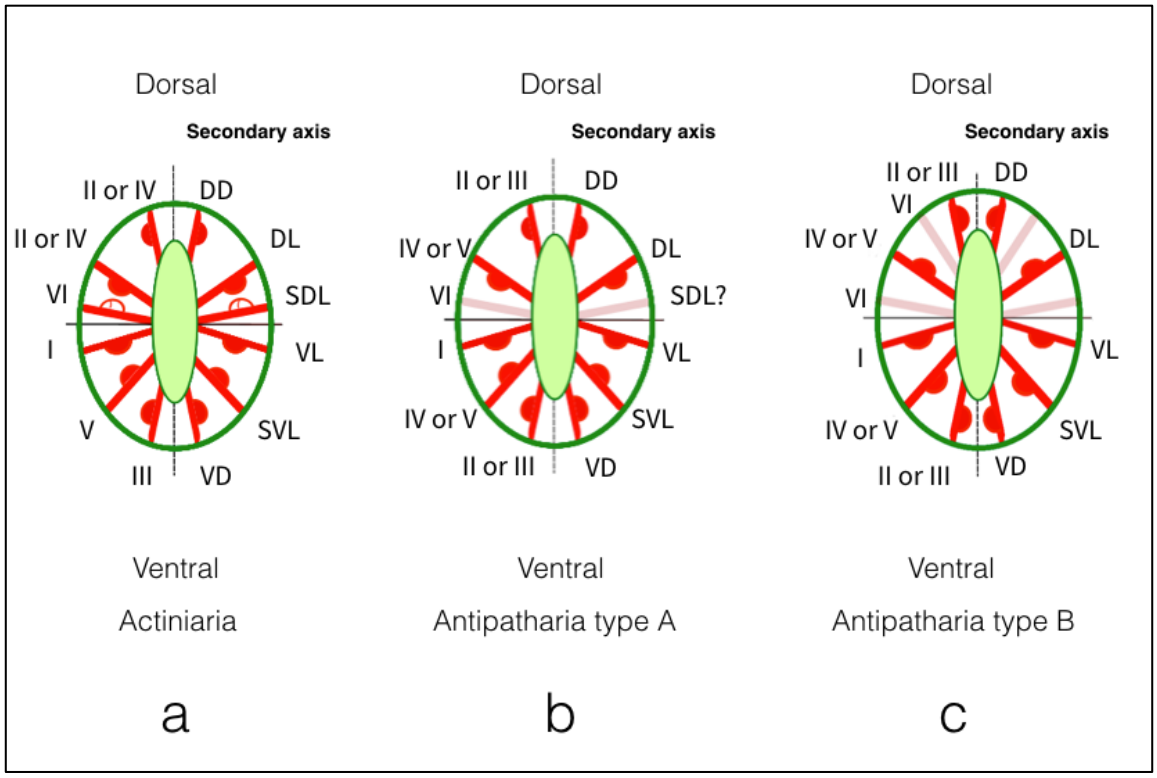

Figure 2. 27. Representation of the 12 mesenteric phase of development of Actiniaria (**a**); Antipatharia type A (**b**) and Antipatharia type B (**c**). In rose there are the possible locations of the 6th mesenteric pair for antipatharians according to available literature, for type B two possibilities are present, as the literature does not allow to discern between both hypotheses.

Homology between the secondary axis of Antipatharia and Actiniaria and its polarity

The homology between the secondary axis (and its polarity) of antipatharians and actiniarians is easily accessed when we compare the different developmental stages between both groups. The order of mesenteric formation is congruent between the findings of Dantan and the first phases of actiniarian development. Also **the dorsal pole and ventral pole are homologous** due to the position of the retractor muscles in the first mesenteric pair to be formed, the different sized compartments that take existence after it's formation and the disposition of the retractor muscles that is easily comparable between type A of Actiniaria and Antipatharia. Dantan (1921) and Brook (1890) defend a homology between the secondary axis of antipatharians and actiniarians, arguing that they do correspond.

Dantan argues that the first pair of mesenteries to form (the ventro-lateral pair **VL**) is homologous between these groups and then compares *Leiopathes glaberrima* with Actiniaria: "*Je crois donc que les mésentères de cet Antipathaire peuvent être considérés comme homologues de ceux du stade Halcampula*".

Brook (1890) also argued for the homology between the first pairs formed in actiniarians and antipatharians. He added that the 6th pair to be formed in *Leiopathes glaberrima* was the last of the six pairs to be developed in *Cereus pedunculatus* (Pennant, 1777). He adds that as the forming pairs are disposed in both sides in relation to the secondary axis we have "*in forms with an elongated stomodaeum a true bilateral symmetry*" with the polyp divided into any number of paired lateral chambers.

Tentacle disposition in Antipatharia

The tentacle disposition of antipatharians do not vary, they always possess six tentacles, two sagittal tentacles corresponding to the directive chambers and that are usually placed at a lower level (van Pesch 1914) and four lateral tentacles corresponding to the four primary lateral chamber of the six mesenteries stage. The secondary

mesenteries that are added, forming new chambers do not imply new tentacles. The relationship between the chambers and tentacles can be seen in **figure 2.28** by van Beneden for antipatharians with 10 chambers (**a**) and antipatharians with 12 chambers (**b**).

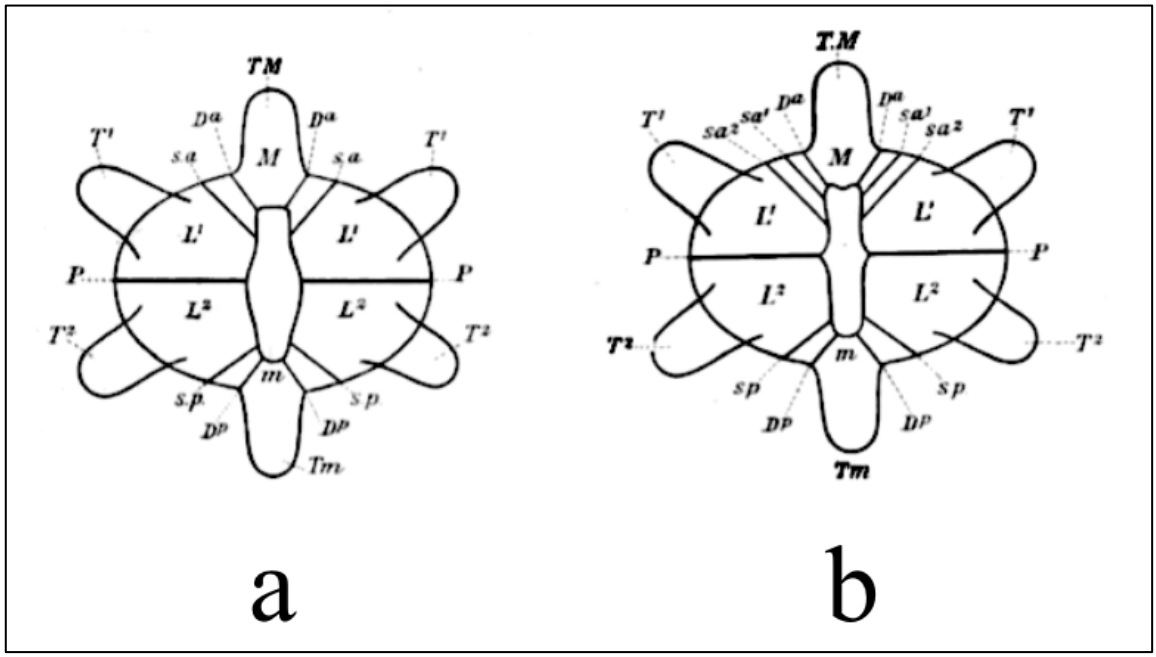

Figure 2.28. Representation from van Beneden (1897) showing the relationship between the tentacles and the mesenteric chambers in **a** – *Antipathella subpinnata* and *Parantipathes larix*; **b** – *Leiopathes glaberrima.* Legend of the figure is as van Beneden described: L^1 and L^2 – Lateral "*loges*"; T.M – *tentacule median anterieur*; T.m – *tentacule median posterieur*; T^1 – *tentacule lateral anterieur*; T^2 – *tentacule laterale posterieur*; M – *loge mediane anterieure*; m – *loge mediane posterieure*; P – *cloisons primaires*; D^a – *cloison primare anterieure*; D^p – *cloison primaire posterieure*; s.a.^{1} – *cloison assessoire anterieure*; s.p. – *cloisson assesoire posterieure*; s.a.^{2} – *cloisson assesoire anterieure supplementaire*.

<u>On the presence or absence of ciliated grooves in Antipatharia</u>

It is important to note that besides van Beneden (1897) no other author identified a special ciliated groove that could correspond to a siphonoglyph in Antipatharia. Van Beneden identified a groove that would continue after the lower part of the pharynx as a hyposulcus (ciliated groove present in some Ceriantharia). No other author found a siphonoglyph or any other specialized groove in an antipatharian (Brook 1889; Dantan 1921). Roule (1905) did not find any specialized groove and said: "*(...) Cette disposition donne à ses deux extrémités l'aspect de rainures, de gouttières, mais ne*

semble point avoir d'autres conséquences. La présence de ces deux gouttières est entrainée par l'aplatissement de l'organe qui les contient; aussi ces sillons, contrairement à leurs similaires de la plupart des autres Anthozoaires, ne paraissent-ils point avoir d'invdividualité bien précise, ni possséder des parois propres qui les délimiteraient latéralement." He also does not see any difference between both poles of the pharynx. In 1989, Goldberg and Taylor, found, by histological means, no differentiated groove or siphonoglyph in *Antipathes aperta* (Goldberg and Taylor, 1989b). More recently Molodtsova (2005) found no siphonoglyph or difference between the two extremities of the pharynx in *Parantipathes euantha*. The description of a difference between both poles of the pharynx by van Beneden might be the consequence of searching for a difference and that he did considered Antipatharia to belong together with Ceriantharia in the Ceriantipatharia and might then have found something that he identified as a hyposulcus. He does not however present any image in his monograph that clearly shows this. It seems rather likely that antipatharians do not present this structure.

2.2.1.3 Scleractinia

Scleractinians (**figure 2.29**) are abundant in the shallow waters of tropical environments and are the main responsible for the formation of coral reefs, one of the world richer ecosystems. More than 1500 species of Scleractinia have been described (Appeltans *et al.* 2012). This group mostly comprises colonial species like *Plerogyra sinuosa* (**figure 2.29a**), but they can also be solitary, attached to the seabed (**figure 2.29b**) or not (**figure 2.29c**). The funding individual of a colony settles on the seabed and starts to secrete calcium carbonate to protect its soft body. In this colony, the polyps reproduce by budding, remaining attached to each other forming a multi-polyp colony with a common skeleton.

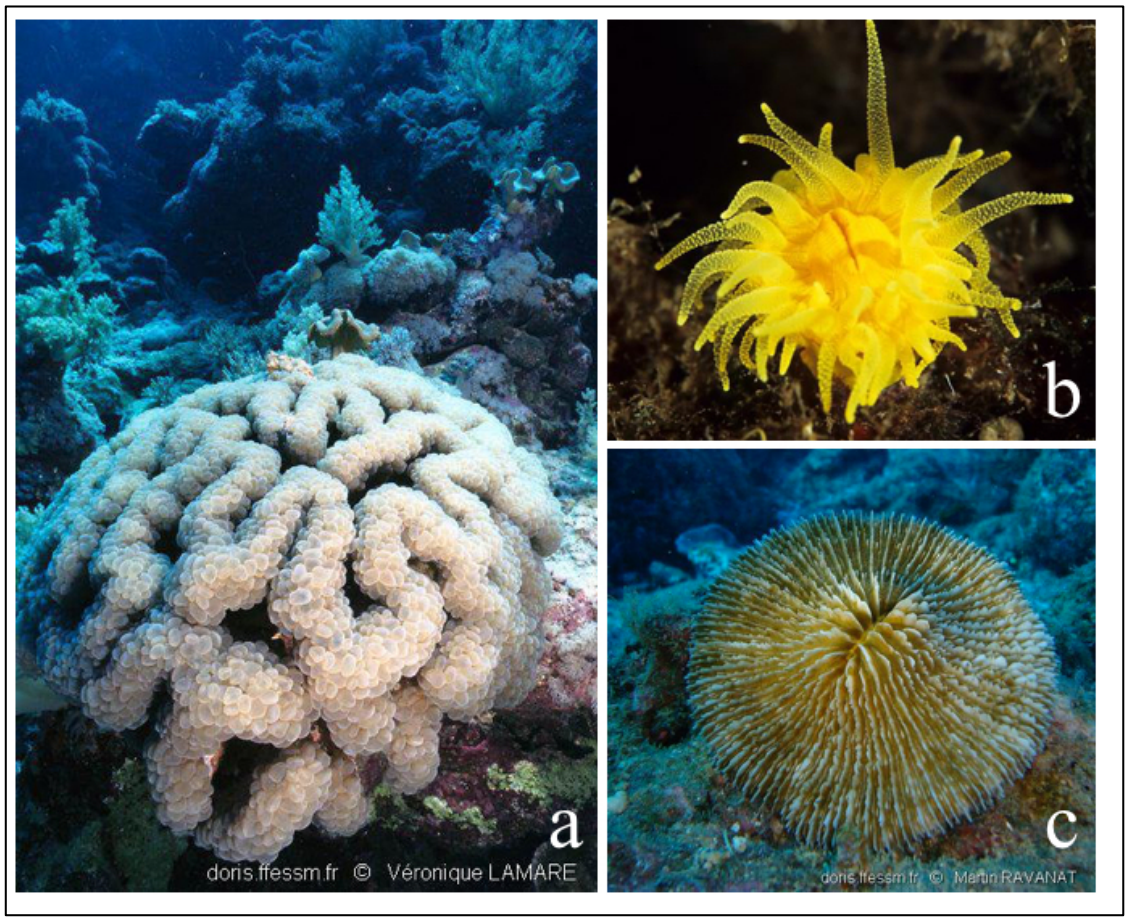

Figure 2.29. Scleractinia representatives: **a** – colony of *Plerogyra sinuosa* (Dana, 1846); **b** – the solitary *Leptopsammia pruvoti* **(**Lacaze-Duthiers, 1897) is attached to the seabed by a calcareous cup; **c** – the solitary *Danafungia horrida* (Dana, 1846) is not attached to the seabed. Images from DORIS (http://doris.ffessm.fr), by Véronique Lamare (a and b) and Martin Ravanat (c).

The development of the Scleractinians (**figure 2.30)** is completely similar to one type of development described in Actiniaria (see **figure 2.31** and **figure 2.32**) in terms of order of mesenteric development and muscle orientation (Wilson 1888 studies in *Manicina areolata*; Duerden 1904 studies in *Siderastrea radians*; Mergner 1971, after Gemmil 1922). There is in the "*Edwardsian stage*" (**figure 2.30**) and the "*Halcampoides stage*" (**figure 2.32**) the same exact order of mesenteric development and the same retractor muscle orientation as in Actiniaria type I (see **figure 2.9**) with the exception of the development order for the secondary lateral pairs for which the order is inverted with the secondary dorso-lateral pair (SDL) to be formed first in Scleractinia. The addition of mesenteries takes place after this phase by the addition of mesenteric couples and not of mesenteric pairs. This addition will take place in the exocoels in a

dorso-ventral fashion, from dorsal to the ventral side (**figure 2.30d** to **2.30f**). This addition of couples will take place in waves of mesenteric couples addition, a second and a third wave are represented in the figure (respectively from **figure 2.30g** to **2.30i**) with a wave of mesenteric couples forming dorsally to the first wave and finally in **figure 2.29j** a wave develops ventrally to the first wave. **Due to important similarities between Scleractinia and Actiniaria in mesenteric development homology between their secondary axis and respective poles is well established**.

The scleractinians polyps attach to the colony by the formation of septa[§§] that will allow the polyp to be correctly attached to the skelet. These septa are skeleton formation directly under the polyp and their form and development are strongly correlated to the mesenteric arrangement. The development of septa, as the mesenteries, also takes place from dorsal to ventral (**figure 2.33a**). In the end of each wave of septal development a radial symmetry seems to take place momentaneously (**figure 2.33b**) but that bilaterality can still be observed in later phases of septal development, even when different septa from different developmental waves start to fuse (**figure 2.33c**). The tight relation with the formation of mesenteries and their disposition can be observed in **figure 2.33d**.

[§§] Vertical divisions of the skelet that sit below the polyp are called septa (singular is septum). The septa are often distinctive and may be used to differentiate between genera.

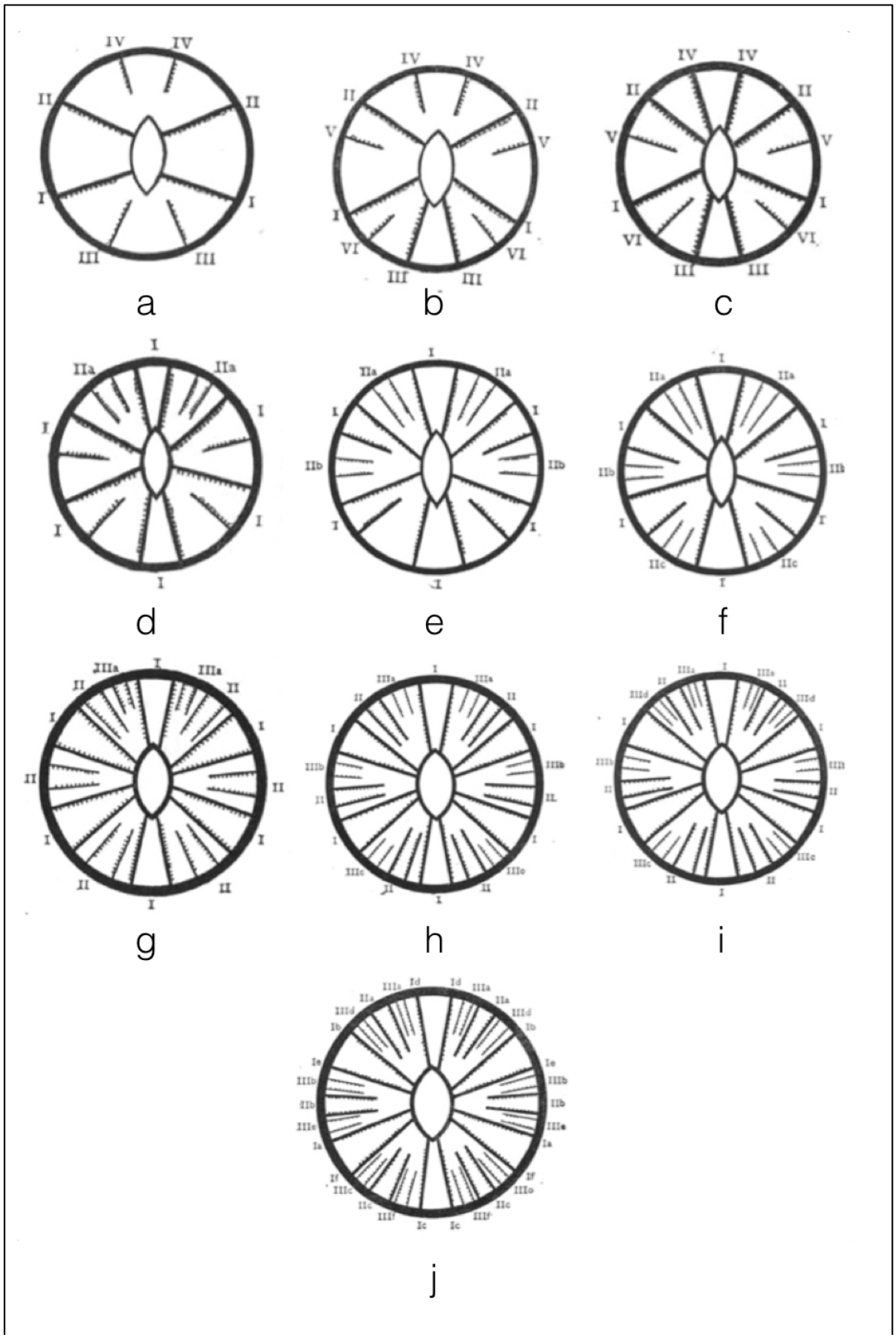

Figure 2.30. Schematic representation of the scleractinian mesenteric development adapted from Duerden 1904 are oriented top to bottom as dorsal to ventral: **a** – "*Edwardsia stage*" with 4 mesenteric pairs (8 mesenteries); **b** – "*Halcampoides stage*" with 6 mesenteric pairs (12 mesenteries); **c** – "*Halcampoides stage*" with the growth of the dorsal directive pair until the pharynx; **d** to **f**: insertion of the first wave of

mesenteric couples from dorsal to ventral; **g** to **i** – insertion of a second wave of mesenteric couples dorsally from the first wave, in g we have also the growth of all mesenteric pairs until the pharynx; **j** – growth of a third wave of mesenteric couples ventrally to the first mesenteric couples wave. From a to c the roman numbers correspond to the order of mesenteric pairs formation; from d to j roman numbers correspond to the order of formation of the endocoels.

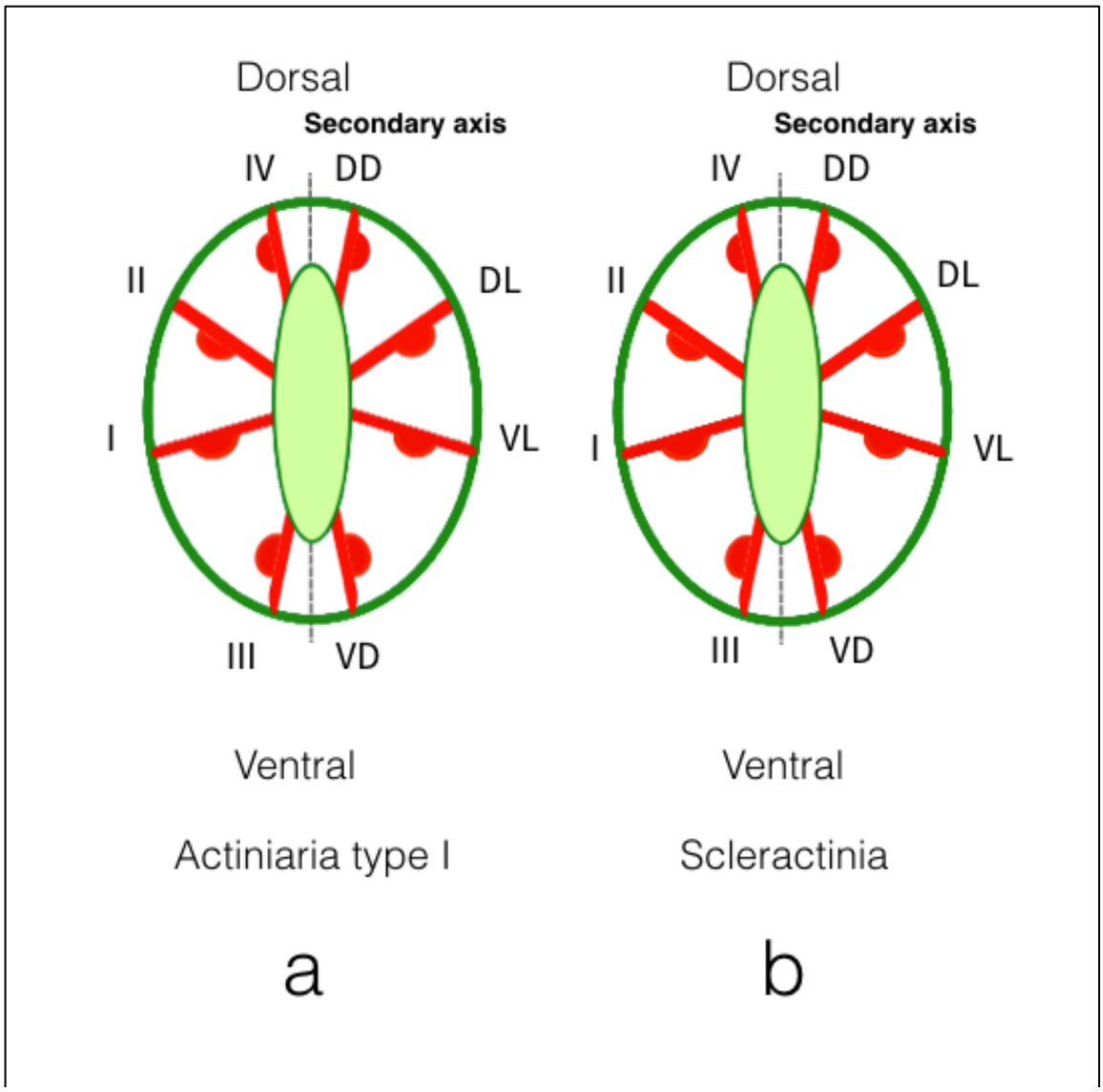

Figure 2.31. Schematic representation of the "*Edwardsia stage*" of development of Actiniaria type I and Scleractinia, **a** – Actiniaria type I; **b** – Scleractinia.

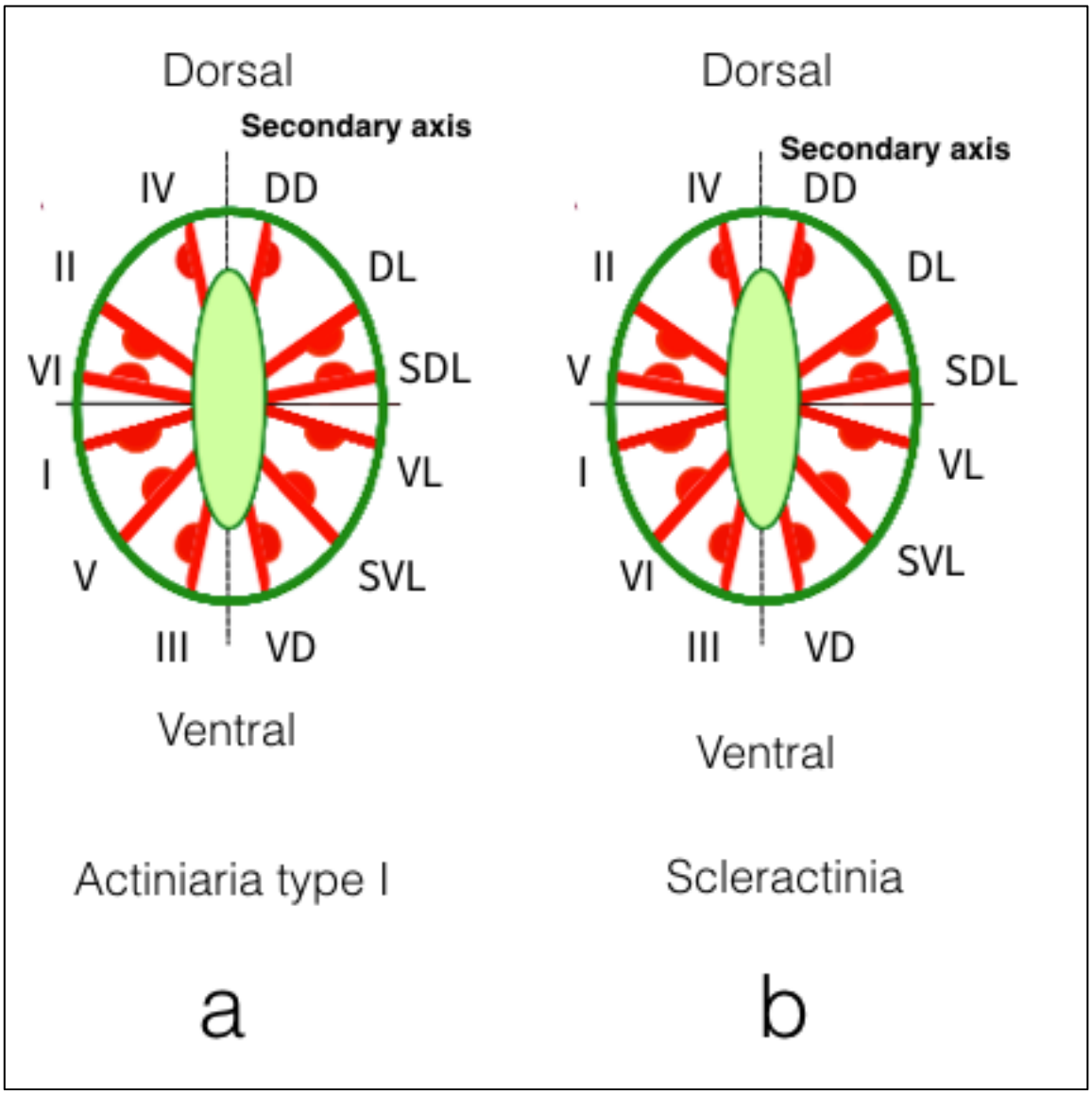

Figure 2.32. Schematic representation of the "*Halcampoides stage*" of development of Actiniaria type I and Scleractinia **a** – Actiniaria type I; **b** – Scleractinia. In **a** and **b** an additional axis the transverse axis is represented in a right angle to the secondary axis, at this stage of development also this axis is a symmetry line for the mesenteric arrangement of scleractinians.

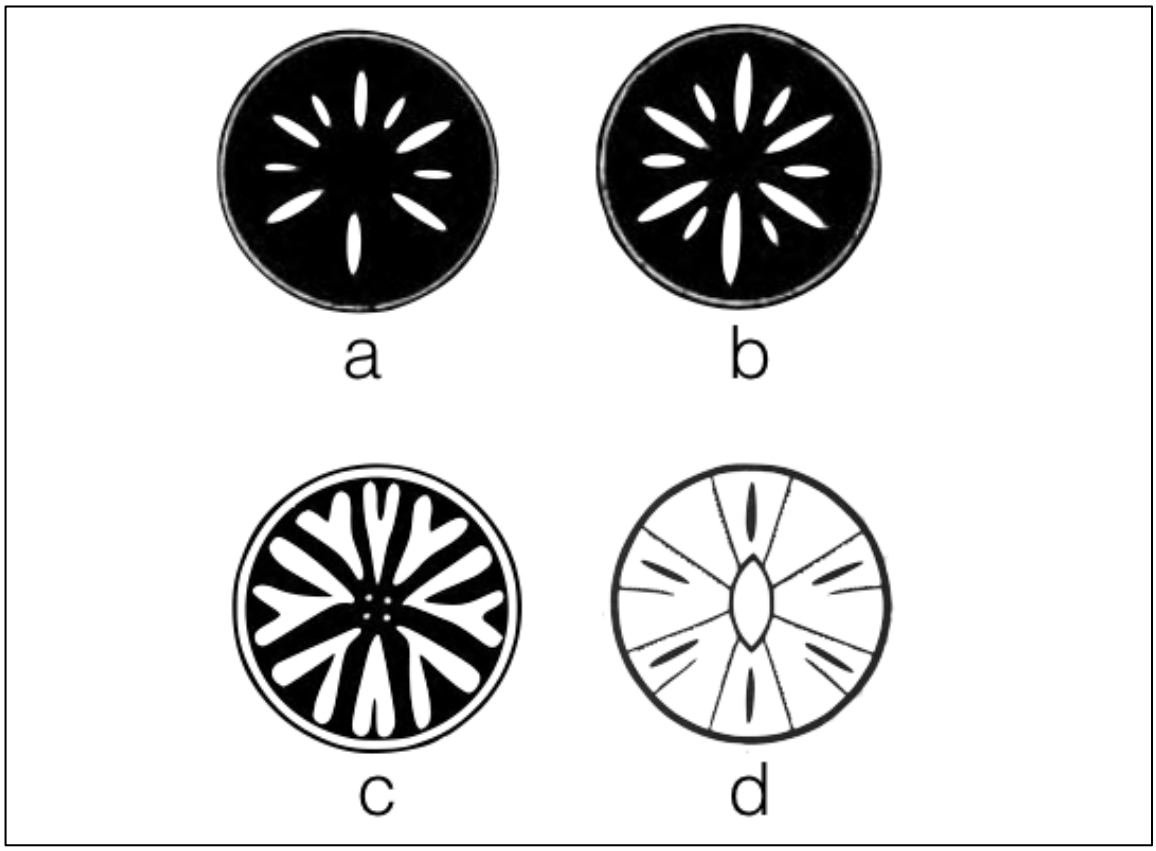

Figure 2.33. Schematic representation of the septa arrangement in the scleractinian species *Siderastrea radians* by Duerden 1904: a – The addition of septa is also done in dorsal to ventral waves with the septa of the same order appearing first in the dorsal parts and only later in the ventral parts; b – end of a wave of septal development; c – later phase of septal development with the septa fusing, still in this phase the bilateral arrangement is visible; d – schematic representation showing how the first wave of septa is placed in relation to the "*Halcampoides stage*" mesenteries.

2.2.1.4 Zoanthidea

With more than 100 described species Zoanthidea are one of the smallest anthozoan groups in terms of species (Appeltans *et al.* 2012). One of the classical species *Parazoanthus axinellae*, the yellow encrusting anemone, from the Atlantic Ocean and the Mediterranean Sea, is illustrated in **figure 2.34**. They present a ventral siphonoglyph (**figure 2.35**).

Figure 2.34. *Parazoanthus axinellae* (Schmidt, 1862) is one of the most common Zoanthidea, being usually called "Yellow encrusting anemone" or "*Mimosa de mer*" in French, can be found in the Atlantic ocean and Mediterranean sea. Photo from DORIS (http://doris.ffessm.fr) by Denis Ader.

Zoanthidea order of mesenteric development is well establish in literature (Herberts 1987) and presents some differences in relation to the actiniarian development, the two main differences are:

- The proliferation of supplementary mesenteries (after the first 6 mesenteric pairs) (**figure 2.35**) – is restrained to two ventral zones (exocoels) just outside the ventral directive mesenteries (VD). This results that the **adult zoanthid polyp always maintains a bilateral symmetry**.
- The dorsal directive mesenteries (DD) form in 6th and then no "*Edwardsia phase*" *stricto sensu* exists in Zoanthidea. As the pair corresponding to the secondary lateral mesenteric pairs (SDL and SVL)

of the "*Halcampoides phase*" are here developed in 4th and 5th and before the dorso-directive pair (DD) that is developed in 6th.

- Their dorsal directive mesenteries (DD) are incomplete, meaning that they do not reach the pharynx (**figure 2.35**).

The dorsal directive pair of mesenteries is incomplete in opposition to what happens in other actiniarian orders. Its developmental order is also different has indicated. These characteristics of the dorsal directive pair are then certainly derived in this group in relation to the rest of Anthozoa.

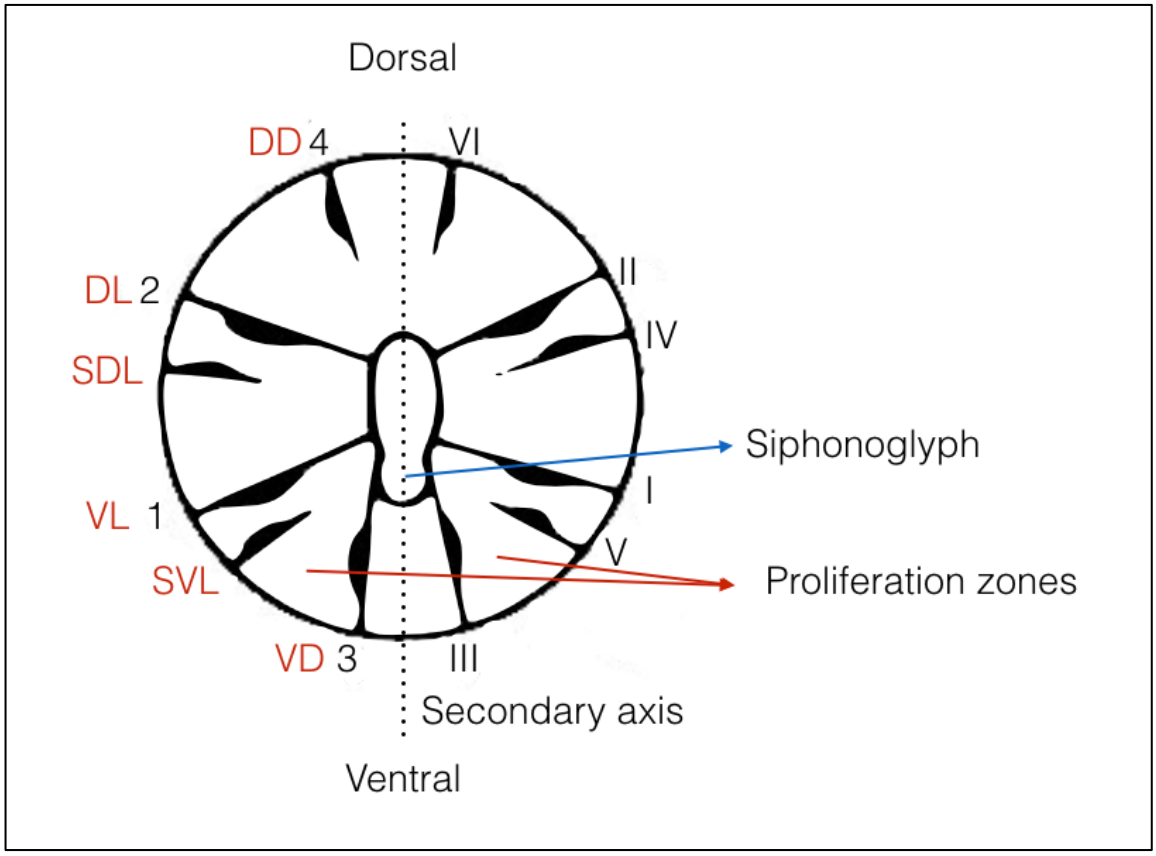

Figure 2.35. Schematic representation of the Zoanthidea mesenteric development. Zoanthidea present a single ventral siphonoglyph, two mesenteric proliferation zones where mesenteries will form after the 12 mesenteric stage (represented in the figure) between the ventro-directive pair (VD) and the ventro-lateral pair (VL). They also present an incomplete dorso-directive pair (DD), as this pair does not reach the pharynx. The order of mesenteric development is in roman numerals. The Arabic numerals correspond to the order of formation of the mesenteric pairs not counting with the secondary lateral pairs (SDL and SVL). Original figure from: Chisholm, H**.** (1911) from *The Encyclopedia Britannica*.

If representing the edwardsian mesenteries of Actiniaria and the corresponding ones in Zoanthidae (**figure 2.36**) we can see that we have a different order of mesenteric pairs development (in the figure, for Zoanthidea the order corresponds to the order of formation between the "edwardsian mesenteries"). However besides this order difference we can see that the disposition of the mesenteries and the orientation of the respective retractor muscles is the same.

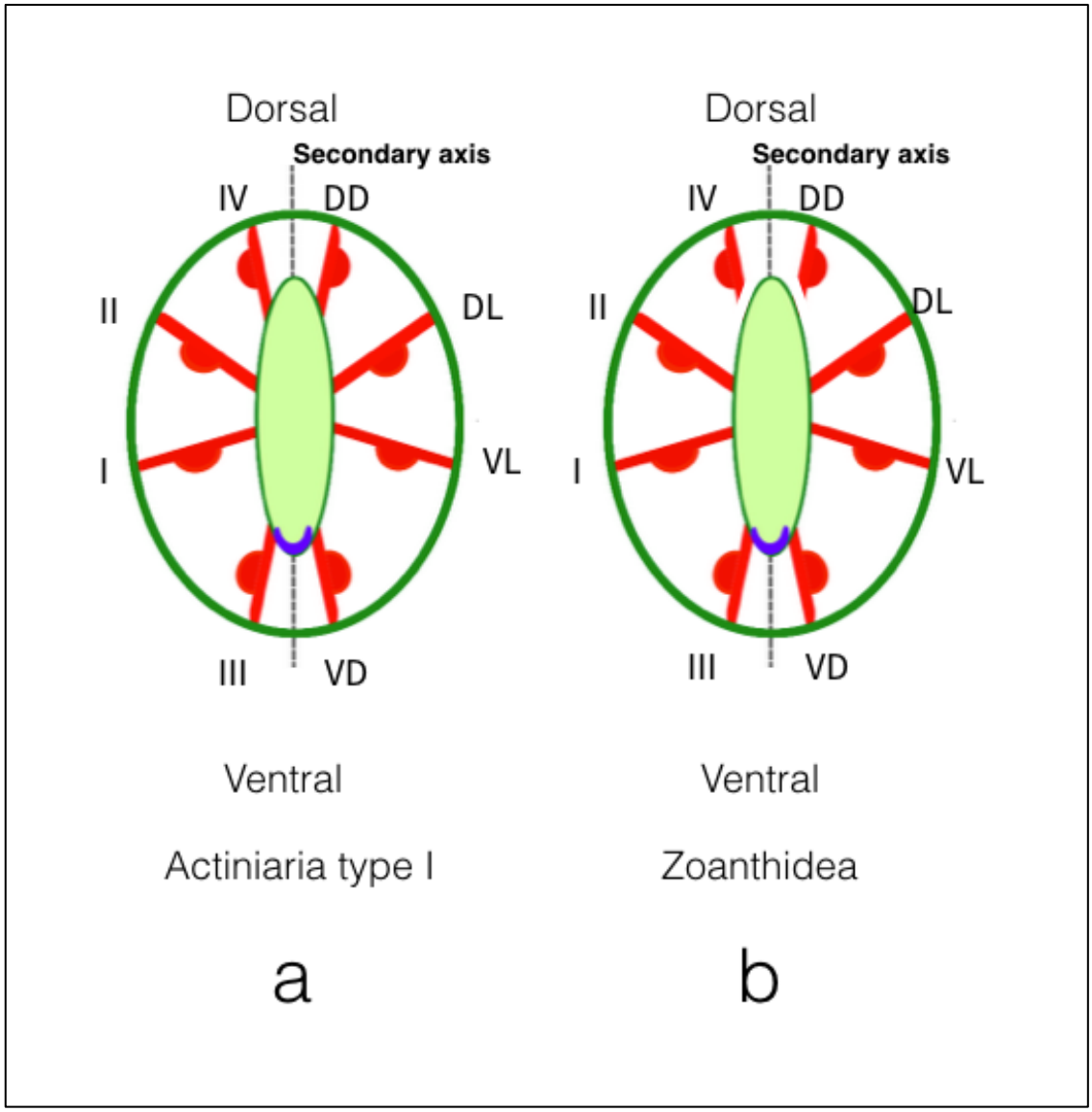

Figure 2.36. Schematic representation of the Actiniaria type I and Zoanthidea mesenteric development of the directive and primary lateral mesenteries, corresponding to the ones present in the "*Edwardsia stage*". The order of mesenteric development varies but the disposition of mesenteries and the orientation of the respective retractor muscles is the same.

Comparing the "*Halcampoides stage*" (**figure 2.37**) we can notice that the orientation of the mesenteric pairs and respective retractor muscles is the same. The only difference is on the order of development of mesenteric pairs, namely it corresponds to the development of the secondary lateral pairs (SVL and SDL) before the Dorso-directive pair (DD) in relation to what happens in Actiniaria type I and in Scleractinians (**figure 2.34**).

The first pair of mesenteries to be form (VL) is as in Actiniaria, asymmetrically formed along the secondary axis, resulting in two different sided compartments as in Actiniaria (**figure 2.36**). The smaller corresponds to the side of the siphonoglyph and the retractor muscles of the first mesenteric pair are directed to the siphonoglyph (**figure 2.36b**). During all their developmental stages Zoanthidae polyps conserve a bilateral symmetry. A radial symmetry is attained at the 12 mesenteries stage ("Halcampoides") but as they possess a single ventral siphonoglyph this bi-radial symmetry is only partial and will be disrupted as soon as new mesenteries form in the proliferation zones.

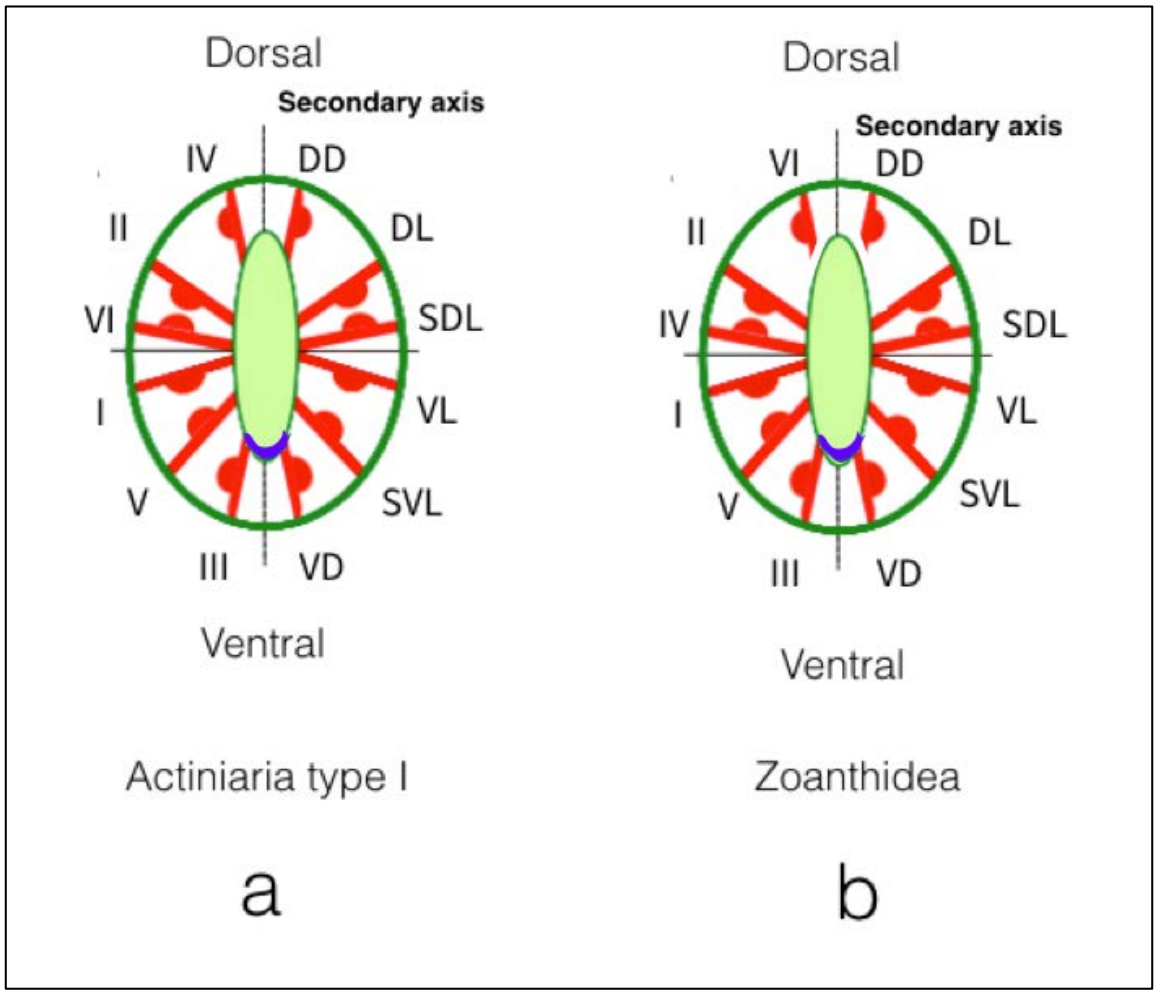

Figure 2.37. Schematic representation of the Actiniaria type I and Zoanthidea mesenteric development at the 6 mesenteric pairs (12 mesenteries) stage ("*Halcampoides stage*"). The order of mesenteric development varies but the disposition of mesenteries and the orientation of the respective retractor muscles is the same. The difference corresponds to the development of the secondary lateral pairs (SVL and SDL) before the Dorso-directive pair (DD). Zoanthidea present a single siphonoglyph and thus a true bilateral symmetry is never attained.

2.2.1.5 Corallimorpharia

Corallimorpharia is a small order of hexacorallians closely related to Scleractinia with less than 50 described species (Appeltans *et al.* 2012). Mostly tropical, they usually have a narrow body column topped by a wide oral disc and short tentacles (**figure 2.38**).

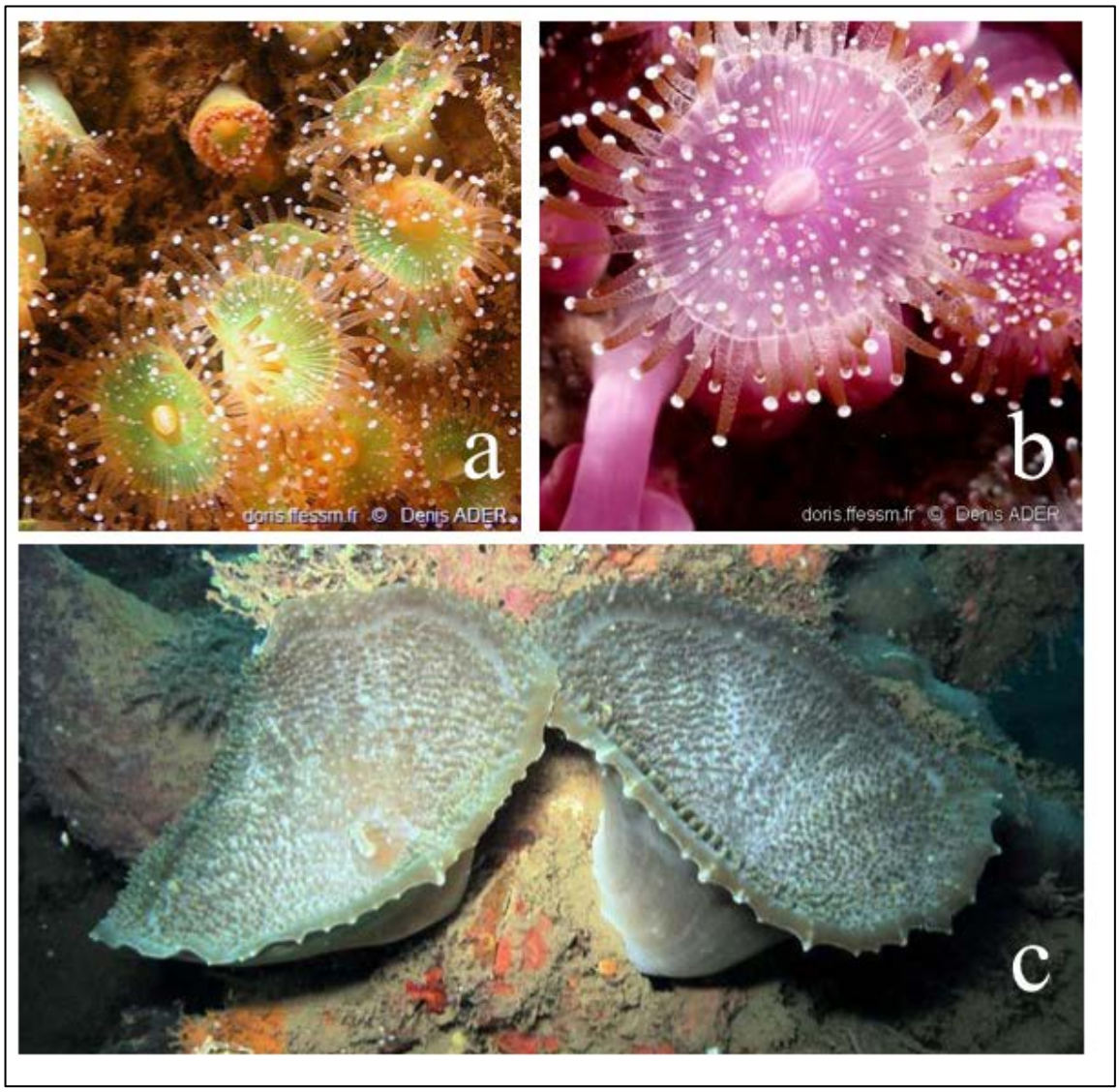

Figure 2.38. Two corallimorpharian species: **a** and **b** show different colour morphs of the species *Corynactis viridis* (Allman, 1846) and **c** shows two polyps of *Amplexidiscus fenestrafer* (Dunn & Hamner, 1980). Photos from DORIS (http://doris.ffessm.fr), a and b by Denis Ader and c by Sylvain Le Bris.

The mesenteric arrangement and retractor muscles disposition for this group has been described as similar to the scleractinian one (Daly 2003). Even if no major studies have been made in members of this order, McMurrich showed in 1891 that the order of formation of the first four pairs of mesenteries of *Rhodactis osculifera* (Le Sueur, 1817) corresponds to the order of formation that its found in Actiniaria type I (McMurrich 1891). First the ventro-lateral pair (VL), followed by the dorso-lateral pair (DL). In third there is the addition of the ventro-directive pair (VD) and finally the dorso-directive pair (DD) (**figure 2.39**).

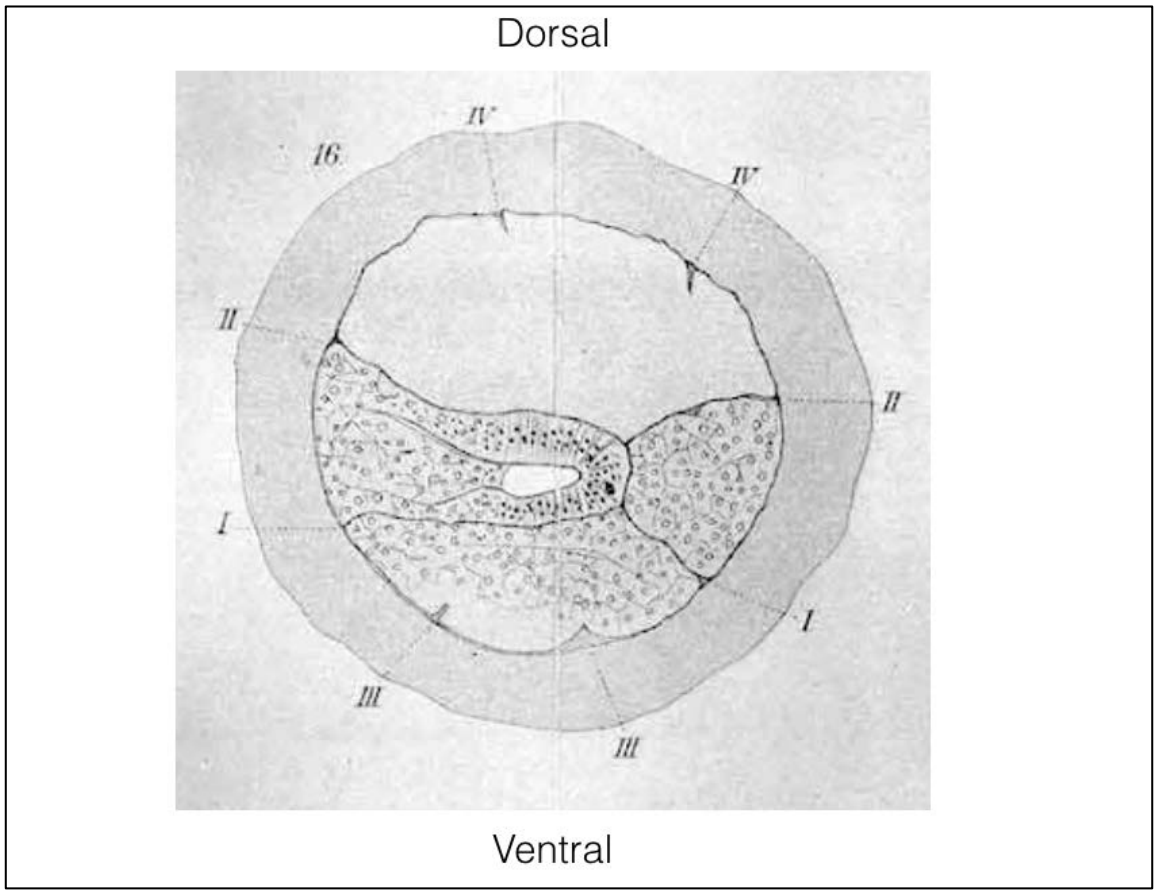

Figure 2.39. Original illustration, representing a phase of the embryogenesis of a corallimorpharian – *Rhodactis osculifera* (Le Sueur, 1817) by McMurrich in 1891, showing the order of mesenteric formation until the 4th mesenteric pair.

The disposition of the retractor muscles is not available in McMurrich descriptions, however as referred it is the same as in scleractinians (Daly *et al*. 2003). It is possible to schematically compare the 4 mesenteric pair stage of development of Corallimorpharia and that of Actiniaria type I (**figure 2.40**). As evident in the figure the development of Corallimorpharia is the same as Actiniaria type I, and in consequence of Scleractinia and almost similar to the development of Zoanthidea.

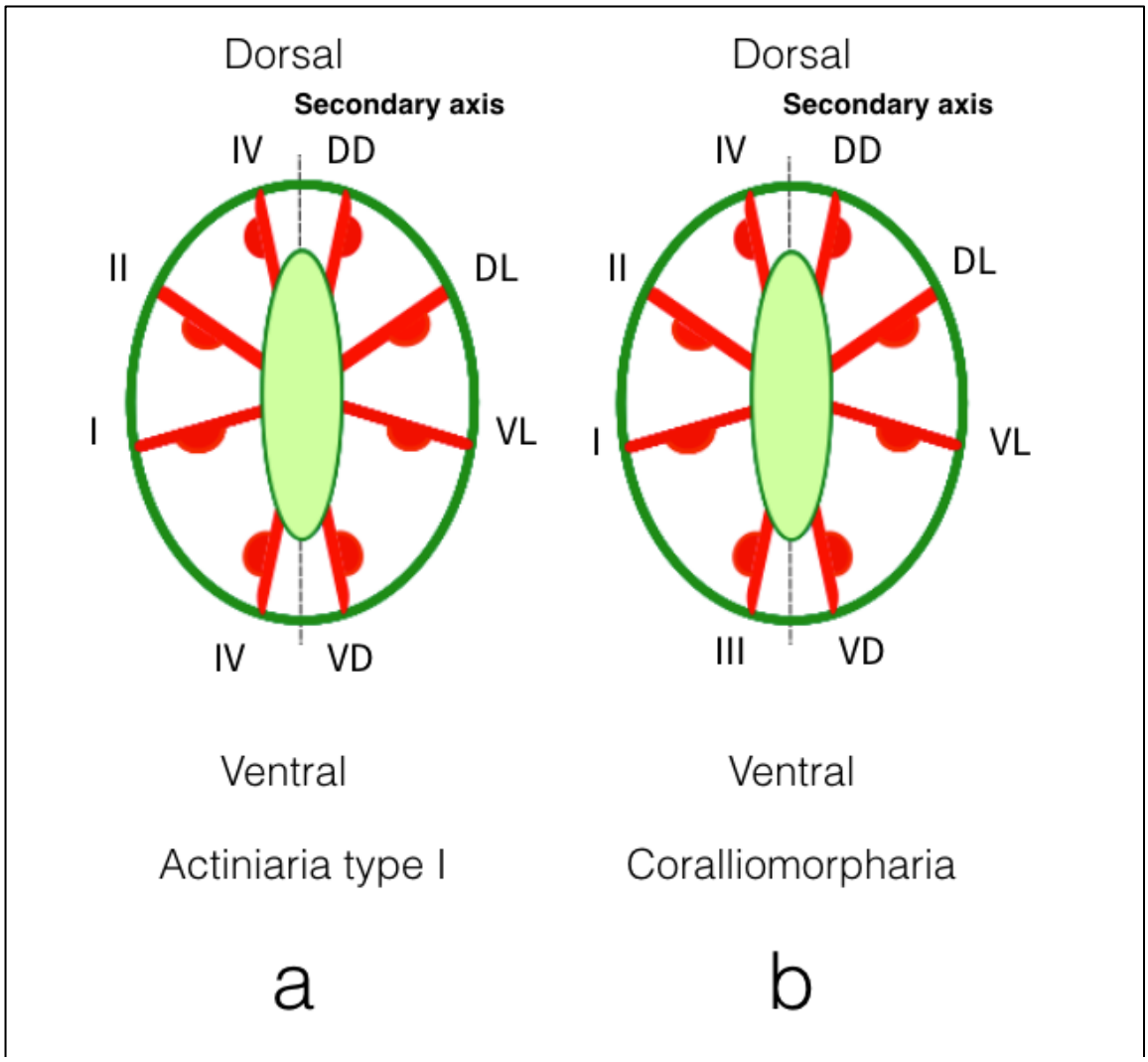

Figure 2.40. Schematic representation of the Actiniaria type I and Corallimorpharia mesenteric development at the 4 mesenteric pairs (12 mesenteries) stage ("*Edwardsia stage*"). The order of mesenteric development is the same (according to McMurrich studies in *Rhodactis osculifera*) and the disposition of mesenteries and the orientation of the respective retractor muscles is the same.

During its development, the corallimorpharian polyp conserved at all stages a bilateral symmetry until the "*halcampoides stage*", the 6 mesenteric pairs stage, with 12 mesenteries. Do to their adult mesenteric arrangement, that is similar to the one found in Scleractinia, it is possible to presume that no difference occurs until the 12 mesenteric stage and thus a schematic representation of this stage follows in comparison to Actiniaria (**figure 2.41**).

The order of development of the 5th and 6th mesenteric pairs, the secondary dorso-lateral (SDL) and secondary ventro-lateral (SVL) pairs has not been accessed by developmental studies. However we know that in Actiniaria and Scleractinia both pairs

form without major time differences and that the addition of one is directly followed by the addition of the other, meaning that no adult forms exist with only one of them. This being said, the order of development of pairs will be represented as V/VI for both in **figure 2.41**, relating to the developmental order of Actiniaria and Scleractinia. Independently of the formation order for these pairs, the result at the 12 mesenteric state will be the same: **a bi-radial symmetry of the mesenteric arrangement and associated retractor muscles**.

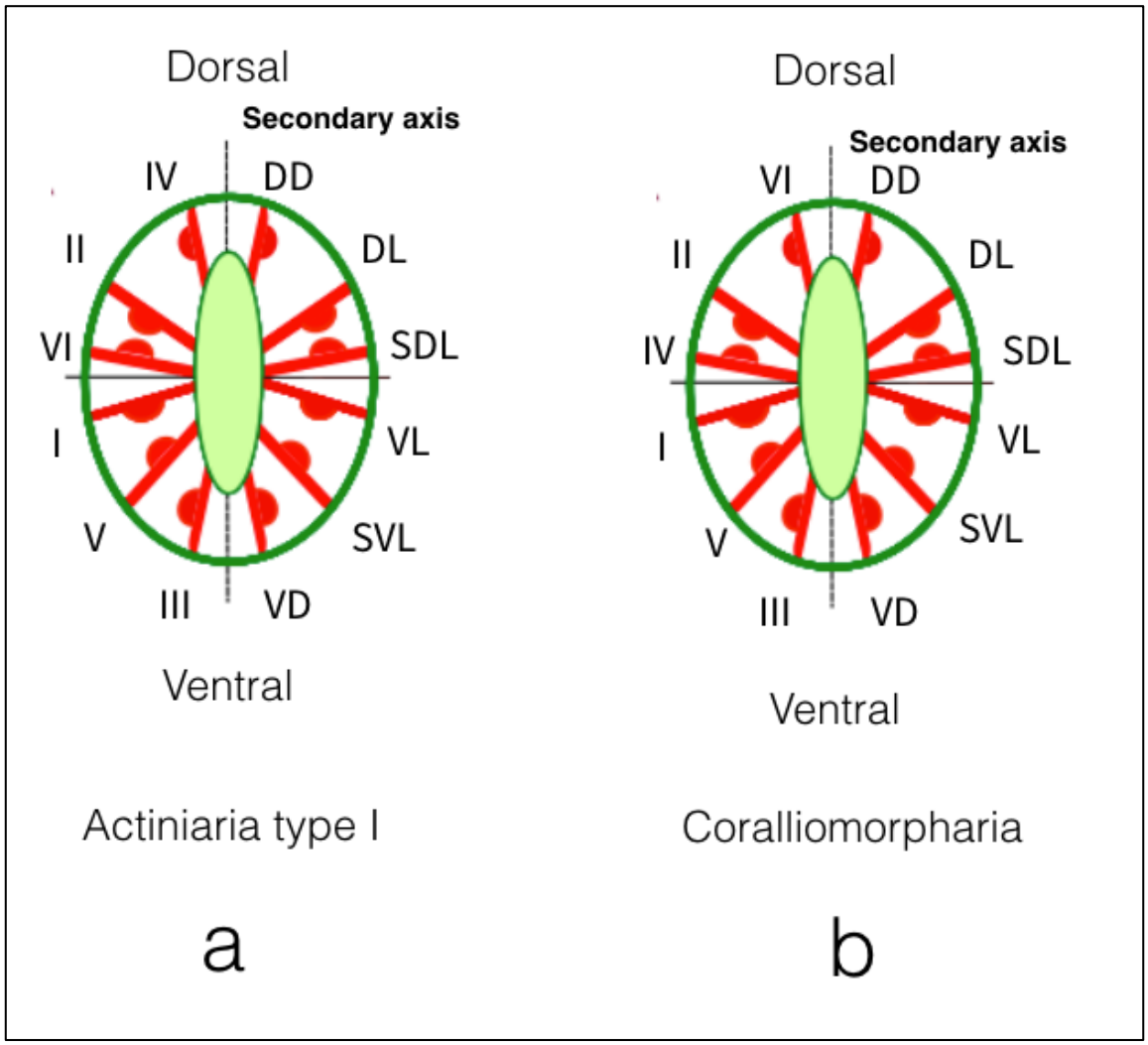

Figure 2.41. Schematic representation of the Actiniaria type I (**a**) and Corallimorpharia (**b**) mesenteric development at the 6 mesenteric pairs (12 mesenteries) stage ("*Edwardsia stage*"). The order of mesenteric development is the same (according to McMurrich studies in *Rhodactis osculifera*) until the development of the 4th pair and the order of development of the 5th and 6th pair has not been accessed but can be presumed to be similar to Scleractinia or Actiniaria (see text). The disposition of mesenteries and the orientation of the respective retractor muscles is the same and leave the polyp with a bi-radial symmetry of the mesenteric and retractor muscles arrangement.

2.2.2 Ceriantharia

Ceriantharia, classically considered as a hexacorallian group, are usually called tube-dwelling anemones. They receive this common name because their external morphology resembles that of an anemone and because these solitary animals usually present their soft body buried in the sediment (**figure 2.42**). Despite this common name they belong to a different order in relation to anemones (Actiniaria) to which they are not closely related. The phylogenetic position of this clade is hardly debated (see section 2.3.2 of this chapter). This clade comprises around 140 described species (Appeltans *et al.* 2012).

The fact that the position of Ceriantharia in the anthozoan tree is disputed and the extremely different modality of mesenteric formation and arrangement justify that we look at them separately from the other classical hexacorallians.

Figure 2.42. Ceriantharia species: **a** – *Arachnanthus oligopodus* (Cerfontaine, 1891) showing the complete animal with its body column outside the substract and **b** – *Cerianthus lloydii* (Gosse, 1859) with its body column surrounded by the subtract and only the mouth and tentacles visible. Photos from DORIS (http://doris.ffessm.fr) by Gilles Cavignaux and David Borg respectively.

Ceriantharia presents special characteristics that distinguish them from the other anthozoan groups. The aboral end contains a pore, they do not possess a basal disc and they have two tentacle types (short labial tentacles around the mouth and long marginal tentacles around the oral disc). They possess a specific type of nematocysts (**ptychocysts**) and have a distinctive larvae form. Their specific pelagic **arachnactis larva** that develop tentacles and mesenteries before settling. All these characteristics, also account to the fact that Ceriantharia is quite distinctive from the other classical hexacorallians in addition to their mesenteric arrangement and development which, as previously said, are distinctive and amongst the most controversial in Anthozoa.

Ceriantharia only present complete mesenteries, meaning that all of them reach the pharynx. Van Beneden (1897) and McMurrich (1910) describe that they pass through a larvae stage that possesses four pairs of bilaterally disposed mesenteries. These eight mesenteries being named **protomesenteries** (or "primitive pairs") (Tiffon 1987) and their formation order is as seen in **figure 2.43a**. First, the lateral pair on the siphonoglyph side (I), then the second lateral pair (II), then the directive pair of the siphonoglyph side (III) followed by a pair opposed to the previous one in the side without siphonoglyph (IV).

Taking the position of this last forming pair one would associate it as a directive pair, as it appears opposed to the directive pair associated to the siphonoglyph (pair III). In fact classical directive pairs are described as having no formation of mesenteries between them (they are endocoels). This is not the case for this pair as the space between them corresponds to the "multiplication chamber" of Ceriantharia, which is the zone of formation of the secondary mesenteries of this group (the **metamesenteries** (Tiffon 1987) (**figure 2.43b**). There is an addition of metamesenteries in the space between the protomesenteries of the 4th pair (**figure 2.43b** IV pair of mesenteries): pairs 5 to 9. **This addition does not stop and will continue as the animal grows.** The addition of mesenteries after the protomesenteries in a single proliferation zone makes that **the polyp of Ceriantharia never loses its bilateral organization**.

Ceriantharia development and body orientation

The controversy in this group for the development and body orientation starts as Carlgren (1906) considers that there are only 6 protomesenteries in Ceriantharia (3 pairs) and that the 4th pair to be formed is already a metamesenteric ("secondary") pair. Its opinion comes from the fact that nothing allows to distinguish the 4th pair of the 5th or 6th and so on. If we consider Carlgren opinion, Ceriantharia would not pass an "*Edwardsia stage*" with four "primary" mesenteric pairs and thus, would not be easily compared with Actiniaria or Octocorallia. I would also mean that this group might have lost a directive pair or that it had never possessed it (depending on the phylogenetic position of Ceriantharia). Another factor that indicates that Ceriantharia does not pass by an Edwardsian stage comes from the fact that even if they do pass a phase with 8 protomesenteries this phase does not comprise two pairs of directive mesenteries as happens in the other hexacorallians and in Octocorallia. Despite this, McMurrich (1910) makes the point that if we consider the siphonoglyph as ventral, the order of mesenteric formation of the first four pairs of mesenteries (primary mesenteries in both groups) would be the same, then pointing towards an homology between the sides that have a siphonoglyph between both groups.

First of all, it is well accepted by authors that there is **a homology of the secondary axis of Ceriantharia and that of the other anthozoans** (see for example: Carlgren 1906; Brook 1890; Berking 2007). The question is how do the poles of the secondary axis of Ceriantharia relate to the poles of the secondary axis of Actiniaria, how do their polarities relate.

The most immediate characters to use in order to access the orientation of the Ceriantharia order would be the siphonoglyph position and the orientation of the retractor muscles associated to the mesenteries as in the other groups.

The siphonoglyph in Ceriantharia can be more or less developed. It presents in most species, more mesenteries attached to the siphonoglyph than the two directive mesenteries (Carlgren 1906). This is a specific characteristic of the Ceriantharia siphonoglyph that may be due to spatial constrains, as the metamesenteries form in the opposite side the existing may be pushed towards the siphonoglyph. However, it can also suggest that the ceriantharian siphonoglyph is not homologous to either the sulcus or sulculus of Actiniaria. Carlgren observed that the longitudinal muscles (retractor muscles) are positioned in the mesenteric side looking away from the siphonoglyph

(**figure 2.43c**), meaning that they are positioned in each mesentery in one side of the mesoglea. In this case, in the side looking away from the siphonoglyph, and so considers this siphonoglyph to be dorsal. This opinion that the Ceriantharia siphonoglyph is dorsal is the one we mostly find today in textbooks (Kotpal 2004; Khanna and Yadav 2005; Sharma 2014). Other textbooks as Brusca and Brusca (2003) prefer not to approach this issue and therefore do not mention the presence of a siphonoglyph in Ceriantharia.

The fact is that, even if it is widely present in textbooks, the reasoning that the siphonoglyph is dorsal due to the retractor muscle disposition is questionable. Numerous authors disagree about the presence of retractor muscles in Ceriantharia associated to the mesenteries. Arai (1972) describes in *Pachycerianthus fimbriatus* that the musculature runs radially on both sides of the mesoglea of the mesenteries and that when it gets closer to the pharynx the musculature of one side turn to run almost longitudinally. This is in agreement with Dr. E. A. Robson's work on *Cerianthus lloydii* as quoted by Pantin (1966). Van Beneden (1897) describes two larvae in which the longitudinal musculature runs in both sides of the mesoglea. In opposition, Carlgren (1893) describes them in the opposite side of the siphonoghyph for each mesentery. The musculature in the Ceriantharia mesenteries is very poorly developed and the fact that it is radial in both sides and that near the pharynx the fibers would turn to run almost longitudinally allows to question if there is an homology to the true retractor muscles presented in the other anthozoan groups.

As the homology of the retractor muscles and of the siphonoglyph is not well established in relation to other groups, **the Ceriantharia body orientation is still very doubtful**.

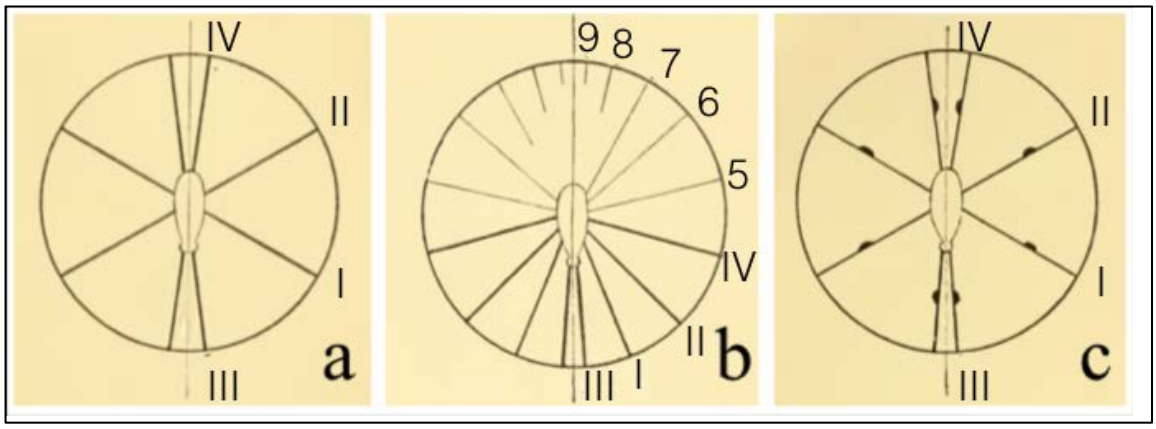

Figure 2.43. Schematic representation of the mesenteric arrangement in Ceriantharia, visible in the figures are the flattered pharynx (central) with the siphonoglyph in one of its poles, the mesenteries, the body column wall: **a** – 8 protomeseteric stage with their developmental order, from pair I to IV; **b** – Development of the metamesenteries (5 to 9) in one pole of the secondary axis, between the 4th pair of protomesenteries (IV); **c** – Carlgren (1893) described the longitudinal musculature to be situated in the mesentery side opposite to the siphonoglyph. Roman numeral to the protomesenteries and Arabic numerals to metamesenteries according to McMurrich (1910). Adapted from Delage and Hérouard (1901).

Regarding the position of the first mesenteric pair to be formed it seems that in Hexacorallian groups (Actiniaria; Antipatharia; Zoanthidea; Scleractinia and Corallimorpharia) this pair divides the body of the polyp in two unequal parts, the smallest being the ventral one. McMurrich in 1889 in "*On the occurrence of an Edwardsia stage in the free swimming embryos of a Hexactinian*" describes the first pairs of mesenteries in Ceriantharia (*Arachnatis brachiolata*) and their order of formation (**figure 2.44**). In his representation, the 5th mesenteric pair is forming in the "multiplication chamber" (between the 4th protomesenteric pair), by his representation it's visible that, in this phase, the first pair of mesenteries to form (I) divides the body in two unequal parts. This asymmetry is also visible in the representation of the 8 mesenteries phase from Delage and Hérouard (1901) (**figure 2.43a**). We have no clear information if this pair divides the body in two unequal parts since it's formation but in a phase with eight mesenteries we can accept that it is the case. The smaller side is the one that bears the siphonoglyph as happens in all other hexacorallian groups.

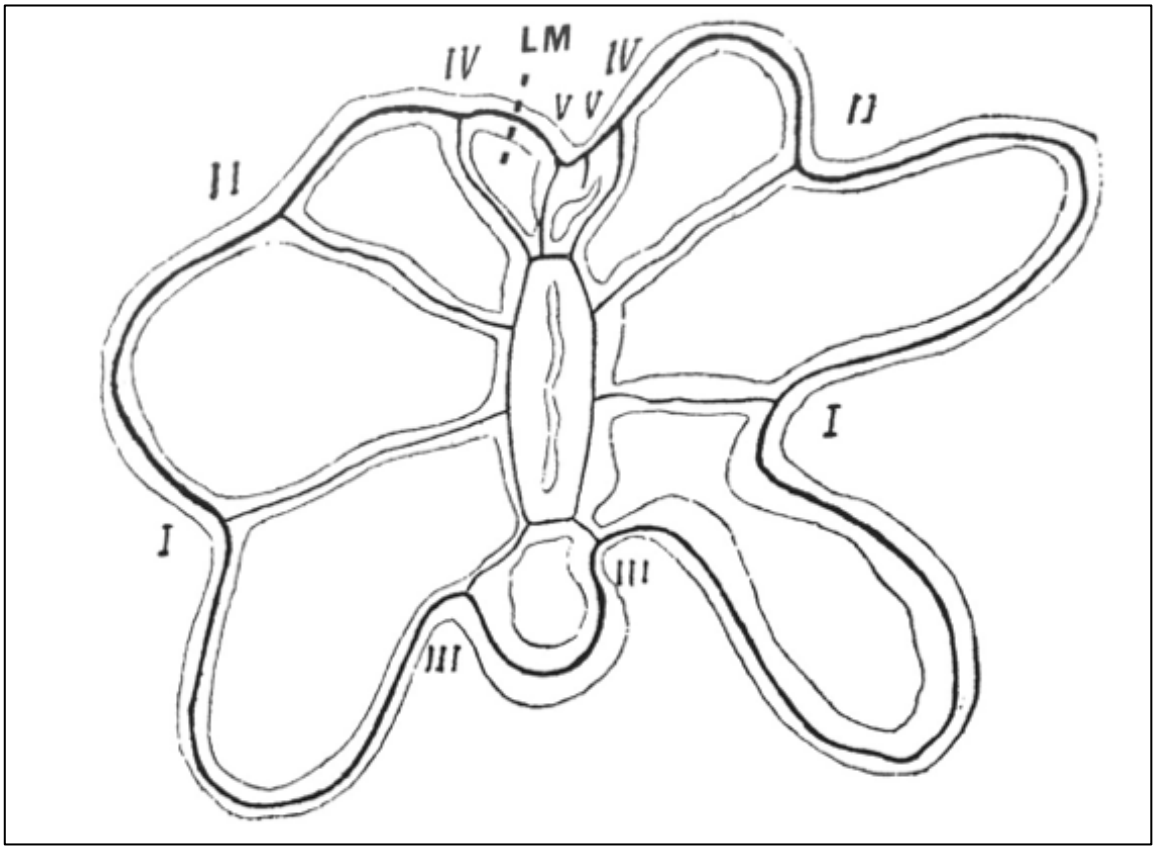

Figure 2.44. Transversal cut of a larvae (Cerinula) of *Aranchnatis brachiolata* by McMurrich (1889). Protomesenteries are represented and numbered by their order of development: I, II, III, IV. The first pair of metamesenteries (V) appears in the "multiplication chamber" (LM) in the opposite site to the siphonoglyph, which is not represented in the figure. Figure from Tiffon (1987).

If we represent the Ceriantharia developmental stage with 8 mesenteries (4 mesenteric pairs) side by side with the actiniarian type I (**figure 2.45**) we can see that that there is an agreement between the position of the pair in relation to the siphonoglyph (blue in the ventral part of the flattered pharynx of Actiniaria and in the same respective side of Ceriantharia) and their order of development between Ceriantharia and Actiniaria type I. As there is an agreement with Actiniaria type I there also is with Scleractinia, Corallimorpharia and Zoanthidae.

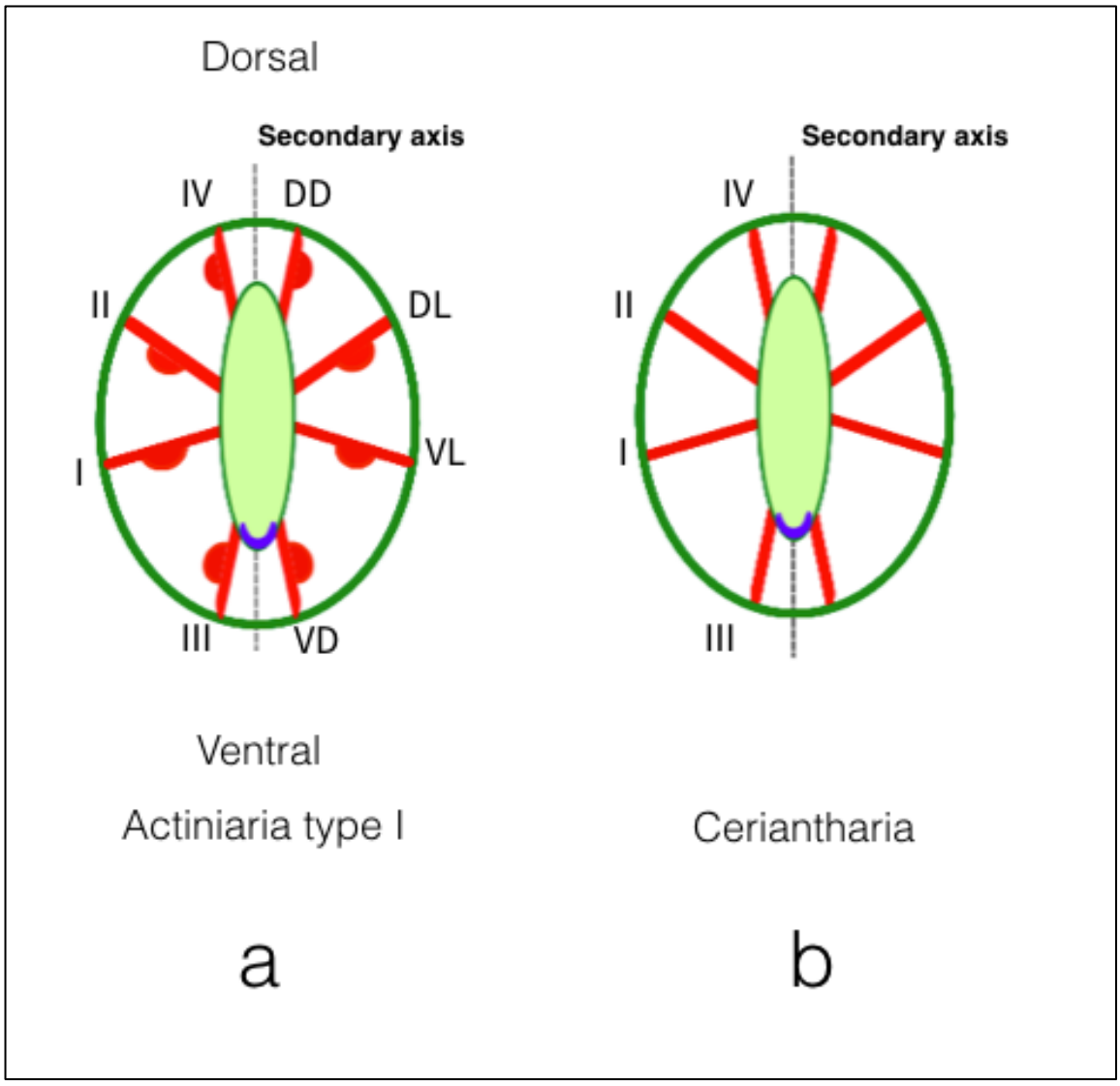

Figure 2.45. Schematic representations of the 8 mesenteric phase of: **a** – Actiniaria type I ("*Edwardsia stage*") and of **b** – Ceriantharia. Both schematic representations have the siphonoglyph represented in in blue in one side of the flattered pharynx. In terms of respective position of the mesenteric pairs the order of development of ceriantharian pairs is in agreement with the order of development in Actiniaria type I.

No clear response is here given to the orientation of the body of the members of Ceriantharia. If there is a homology between the longitudinal muscles described in the mesenteries, then the dorsal siphonoglyph hypothesis retains its sense. Nevertheless, the ventral siphonoglyph hypothesis is also valid at this point because no clear reason exists (other that the possible retractor musculature disposition) to why this siphonoglyph is not ventral as in all other anthozoans. The division of the body by the first mesenteric pair (forming two unequal) parts seems to be in agreement with what happens in the other hexacorallian groups and with a ventral position of the

siphonoglyph. Also the developmental order for the mesenteric pairs is in agreement with this hypothesis. In my opinion the muscle disposition is not a strong enough argument to dorsalize the Ceriantharia siphonoglyph specially because its homology in relation to the other actiniarian retractor muscles is at least doubtful. The other arguments presented here point to a ventral siphonoglyph, which with the available knowledge to the date is the hypothesis that I favour. **These facts will hopefully revive the debate about the body orientation of Ceriantharia**.

2.2.3 Octocorallia

Octocorallia comprise around 3100 described species (Appeltans *et al.* 2012) and are colonial organisms, represented in **figure 2.46**. They include three orders: **Alcyonacea** (soft corals – **figure 2.46a**), **Helioporacea** (blue coral – figure **2.45b**), and **Pennatulacea** (sea pens – **figure 2.46c**). These organisms have an internal skeleton secreted by the mesoglea and polyps with eight tentacles (figure **2.46d**) and eight mesenteries (**figure 2.47**).

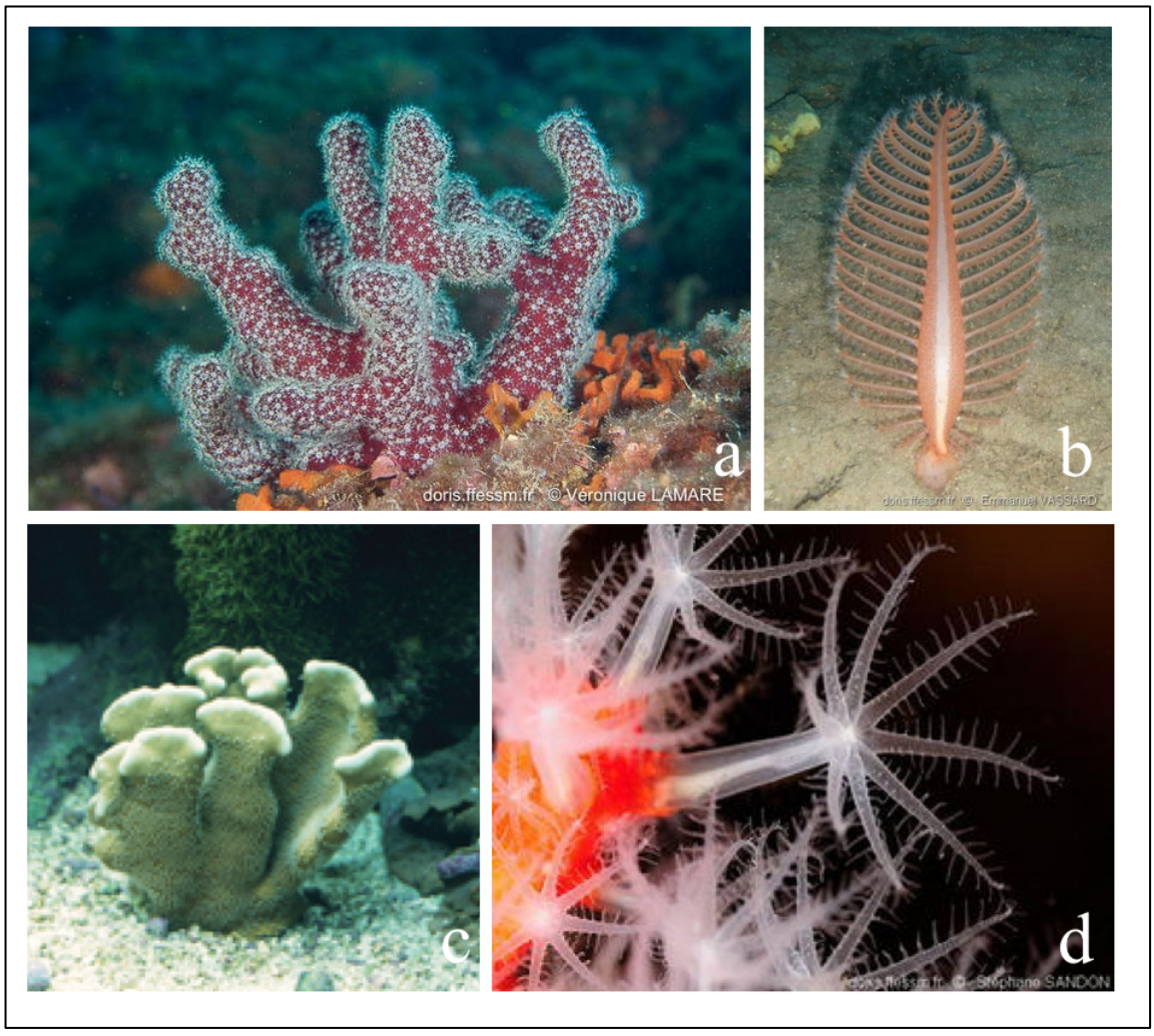

Figure 2.46. Octocorallia diversity: **a** – Soft coral (Alcyonacea) *Alcyonium acaule* (Marion, 1978); **b** – Sea Pen (Pennatulacea) *Pennatula rubra* (Ellis, 1761); **c** – Blue coral (Helioporacea*) Heliopora coerulea* (Pallas 1766); **d** – Detail of *Corallium rubrum* (Linnaeus, 1758), a soft coral (Alcyonacea), allowing to distinguish the characteristic eight tentacles of octocorallians. Photos: a, b and d from DORIS (http://doris.ffessm.fr) by Véronique Lamare, Emmanuel Vassard and Stéphane Sandom respectively; d by Bob Goemans form the Tropical Fish Hobbyst Magazine website (http://www.tfhmagazine.com).

In octocorallians, despite some differences in the formation of mesenteries, they seem to always form in a simultaneous fashion, at the second day after fixation of the planula larvae in *Alcyonium digitatum* (Linnaeus, 1758) (Tixier-Durivault 1987 after Matthews 1916) and for *Fenicullina sp.* before fixation of the larvae (Tixier-Durivault 1987). In the Pennatulacea *Renilla sp.* (Wilson 1883) the formation of the radial mesenteries starts immediately after the formation of the "stomodeum", and even if the formation of the mesenteries happens at once, their development isn't similar, giving us four pairs of mesenteries (Tixier-Durivault 1987).

It's usually accepted that the four mesenteric pairs are homologous to the first four pairs to develop in Actiniaria and that the their body axis coincide. A single siphonoglyph develops in the ventral part of the pharynx. There are two pairs of directives pairs and two pairs of lateral mesenteries. Octocorallians possess then no mesenteric couples. Also all retractor muscles are displayed in the ventral side of the mesenteries. As seen previously the ventral part of anthozoans has been regarded as the one presenting the siphonoglyph and the side bearing the retractor muscles for the primary lateral mesenteries (DL and VL). We have then Octocorallia in complete agreement between muscles disposition and siphonoglyph presence. The four pairs are then as in Hexacorallia named as dorso-directive pair (DD), dorso-lateral pair (DL), ventro-lateral pair (VL) and ventro-directive pair (VD).

Observing a transversal cut passing by the pharynx of an anthozoan polyp (**figure 2.47a**), it can be clearly observed that the organism attends to a clear bilateral organization due to the bilateral disposition of the retractor muscles and the presence of the single siphonoglyph in the pharynx. In this figure, it's represented the only symmetry plan for octocorallians, plan that defines its bilateral organization. The octocorallians also present symmetry elements below the pharynx level. The dorso-directive mesenteric pair (DD) has enteroid pads in their free extremities while the other mesenteries have gonads, leaving us with a bilateral organization also at this level of the polyp (**figure 2.47b**).

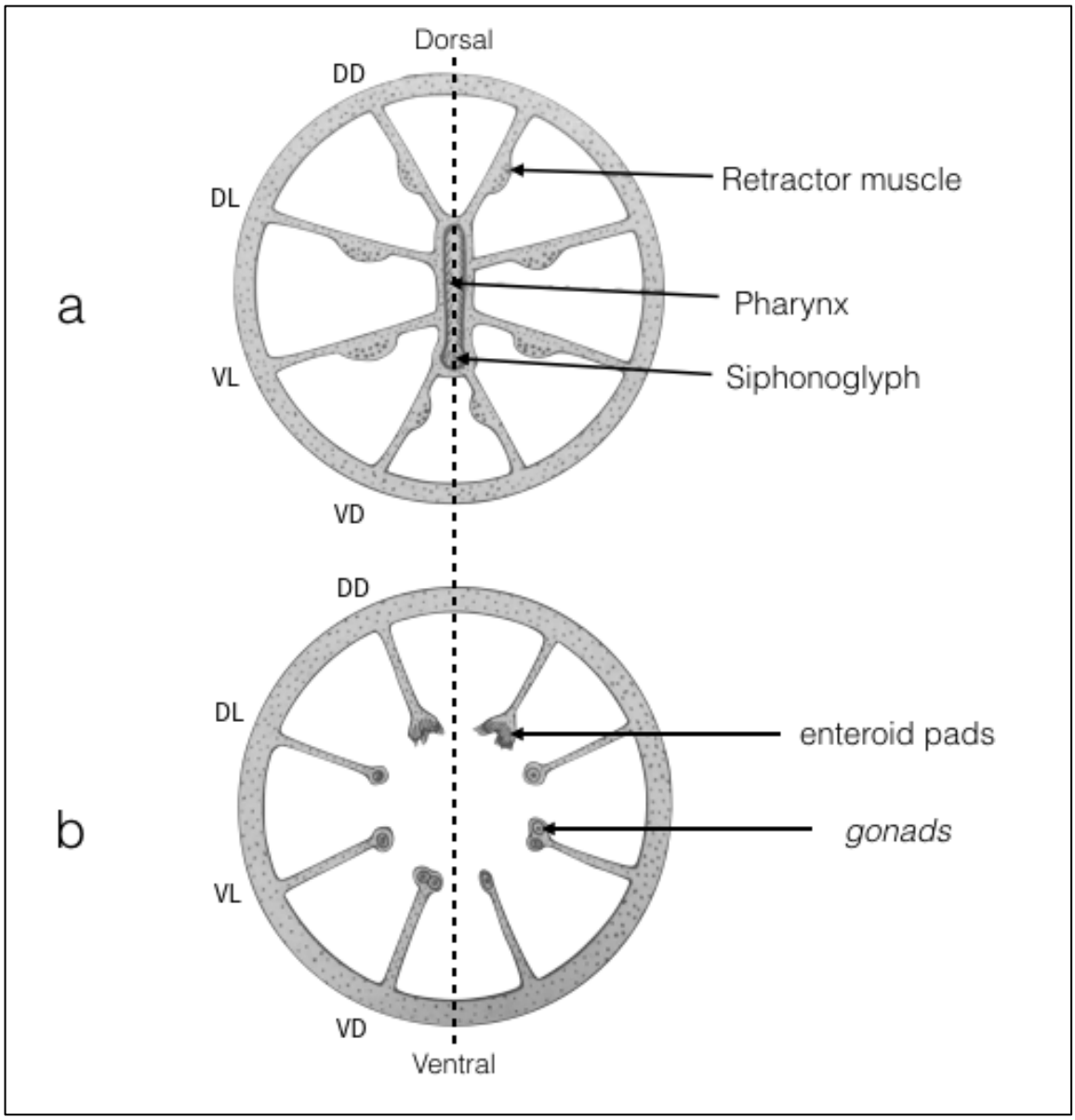

Figure 2.47. Schematic representations of an octocorallian polyp: **a** – at the level of the pharynx; **b** – below the pharynx level. Adapted from Tuzet (1961).

The schematic representation of an actiniarian polyp transverse cut (**figure 2.48a**) along the pharynx level and one of an octocorallian (**figure 2.48b**), allow the visualization that the only difference between their arrangement is the orientation of the retractor muscles in the ventro-directive pair (VD). In the octocorallians this pair is disposed in a ventral position in relation to the mesenteric pair, it is in the endocoel formed between the ventro-directive pair. Octocorallians have then all their muscles oriented towards the ventral side. The homology between the first four mesenteric pairs of Actiniaria and Octocorallia seems evident by due to the important homologies they

have, such as the presence of polarized retractor muscles and their congruent distribution, this homology has been considered by authors such as McMurrich (1894).

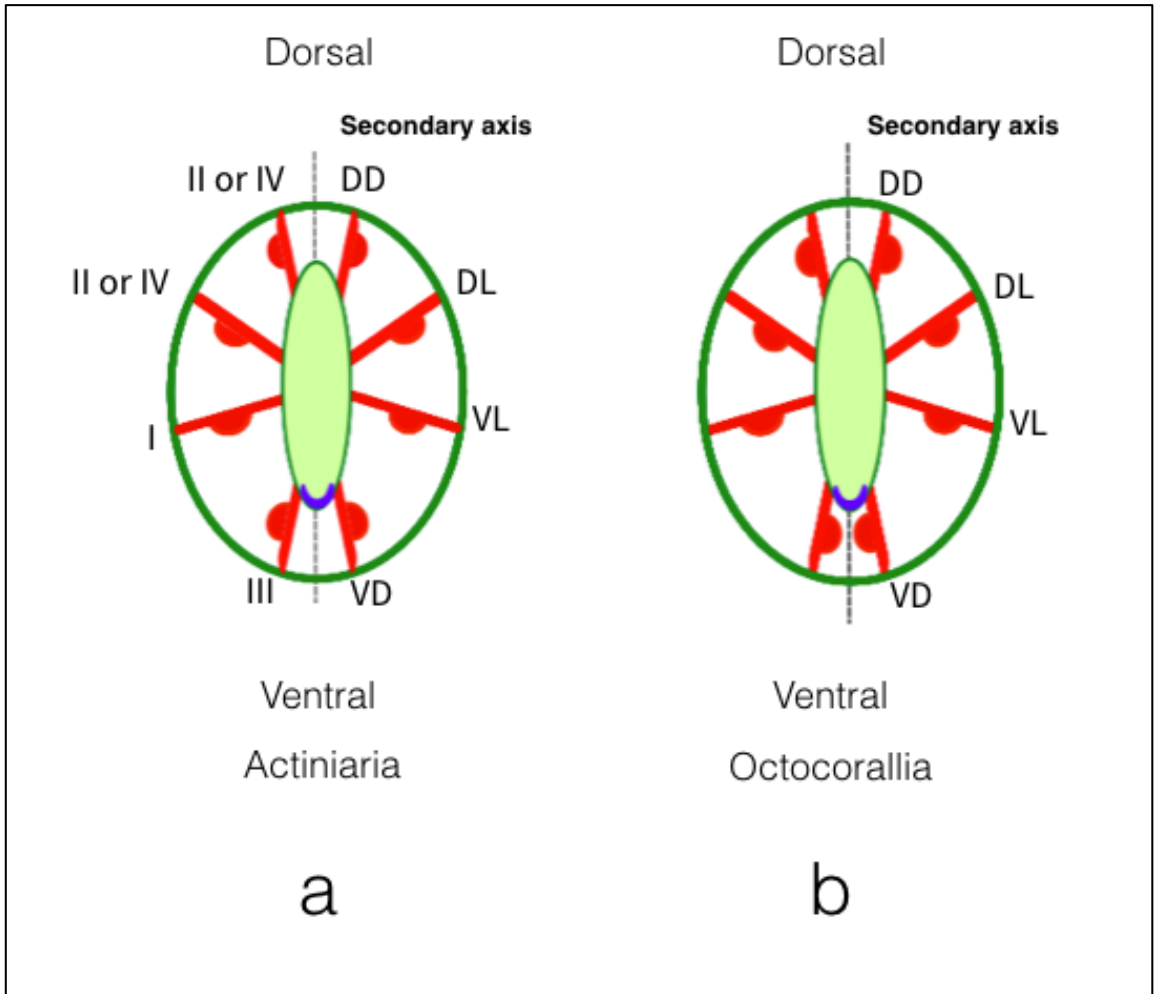

Figure 2.48. Schematic representations Actiniaria at the "*Edwardsia stage*" and Octocorallia: **a** – Actiniaria type I ("*Edwardsia stage*"); **b** – Octocorallia. Both schematic representations have the siphonoglyph represented in in blue in one side of the flattered pharynx. The number of mesenteries and the disposition of retractor muscles is the same with the exception of the retractor muscles associated with the ventro-directive pair (VD). The order of mesenteric development is not indicated for Octocorallia as they develop all their mesenteries simultaneously.

2.2.4 Final considerations for the homology of mesenteric development across Anthozoa

In **figure 2.49** there is a schematic view that represents the generalization of what was discussed previously in this chapter. Details can be found in the picture and respective legend. This schematic view makes visually accessible the big quantity of literature data discussed.

In general terms, the anthozoan polyps pass by a synchronous formation of two first mesenteries (I) or ventro-lateral pairs that subdivide the uniform gastric cavity into two asymmetric compartments. At this initial developmental stage, all anthozoans are bilateral. Then the bilateral disposition of mesenteric pairs conserves the bilateral symmetry in all groups. Ceriantharia (not shown), Octocorallia, Zoanthidea, and Antipatharia never lose their bilateral organization. Scleractinians, Corallimorpharia and Actiniaria (although not all) become bi-radial later in their development. From this comes that the bilateral morphology in Anthozoa is a common to all groups. It is associated with a flattered pharynx in all groups with the exception of Octocorallia. Their mesenteric development can be compared around the same axis (directive axis).

As Brook (1890) said "*the mesenteric pairs are added in both sides of the elongated pharynx, that the two pairs of directive mesenteries limit an "anterior" and a "posterior" chamber and that between those two chambers the polyp is divided into any number of paired lateral chambers*". Brook makes the point that the arrangement in Octocorallia, Ceriantharia and hexacorallians is easily understood, as all are modification of one plane.

From the discussion of every group in relation to Actiniaria the dorsal and ventral sides between the different groups can be compared and are correspondent. Grebel'nyi (1981) states that the lateral proto-mesenteries (Edwardsia stage) of anthozoans present their retractor muscles distributed in a bilateral way.

From the synthesis of the literature presented in this chapter, general conclusions can be made:

- **The secondary axis and bilaterality are homologous amongst anthozoans;**
- **The ventral and dorsal sides of the anthozoans correspond between the different orders** (Ceriantharia is problematic as seen in this chapter);
- **Antipatharians and actiniarians have the same body orientation;**
- **The ancestor of anthozoans was most probably bilateral.**
- **Radial symmetry seems a derived trait in Anthozoa that is only present in some adult forms of hexacorallians.**

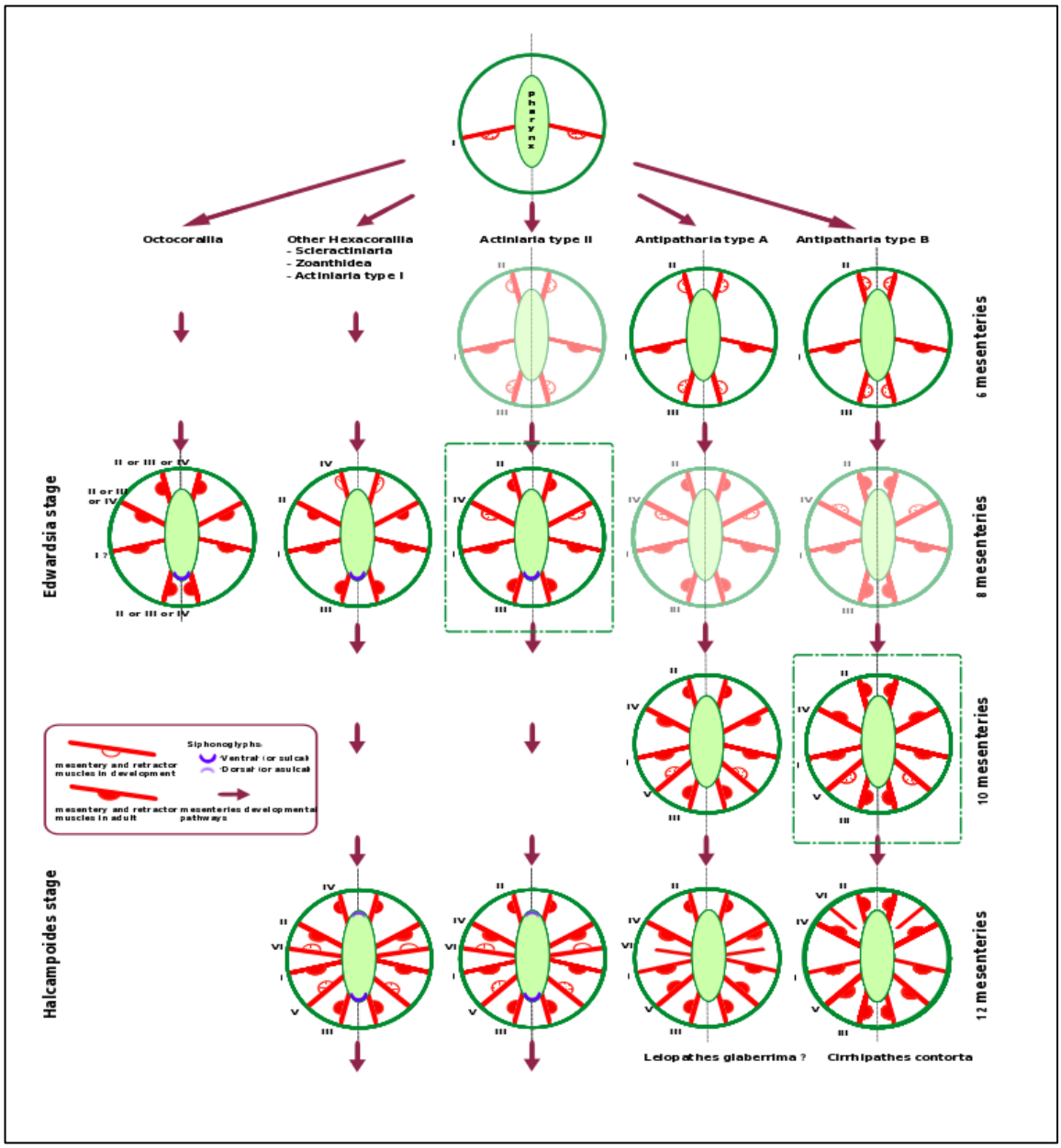

Figure 2.49. Schematisation of cross-sections at pharynx level of developing polyps displaying the different sequences of mesenteries formation in anthozoan orders. Zoanthidea have been represented together with the other hexacorallians, however their dorsal-directive mesenteric pair does not reach the pharynx and is the last mesenteric pair to form before the Halcampoides stage. Ceriantharia are not taking into account here because their body orientation is disputed. Shadowed schemes represent transitory stages of development while other representations correspond to the adult organisation (and then, belong to a species or a group of species). The roman numbers corresponds to the chronology of mesenteric pairs development.

2.3 Symmetry and the origin of bilaterality in Metazoa

As a rule in comparative biology no clear understanding on the analysis of a character, for a given group, will come without looking for that same character outside of the given group and without the understanding of phylogenetic relationships inside and outside of the group. To discuss the evolution of symmetries we must then take in account the symmetries in the other metazoan phyla and the relative phylogenetic positions between them if we want to study the origin of bilaterality in Anthozoa.

2.3.1 Metazoan phylogeny

The phylogeny of the major five extant lineages of metazoans (Bilateria, Cnidaria, Placozoa, Ctenophora and Porifera) has been one of the most challenging and exciting themes in modern zoology. New metazoan phylogenies are constantly being proposed with the evolutive consequences that the change of positions in the metazoan tree brings with it. Lanna (2015) called it as a forest of trees with very conflicting scenarios being published over the last two decades. Regularly a new tree topology is proposed and brings along with it the discussion of the acquisition or lost of several characters, including groundbreaking subjects as the acquisition or loss of nervous system, molecular pathways, genes and body plans.

The monophyly or paraphyly of sponges and the position of Ctenophores have been some of the most erratic factors on the metazoan tree. Based on morphology, the ctenophores have previously been considered as the sister phyla to Bilateria (Nielsen *et al*. 1996, Martindale and Henry 1999). Nevertheless ctenophores have been showed to suffer a severe bottleneck effect and to present a high rate of molecular evolution (Podar *et al.* 2001; Simion *et al.* 2015). In result they present an important branch length that makes this lineage susceptible of Long Branch Attraction (Felsenstein 1978; Philippe *et al.* 2011) and make their position hard to be accessed. The paradigm of Ctenophora sister group to Bilateria was challenged in the beginning of the century by the recent molecular phylogenetic analysis that supported the Cnidaria as the sister group of Bilateria (Collins 1998, Kim *et al.* 1999, Medina *et al.* 2001). With the emergence of

phylogenomics, a number of studies that did not include Ctenophora, found a sister-group relationship for Cnidaria and Bilateria (Srivastava *et al.* 2008, 2010; Sperling *et al.* 2009; Erwin *et al.* 2011). This status was then questioned by the results of Phillipe *et al.* 2009 and Schierwater *et al.* 2009 that showed Coelenterata (Ctenophora and Cnidaria) to be monophyletic and sister to Bilateria. Around the same time, Hejnol *et al.* (2009) recovered Cnidaria as sister to Bilateria and Pick *et al.* (2010) proposed Cnidaria as sister to Bilateria and Placozoa. Since 2010 however, most phylogenomic studies point to the sister group relationship between Bilateria and Cnidaria (Ryan *et al.* 2013, Moroz *et al.* 2014, Borowiec *et al.* 2015, Pisani *et al.* 2015, Whelan *et al.* 2015) regardless of the problematic position of Ctenophora, with exception like Nosenko *et al.* 2013 that found Coelenterata when using ribosomal genes in their analysis and found Cnidaria sister to Bilateria when ribosomal genes were not considered for the analysis. In **figure 2.50** we can see the position of Cnidaria and Bilateria found with phylogenomic reconstructions, the papers from Srivastava *et al.* 2008*,* Sperling *et al.* 2009 and Erwin *et al.* 2011 that found Cnidaria sister to Bilateria were not included as they did not include Ctenophora in their analysis. As we can see in the figure, the vast majority of authors identifies Cnidaria as the sister group of Bilateria, and only some studies from 2009 and 2013 do not. Additionally, Paul Simion, during his PhD thesis in our lab, with an approach that combined a large quantity of data with a rigorous control of quality, also found Cnidaria to be sister to Bilateria. As evidenced by the figure this relationship between Bilateria and Cnidaria comes out at the present day as robust and makes Cnidaria a key group to understand the origin of bilaterian features.

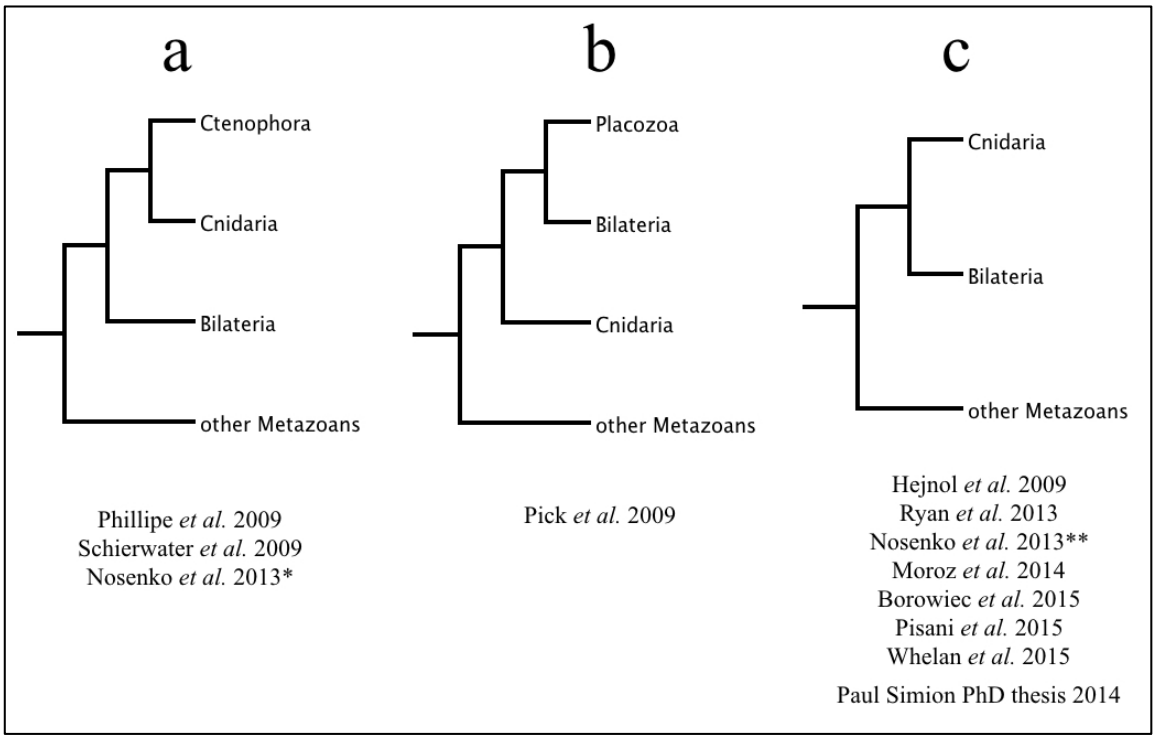

Figure 2.50. Cnidarian position in the Metazoan tree according to different recent phylogenomic studies: **a** – Coelenterata (Cnidaria + Ctenophora) sister to Bilateria; **b** – Cnidaria sister to a clade consisting of Placozoa and Bilateria; **c** – Cnidaria and Bilareria as sister groups. */** With and without ribosomal genes in the analysis, respectively. References corresponding to the three main topologies are indicated under each tree.

2.3.2 Anthozoan phylogeny

For one to understand the evolution of symmetry in Anthozoa or in any other animal group it is essential to understand the evolutionary relations between the objects of comparison. This has been the case for some comparisons made in the pass for the formation of mesenteries in Anthozoa, where authors took into account the believed topology at the time (Brook 1890; Grebel'nyi 1981).

Classically, two subclasses of anthozoans are considered: Octocorallia (gorgonians, soft corals and sea pens) with an eight-fold symmetry and Hexacorallia with a n-six-fold symmetry (sea anemones, black corals, scleractinian corals, tube anemones and corallimorpharians). This difference is easily observed with a simple external observation of the tentacles of both groups, the first one with 8 tentacles (**figure 2.46d**) and the second having 6 (**figure 2.27**) or multiples of 6 tentacles (**figure 2.7**). Octocorallian tentacles are also easily distinguished from those of hexacorallians

for their feather-like shape, as they have lateral branches in contrast with the non-branched tentacles of hexacorallians. This eight and six-fold rule is also present to a certain degree in the internal organization of these animals. If for octocorallians this rule is easily observed and constant for all members of this class with their eight mesenteries, for hexacorallians exceptions to the six-fold rule exist (*e.g.* "*Edwardsia stage*"; see 2.2.1.1 – Actiniaria, in this chapter) and thus the analysis for this "rule" must be done at the level of each hexacorallian order. As the symmetry and mesenteric pattern is homogeneous inside the Octocorallia subclass, the relationships inside this subclass won't be approached, as no information for the evolution of symmetry and mesenteric display would come from it.

As the morphology of the hexacorallian polyps is more variable than that of octocorallian polyps, the monophyly of the group and the relationships within it are harder to interpret (Daly *et al.* 2007). For example, due to the similarities of the morphology of the "*cerinula*" larvae of the cerianthid Arachnactis with the antipatharian polyps, Antipatharia and Ceriantharia have been separated from the remaining orders as a distinct subclass, the Ceriantipatharia (van Beneden 1897; Hyman 1940; Wells and Hill 1956), within Hexacorallia.

More recently France *et al.* (1996) and Berntson *et al.* (1999), with phylogenetic studies with rRNA sequences, and Brugler and France (2007), with the study of the full mitochondrial genome of the antipatharian *Chrysopathes Formosa*, showed that antipatharians and Ceriantharia are not sister taxa and showed Ceriantharia as sister or first branching order of Hexacorallia, and Antipatharia to belong to Hexacorallia. Other studies with molecular phylogenetic approaches on the octocorallian and anthozoan relationships corroborate these conclusions (Berntson *et al.* 2001; Won *et al.* 2001). However in some recent zoology textbooks the Ceriantipatharia are still considered a group (*e.g.* Hickman *et al.* "*Integrated Principles of Zoology*" 2006).

The position of Ceriantharia has also been accessed by phylogenomic studies. In 2015, Zapata *et al.* founded low support for the monophyly of Hexacorallia due to the phylogenetic instability of Ceriantharia that either branched as sister to Hexacorallia (or fist branching hexacorallian) or branched together with Octocorallia. Simion (2014) has found the same instability (ancient PhD student in the lab) in his PhD thesis, also finding Ceriantharia either branching as sister to Hexacorallia (or fist branching hexacorallian) or with Octocorallia.

Ceriantharia seems then to be phylogenetically instable either by molecular phylogenies of rRNA or molecular phylogenies done by phylogenomics. This uncertainty makes us consider the position of Ceriantharia in relation to Hexacorallia as a polytomy (**figure 2.51**).

All groups approached in this chapter (Actiniaria, Antipatharia, Ceriantharia, Corallimorpharia, Scleractinia and Zoanthidea) have been shown to be monophyletic (Daly *et al.* 2003).

Within hexacorallians, Corallimorpharia have been shown to be sister to Scleractinia by mitogenomics (Kayal *et al.* 2013) and by molecular and morphological characters analysis (Daly *et al.* 2003). Antipatharia has been shown to be sister to the Scleractinia and Corallimorpharia group by mitogenomics (Kayal *et al.* 2013), by molecular and morphological characters analysis (Daly *et al.* 2003) and by phylogenomics (Simion Ph.D. thesis, 2014; Zapata *et al.* 2015).

Within this "hexacorallian" group – excluding the Ceriantharia – the only controversial position is the first branching position that has either been found to belong to Actiniaria or to Zoanthidea. Zoanthidea have been found as first branching by mitogenomics (Kayal *et al*. 2013) but Daly *et al*. 2003 found Actiniaria to be the first branching clade with molecular and morphological characters. With phylogenomics, Simion found in his PhD thesis (2004) the position of Zoanthidea to be phylogenetically instable in his analysis. We have then for the Zoanthidea and Actiniaria a polytomy as the first branching clades within the "Hexacorallia excluding Ceriatharia" clade (**figure 2.51**).

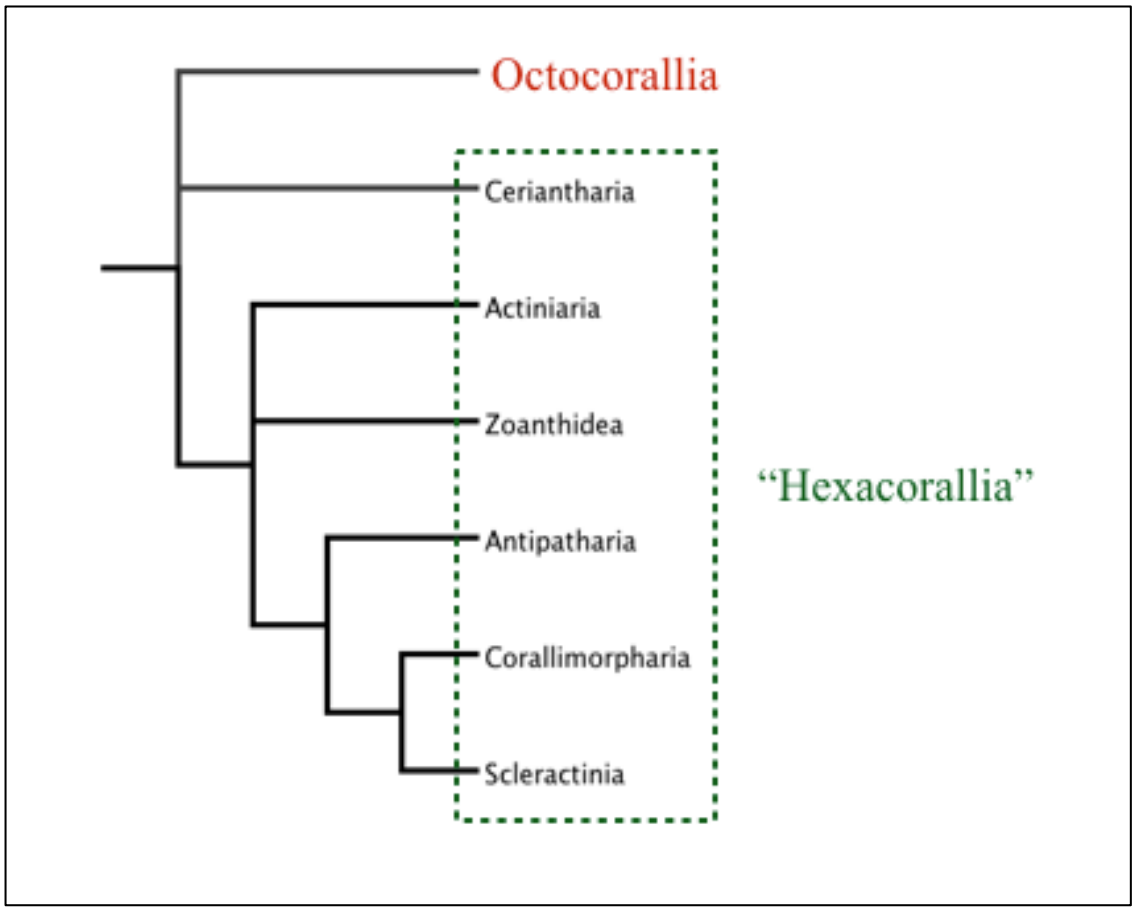

Figure 2.51. Consensus tree for the phylogenetic relations within Anthozoa: The classical group Octocorallia confirms it's status as a monophyletic group but the Hexacorallia (green box) have two problematic clades for which the phylogenetic position is not yet clear: Ceriantharia branches as the first clade of Hexacorallia or as sister to Octocorallia; in a consensus tree Zoantharia branches either as the first branching Hexacorallia (not considering Ceriantharia) or as the second branching hexacorallian after Actiniaria, but most studies place Zoanthidea as branching after Actiniaria.

2.3.3 The anthozoan position within Cnidaria and the evolution of bilaterality

Symmetries present in cnidarians and bilaterians are of the same types: bilateral and radial.

Symmetries in Bilateria

In Bilateria, the bilateral symmetry is the dominant one and the radial symmetry (5-n radial) is confined to the adult phase of most Echinodermata. In Echinodermata the larval phases still present the same bilateral symmetry found in Bilateria and no doubts persist nowadays that the common ancestor of bilaterians presented a bilateral symmetry. The radial symmetry of the adult form in Echinodermata, like the well known starfishes, is an example that bilaterality can be lost, possibly because radial symmetry allows equal opportunities for food gathering and defence in all directions around the body in compromising the direction of movement or the formation of a specialized head (Hinde 1998). However "remains" of bilateral symmetry can still be found in adult Echinodermata due to the presence of a madreporite that breaks the penta-radial symmetry and implies a bilateral symmetry; or by other anatomical elements as shown by Morris (2007) that describes a bilateral plane of symmetry during the adult development of *Holopneustes purpurescens* through the podia, the mouth, the archenteron and the blastopore and that this adult bilateral plane is homologous with the bilateral plane of the remaining Bilateria. Ji *et al.* 2012 showed bilateral tendencies in the behavior patterns of Echinoderms. The radial echinoderms indicate that bilaterality can be lost secondarily and give rise to a radial symmetry and also shows that in radial animals that "vestigial" bilateral features can be consequence of a previous bilateral state in evolution.

Symmetries in Cnidaria and cnidarian phylogeny

Cnidarians can be divided in two groups, the class Anthozoa presenting either a radial or bilateral external symmetry and a bilateral or radial internal symmetry (as detailed previously in this chapter) (Dougherty and Brown 1963; Daly *et al.* 2003; Khanna and Yadav 2005) and the Medusozoa comprising all classes of Cnidaria that form a medusa phase and that possess a more "classical" n-radial symmetry, altrough some rare but clear instances of bilateral symmetry exist in the hydrozoan class of Medusozoa (Manuel 2009).

However, all classes of anthozoan polyps possess a bilateral symmetry in the adult form or pass by a bilateral phase during their development (as seen previously in this chapter). The present view of the phylogeny of cnidarians divided in Anthozoa and Medusozoa has not been particularly challenged with the exception of recent papers that revealed Anthozoa to be paraphyletic with mitogenomic approaches *(*Kayal and Lavrov 2008*;* Kayal *et al.* 2013*;* Zou *et al.* 2012; Park *et al.* 2012), otherwise papers using morphological characters (Bridge *et al.* 1995; Marques and Collins 2004), molecular phylogenies (Bridge *et al.* 1992; France *et al.* 1996; Song and Won 1997; Brentson *et al.* 1999; Collins *et al.* 2006; Stampar *et al.* 2014) and phylogenomics analysis (Zapata *et al.* 2015) have shown this vision of the relationships within Cnidaria to be correct – corresponding to phylogeny represented in **figure 2.52**).

Evolutionary scenarios for the evolution of symmetries in the Cnidaria and Bilateria clade

Cnidarians present two different types of symmetry. If before they were placed with ctenophores as radiata and their bilaterality was seen as a derived trait. Nowadays, knowing that the sister group of cnidarians is Bilateria and that the bilateral symmetry is well present in the first branching class of cnidarians, the anthozoans, one should wonder about the ancestral symmetry of the Cnidarian ancestor and in consequence of the last common ancestor between Cnidaria and Bilateria.

In order to test evolutionary scenarios about the gain and loss of bilaterality in cnidarians and Bilateria, the homology between their bilaterality needs to be accepted. Meaning that the bilaterality present in both groups can be considered as the same character. Indeed it can as bilaterality results from the existence of one or more elements (whatever they may be) that break a radial or cylindrical symmetry. These elements can then be considered a character like any other; bilaterality is then the presence of one or more elements that break a radial (or cylindrical) symmetry, and radial symmetry their absence.

If, as in **figure 2.52**, we place the origin of bilaterality in the metazoan tree we easily see that we have two hypotheses for the evolution of bilaterality and radiality in the clade comprising Bilateria and Cnidaria:

1. The Cnidaria and Bilateria last common ancestor presented a bilateral symmetry and bilaterality has been lost in Medusozoa – **1** in **figure 2.52**.
2. The Cnidaria and Bilateria last common ancestor presented a radial symmetry and there was independent evolution of a bilateral symmetry in Bilateria and in Anthozoa – **2** in **figure 2.52**.

The idea that the last common ancestor of Cnidaria and Bilateria presented a bilateral symmetry – hypothesis 1 – implies a single step of symmetry change, in opposition to two steps in hypothesis 2. Hypothesis 1 is then more parsimonious. Although this fact alone is not enough to estimate the veracity of the hypothesis.

Having accessed that the anthozoan last common ancestor was almost certainly bilateral (as shown in this chapter), and knowing the close relation of this cnidarian group to Bilateria, comparative studies of molecular pathways responsible for the establishment of symmetries in both groups can give important insights to discern between both hypothesis that have been presented.

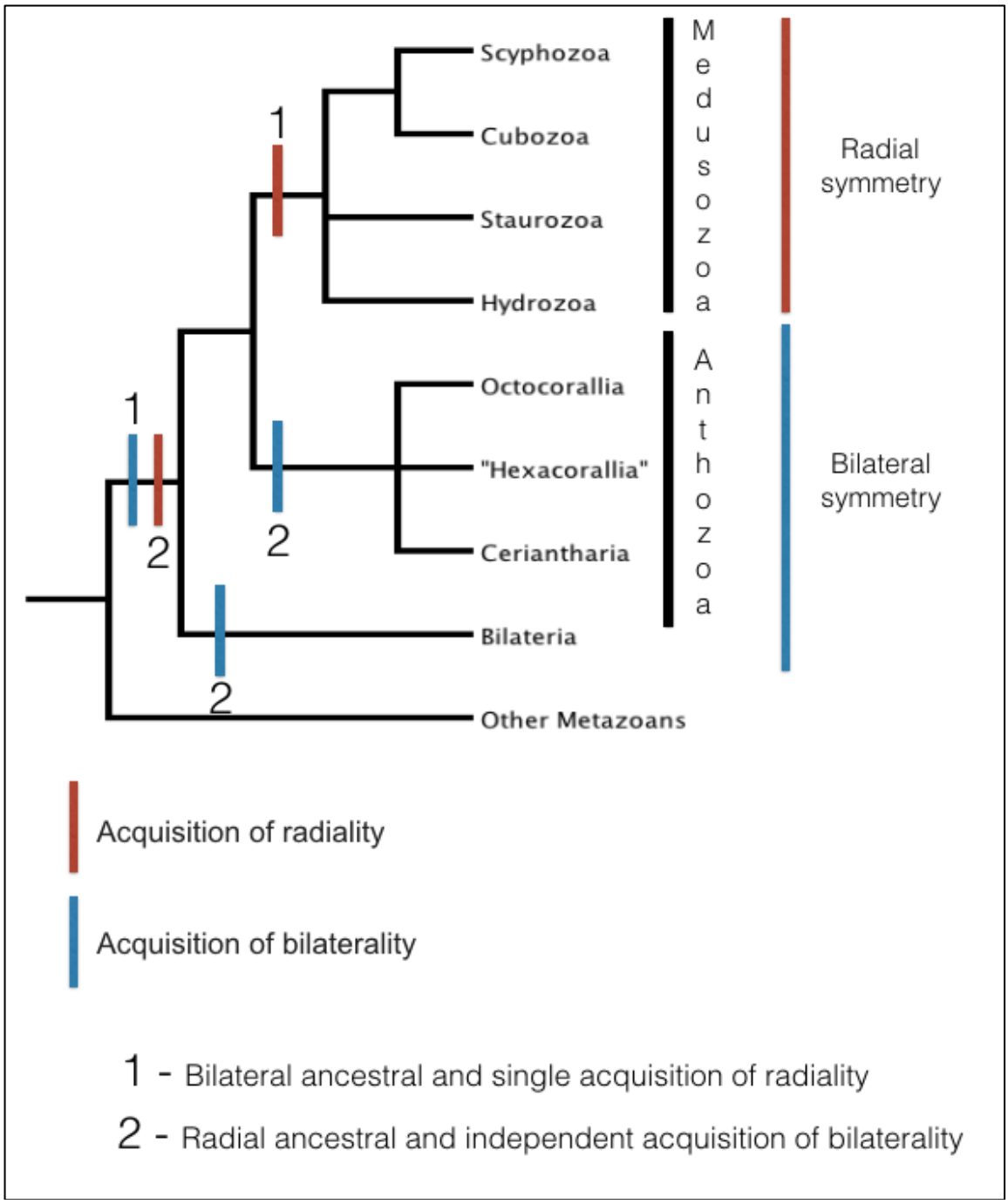

Figure 2.52. Hypothesis for the evolution of bilaterality and radiality in Bilateria and Cnidaria.

2.3.4 Opinions on the origin of bilaterality

The origin of a bilateral symmetry has for long been considered to be associated with the movement of animals, namely it is widely discussed that the bilateral symmetry in Metazoa evolved from radial symmetry due to selection for directed locomotion (Beklemishev 1969; Willmer 1990; Ruppert *et al*. 2004). This idea has also been reinforced by the idea that this symmetry is the only that, according to the laws of physics, can allow sufficient force for changes of direction in a three-dimensional macro-world (Holló and Novák 2012).

Recently this classical idea has been challenged by the hypothesis that bilaterality could have originated before the common ancestor of Cnidaria and Bilateria, and specially before the common ancestor of Anthozoa and Bilateria, leading Finnerty (2005) to argue that a possible alternative explanation to the origin of bilaterality is that bilateral symmetry evolved to improve the efficiency of internal circulation by affecting the compartmentalization of the gut and the location of major ciliary tracts (Finerty 2005). Despite the reasoning of Finnerty that gave a credible explanation to the origin of bilaterality, it is possible that bilaterality evolved due to locomotion even if it evolved before the ancestor of Cnidaria and Bilateria.

3 MORPHOLOGICAL CHARACTERIZATION OF *A. CARIBBEANA* POLYP BILATERAL ORGANIZATION AND MOLECULAR INSIGHTS ON THE ORIGIN OF ITS BILATERALITY

Are the secondary axis of *Nematostella* (Actiniaria) and Antipatharia homologous? Are the genes associated with the establishment of *Nematostella* secondary axis also polarized in an adult Anthozoan, the antipatharian polyp that has a bilateral adult symmetry? A characterization of the expression of the BMP-pathway and the *HOX* genes will be done in an antipatharian species in order to compare the expression of this developmental genes that are differentially expressed along the secondary axis of *Nematostella* so that we can test if their directive axes are homologous. Understand then if the biltaterality of the antipatharian polyp homologous

to the *Nematostella* bilaterality? Is this bilaterality homologous to the one found amongst Bilateria.

To address this question the detailed anatomy of an antipatharian polyp will be studied in order to confirm its previously doubtful bilateral organization together with a detailed morphological study of *Antipathes caribbeana*. This detailed anatomical study is needed to gain new information's on the morphology of this species and antipatharians in general and specially in order to understand the gene expression patterns, as those cannot be interpreted without a good knowledge on the morphology of the species. This detailed anatomical study will be presented in the first section of this chapter (section 3.1).

As gene expression studies in antipatharians have never been done, the establishment of a protocol of *in situ* hybridization was one of the major aspects of this work. The establishment of a working protocol in *Antipathes caribbeana* is present in the second part of this chapter (section 3.2).

In section 3.3 a brief review on the cnidarian developmental genes important to this study will take place, anticipating the section 4.4 that corresponds to the paper with the main results from the gene expression of the developmental genes involved in the secondary axis of the antipatharian polyp and the discussion of those patterns.

3.1 Detailed anatomy of *Antipathes caribbeana*

The study of the detailed anatomy of *Antipathes caribbeana* had two main objectives: to gain sufficient insight on the tissue level organization of the species in order to be able to interpret gene expression patterns and to study the existence and distribution of symmetry core elements.

Although the presented reasons were the main ones behind this morphoanatomical detailed study, the fact that the detailed anatomy of antipatharians has not been studied enough at several different levels also gives importance to this study and to the findings that came out of it.

The study of the detailed morphology of antipatharians presents some special challenges: the small size of the polyps (1 mm), its fragile tissues and the presence of the skelet pose a big challenge that needs to be overcome.

As examples of morphological elements important for the symmetry of antipatharians have[***]:

- **Flattered pharynx**. External organizations gave us access to the flattered mouth and let us presuppose a flattered pharynx but this needed to be confirmed.
- **Siphonoglyph**. As in literature there are contradicting elements about the presence of a siphonoglyph, its absence or the presence of two different siphonoglyphs, one objective is to understanding whether *Antipathes caribbeana* possess this morphological element.

[***] The morphological elements that are important in terms of symmetry of antipatharians and anthozoans in general have been approached in chapter 1 and chapter 2.

- **Mesenteries**. An extremely important element in the organization of the polyps. The mesenteries and their formation are extremely important in terms of the establishment of the bilateral symmetry found in the internal organization of antipatharians. Here we studied the number and disposition of these elements.
- **Retractor muscles**. The disposition of the retractor muscles is associated with mesenteries. They are disposed in anthozoans in a polarized fashion in a single side of each mesentery and are then an important symmetry element. The disposition of retractor muscles in antipatharians was only successfully accessed by van Pesch (1914) and all other authors failed to identify them. This includes electro-microscopy studies that either did not approach the subject (Goldberg and Taylor 1989a, b). Here we confirm the presence of retractor muscles in *Antipathes caribbeana* and their distribution in the species mesenteric system.

The main elements of the organization of the antipatharian polyp have been approached in chapter 1. Here, with the results obtained by staining and immunohistochemistry we will address the detailed anatomy of the polyp. We investigated: i) the muscle system of the entire polyp with focus on the oral cone and tentacles; ii) the oral cone organization with a special focus on symmetry elements as the retractor muscles, mesenteries and the presence or absence of siphonoglyphs; iii) then the tentacle anatomy will be discussed and finally the disposition and presence of reproductive organs in the polyps.

3.1.1 Musculature of the polyp

This work is the first to give a detailed overview of the musculature system of an antipatharian polyp, in this case of the species *Antipathes caribbeana*. The cnidarian muscle systems are structures derived from epitheliums and contractile (myonemes) and are then called epitheliomuscular systems, no mesogleal derived muscle exists. This study of the musculature was done by phalloidin staining (protocol in chapter 1, section 1.6.4) on non-dehydrated fixed polyps. Phalloidin is a bicyclic peptide that belongs to a

family of toxins isolated from the deadly *Amanita phalloides* mushroom and is commonly used in imaging applications to selectively label F-actin.

The muscle system of antipatharians has been quite hard to be studied by the main authors that studied the detailed morphology of antipatharians (Brook 1889; van Pesch 1914). Pax reviewed the knowledge of the musculature system of antipatharians in 1987 and in general terms what we know is that there are longitudinal ectodermal muscles in the tentacle and in the oral cone as well as a ring system of endodermal origin at the base of the tentacles and around the mouth (Pax 1987). The presence of retractor muscles has only been discriminated by van Pesch in 1914.

Here, by phalloidin staining, we were able to discriminate the muscle system of *Antipathes caribbeana* at a much more detailed level. It is important to note that phalloidin also stains the apex of the nematocyst batteries (due to be rich in actin) in the ectoderm and so this will also be visible in several images. In **figure 3.1a** we can see that the musculature is present in all the polyp area including the tentacles and the oral cone but also in the remaining polyp body. Visible is the presence of retractor muscles (**RM**) confirming that ***Antipathes caribbeana*** **presents retractor muscles**. These **retractor muscles** do extend to the base of the oral cone to the area where it **connects with the muscle system at the base of tentacles** but it does not continue in the tentacle itself (**figure 3.1b**). This result confirms van Pesch (1914) that was the only author to distinguish retractor muscles in antipatharians until the present day. **Antipatharians species do have retractor muscles**.

In **figure 3.1c** its visible that in the **oral cone the muscle system is denser in its apex**, probably due to the smaller circumference of the apex. As evident in this figure the retractor muscles have a different nature. In **figure 3.1d** we can see the detailed anatomy of the retractor muscles, in this case, as in other anthozoans, the retractor muscles stand out from the rest of the musculature as more developed muscles, presenting a different orientation that the rest of the musculature, starting in the apex of the oral cone. In **figure 3.1e** we identify that in the **oral cone there are longitudinal and circular muscle fibres** (arrow correspond to the mouth apex).

The longitudinal muscle fibres and circular muscle fibres are not in the same plane; the longitudinal are in the ectoderm while the circular are associated to the endoderm. This can be visualized in **figure 3.2a** where we can see that there are muscle fibres in both senses. By using the focus in the microscope we were able to determine

their relative position and their association to the endoderm or ectoderm was later confirmed by transversal cuts performed in the oral cone (later in this chapter). At the level of the circular muscle fibres (**figure 3.2b**) we can see that they do not go all around the mouth as there are interruptions that correspond to the mesenteric spaces, actually the circular muscle system will be around each of the endoderm compartments delimitated by the mesenteries. This information itself is also new as this muscle system has been described as being all around the mouth and no interruptions had been described (Pax 1987).

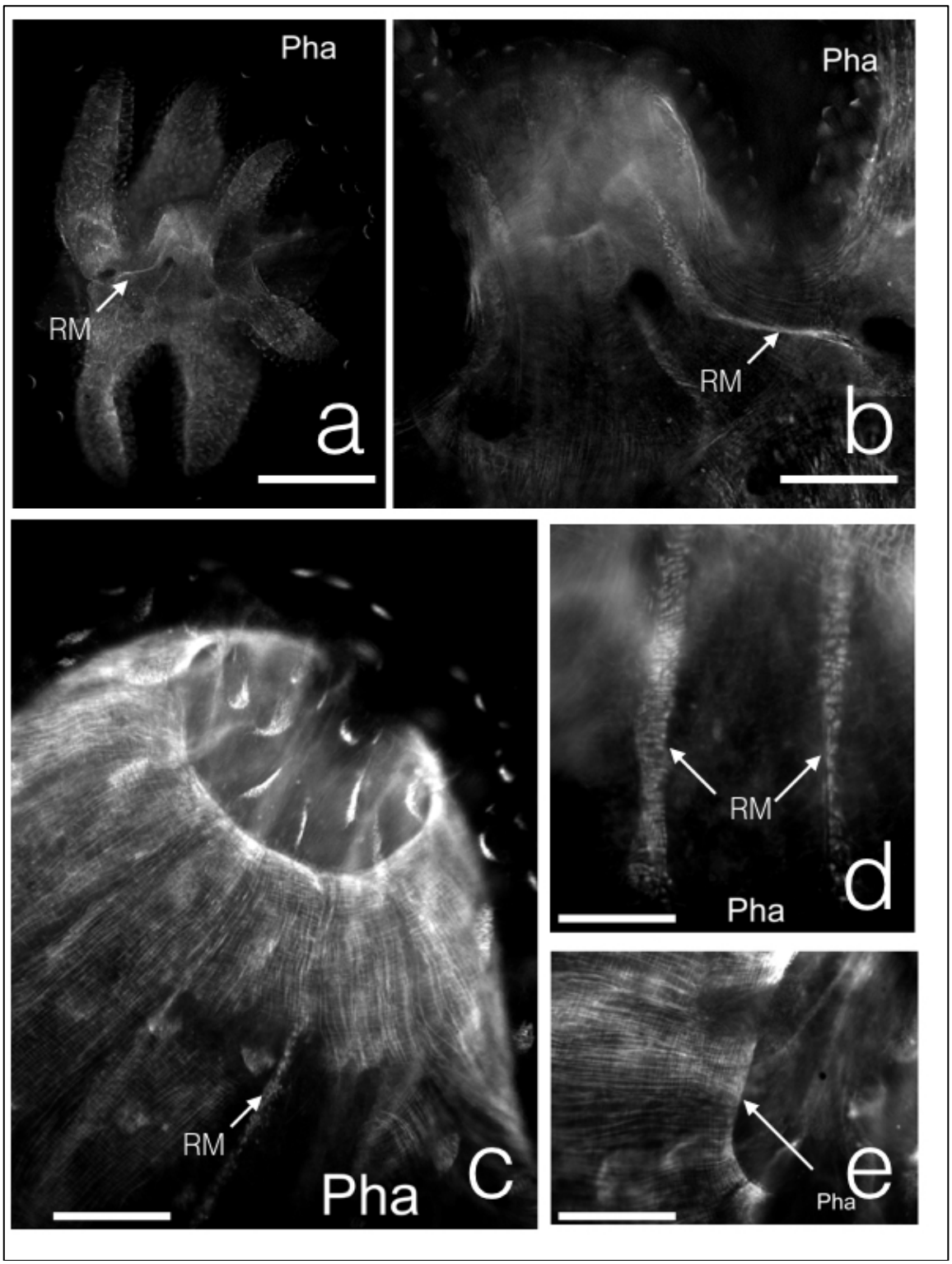

Figure 3.1. The muscle system of *Antipathes caribbeana*. In all figures Pha corresponds to phalloidin staining and RM to retractor muscles. **a** – oral cone in the centre, surrounded by the six tentacles; **b** – oral cone with a retractor muscle going to the base of a tentacle situated in the right of the figure; **c** – oral cone musculature, apex in the top of the figure; **d** – detail of the retractor muscles in the oral cone; **e** – presence of longitudinal and circular muscle fibers in the oral cone. The arrow represents the apex of the oral cone. Scale bars: a – 600 μm; b – 150 μm; c – 100 μm; d – 70 μm; e – 60 μm.

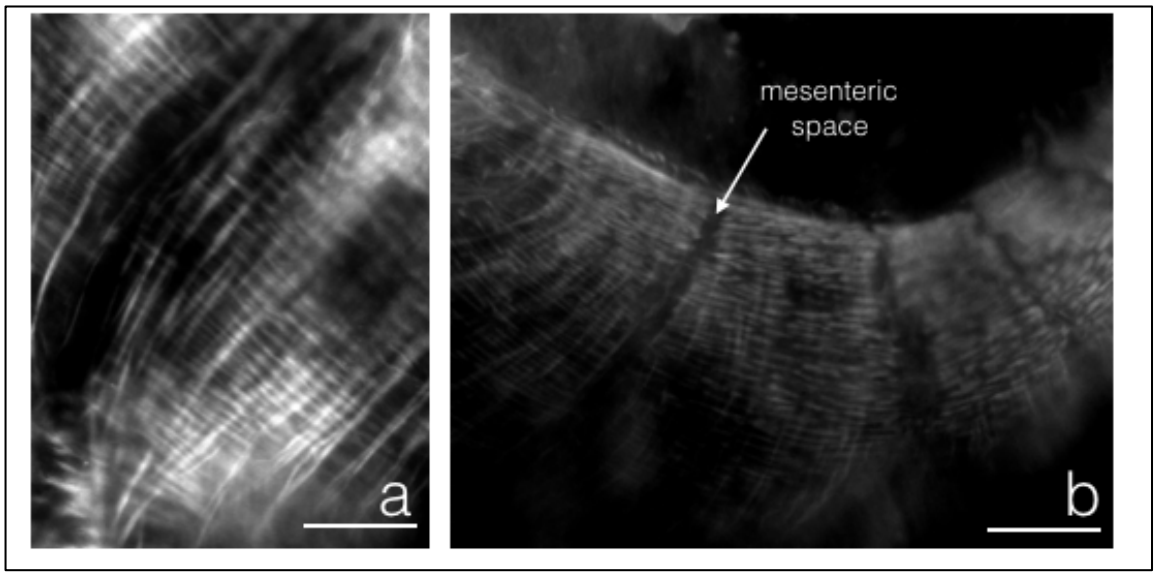

Figure 3.2. Details of the muscle system in the oral cone of *Antipathes caribbeana*: a – detail allowing the visualization of longitudinal ectodermal and circular endodermal muscle fibers; b – detail at the level of the circular muscle fibers showing that they do not go all around the mouth and are interrupted at the mesenteric spaces. Scale bars: a – 25 μm; b – 50 μm.

Longitudinal sections of the whole-mouth phalloidin staining polyps allowed the confirmation that the endodermic circular muscle system is denser in the oral cone apex in relation to the rest of the oral cone (**figure 3.3**).

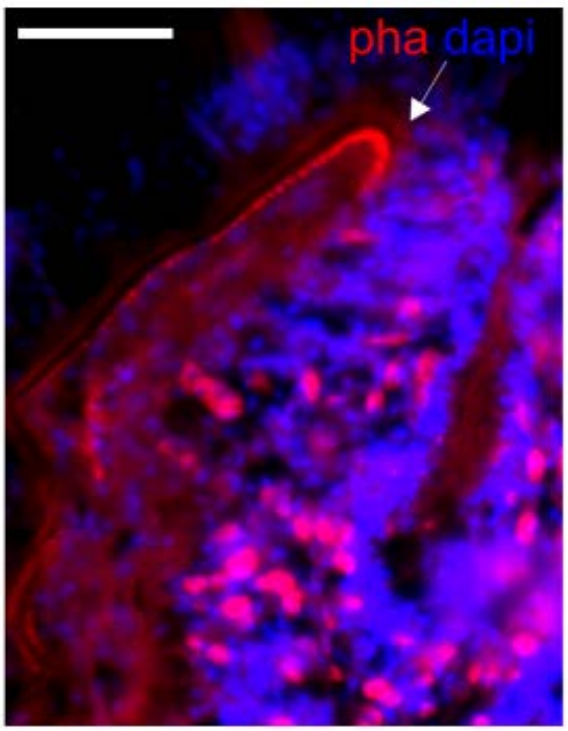

Figure 3.3. Longitudinal section of the oral cone confirms that the endoderm circular muscular system is denser at the oral cone apex. Scale bar: 100 μm.

Tentacle muscle system

The epitheliomuscular muscle system of the tentacles is as in the oral cone. The longitudinal fibers go from the base of the tentacle to its apex (**figures 3.4a and 3.4b**) at the base of the ectoderm. At the base of the tentacles the longitudinal fibers do not continue to the rest of the colony, ending in a quite abrupt way (**figure 3.4c**). This lack of connectivity to the rest of the polyp may be one of the reasons behind the fact that antipatharian tentacles are not retractable and only partially contractible. In **figure 3.4d** and **3.4e** there are respectively the longitudinal and circular muscle fibers that are arranged in an orthogonal fashion between them.

In **figure 3.5a** there is a longitudinal cut of a tentacle showing the longitudinal muscle fibers. In transverse cuts of a proximal lateral tentacle and of a distal lateral tentacle (those that have different sizes – see chapter 1) (**figure 3.5b** and **3.5c**), we can see that no difference exists between these tentacles in terms of muscle distribution. In both figures the relative position of both muscle systems its visible, the longitudinal muscle fibers being at the base of the ectoderm and the circular muscle fibers in the other side of the mesenchyme associated with the endoderm. The longitudinal fibers are larger and more developed than the circular fibers.

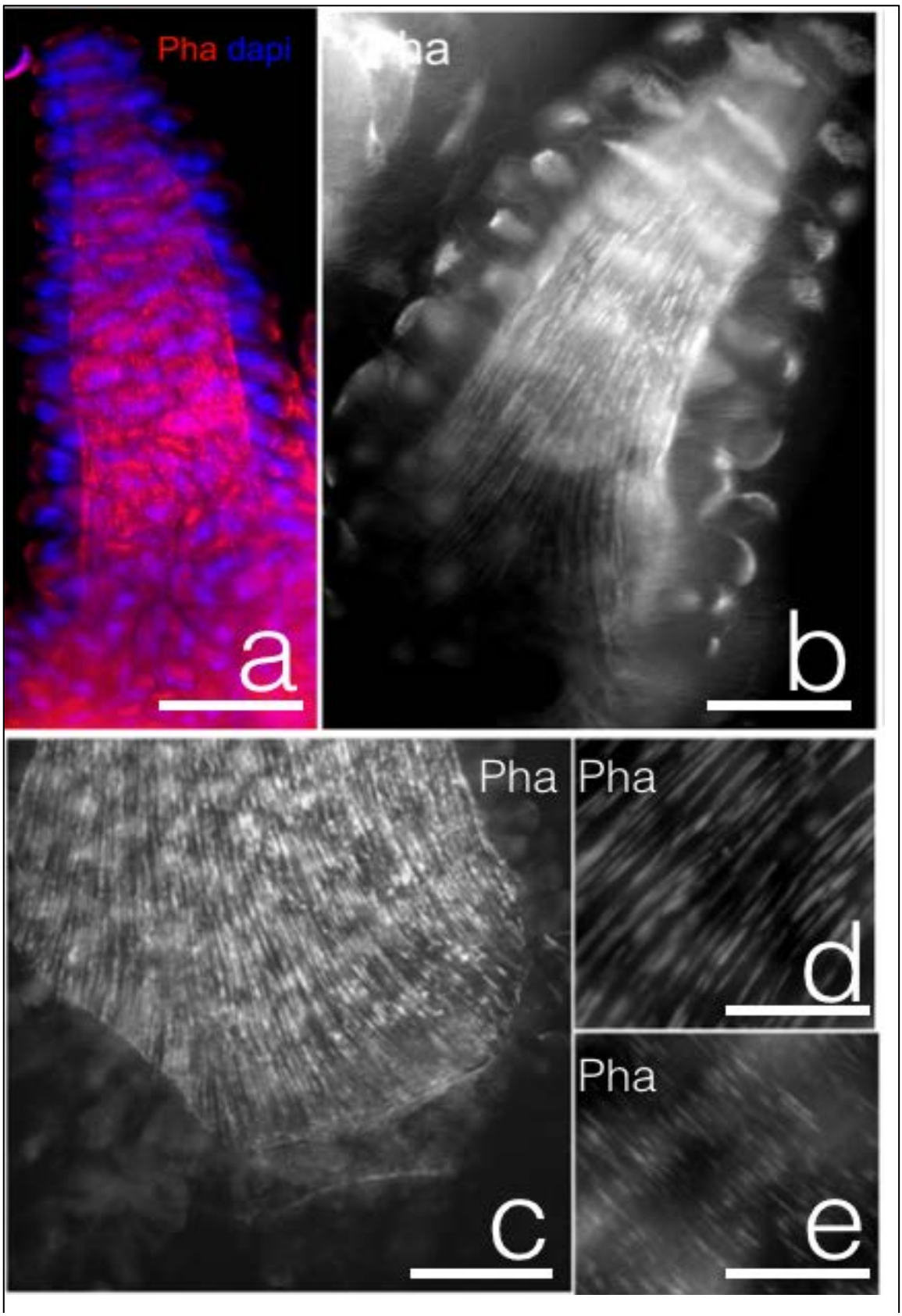

Figure 3.4. Epitheliomuscular system of the *Antipathes caribbeana* tentacles: **a** – phalloidin and dapi staining allow to position the muscle fibers at the base of the ectoderm; **b** – the longitudinal muscle fibers go from the base of the tentacle to its apex; **c** – the longitudinal muscle fibers end in an abrupt way at the base of the tentacle; **d** and **e** – the longitudinal and circular muscle fibers are disposed in an orthogonal way in the tentacle. Scale bars: a – 200 μm; b – 150 μm; c – 100 μm; d – 50 μm; e - 50 μm.

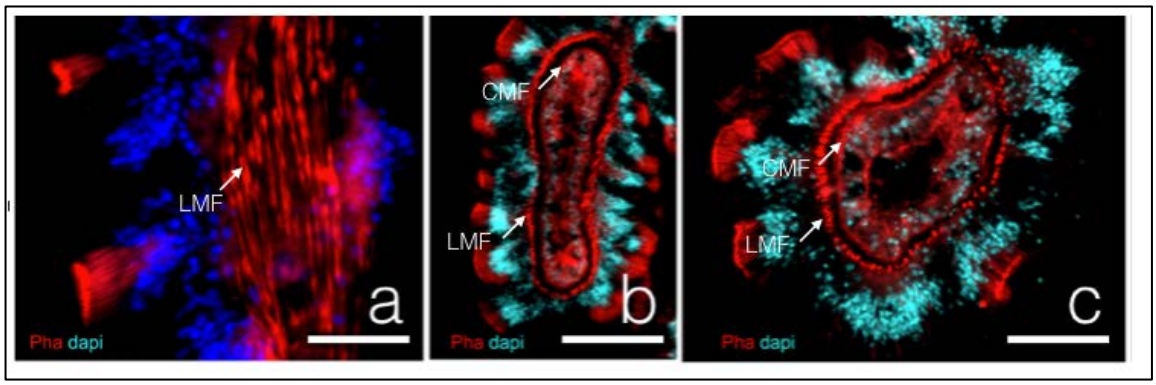

Figure 3.5. Detailed visualization of the muscle system in *Antipathes caribbeana* tentacles; **LMF** is longitudinal muscle fibers and **CMF** is circular muscle fibers: **a** – longitudinal cut of a tentacle showing the longitudinal muscle fibers; **b** – transversal cut of a proximal lateral tentacle; **c** – transversal cut of a distal lateral tentacle. Scale bars: 100 μm.

Polyp body muscle system

The muscle system outside the oral cone and the tentacles is poorly developed. It's visible in phalloidin staining that fibers do occur it is however hard to access their orientation and organization. Apart from the longitudinal muscle fibers of the ectoderm of the polyp no more has been described.

In the space between the lateral tentacles however we were capable of distinguish the muscle organization. The longitudinal muscle fibers are ectodermal and are present from the base of a lateral tentacle to the base of the lateral tentacle of the same pair (**figure 3.6a**).

The endodermal circular musculature is also present in the space between lateral tentacles but does not communicate between both tentacles as it is separated by the ventro-lateral mesentery (also called primary transverse mesentery in some antipatharian literature) that passes between both lateral tentacles (**figure 3.6b**).

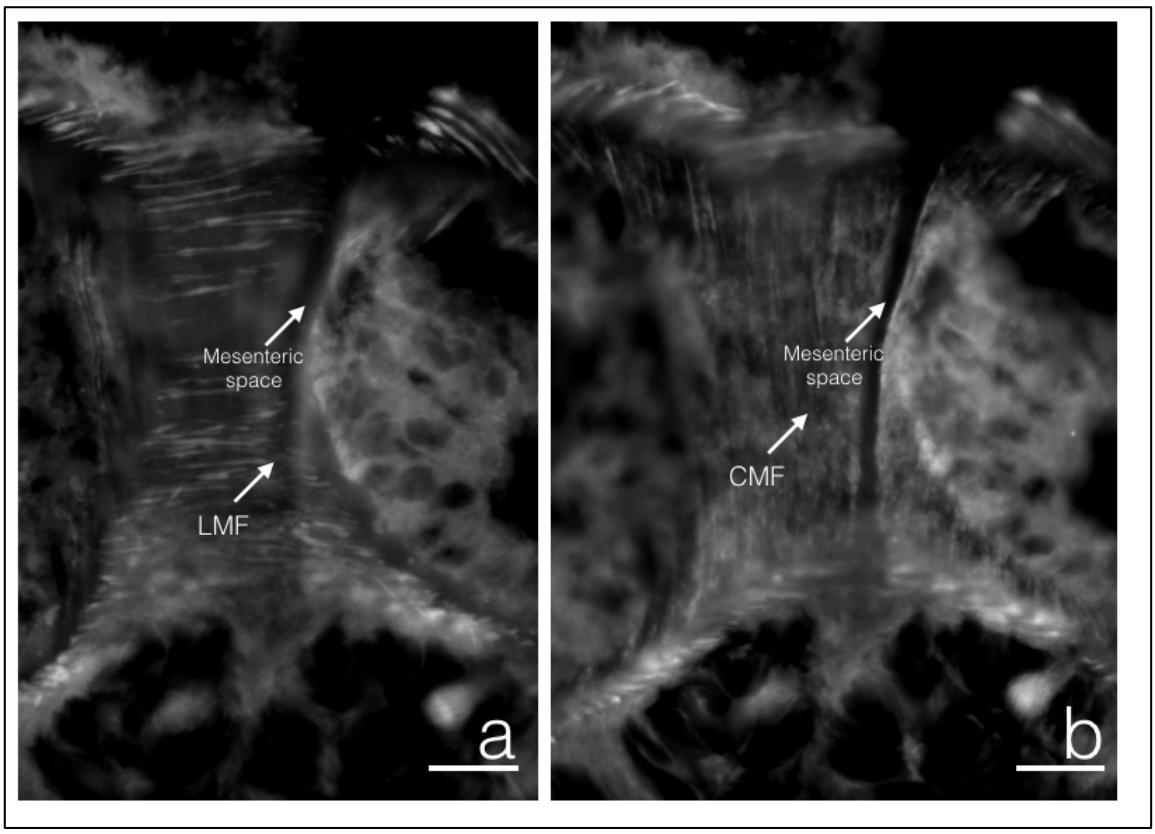

Figure 3.6. Muscle distribution between the pairs of lateral tentacles: **a** – ectodermal longitudinal muscle fibers (**LMF**); **b** – endodermal circular muscle fibers (**CMF**). Scale bars: 75 μm.

3.1.2 Detailed anatomy of the oral cone

General anatomy of the oral cone

Transversal sections of the oral cone were stained with toluidine blue for histology, with phalloidin and dapi and used for immunohistochemistry in order to detail the anatomy of this part of the polyp.

The histological sections give us alone much information about the organization of the oral cone. The first and most striking is that **the pharynx of the *Antipathes caribbeana* is flattered along the secondary axis**. This is visible in **figure 3.7a** (black arrows show the flattered pharynx). From the pharynx outward we have the pharynx ectoderm, a sheet of mesoglea, a layer of endoderm, another sheet of mesoglea and finally the oral cone ectoderm. This elements will be better visualized in more figures that follow and that are more magnified that this one.

The pharynx ectoderm

In **figure 3.7b** we have a magnified plan of the pharynx ectoderm. In *Antipathes caribbeana* we observe a pseudostratified organization of the pharynx ectoderm as has been described for *Antipathes aperta* (Goldberg and Taylor 1989b).

Goldberg and Taylor (1989b) described the cellular types of the *Antipathes aperta* pharynx ectoderm by means of toluidine blue histological sections and electromicroscopy sections.

Electromicroscopy is necessary to precisely identify all the cellular types. However, by comparison of our histological sections with toluidine blue to Goldberg and Taylor (1989b) sections with the same technique we are able to identify due to the different staining and cell forms the majority of described cell types. Although, once again, to be sure of the identifications electromicroscopy would be needed.

In **Figure 3.7b** and in **figure 3.7c** (that corresponds to a magnified view of the squared section in **figure 3.7b**) we have then identified:

- **Zymogen cells** (zym): active in the process of extracellular digestion, releasing their contents into the pharynx cavity (Haynes 1973);
- **Cup-collar cells** (ccc): cells that reach the gastric cavity and have cup-like cytoplasmatic extensions, they bear a single flagellum. They play a major role in endocytosis (Goldberg and Taylor 1989b);
- **Spumous mucus cells** (spm): more abundant in the basal part of the pharynx ectoderm, they are expanded basally and constricted apically, their main function can be presumed to be the formation of the mucus (Vader and Lönning 1975) produced by antipatharians (as mucus nets used for capturing preys (Lewis 1978));
- **Vesicular mucus cells** (vm): another mucus producing cell type, this one is present in a more apical position in the ectoderm and are not basally expanded (Goldberg and Taylor 1989b);
- **Microbasic mastigophores type A** (mbm.a): stinging cell (cnidocyte) characterized by a drop-shaped capsule, round and sharp at the opposite ends; the shaft arises from the round side of the capsule, reaching the 2/3 of the undischarged capsule length; it distally presents a V- shaped notch (Bo 2008).
- **Microbasic mastigophores type B** (mbm.b): stinging cell (cnidocyte) characterized by an almond shaped capsule, round and slightly sharp at the opposite ends; the shaft arises from the round side of the capsule, reaching the 2/3 of the undischarged capsule length; it distally presents a V-shaped notch (Bo 2008).
- **Ganglion cells** (n.pl): The ganglion cells occur as a plexus at the base of the pharynx ectoderm, in the frontier with the sheet of mesoglea.

The description of the cnidocyte types present in antipatharian species is important at this point as more and more descriptions arise. At the moment it is not an element used for taxonomy of antipatharian species but it may be in the future as the current used elements have proven to result in polyphyletic families (see chapter 1). Bo (2008) already compared the presence of cnidocyte types between some families but the present knowledge of the cnidocyte composition of the different families does not allow a full comparison of this trait

No specialized groove was detected in the pharynx of *Antipathes caribbeana*. **There is then no siphonoglyph in *Antipathes caribbeana***. This shows that **Antipatharia has no siphonoglyph** together with the results of Goldberg and Taylor (1989) in *Antipathes aperta* and the results of Molodtsova (2005) in *Parantipathes euantha*. It's coming the time to take the presence of siphonoglyphs in Antipatharia out of the textbooks that announce their presence (*e.g.* Kotpal 2004; Sharma 2014).

As pointed by Goldberg and Taylor in 1989b, the Antipatharia present a simple, rather uniform pharynx (without siphonoglyph) pharynx, as do scleractinians and corallimorpharians. The authors point that these orders are not closely related to antipatharians but recent studies (Daly *et al.* 2003; Kayal *et al.* 2013; Simion Ph.D. thesis, 2014; Zapata *et al.* 2015) have pointed Antipatharia as sister-group to the Scleractinia and Corallimorpharia clade. Here we can propose that **the uniform and simple pharynx lacking a siphonoglyph can be considered as a synapormorphy of the group comprising Antipatharia, Scleractinia and Corallimorpharia** (all other anthozoan orders possess siphonoglyphs).

Endoderm

The endoderm of antipatharians is subdivided by mesenteries forming endodermic compartments. In **figure 3.7d** we can see one compartment between the oral cone ectoderm and the pharynx ectoderm. The endoderm compartments are surrounded by sheets of mesoglea all around, between them and the pharynx ectoderm, between them and the oral cone ectoderm and between each other by the mesenteries mesoglea. A special type of cells is present and dominates in the endoderm, the **lipoidal cells** (lip) (Goldberg and Taylor 1989b): cells with large lipoidal inclusions.

Oral cone ectoderm

The oral cone ectoderm has a different identity in comparison to the pharynx ectoderm (Goldberg and Taylor 1989a, b). It presents both spumous mucus cells and vesicular mucus cells in a big quantity, in the oral cone ectoderm they stain so strongly with toluidine blue that they seem to dominate the entire ectoderm. In figure **3.7e** we can see the presence of wart-like structures, marked with an asterisk in the figure, mainly constituted by **spirocytes** (type of nematocytes with a thin, single-wall capsule that is acidophilic and contains a long, spirally coiled, unarmed thread of uniform diameter) but also by mastigophores in a smaller number, these structures are surrounded by both types of mucus cells. The wart-like structure can be observed better in **figure 3.7f** where the coiled aspect can be perfectly discerned.

Goldberg and Taylor (1989a) refer a zone of spirocytes and nematocytes proliferation in the basal area of the ectoderm below the wart-like structures. In our observations we did find mastigophores and spirocytes outside the wart-like structure environments, both in the endoderm and in the basal area of the ectoderm away from the wart-like structures (spi and mbm.a in **figure 3.7e**). Sectioning of the sample can have displaced these elements but their intact aspect and the reproducibility of these observations suggest that either mature spirocytes or mastigophores are present in the endoderm or that there is production of both cell types in the endoderm and that they would later migrate to the wart-like structures.

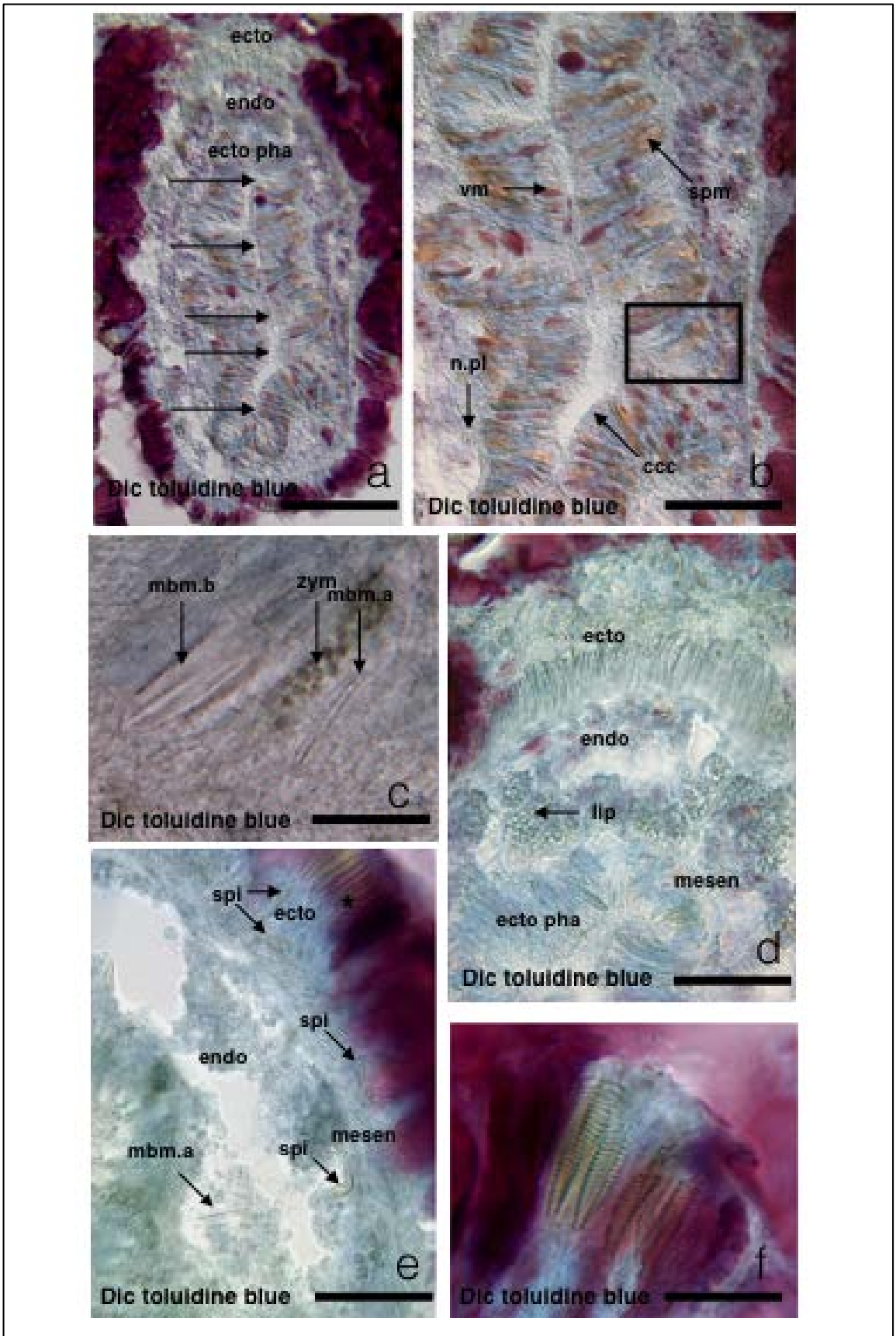

Figure 3.7. Histological transverse sections of the oral cone of *Antipathes caribbeana* stained with toluidine blue: **a** – complete oral cone section comprising the endoderm layer and the oral cone and pharynx ectoderms; **b** – magnified view of the pharynx ectoderm; **c** – magnified view of part of the pharynx ectoderm; **d** – magnified view of the endoderm; **e** – magnified view of the endoderm and the oral

cone ectoderm; **f** – magnified view of the wart-like structures of the oral cone ectoderm. Abbreviations: **ecto** – oral cone ectoderm; **endo** – endoderm; **ecto pha** – pharynx ectoderm; **vm** – vesicular mucus cell; **spm** – spumous mucus cell; **ccc** – cup-collar cell; **n.pl** – nervous plexus (ganglion cells); **zym** – zymogen cell; **mbm.a** – Microbasic mastigophores type A; **mbm.b** – Microbasic mastigophores type B; **mesen** – mesenchyme (mesoglea); **lip** – lipoidal cell; **spi** – spirocyst. Scale bars: a – 200 μm; b – 100 μm; c – 10 μm; d – 50 μm; e – 50 μm; f – 10 μm.

Autofluorescence

To further investigate the organization of the oral cone different sections were subject to immunohistochemistry detected by means of immunofluorescence. It is then important to note that the pharynx ectoderm presents autofluorescent cells in both the wavelengths used in this study, 488nm and 568nm. The same cells presented autofluorescence in both the wavelengths. Here we present the autofluorescence with the 568 wavelengths as an example in **figure 3.8**. Part of the pharynx ectoderm cells present autofluorescence. Their identification with dic in light microscopy is problematic but both nematocysts and zymogen cells seem to present autofluorescence. The zymogen cells only occur in the pharynx ectoderm but the nematocysts are also present in the rest of the ectoderm and so their autofluorescence can be observed in tentacle section presented in **figure 3.8d**.

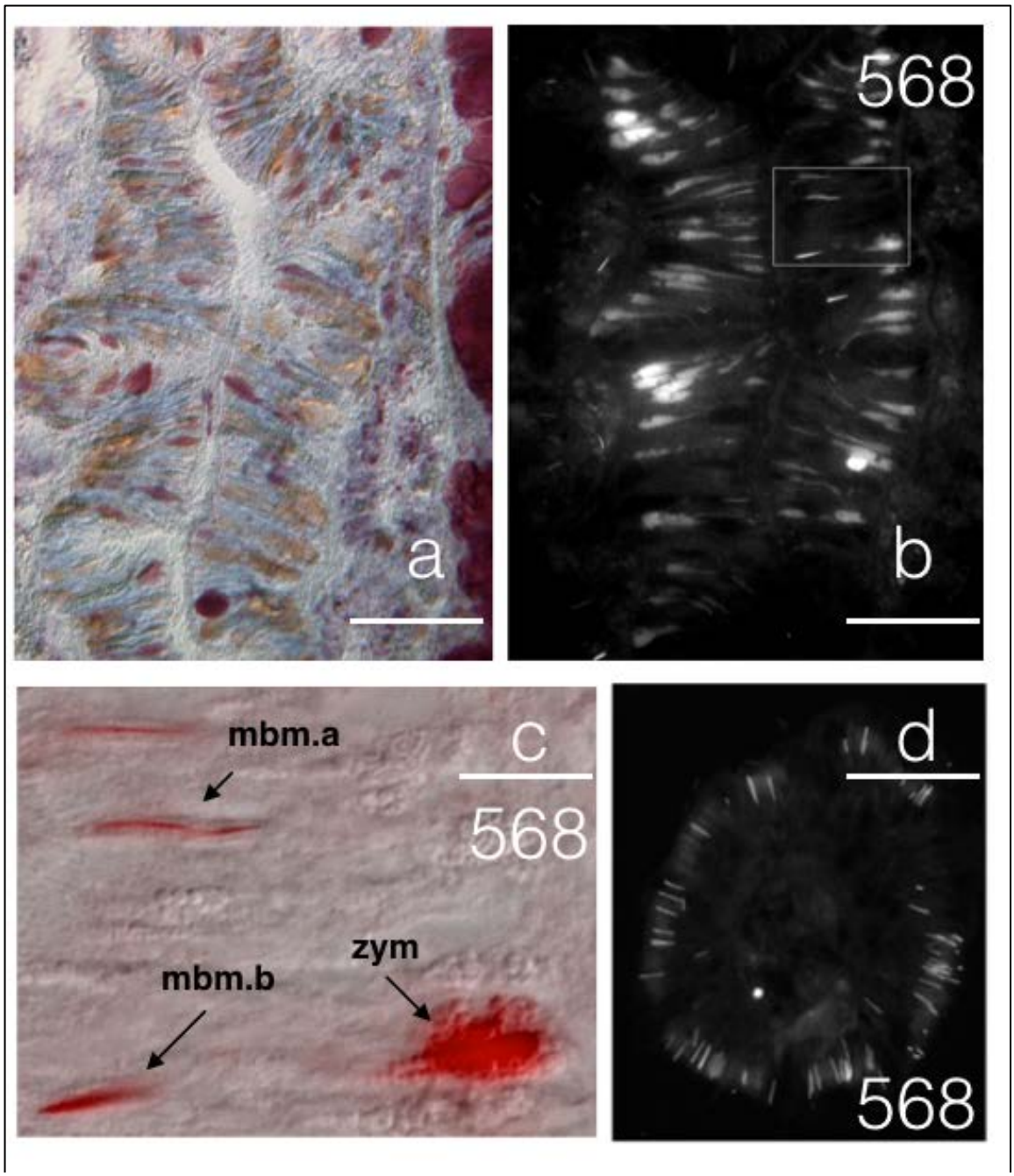

Figure 3.8. Autofluorescence in *Antipathes caribbeana*. **a** – the pharynx ectoderm; **b** –autofluorescence in the pharynx ectoderm at a wavelength of 568nm; **c** – a magnified view of the pharynx ectoderm with dic allows to distinguish that the cell types presenting autofluorescence are the mastigophores and the zymogen cells; **d** – tentacle ectoderm also present mastigophores and then autofluorescence. Abbreviations: **zym** – zymogen cell; **mbm.a** – Microbasic mastigophores type A; **mbm.b** – Microbasic mastigophores type B. Scale bars: a – 100 μm; b – 100 μm; c – 10 μm; d – 100 μm.

IHC confirms that *Antipathes caribbeana* has no siphonoglyph

The use of the commercial antibody 6-11B-1 (mouse monoclonal anti-acetylated α-tubulin (6-11-B1, Sigma, 1/1000)) against acetylated α-tubulin that binds primarily to primary cilia, centrioles, mitotic spindles, midbodies and to subsets of cytoplasmic microtubules in 3T3 and HeLa cells we were able to reveal the presence of ciliature in the oral cone of *Antipathes caribbeana*. The nucleus was stained with DAPI that emits fluorescence upon binding to A/T regions of DNA.

All the ectoderm is ciliated, including the pharynx ectoderm (**figure 3.9**). The endoderm presents ciliated spaces. They can be closed or open as seen in **figure 3.9**, when closed the ciliature is visible as a straight line caused by the space being closed.

In addition to no clear grooves detected by histology on the pharynx, we can see that no special ciliature exists at any point of the pharynx as there is a homogeneous dense ciliature in all pharynx ectoderm. **The homogenous ciliature of the pharynx confirms that no siphonoglyph is found in *Antipathes caribbeana***.

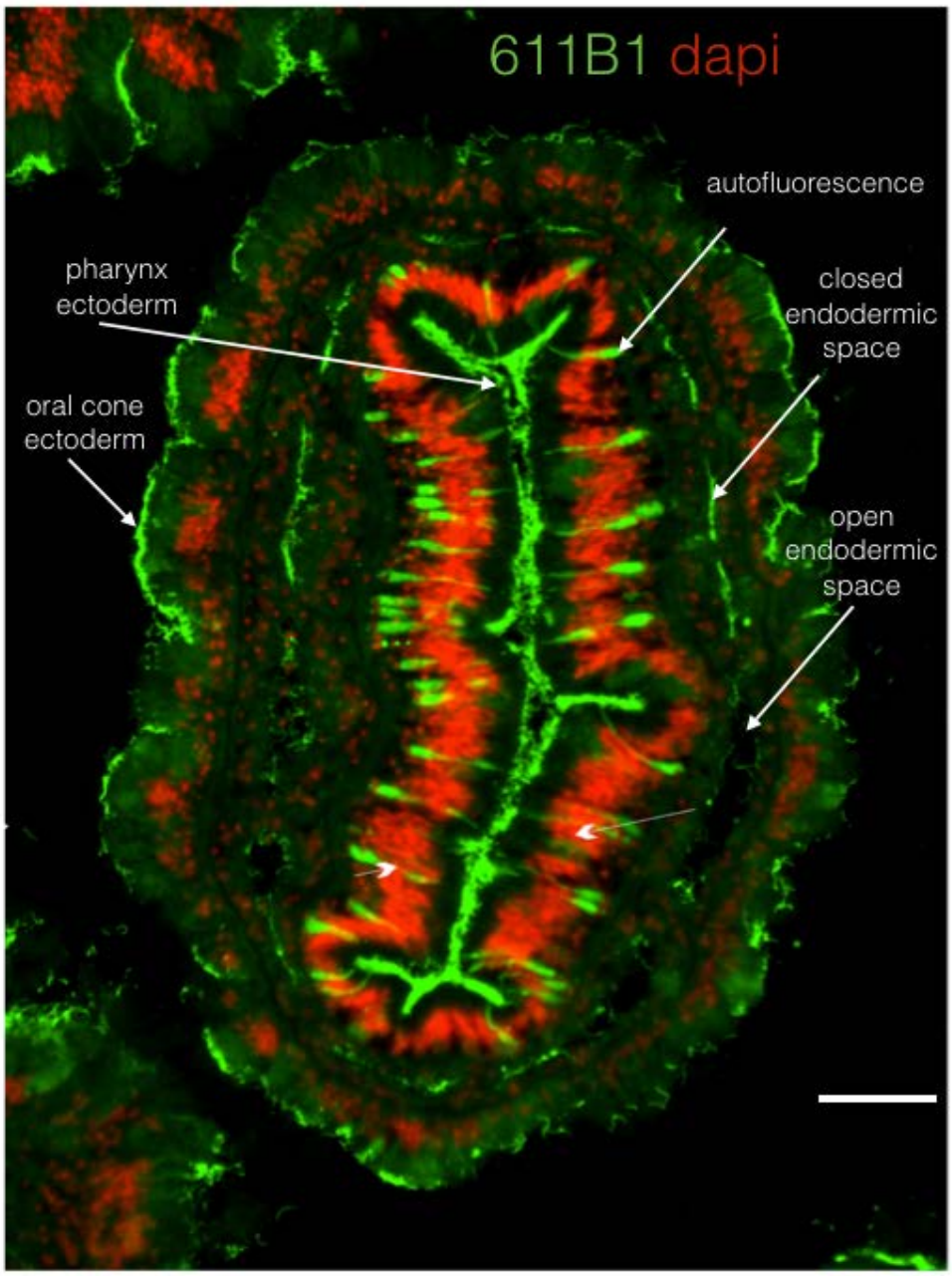

Figure 3.9. Transverse section of the oral cone of *Antipathes caribbeana* with the ciliature revealed by IHC against acetylated alpha tubulin and the nucleus stained by DAPI. Notice the autofluorescence of the nematocytes tubules (nematocysts). Scale bar: 70 μm.

The oral cone of *Antipathes caribbeana* presents 10 mesenteries

The use of an antibody against beta-actin allowed to clearly distinguish together with a DAPI staining that the **endoderm of the oral cone is divided by 10 mesenteries**. The sheets of mesoglea of the mesenteries are thin and in histology it is complicated to be absolutely sure of the number of mesenteries (**figure 3.10a**). The use of an antibody against beta-actin that allows the visualization of the cellular structure permits a clear visualization of the endodermic compartments (**figure 3.10b**). After a clear visualization of the organization it is possible to count the number of endodermic compartments either by DAPI staining or by beta-actin IHC (**figures 3.10c and 3.10d**). This endoderm compartments are separated by mesenteries that are then 10. This number of mesenteries is the most common number in antipatharians and its characteristic of the Antipathidae family (Wagner 2015) to which *Antipathes caribbeana* belongs (Bo 2008).

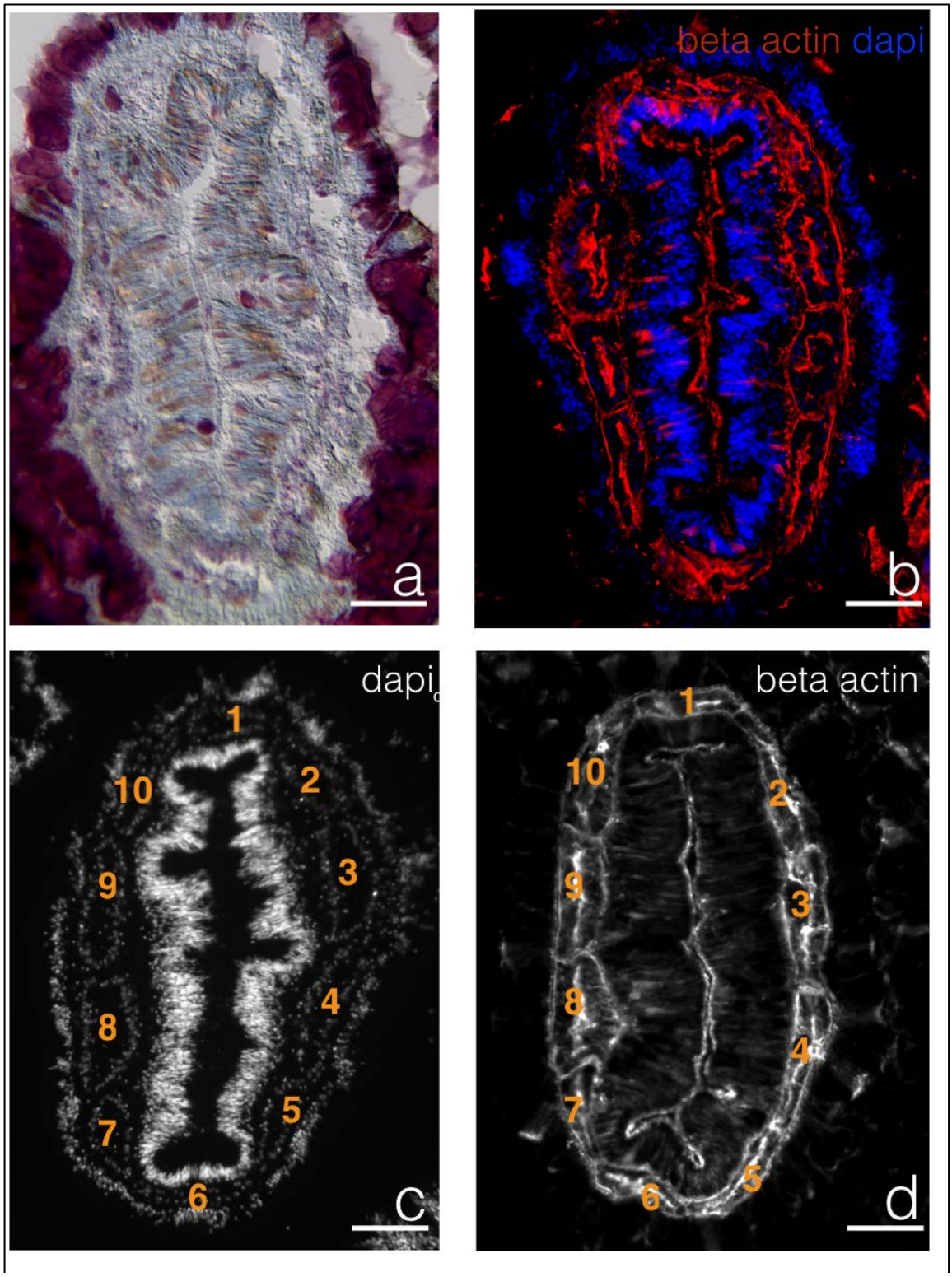

Figure 3.10. Transverse sections of the oral cone of *Antipathes caribbeana*: the oral cone presents 10 mesenteries; **a** – histological section of the oral cone; **b** – beta-actin/dapi allow the visualization of the ectoderm and endoderm layers; **c** – DAPI allows the visualization that the nucleus of the endoderm are organized in 10 compartments separated by mesenteries (mesoglea); **d** – the beta actin allows the visualization of the mesenteric limits of the 10 endodermic compartments. Scale bars: 50 μm.

The retractor muscles of *Antipathes caribbeana* are disposed in a polarized manner along the secondary axis

Whole mount polyps stained with phalloidin and DAPI were cryosectioned in order to determine the position of the retractor muscles associated with the mesenteries and their precise number.

As visible in **figure 3.11a** there are retractor muscles (red arrows) associated to the mesenteries in *Antipathes caribbeana*. These retractor muscles had been previously observed in all-mount samples (**figure 3.1**), the cryosections allow to easily precise their number, to which mesenteries they are associated and how they are distributed in relation to each mesentey. They are present in the lateral mesenteries and absent in the directive (dorsal and ventral) mesenteries. The directive mesenteries are easily identified, as they are disposed in the extremities of the flattered pharynx of antipatharians.

***Antipathes caribbeana* presents then 6 retractor muscles**. As seen in chapter 2 the central lateral mesenteries in antipatharians with 10 mesenteries are the ventro-lateral pair (VL) marked in the **figure 3.11a** with green arrows. This pair of mesenteries possesses its retractor muscles disposed in a polarized fashion. In **figure 3.11a'** the lateral mesenteries are represented by green arrows and the retractor muscles as red arrows. The position of the retractor muscle associated to the ventro-lateral pair makes a polarity in the secondary axis of *Antipathes caribbeana*. The **disposition of the retractor muscles makes then that there is only a single plane of symmetry for the *Antipathes caribbeana* polyp and thus the polyp has a bilateral symmetry**: the plan that passes along the flattered pharynx. The polarised muscle disposition gives at the same time a polarity to the secondary axis.

This result confirms van Pesch (1914) that was the only author to distinguish retractor muscles in antipatharians until today and that did discover that they are disposed in a polarized manner along the secondary axis of antipatharians.

The transverse sections of the oral cone confirm the association of the longitudinal muscle fibers to the ectoderm and of the circular muscle fibers to the endoderm (**figure 3.11b**).

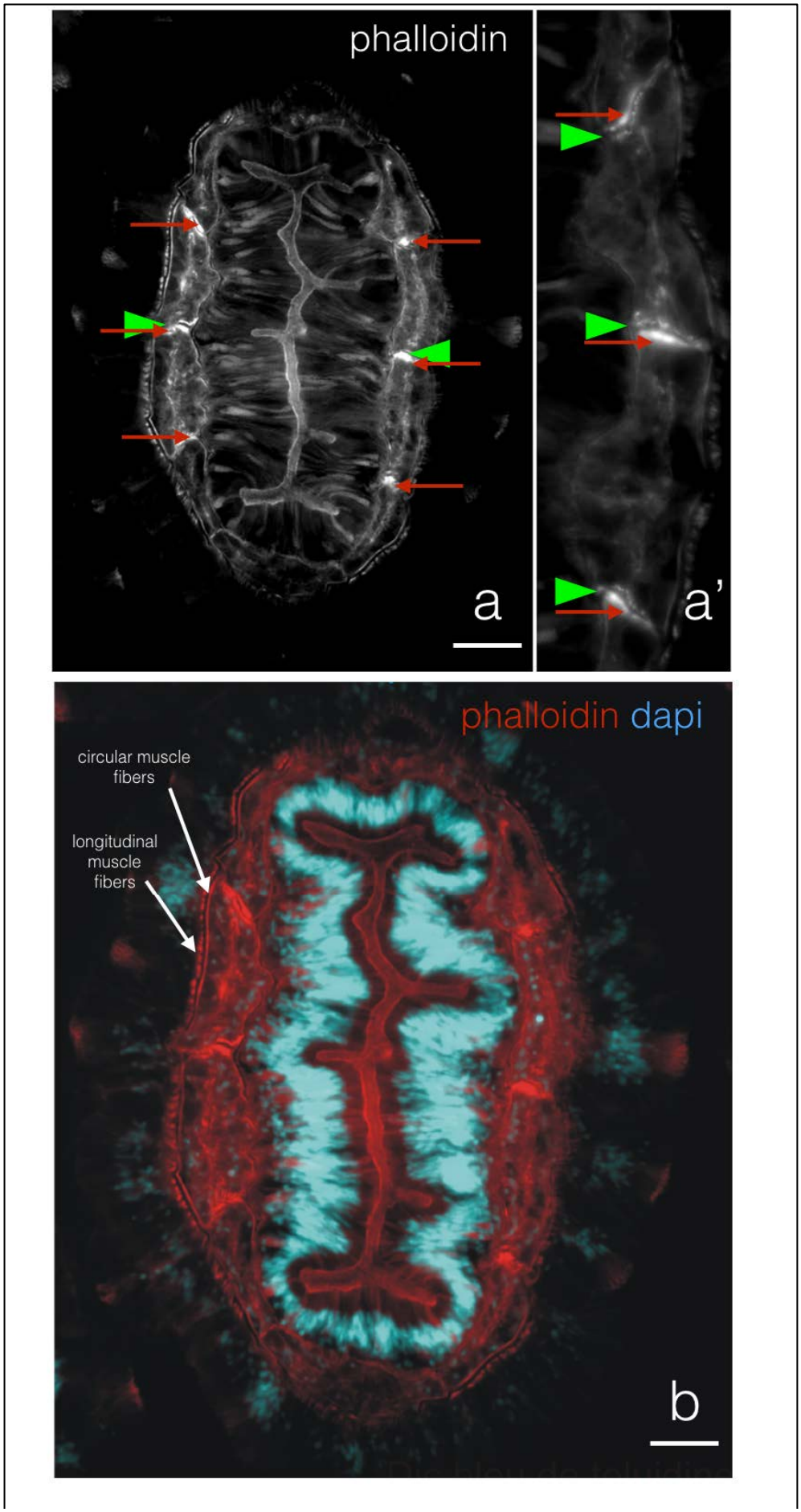
phalloidin
a
a'
phalloidin dapi
circular muscle fibers
longitudinal muscle fibers
b

Figure 3.11. Transverse sections of the oral cone showing the disposition of the retractor muscles in *Antipathes caribbeana*: **a** – *Antipathes caribbeana* presents 6 retractor muscles associated to the lateral mesenteries, green arrowheads marks the ventro-lateral mesenteric pair and red arrows mark the retractor muscles; **a'**: magnified view of the lateral mesenteries and respective retractor muscles, mesenteries marked with green arrowheads and retractor muscles with red arrows; **b** – the transverse sections confirm the association of the longitudinal muscle fibers with the ectoderm and of the circular muscle fibers with the endoderm. Scale bars: a – 70 μm; b – 50 μm.

Review of the main elements of the oral cone morphology

A clear understanding of the oral cone general morphology is difficult in a first approach. A general schematic representation is proposed in **figure 3.12** as a review of the main elements presented so far and to allow a clear interpretation of the main morphological charecters.

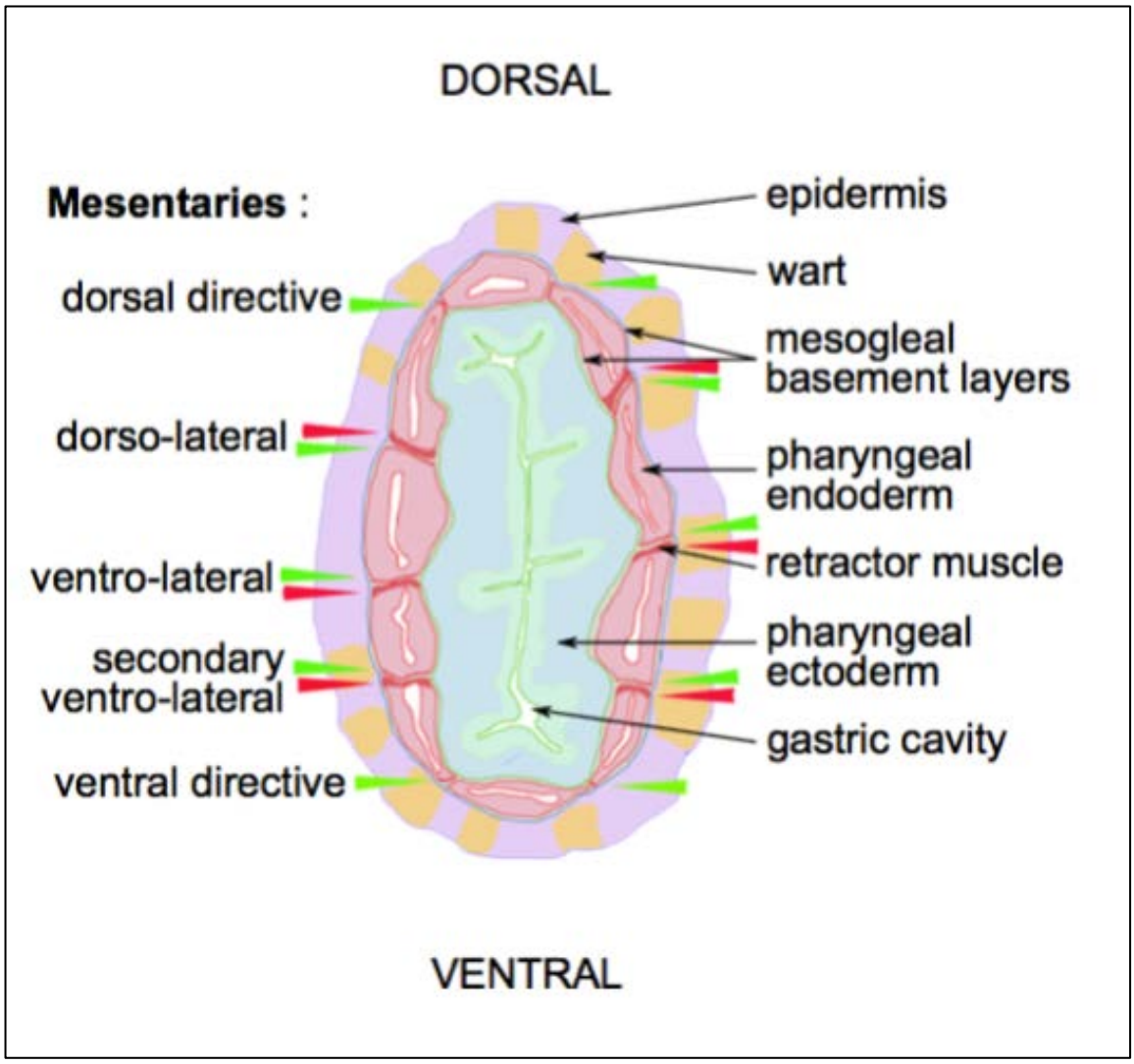

Figure 3.12. Schematic representation of the oral cone of *Antipathes caribbeana*. Red arrowheads represent the retractor muscles and green arrowheads the mesenteries.

Schematic representation of *Antipathes caribbeana* retractor muscle and mesenteric systems

The disposition of the retractor muscles in the ventro-lateral mesenteric pairs gives us the orientation of the antipatharian body as these muscles are always (see chapter 2) disposed in a ventral position in relation to this mesenteric pair.

A schematic representation of the retractor muscle and mesenteric systems of *Antipathes caribbeana* can be found in **figure 3.13**. With this schematic representation is easy to see that the muscle system of this species corresponds to the muscle system B

of Antipatharia as the muscles in the Dorso-lateral pair and in the secondary ventro-lateral pair are pointing towards the closer directive mesenteries (see chapter 2 for details on the Antipatharia type B muscle system).

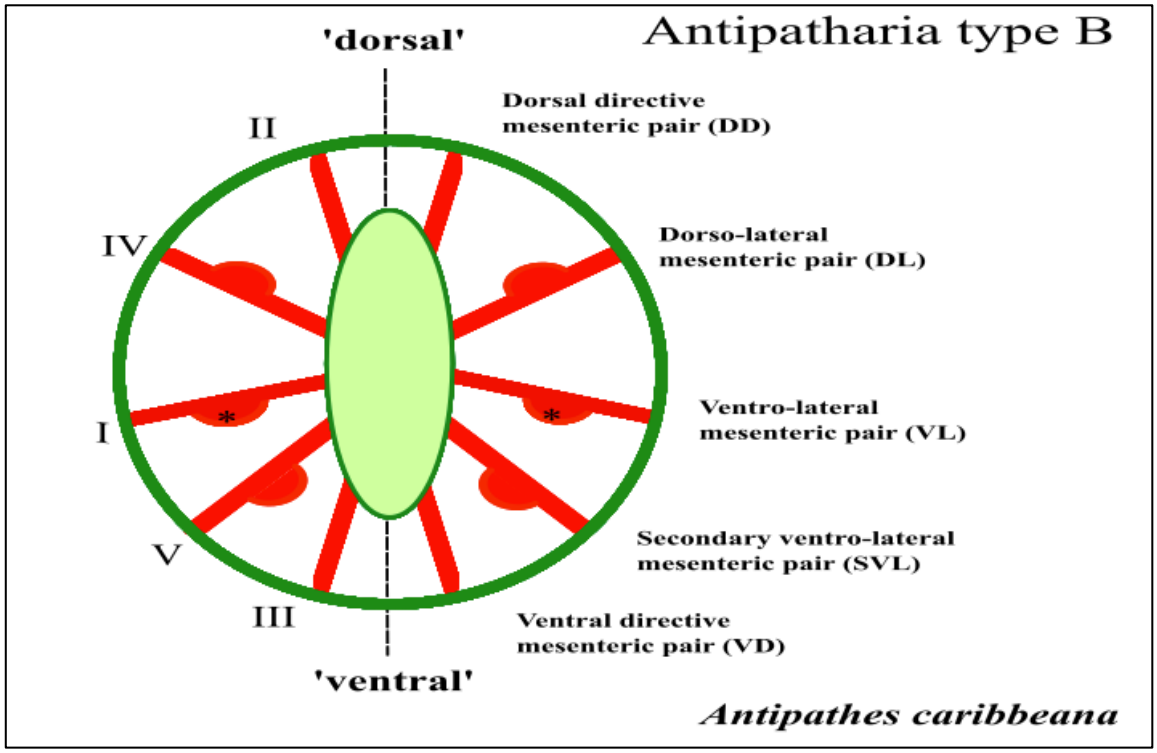

Figure 3.13. Schematic representation of *Antipathes caribbeana* retractor muscle and mesenteric systems

Antipathes caribbeana nervous system in the oral cone

The use of an antibody against FMRFamide (rabbit polyclonal anti-FMRFamide (ABcam, 1/1000)) allowed the visualization of FMRFamide positive neural cells. It revealed the existence of two circular neural nets, one, larger, at the base of the pharynx ectoderm and a second at the base of the oral cone ectoderm (arrows in **figure 3.14a**). The oral ectoderm circular neural net is much thinner that the one from the pharynx ectoderm. There is less density of nerve cells and can only be well visualized at a higher magnification (**figure 3.14b**), being especially visible and more consistent below the wart-like structures.

In our observations the pharynx ectoderm nerve layer sometimes seemed to have prolongations to the ectodermal layer, although no convincing images could be done and no certainty exists for this observation (**figure 3.14c**).

It is however clear that FMRFamide positive neural cells come from the oral cone ectodermal neural layer and innervate the wart-like structures (**figure 3.14d**). The YL1/2 antibody marks part of the neural system of other cnidarians as *Clytia hemisphaerica* (Jager *et al*. 2011) but does not seem to mark the antipatharian neural system in an effective way. In **figure 3.14d** we can see neural cells (arrow) that are marked partially by both YL1/2 and FMRFamide, it is however an exception.

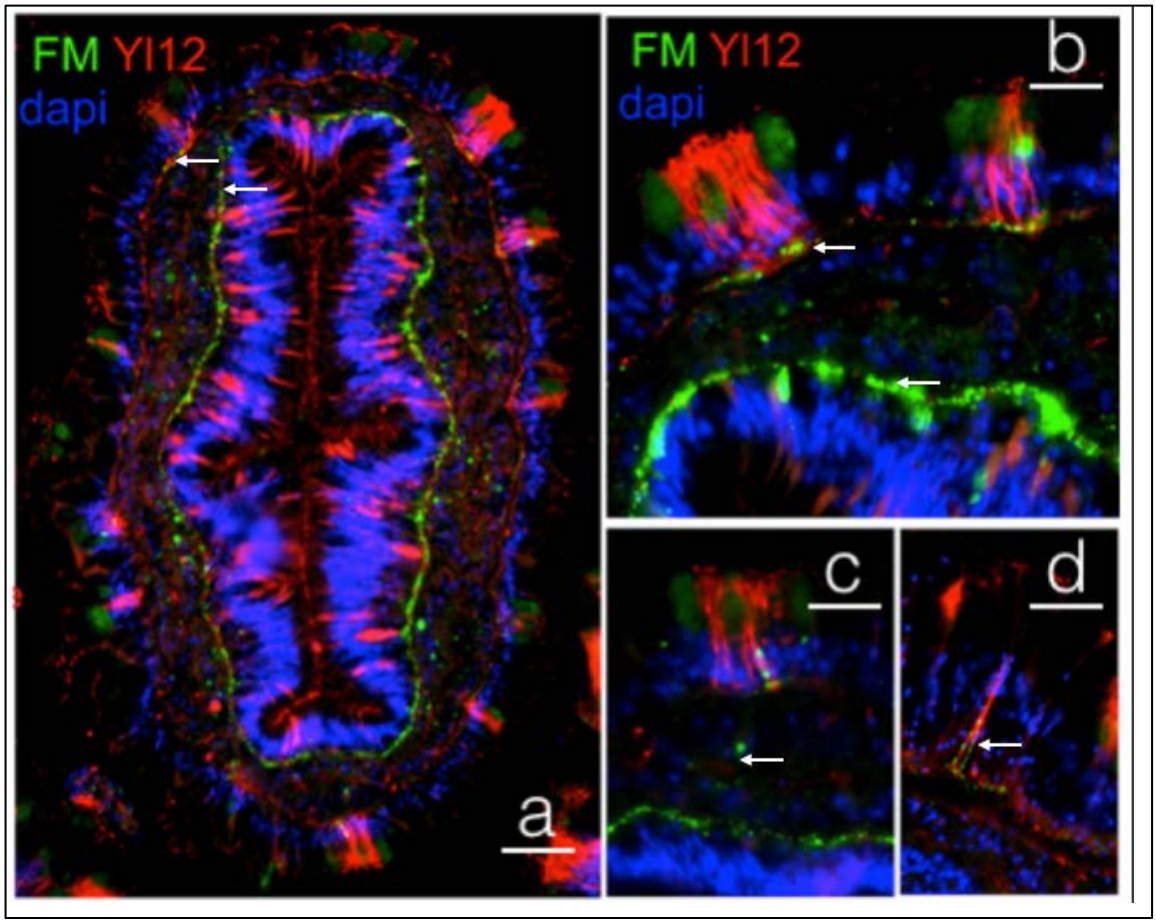

Figure 3.14. Transverse section of the oral cone of *Antipathes caribbeana* with the nervous system revealed by IHC against FMRFamide and tubulin (YL1/2), nucleus stained by DAPI: a – the oral cone presents two neural areas, one at the base of the pharynx ectoderm and a thinner are at the base of the oral cone ectoderm (arrows); b – detail on the two neural nets of the oral cone; c – the presence of FMRFpositive cells in the endoderm; d – YL1/2 does not seem to mark the neural cells of *Antipathes caribbeana*, only rarely FMRFamide and YL1/2 positive cells have been detected (arrow). Scale bars: a – 50 μm; b, c and d – 20 μm.

Isolated FMRFamide positive cells were observed extending from the pharynx neural area to the pharynx ectoderm (**figures 3.15a** and **3.15b**). FMRFamide positive cells were also detected around the spirocytes batteries (figure 3.14c), at the base of the spirocytes batteries (**figure 3.15d**) and innervating the spirocytes batteries (**figure 3.15e**).

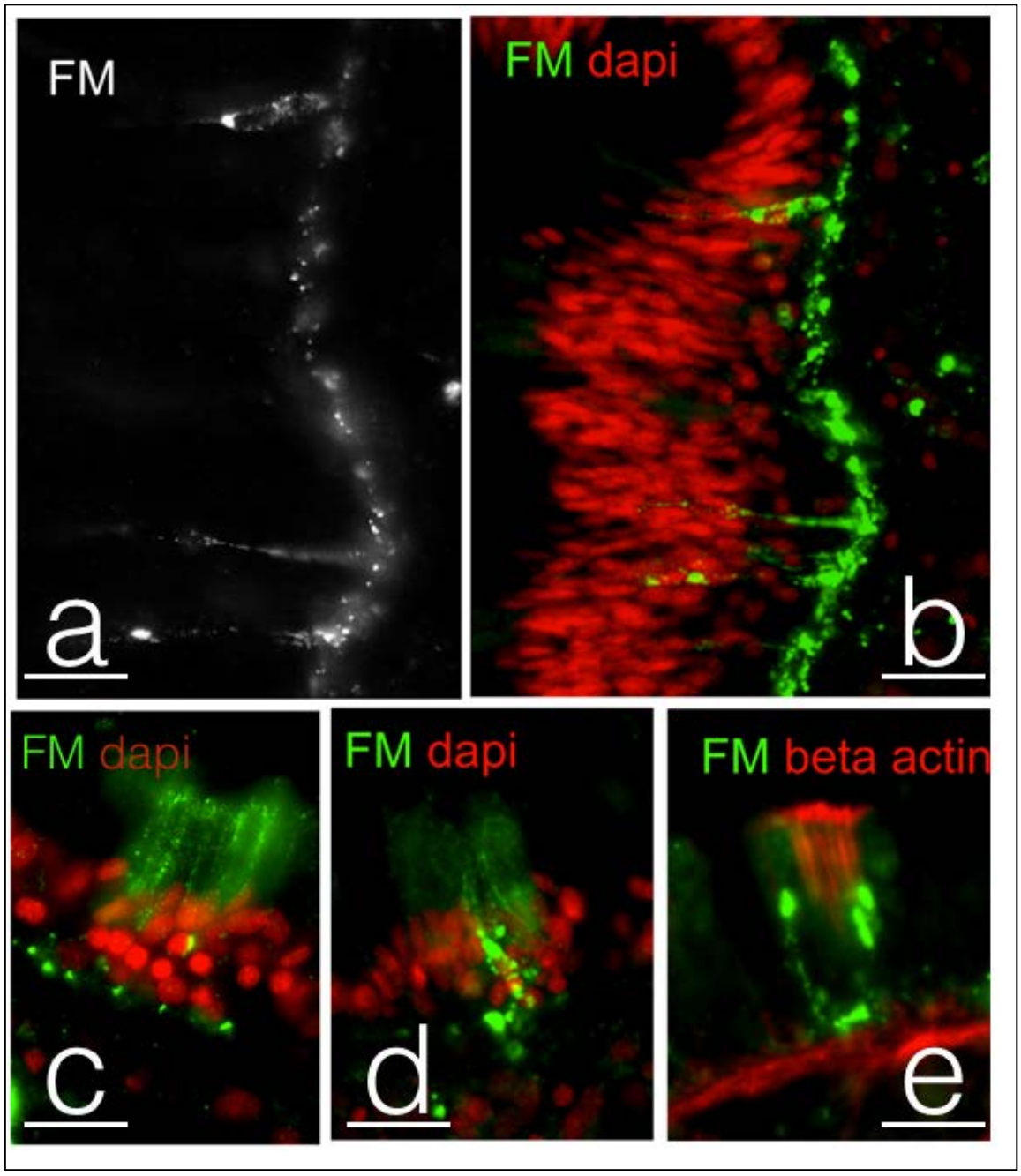

Figure 3.15. Details of the pharynx ectoderm and oral cone ectoderm nervous systems revelled by IHC against FMRFamide; beta-actin and DAPI staining: **a** and **b** – detail of the pharynx ectoderm nervous ring with FMRFamide positive extensions going towards the pharynx ectoderm; c – FMRFamide positive cells surround the spirocytes; d – FMRFamide positive cells at the base of the wart-like structures; e – FMRFamide positive cells enervate the spirocytes wart-like structures. Scale bars: 20 μm.

Differences between the pharynx ectoderm and the remaining ectoderm

Whole-mount IHC was performed in the *Antipathes caribbeana* polyps in order to study the difference between the pharynx ectoderm and the oral cone ectoderm. In **figure 3.16a** we can see that the wart-like structures (marked by beta-actin) are absent after a certain point towards the opening of the mouth (in the centre), this point corresponds to the beginning of the pharynx ectoderm that is then deprived of such structures.

The use of YL1/2 and 6-11B-1 show that the ciliature of the pharynx ectoderm is more dense and developed that that of the oral cone ectoderm (**figure 3.16b**). The structural limit between both ectoderms is not abrupt but can be visualized as a structural differentiated zone marked by YL1/2 and ciliated as seen by the 6-11B-1 marking. It is however no especially ciliated in comparison to the pharynx ectoderm (**figure 3.16b**).

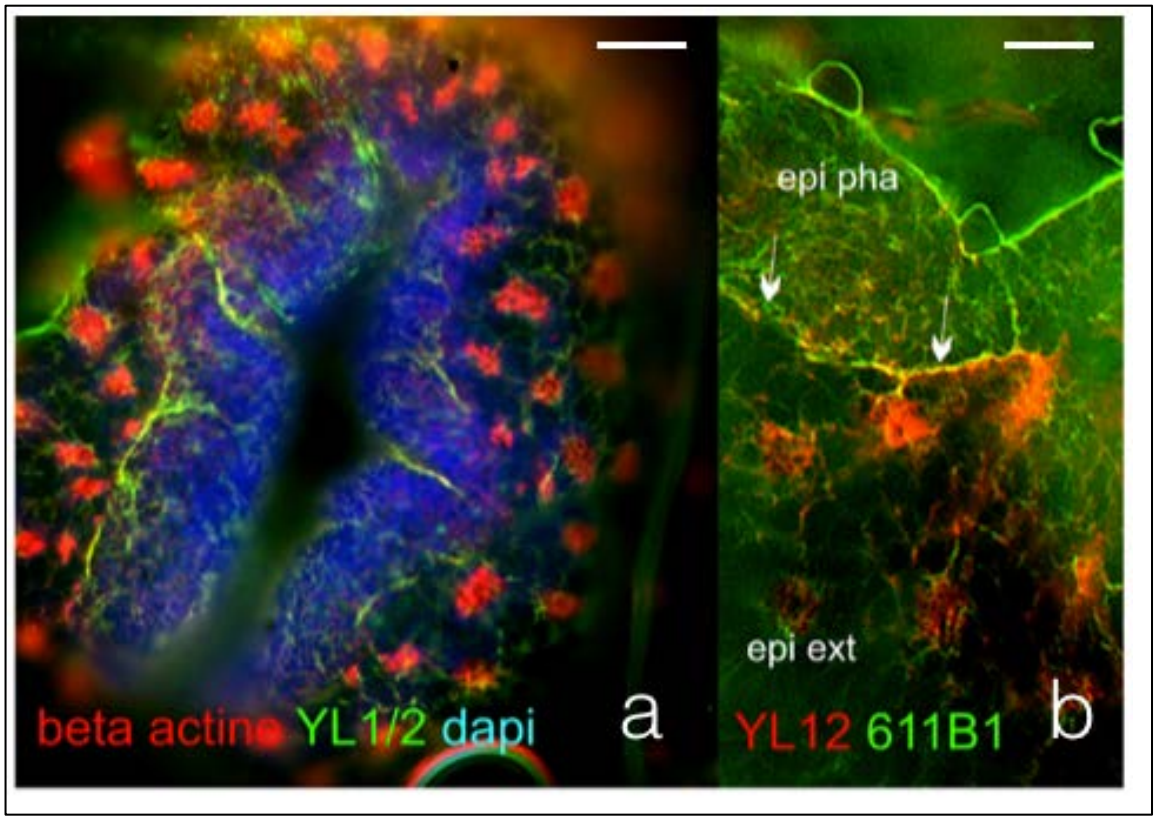

Figure 3.16. Different identities of the oral cone ectoderm and pharynx ectoderm: a – pharynx ectoderm does not have wart-like structures; b – no abrupt limit is evident between both ectoderms. Scale bars: a – 50 μm; b – 25 μm.

The limit between the pharynx ectoderm and the oral cone ectoderm can also be perceived in longitudinal cuts of the oral cone, both by histology and by IHC. By histology (**figure 3.17a**) we can see that the spumous mucus cells of the oral cone (violet stained) are much more abundant in the oral cone ectoderm and that their dimensions in this tissue are larger. The limit between the two ectoderms is marked with an arrow, this limit is clear and the difference between the ectoderms is astonishing. IHC (**figure 3.17b**) with YL1/2 and 6-11B-1 reveals that the pharynx ectoderm is much more ciliated that the oral cone ectoderm and that it does not possess wart-like structures, limit between both ectoderms marked with an arrow.

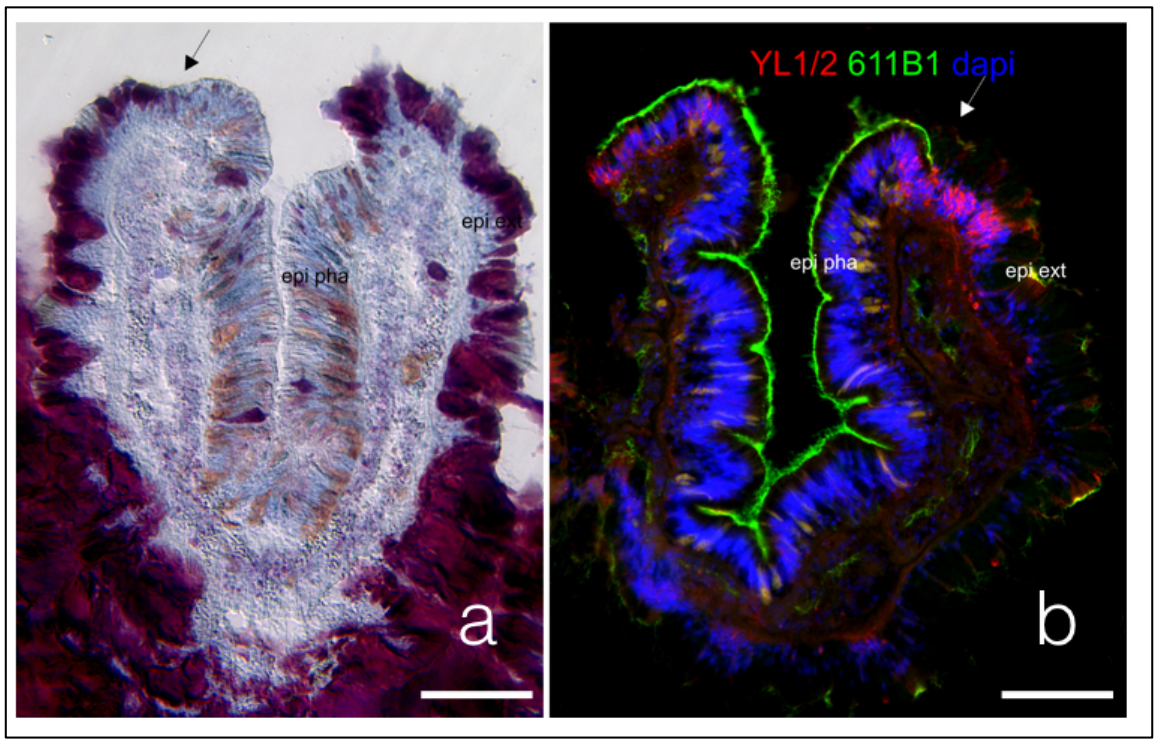

Figure 3.17. Longitudinal cuts of the oral cone showing the differences between the pharynx ectoderm and the oral cone ectoderm: a – toluidine blue staining shows the different composition in mucus cells between both ectoderms; b – IHC with YL1/2 and 6-11B-1 show that the pharynx ectoderm is much more ciliated that the oral cone ectoderm, it also lacks wart-like structures. Scale bars: 100 μm.

The neural areas of both ectoderms are connected

Longitudinal cuts of the oral cone allowed by FMRFamide staining to see that both neural areas of the oral cone of the antipatharians seem to be connected as there are FMRFamide positive cells at the base of the frontier between both ectoderms (white arrow, **figure 3.18**).

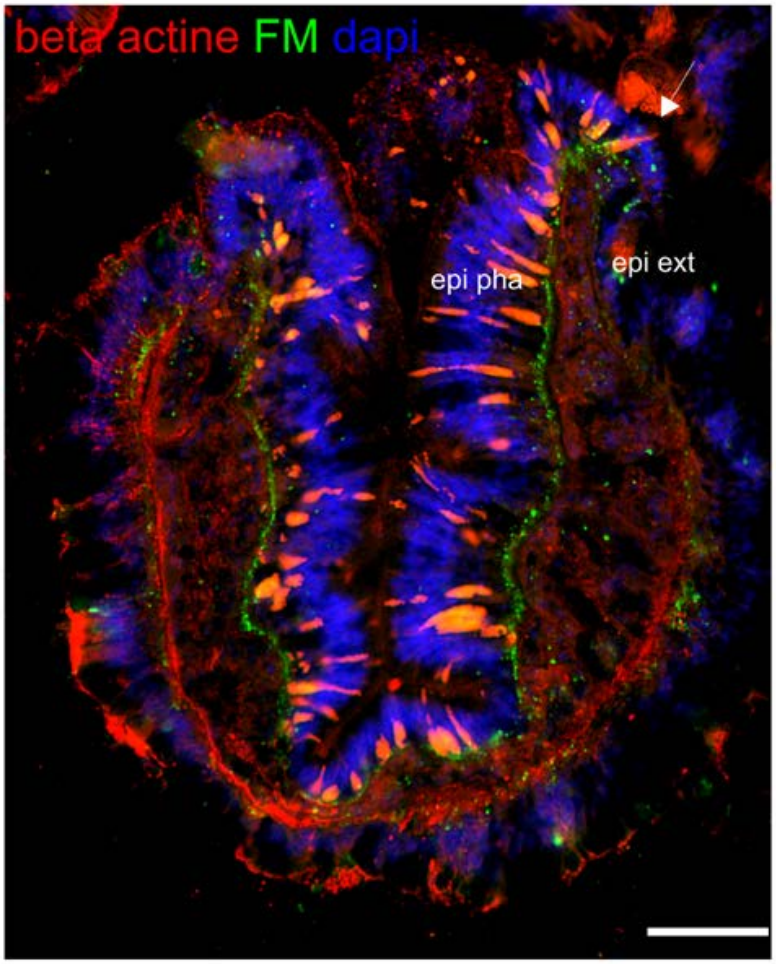

Figure 3.18. Longitudinal section of the oral cone of *Antipathes caribbeana*: both neural areas are connected by FMRFamide positive cells (white arrow). Scale bar: 100 μm.

3.1.3 Detailed anatomy of the tentacles

All tentacles of *Antipathes caribbeana* have the same morphology. They have an external ectodermal layer similar to the oral cone ectoderm but richer in wart-like structures. As visible in figure 3.19 they have an ectodermal layer and an endodermal layer.

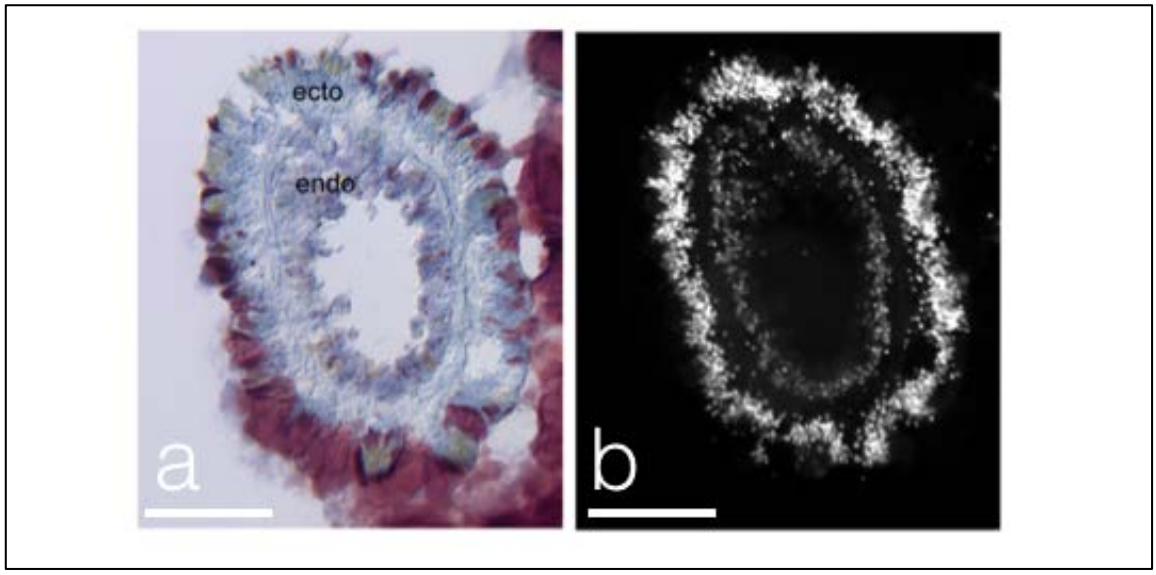

Figure 3.19. Transverse cut of a tentacle with toluidine blue staining allowing to discern an ectodermic and an endodermal layer. a – histological section; b – DAPI of the histological section showing two nuclear zones: an endodermic zone in the centre and an ectodermic zone around it. Scale bars: 100μm.

The limits between these layers are well revealed by immunohistochemistry with a beta actin antibody together with a DAPI staining (**figure 3.20**).

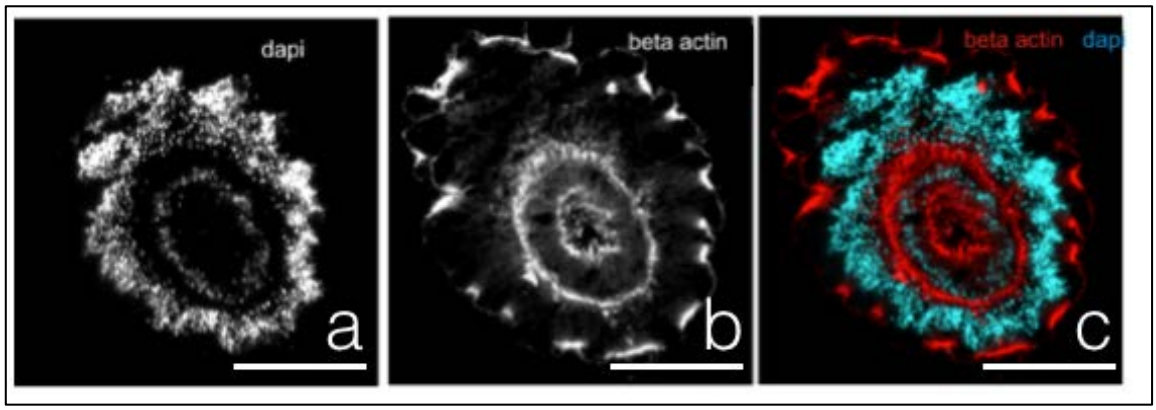

Figure 3.20. Transverse sections of a tentacle showing two nuclear zones separated by a layer of actin staining that marks the limit between the endoderm and the ectoderm. Scale bars: 100μm.

Longitudinal sections of tentacles reveal that the endodermal section is hollow in its interior (**figure 3.21**).

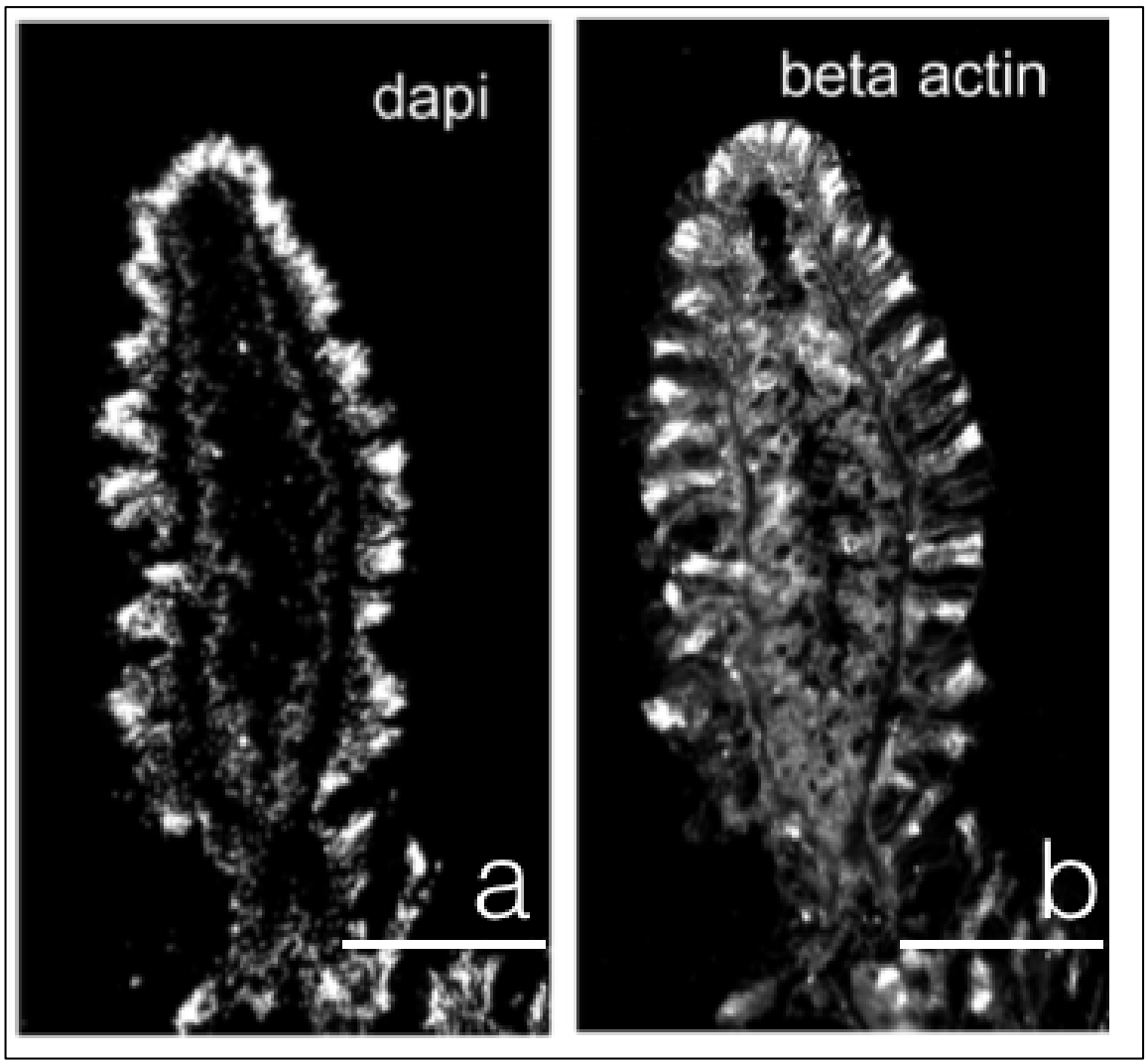

Figure 3.21. Longitudinal sections of a tentacle showing that the endoderm is hollow in its centre. Scale bars: 200μm.

The endoderm and ectoderm layers of the tentacle are separated by a sheet of mesoglea as visible in **figure 3.22a and 3.22b**. The endoderm is rich in lipoidal cells and the ectoderm presents both types of mucus cells, spirocytes, ganglion cells and mastigophores (**arrow in figure 3.22c**) (Goldberg and Taylor 1989a).

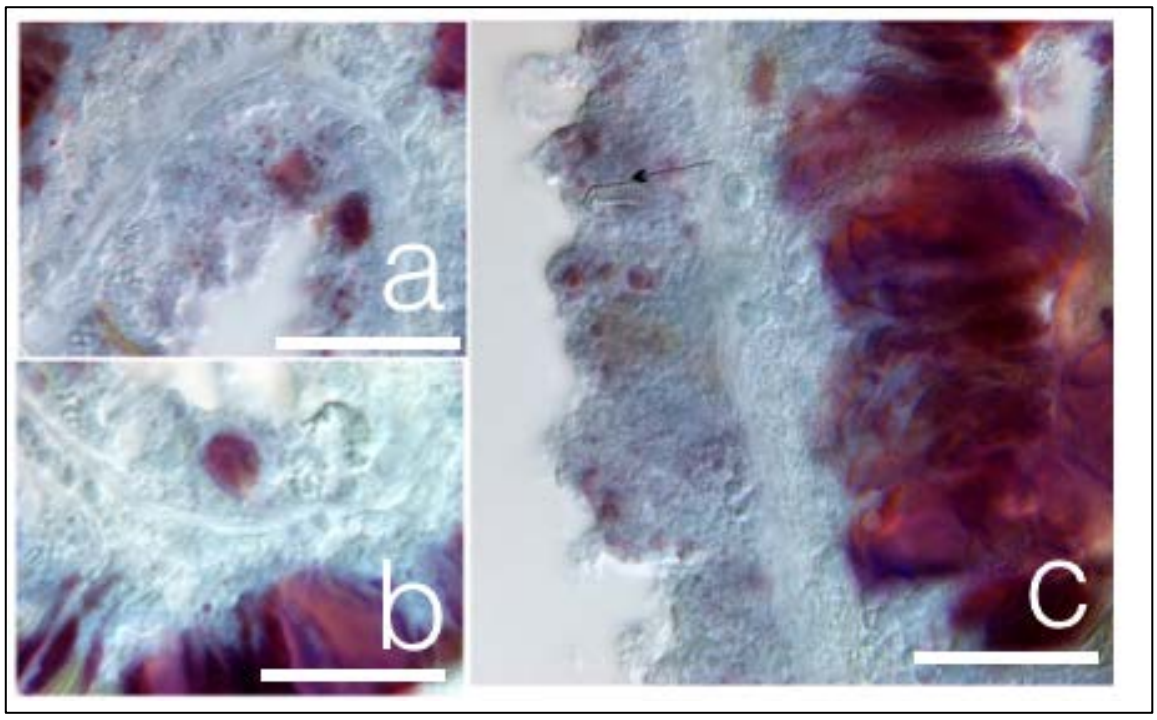

Figure 3.22. Details of transverse sections of a tentacle showing that the mesoglea separating the endoderm and the ectoderm: **a** and **b** – the mesoglea sheet is always present between the endoderm and the ectoderm; **c** – mastigophores in the tentacle ectoderm. Scale bars: a – 25 μm; b – 25 μm; c - 50μm.

The ectoderm of the tentacles is also ciliated, especially in the wart-like structures (**figure 3.23**). The top of the wart-like structures is rich in actin (**figure 3.24**) as the cilia present in the apex of nematocytes is formed by parallel actin filaments (Beckmann 2013).

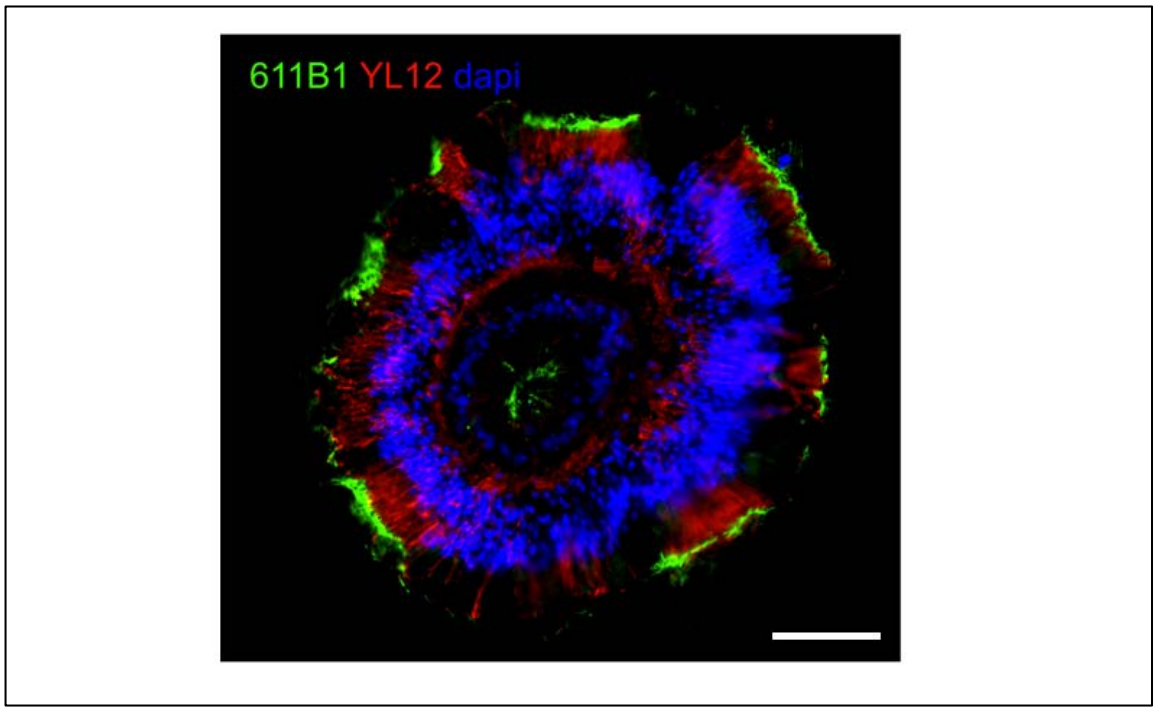

Figure 3.23. Ciliature of the tentacle ectoderm. Scale bar: 200 μm.

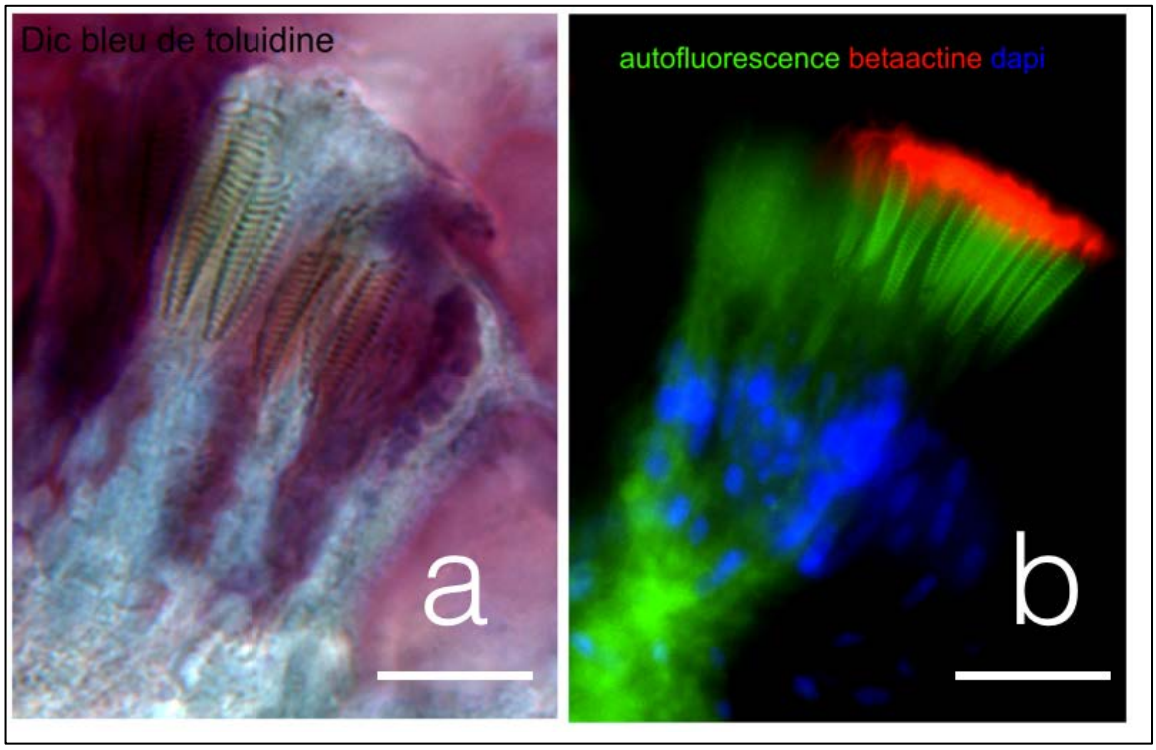

Figure 3.24. Organization of the wart-like structures. Scale bars – 10 μm

3.1.4 First description of hermaphroditism in an antipatharian polyp

The only mesenteries to bear reproductive organs in most antipatharians are the ventro-lateral mesenteries (also called in antipatharian literature as primary transverse mesenteries, for example by Wagner *et al*. 2011). This can be observed by longitudinal cuts along a branch of a colony as done four our species *Antipathes caribbeana* (**figure 3.25a**). The reproductive organs are then disposed in both sides of the gastric cavity until the limit of the polyp.

In 2011, Wagner *et al*. published a review on the antipatharian reproduction and pointed that for most species the colonies are gonochoric, meaning that a colony is either male or female. From more than 50 species studied or reviewed by the authors only two exceptions to this rule came out: sequential hermaphroditism in *Cirripathes sp*. Described by Bo (2008) and simultaneous hermaphroditism described by Pax (1987) in *Stichopathes saccula*.

Bo (2008) describes the sequential hermaphroditism as her findings showed that the colonies of *Cirripathes sp*. may change sex with age. The simultaneous hermaphroditism described by Pax in *Stichopathes saccula* is the only simoultaneous hermaphroditism described for the Antipatharia and the species is described has having in some colonies both male and female polyps. However van Pesch (1914) only found male colonies of this species. Pax (1987) only refers the existence of hermaphrodite colonies in an antipatharian review of anatomy and does not present true evidence of this hermaphroditism, not detailing if the analyzed polyps are from the same branch. It can be that the polyps came from different branched and thus they can easily belong to different colonies, as the collection of antipatharians is most of the times complicated and done by chance. Without details it is impossible to confirm this findings of Pax (1987) and Wagner *et al*. (2011) consider that there is need of more studies in the species in order to confirm this findings and that simultaneous hermaphroditism really can exist in antipatharians.

In *Antipathes caribbeana* we found both male and female sexual organs in the same colony and more stickling we found them in the same polyp, meaning that both the colony and the polyp are simultaneous hermaphrodites. The presence of both mature and immature oocytes (red arrows) in a polyp of Antipathes *caribbeana* can be observed in **figure 3.25b**, together with the presence in the same polyp of spermatocysts (green

arrow). The anatomy of these reproductive organs in our sample leave no doubt about their true identity when compared with the morphologies described by Bo (2008) and Wagner *et al*. (2011).

With this finding we **confirm that simultaneous hermaphroditism does exist in Antipatharia** (as previous descriptions were dubious) and we **describe for the first time the existence of hermaphroditism at the polyp level**.

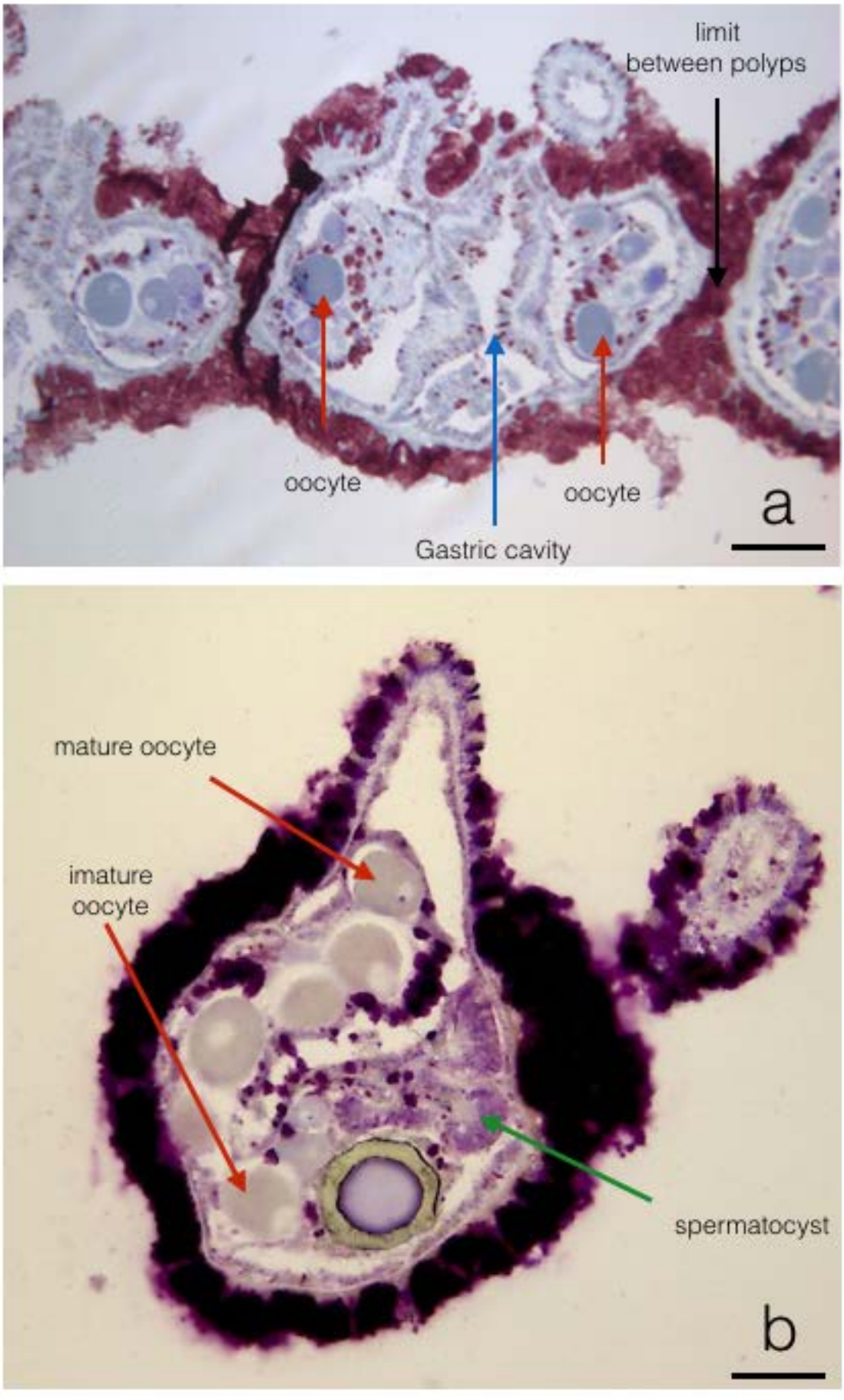
limit
between polyps
oocyte
oocyte
Gastric cavity
a
mature oocyte
imature
oocyte
spermatocyst
b

Figure 3.25. *Antipathes caribbeana* polyps may present both male and female reproductive systems: a – sexual cells are associated to the ventro-lateral mesenteries between the gastric cavity and the polyp limit; b – presence of both male and female reproductive systems in an *Antipathes caribbeana* polyp. Scale bars: a - 200 μm; b – 120 μm.

3.2 Development of an *in situ* hybridization protocol for antipatharian species

In order to study the gene expression of the developmental genes in antipatharians species a protocol for *in situ* hybridization (ISH) needed to be developed. The developed protocol corresponds to the first protocol developed and executed in antipatharian species as well as in a colony of anthozoan polyps.

Several essays have been performed in order to optimize a working protocol for both *Antipathes caribbeana* and *Plumapathes pennacea*.

In **table 3.1** is presented a brief overview of the conditions that were tested and in green the selected condition. Several possible combinations of the presented conditions needed to be tested in order to obtain the final protocol.

The antipatharians presented a staining reaction time unusually long with some genes presenting times of several days (up to 15 days). This conditioned in time the development of the optimal protocol.

Plumapathes pennacea presented encouraging preliminary results in the first performed *in situ* hybridization. However this species presents in comparison to *Antipathes caribbeana* smaller and more fragile polyps. All the consequential in situ hybridizations performed in this species turned out to be compromised by the conservation state of the branches of this species. The fixation method performed in Guadeloupe was not ideal for the preservation of this species and unfortunately the study of the expression patterns for this species had to be abandoned.

The detailed protocol of *in situ* hybridization for *Antipathes caribbeana* can be found in the supplementary materials.

Hybridization temperature	Permeabilization	Acetylation	Fixation
60ºC	No permeabilization	Glacial acetic acid	PFA* 4% 2 hours of fixation
58ºC	10 minutes of Proteinase K 0.01mg/ml	No acetylation	F+G**
55ºC	10 minutes of Proteinase K 0.001mg/ml		

Table 3.1. Tested conditions for the development of an ISH protocol for antipatharians. *PFA - Paraformaldehyde; ** F+G: Formaldehyde 3.7% and Glutaraldehyde 0.2%

3.3 Paper: Black corals (Antipatharia, Anthozoa) insights on putative homology of the polyp secondary axis across Anthozoa (Cnidaria).

To be submitted in September to the journal EvoDevo (BioMed Central).

Preface to the paper:

Molecular establishment of the Bilateria and Cnidaria body axes

Due to the extreme diversity of morphologies present in metazoans, and to the fact that the morphological elements across metazoans are extremely diverse and not easily comparable, biologists have recurred to the comparison of the molecular establishment of their body axis to understand the possible homology between those axes in what is called an evo-devo approach.

As bilaterians have two defined body axis, the primary body axis (antero-posterior) and the secondary axis (dorso-ventral) and has their molecular characterisation has been studied in many different phyla of Bilateria, authors started comparing the primary body axis of this group with the primary body axis present in other metazoans (cnidarians, ctenophores; placozoans and poriferans.

Cnidarians present a main body axis, the oral-aboral axis (Manuel 2009, Houliston *et al*. 2010). However, as seen in chapter 2, anthozoans possess a secondary body axis, orthogonal to the primary body axis as bilaterians have. The existence of a class (Anthozoa) with two body axes in Cnidaria, the sister group of Bilateria, makes morphological and molecular comparisons of those axes at once more complex and more interesting.

The major genes implied in the establishment and patterning of the body axis of bilaterians and cnidarians will be addressed here in a comparative fashion in order to

evidentiate the present knowledge on the homology between the primary body axis of Cnidaria and Bilateria and of the secondary body axis of Anthozoa and Bilateria.

Wnt/β-catenin signalling strongly suggests the homology between the primary body axis of cnidarians and bilaterians

The primary body axis of cnidaria has been compared to Bilateria mostly by the comparison of the Wnt/β-catenin signalling expression that defines the polarity of this axis in Bilateria.

Wnt/β-catenin signalling has been shown to be indispensable for the establishment of the oral-aboral axis in many cnidarians. This is also the case in diverse deuterostomes and in planarians (protostomes) (reviewed in Peterson and Reddien 2009) in Bilateria. In cnidarians previous studies indicate that *Wnt* genes are expressed in staggered dommains in the oral half of the embryo, for example there has been considerable progress in the understanding of the axial patterning in *Nematostella* (Kusserow *et al*. 2005 ; Kumburegama *et al*. 2011 ; Rottinger *et al*. 2012) but many questions reamin to be addressed. However, the similarities lead most authors to admit a probable homology between the cnidarian and bilaterian primary body axis (Manuel 2009; Petersen and Reddien 2009; Ryan and Baxevanis 2007; Lanna 2015; Technau *et al*. 2015).

The formation of the secondary axis of anthozoans seems to be mediated by the BMP-pathway

The asymmetry along the secondary axis of bilaterians, creating dorsal and ventral poles, occurs by a gradient of BMP (bone morphogenetic protein – a member of the TGF-β family (Derynck and Miyazono 2008)) signalling activity as in Bilataria numerous studies strongly suggest that the Bilateria last common ancestor had its dorso-ventral axis (secondary axis) patterned by the BMP-pathway (Arendt and Nübler-Jung

1994; De Robertis 2008; Genikhonovich *et al*. 2015) and, exception for vertebrates, its polarity is conserved (Holley *et al*. 1995; Arendt and Nübler-Jung 1999).

In Cnidaria, while in *Hydra*, a radial hydrozoan (without a secondary body axis) an asymmetric expression of BMP signalling pathway components was not observed (Rentzsch *et al*. 2007), in two anthozoan species, *Nematostella vectensis* and *Acropora millepora*, an asymmetric expression of this pathway exists along the secondary axis of these animals.

In 2001, Samuel *et al*. discovered that the "dpp/BMP" signalling pathway was conserved in the coral *Acropora millepora*. The next year Hayward *et al*. (2002) discovered that the *dpp/BMP2/4* was expressed in a localized way in the coral embryo in one side of the blastopore. In 2003, Hwang *et al*. showed the conservation of the *Bmp2/4* gene between bilaterians and anthozoans, detecting its presence in four species of scleractinians and in an actiniarian (*Actinia equina*).

In more recent years, in the sea anemone, *Nematostella vectensis* (Actiniaria, Hexacorallia), it was discovered that the *BMP2/4* and *BMP5-8* and the antagonist *chordin* are expressed at the gastrula and planula stage in largely overlapping domains on the same side of the body (Finnerty *et al*. 2004; Matus *et al*. 2006a, Matus *et al*. 2006b; Rentzsch *et al*. 2006; Ryan *et al*. 2007) in an asymmetric way on the animal secondary axis. This, together with the results in *Acropora millepora* (Scleractinia), where *chordin* expression begins early on one side of the blastopore, then becomes localized on ectoderm in the early planula on one side toward the oral end and finally disappears before settlement (Hayward *et al*. 2002; Hayward *et al*. 2015; Okubo *et al*. 2016), lead authors (Finnerty *et al*. 2004; Finnerty 2005; Matus *et al*. 2006a) to propose a homology between the secondary axis of bilaterians and anthozoans.

Contrarily to Bilateria the *BMP2/4* and its antagonist *chordin* are expressed in *Nematostella* in the same side of the secondary axis. The same actors of the BMP-pathway seem to be expressed differently (or plus precisely in different positions) in a similar regulation network (Saina *et al*. 2009). However the interpretation of *Acropora millepora* expression images in Hayward *et al*. 2002; Hayward *et al*. 2015; Okubo *et al*. 2016 for *chordin* and *BMP2/4* are problematic as no morphological elements allow at the presented stage of development to distinguish both poles of the secondary axis. As no double ISH with both genes is presented it is impossible to interpret weather they express in the same side of the secondary axis or in different points of that axis.

Leclère and Rentzsch (2014) showed that RGM (repulsive guidance molecule) is a positive regulator of Nematostella BMP signalling and is required for the formation and orientation of a phosphorylated Smad1/5/8 gradient along the secondary axis. By knockdown of RGM, they showed that this gradient was responsible for the establishment of the pSMAD1/5/8 gradient and the symmetry break with establishment of the directive axis, perpendicular do the oral-aboral axis. The knockdown of RGM in absence of a pSMAD1/5/8 gradient and resulted in a radial symmetry of the planula larvae.

Hox genes are expressed along the primary and secondary body axis of *Nematostella vectensis*

Hox genes define the antero-posterior axis in bilaterians where they have a collinear expression along that axis (reviewed in Gehring *et al*. 2009). As the sister group to Bilateria, cnidarians are important to understand when the *Hox* patterning appeared. However the orthology between their *Hox* genes is disputed with the exception of the anterior *Hox* genes (Chourrout *et al*. 2006; Ryan *et al*. 2007; Chiori *et al*. 2009; Thomas-Chollier *et al*. 2010).

It is now admitted that in cnidarians *Hox* genes seem to play no major role in the establishment of the primary body axis (Houliston *et al*. 2010). In fact in *Clytia hemisphaerica Hox* and *ParaHox* genes, only two subgroups exhibit restricted expression along the oral–aboral axis during development (Chiori *et al*. 2009).

In *Nematostella,* that as an anthozoan possess two body axis, *Hox* genes are differentially expressed in both axes (Finnerty *et al*. 2004; Matus *et al*. 2006a; Ryan *et al*. 2007; Leclère and Rentzsch 2014; Arendt *et al*. 2015). This contrasts with Bilateria for which the *Hox* genes are expressed only along the primary axis. In 2004 Finnerty *et al*. showed that in *Nematostella* development one *Hox* gene – *Anthox6* – was expressed in the pharyngeal endoderm, corresponding to an expression towards the oral pole, while another *Hox* gene – *Anthox1* – is expressed in the aboral ectoderm, while three other *Hox* genes (*Anthox1a*; *Anthox8* and *Anthox7*) were expressed in middle region (body column) of the planula larva of *Nematostella*. Finnerty results and additional

results by Ryan *et al*. 2007 showed that those Hox genes expressed in the middle body endoderm were differently expressed along the secondary axis, being expressed in the opposite side of the siphonoglyph.

Importantly, the expression of *Anthox6* (Leclère and Rentzsch 2014) and *Gbx* (Ryan *et al*. 2007; Arendt *et al*. 2015) is coincident with the place of formation of specific mesenteries or associated to specific mesenteric compartments leaving the idea that *Hox* genes might have a patterning role in the secondary axis of *Nematostella* and possibly of anthozoans.

As proposed in the preface of this work we used a molecular approach: a characterization of the expression of the BMP-pathway and the *Hox* genes pattern to compare the expression of these developmental genes that are differentially expressed along the secondary axis of *Nematostella*[†††]. This comparison of gene expression patterns will allow us to understand if the results obtained in *Nematostella* can be generalized to another group of anthozoans that has a bilateral adult symmetry. This will allow to gain insights important to the discussion of the hypothesis that bilaterality may have originated in the common ancestor between Anthozoa and Bilateria.

[†††] In addition to the genes presented in the paper, other genes were tested for *ISH* in *Antipathes caribbeana*, including RGM; neogenin; activin; noggin and gdf5-like. However, for those, no distinguishing pattern was obtained.

4 MORPHOLOGICAL DIFFERENCE IN TENTACLE SYMMETRY IN TWO ANTIPATHARIAN SPECIES

As seen in chapter 1, the polyp of antipatharians usually exhibit a radial symmetry for its tentacle disposition as happens for the tentacles of *Plumapathes pennacea* (**figure 4.1a**). However, the existence of a symmetry plane for the tentacles of *Antipathes caribbeana* (**figure 4.1b**), orthogonal to the directive axis, makes that the polyps of this species exhibit an asymmetry between the distal ("left") and proximal ("right") sides of the polyp.

The asymmetry created by the different tentacle size in *Antipathes caribbeana* can be seen in **figure 4.2** by comparison with *Plumapathes pennacea*, directive axis is drawn to help visualization of the asymmetry. How did the asymmetry in tentacle size arise in *Antipathes caribbeana*?

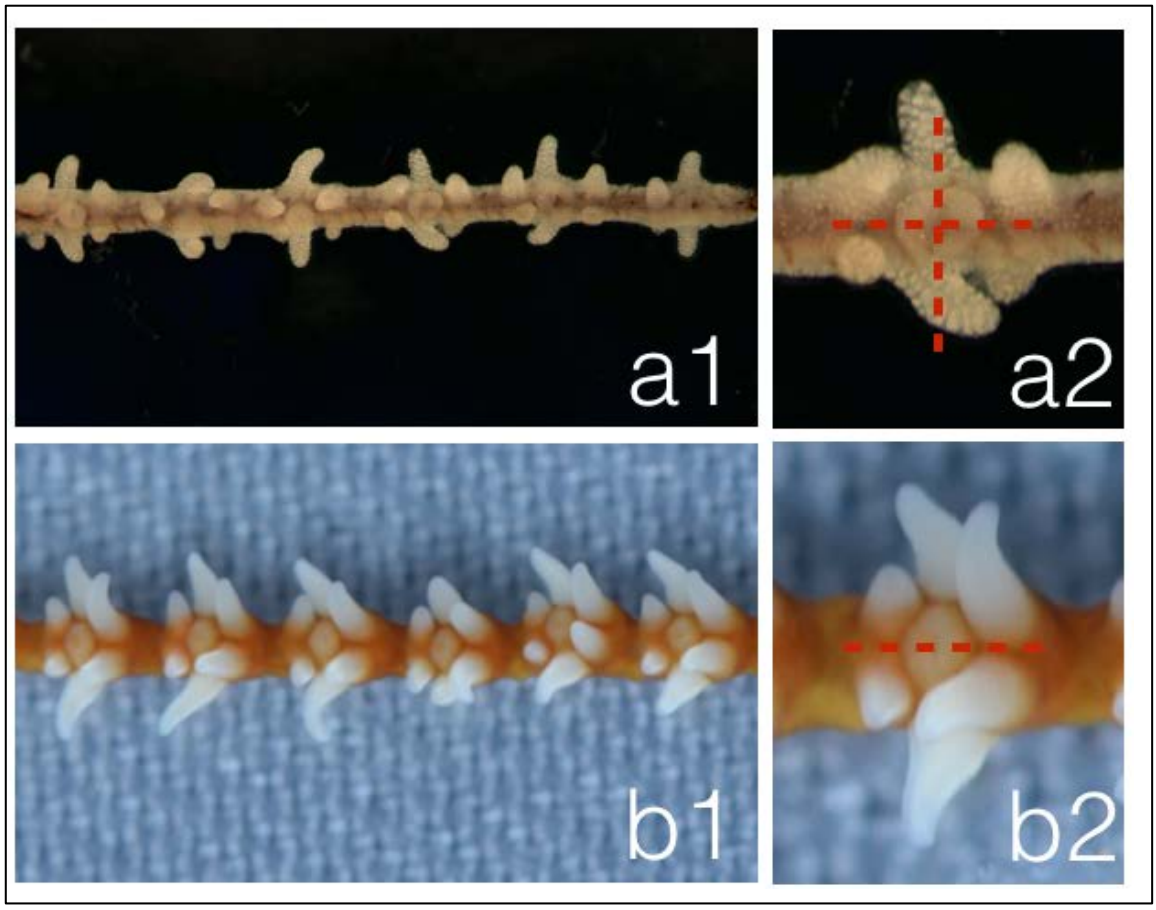

Figure 4.1. The antipatharian species *Plumapathes pennacea* and *Antipathes caribbeana* from Caribbean waters present different types of symmetry in their tentacle disposition: **a1** and **a2** – *Plumapathes pennacea* presents a bi-radial symmetry in their tentacle organization; **b1** and **b2** – *Antipathes caribbeana* present a single plane of symmetry for their tentacle organization because of the longer tentacle size of their proximal lateral pair in relation to the distal lateral pair. The branches and polyps are oriented with their distal sides on the left and with their proximal sides on the right. Original photos from b1/b2 by Michaël Manuel.

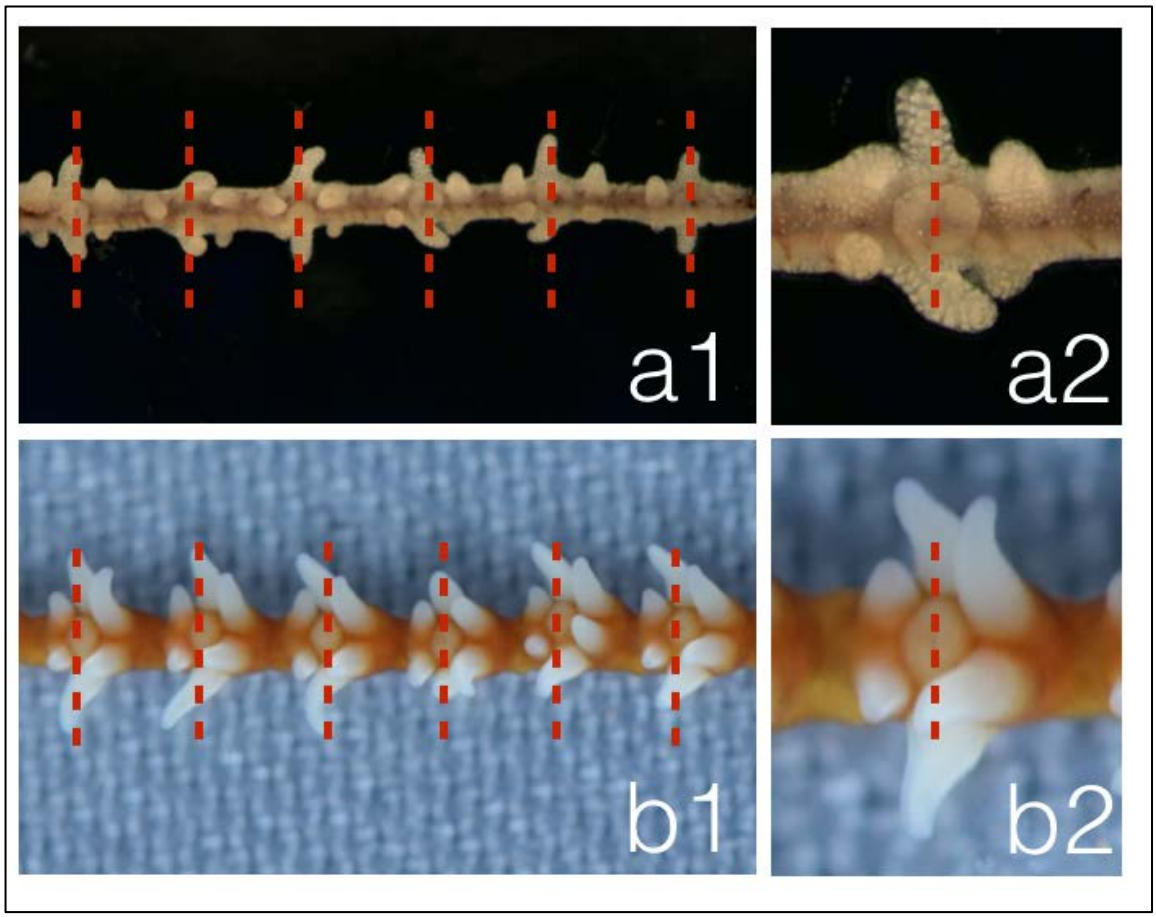

Figure 4.2. The antipatharian species *Plumapathes pennacea* and *Antipathes caribbeana* from Caribbean waters present different types of symmetry in their tentacle disposition: **a1** and **a2** – *Plumapathes pennacea* lateral tentacles are symmetric in relation to their directive axis (red discontinuous line); **b1** and **b2** – *Antipathes caribbeana* lateral tentacles are asymmetric in relation to their directive axis (red discontinuous line) with its lateral proximal tentacles (right side of the polyp) being bigger than their distal lateral tentacles (left side of the polyp). As in precedent figures the branches and polyps are oriented with their distal sides on the left and with their proximal sides on the right. Original photos from b1/b2 by Michaël Manuel.

4.1 Experimental procedure

A RNA-seq analysis was designed to answer the question of the molecular origin of the tentacle asymmetry. Samples were prepared comprising the lateral tentacles and respective basis of *Antipathes caribbeana* and *Plumapathes pennacea*, resulting in 4 different samples:

- Distal lateral tentacles and respective basis of *A. caribbeana* (A.D. in **figure 4.3**);
- Proximal lateral tentacles and respective basis of *A. caribbeana* (A.P. in **figure 4.3**);
- Distal lateral tentacles and respective basis of *P. pennacea* (P.D. in **figure 4.3**);
- Proximal lateral tentacles and respective basis of *P. pennacea* (P.P. in **figure 4.3**);

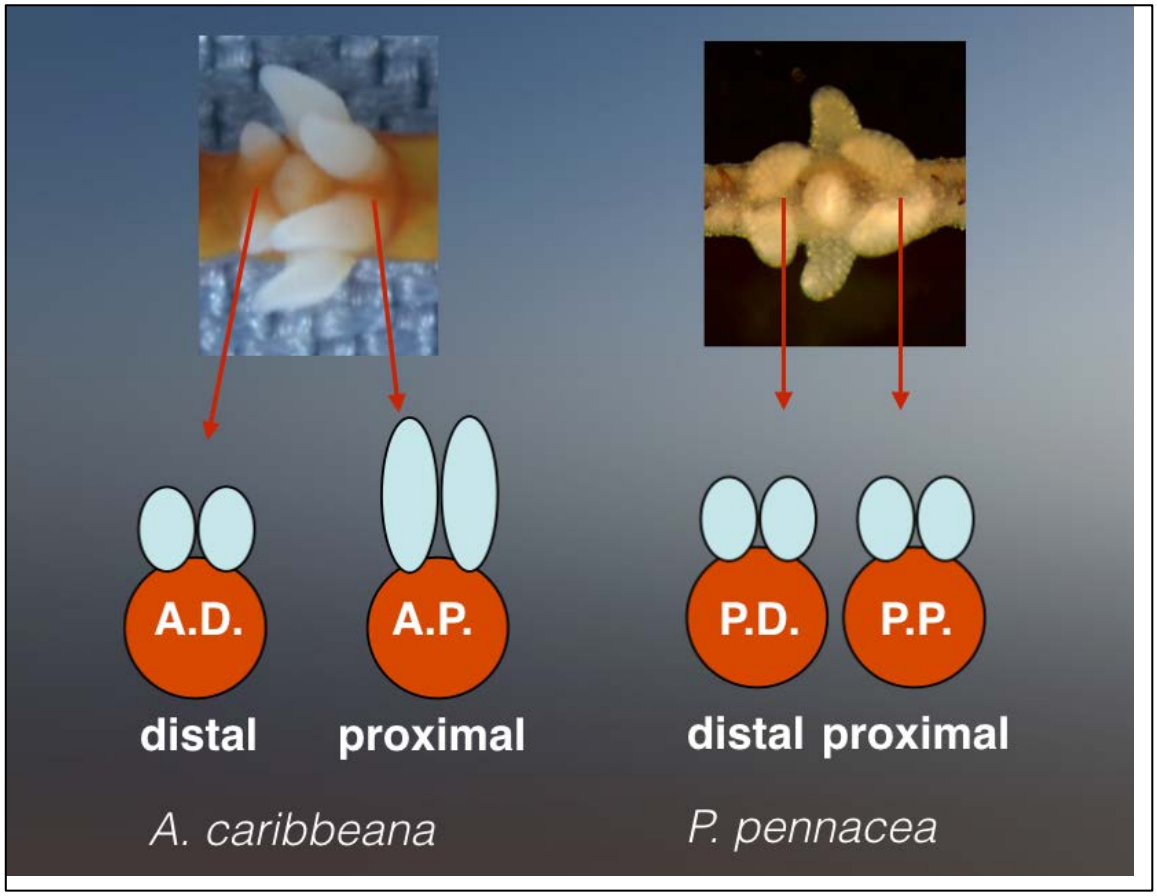

Figure 4.3. Schematic representation of dissections performed to obtain each sample.

Total RNA from each condition was prepared in duplicates in a quantity of 1 µg (0.5 µg of tentacles RNA and 0.5 µg of basis RNA). As the polyps are really small (1 mm or less), to obtain these quantities of RNA hundreds of tentacles and basis needed to be micro-dissected. Extreme care was taken in order to not include any part of the oral cone in the samples.

RNAs were prepared with the RNAqueous-Micro Kit (thermofisher) and purified with a DNAse treatment (37ºC, 30 minutes). Finally they were concentred with the kit RNeasy MinElute Cleanup (Qiagen).

The sequencing and bioinformatics analysis were performed in collaboration with the platform "Plateforme de Séquençage à Haut Débit" of the I2BC "Institut de Biologie Intégrative de la Celulle". Collaborators from the platform were: D. Naquin, C. Dard-Dascot, M. Silvain, E. van Dkik, Y. Jaszczyszyn, H. Auger and C. Thermes.

Ribominus technology (thermofisher) was performed to selectively deplete the ribosomal RNA molecules.

Sequencing was performed by NextSeq 500 of Illumina (High output Kit 75 cycles) with 32 to 54 cycles (pair-ended). Data analysis pipeline included bcl2fastq2 v2.15.0 for demultiplexing, Fastqc 0.11 for accessing read quality, Cutadapt-1.3 for adaptor trimming and TopHat2 for mapping with a seed length of 8. Post-trim reads varied from 37 352 447 to 74 028 267 wich correspond to an apropiate number of reads.

Differential analysis was performed to compare the transcripts between the distal and proximal lateral pairs of each species with DESeq2 (Love *et al*. 2014) method for differential analysis of count data. Comparisons were made with the reads that mapped without ambiguity. Blasts were performed with e-value of 1e-20 against *Nematostella vectensis* and *Homo sapiens*. Transcripts with a padj[‡‡‡] value below 1% were considered to be differentially expressed.

[‡‡‡] Padj corresponds to the p-value adjusted for multiple testing using the Benjamini-Hochberg method (corrected p-value).

4.2 Results and discussion

The tables of the significantly differently expressed transcripts that came out of the analysis are found in the supplementary materials, with the name of the sequence, the base mean number of reads, the log 2 of the comparison, lfcSE (standard error), stat, p-value, padj, blast against *Nematostella vectensis* and blast against *Homo sapiens*.

In table 4.1 can be found the number of differentially expressed genes for the comparison between the distal pair of lateral tentacles against the proximal lateral pair of each of the two species. The first observation is that **there are more differently expressed genes for the comparison between the pairs of *Antipathes caribbeana***, the species that has the difference in tentacle size: 145 differently expressed transcripts against only 27 for *Plumapathes pennacea*. The second observation is that **there are more genes up-regulated in the distal pair of tentacles of *A. caribbeana* than in its proximal pair** (112 against 33). We have then more genes up-regulated in the side of the lateral distal tentacles of A. caribbeana wich corresponds to the side with the smaller tentacles. Having studied the organization of both distal and proximal lateral pairs of tentacles of A. caribbeana and knowing that no morphological difference exists other than their size, expected differentially expressed genes related to growth might be expected togheter with genes envolved in positional information. Genes related to described left-right described asymmetries, specifically Nodal and Lefty, involved in the left-right asymmetry establishment in bilaterians have not been found in our reference transcriptomes for both antipatharian species.

Comparison between the distal and the proximal pairs	*Antipathes caribbeana*	*Plumapathes pennacea*
Up-regulated	112	16
Down-regulated	33	11

Table 4.1. Table with the number of differentially expressed transcripts, between the distal and proximal pairs of lateral tentacles and respective bases for *Antipathes caribbeana* and *Plumapathes pennacea*.

The results arrived quite late in the course of this Ph.D., however we proceeded for validating this results by ISH. We aimed to synthetize 8 probes for each condition, totalizing 16 probes per species. We chose the transcripts with more differently expressed for each condition that were big enough to produce a probe. For *Plumapathes pennacea* not enough transcripts had this condition and then only 10 probes were aimed for. In the end only 4 probes for each species were used for ISH. *Plumapathes pennacea*, as discussed in chapter 3, poses a problem for ISH as its conservation state is bad and so it is not surprising that no pattern was obtained. However we have obtained two expression patterns that correspond to the RNA-seq analysis results for *Antipathes caribbeana*.

Transcript *Anspe_C516442*, identified by reciprocal blast as an HLX-related gene (homeobox transcription factor) has its expressed in the oral cone and tentacles but outside this areas it is only expressed in the distal part of the polyps (**figure 4.4a** – white arrows), which is in agreement with the results from RNA-seq. This is the more differently expressed gene from the analysis, with a log2 of 4.16, which can explain why the difference is visible by ISH.

Transcript *Anspe_4761*, identified by reciprocal blast, as a chitinase-like gene is also more expressed in the distal part of the polyps, especially in the space between the small tentacles (**figure 4.4b** – white arrows). This gene expression is also in agreement with the RNA-seq results. The log2 for this gene (2.15) is smaller and comparable to the value of other genes for which no polarized pattern was found. It may be visible in ISH because of the precise location of its expression between the distal tentacles. Our aims to obtain a pattern for a transcript up-regulated in the proximal lateral pair side of *Antipathes caribbeana* were not fructiferous (possibly due to the

absence of transcripts with differences of expression, log2, of sufficient magnitude to be detected by ISH).

In a cross section of the oral cone of *Antipathes caribbeana* ISH for **Anspe_C516442** (HLX-related gene) (**figure 4.5**) we discovered that it was **expressed specifically in the four distal lateral endodermic compartments**. This result shows that also in the oral cone there is a molecular asymmetry and that the molecular asymmetry suggested for *Antipathes caribbeana* is not restricted to the tentacle areas. A schematic representation of the pattern in the endodermic compartments can be seen in **figure 4.6**, in this representation we see that the expression is asymmetric in the distal/proximal axis (colony axis) in the oral cone. Cases of left/right asymmetry have not been documented in cnidarians. Here we present this asymmetry as a distal proximal asymmetry, however it is similar to a left/right asymmetry of bilaterians as it is orthogonal to symmetry plane of the body.

The fact that no transcripts are differently expressed in both species limits the capacity of the results to answer the original question (small number of differently expressed transcripts in *P. pennacea*). However it can be said that there is a size asymmetry of the tentacles in *Antipathes caribbeana* but also a molecular asymmetry in the same axis as the tentacle asymmetry. In contrast with P. pennacea for winch no tentacle asymmetry or "relevant" molecular asymmetry was found.

More importantly **these results confirm that the polyp of *A. caribbeana* possess 3 body axis that have implications in its morphology, the oral-aboral axis, the directive axis and the proximal distal axis. This is a unique case in cnidarians.**

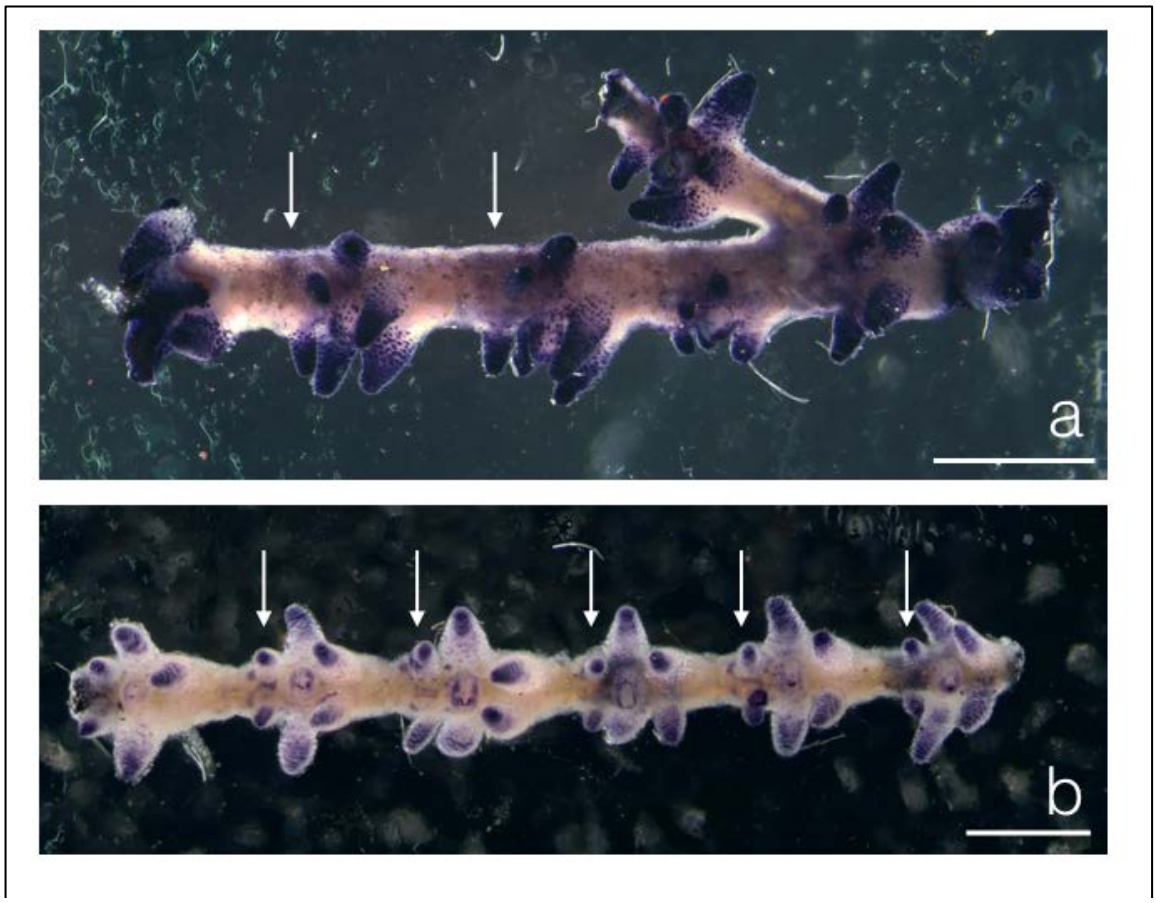

Figure 4.4. *In situ* hybridization patterns: **a** – Anspe_C516442 (HLX-related) and **b** – Anspe_4761 (chitinase-like). Both genes are up-regulated in the distal lateral pair of tentacles (with basis). Scale bars: 1 mm.

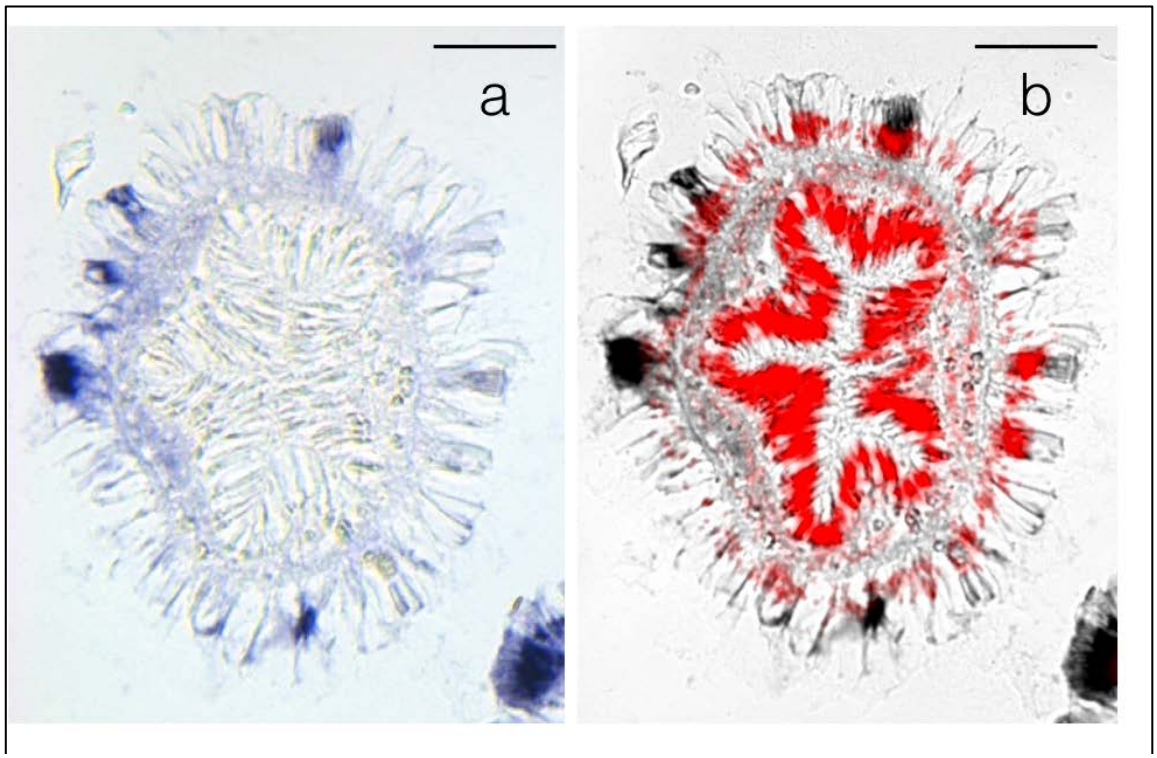

Figure 4.5. Cross-section of the oral cone of *Antipathes caribbeana* showing the expression of Anspe_C516442 (HLX-related) in the four lateral endodermic chamber of the distal side of the polyp. Distal –left, proximal – right, Scale bars: 100 mm.

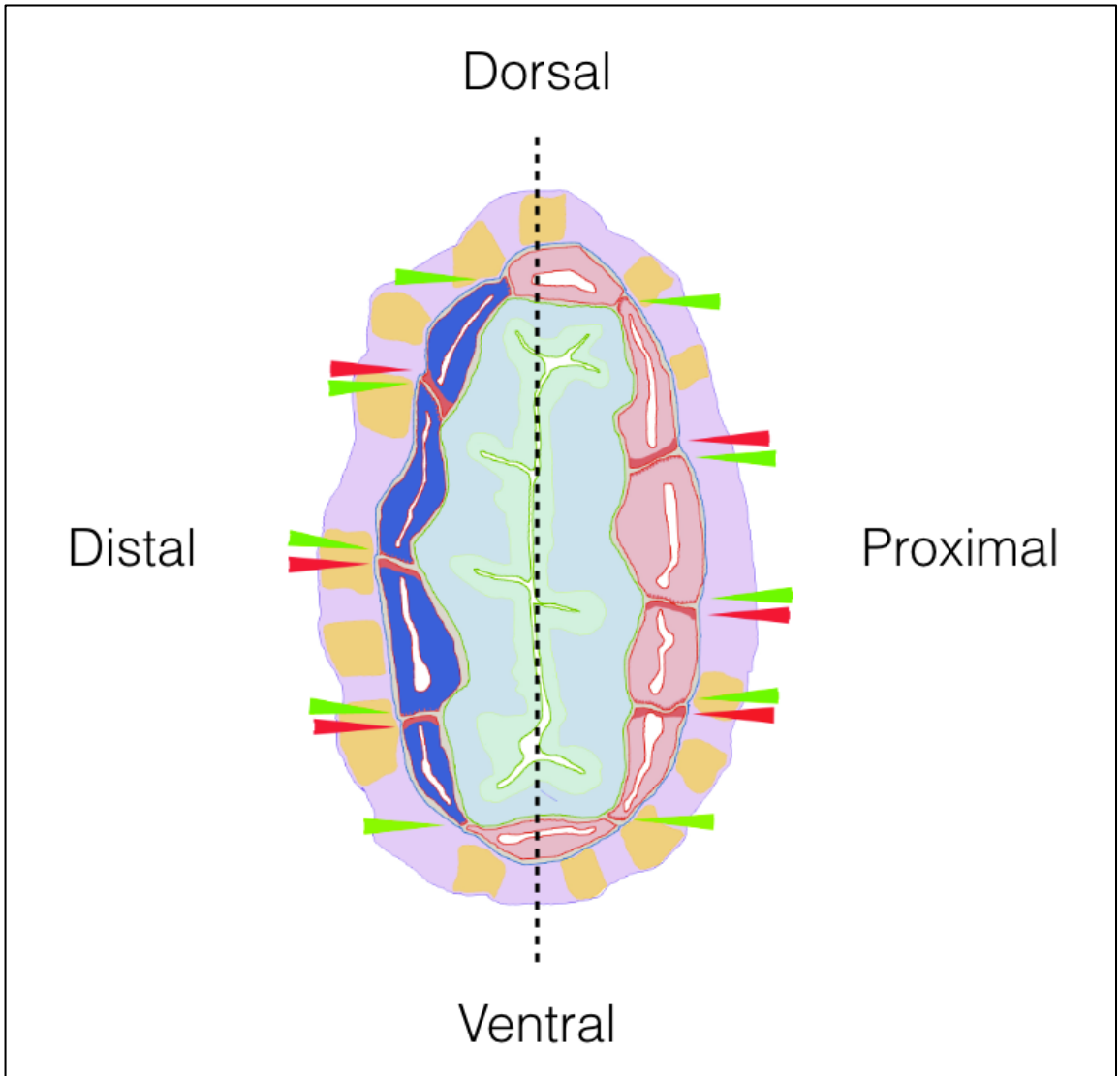

Figure 4.6. Schematic representation of the expression of Anspe_C516442 (HLX-related) in the oral cone of *Antipathes caribbeana* in relation to the directive axis.

4.3 Observation of asexual reproduction

When dissecting the tentacles of *Antipathes caribbeana*, by the tentacle base, we observed that after some seconds they gained movement at started moving in the petri dish with filtered seawater. Their movement was constant and directed as a torpedo. They appeared to possess neutral buoyancy but as their movement was so strong and quick this aspect was difficult to access with certainty. At times tentacles stopped or inversed their movement.

It is important to note that immediately after dissection the tentacle would stand still, neutrally buoyant, for some seconds and would then start with the directed movement at once. At this stage the tentacle open part (open due to the dissection) would already be at least partially closed and present no clear opening in contrast to when it was dissected. The movement of all tentacles was oriented with the basal part (dissection region) of the tentacle up-front in an anterior position.

In opposition the dissected tentacles of *Plumapathes pennacea* when dissected showed movement to a much smaller degree and their movement was not directed as they oscillated around the same point. The dissected tentacles of *Plumapathes pennacea* did not show the same capacity to close the open part after dissection.

Confronted to this huge difference between the dissected tentacles of both species we have maintained dissected proximal lateral tentacles of *Antipathes caribbeana* in petri dishes with filtered seawater.

In **figure 4.5a** there is the a branch of *Antipathes caribbeana* with all their tentacles and in **figure 4.5b** there is a branch without two lateral proximal tentacles, one of each polyp, that have been dissected, the place of where the tentacle was is marked with an asterisk.

Figures 4.5c to 4.5e are photos of tentacles 24 hours after dissection, even if the microscopy material we had at our disposal in Guadeloupe did not allow good quality photos, we can observe that there are two cellular layers, the ectoderm and the endoderm. In the large extremity, corresponding to the dissection point and to the base of the tentacle, is visible a pigmented (orange) tissue. The tentacles are at this point completely closed in the dissection area. In **figure 4.5.d** is visible that the tentacles are still moving.

Six days after dissection two tentacles "*settled*" at the bottom of the petri dish. In **figure 27.f** we can see that when settled the tentacle chanced its conformation adhering perfectly to the petri dish and suffering severe modifications in its morphology. The settled tentacles had natural fluorescence at a wavelength of 568nm, observed by this fluorescence microscopy. This natural fluorescence was not present in tentacles observed before settlement and can be observed in **figure 4.5g**. The fluorescence and aspect of the settled tentacle are congruent with the observation for the settled larvae of the scleractinians *Acropora millepora* (Kenkel *et al*. 2011).

From the only study that described larvae of antipatharians settling, from Kai *et al*. (2001), we have that these larvae from *Antipathella fiordensis* settled after four days of development, that they were bullet shaped and they "swam actively", including typical spiral and straight-line movements, circling, stopping and reversing.

All the observations point to the fact that the tentacles of *Antipathes caribbeana* seem to have the capacity of becoming asexual larvae that can settle and give rise to new colonies as an asexual manner of reproduction.

This observation gives force to the idea that asexual reproduction has a major role in antipatharian reproduction (Wagner *et al*. 2012)

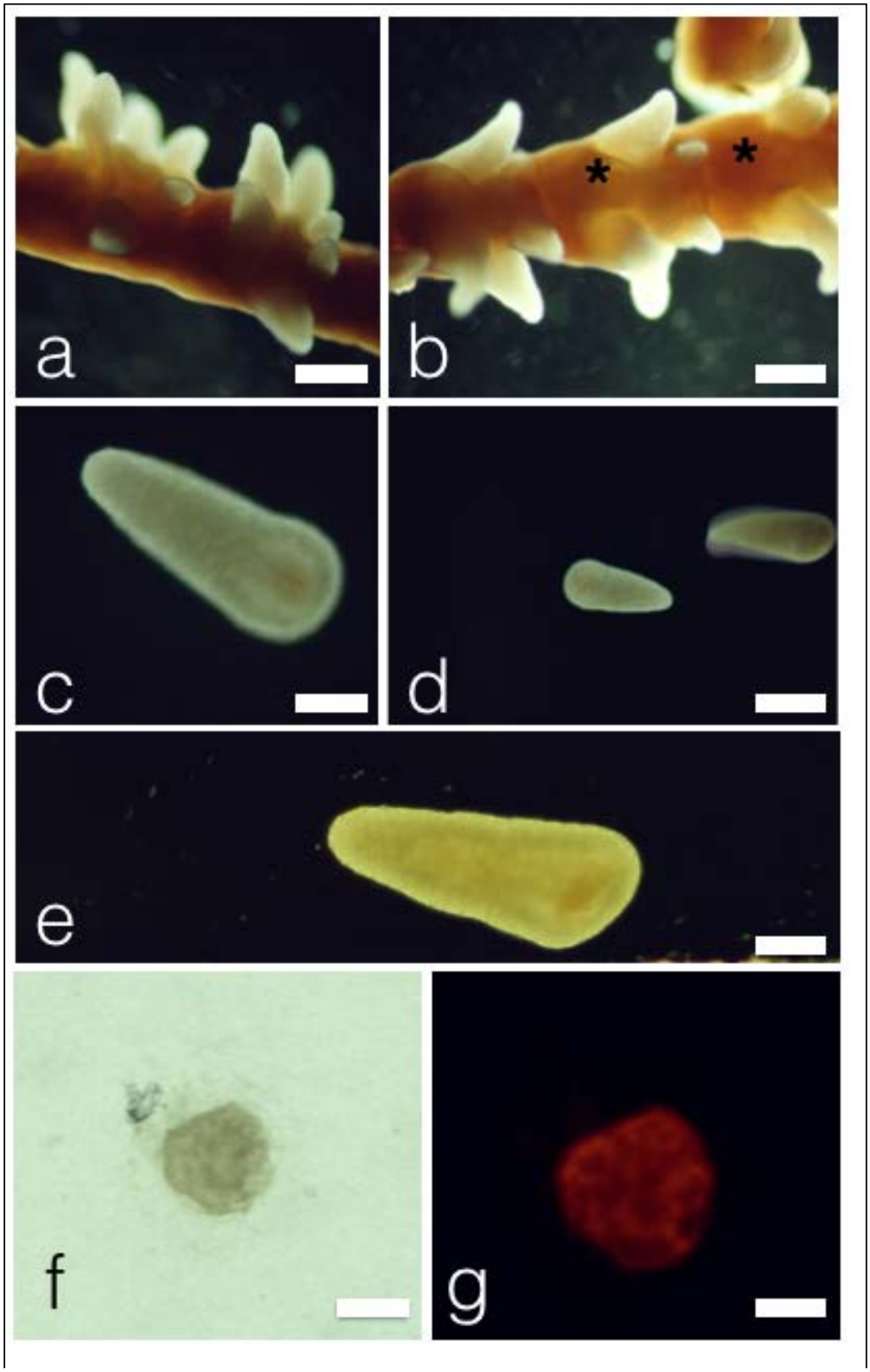
a
b
c
d
e
f
g

Figure 4.5. Asexual reproduction in *Antipathes caribbeana*: **a** – *Antipathes caribbeana* branch after collection, branch with two polyps with 6 tentacles each; **b** – the proximal lateral tentacles where dissected at their base, this image shows the polyps after the dissection of a proximal lateral tentacle, marked with an asterisk; **c** – 24 hours after dissection the tentacle (asexual larvae) no longer presented the opening caused by the dissection; **d** – 24 hours after dissection the tentacle (asexual larvae) presented a directed torpedo like movement; **e** – 24 hours after dissection the tentacle presented two cellular layers the ectoderm and the endoderm, it also presents a pigmented tissue in their basal part of the tentacle; **f** – 6 days after dissection the asexual larvae resulting from the dissected tentacles started to settle in the bottom of the petri dish; a complete cellular and tissue reorganization could be observed; **g** – The settled asexual larvae (tentacles) showed natural florescence at a wavelength of 568nm.

5 GENERAL CONCLUSIONS

This project on antipatharians had it main axis on the understanding of bilaterality at the anatomical level of antipatharians, its molecular origin and how that bilaterality related to the bilaterality that exists in other animals both at the morphological and molecular levels.

One of the objectives proposed was to understand how the morphological bilateral organization of antipatharians polyps relate to the organization of the polyps of the other anthozoan orders. Another related objective was to address if the anatomical bilaterality is derived or pleisiomorphic in Anthozoa.

The detailed review of the literature allowed to conclude that **the secondary axis and bilaterality are homologous amongst anthozoans**. That the ventral and dorsal sides of anthozoans correspond between the different orders (with the possible exception of Ceriantharia for which the body orientation is problematic as seen in chapter 2); **Antipatharians and actiniarians have then the same general body orientation (polarity)**.

By the analysis of the delopmental stages passed by the diverse anthozoans in terms of mesenteric formation it was possible to affirm that in the end the process of mesenteric development is, dispite some changes in their developmental order, quite consistent between groups and comparable. This also allowed the visualization that all anthozoans share bilateral stages during development. Most being bilateral in the adult polyp form and some members of the Hexacorallia subclass acquiring a radial

symmetry in the adult polyp. The ancestor of anthozoans was then most probably bilateral. Radial symmetry seems a derived trait in Anthozoa that is only present in some adult forms of hexacorallians**.**

This is already of major importance as it accounts to the fact that bilaterality is not a derived trait in different anthozoans. However there are still things that need to be addressed because present literature do not allow to uncover. The major case is the body orientation of ceriantharians as discussed in chapter 2. In literature it's usually considered that their siphonoglyph is dorsal meaning that the poles of its secondary axis do not correspond to those of other anthozoans beacause of the disposition of their retractor muscles. Here it's proposed that in fact the ventral side of ceriantharians corresponds to the siphonoglyph side as in other anthozoans based on the developmental order of mesenteric formation.

The detailed anatomy of the *Antipathes caribbeana* polyp has been studied in order to establish very precisely its anatomical organization and in particular its polyp symmetry and orientation. This was achieved by histology and immunohistochemestry in all-mount and cryosectioned polyps. It allowed an understanding of the precise morphology and organization of the polyp revealing new details on the Antipatharia morphology. In terms of symmetry the major findings have been the discovery of the presence of retractor muscles associated to the mesenteries and that they were polarized along the secondary axis. This allowed the orientation of the *Antipathes caribbeana* secondary axis in relation to the secondary axis of *Nematostella*. Additionally it confirmed that no siphonoglyph is present in the flatered pharynx of *A. caribbeana* polyps. In relation to the present knowledge of the hexacorallian tree, we proposed that the absence of a siphonoglyph might be a synapormorphy of the group comprising Antipatharia, Corallimorpharia and Scleractinia.

The characterization of the expression of the BMP-pathway and the *Hox* genes in the adult polyp of *Antipathes caribbeana* allowed to compare the expression of these developmental genes with the expression known for the developmental stages of *Nematostella*. This comparison between the adult polyp of *A. caribbeana* and the developmental stages of *Nematostella* was not obvious but was possible due to the fact

that the secondary axis of both polyps are homologous and that polarities of those axes could be determined.

It allowed to study for the first time the expression of developmental genes in a species of black corals. To discover that developmental genes as *Bmp2/4* are asymmetrically expressed along the secondary axis of *Antipathes caribbeana* adult polyp. This maintenance of gene expression in the adult for developmental genes might be justified by the precise orientation of each polyp in a branch as polyp multiplication takes place from a existing polyp in a budding process and so the maintenance of gene expression for developmental genes might be important for the orientation of the forming polyps.

Despite some differences in the tissular expression between both species, as in *N. vectensis* both *BMP2/4 and chordin* are co-expressed in the ventral side of the polyp. Confirming that the body orientation of the polyps is comparable and that the BMP-pathway is also in this case differently expressed along the secondary axis.

Both *Anthox1a* and *Anthox6a* are differently expressed along the secondary axis of the adult *A. caribbeana* polyp as happens in the polyp of *N. vectensis*, however the polarized expression happens in a different tissue, ectoderm instead of endoderm and its expression is localized in different poles. Both *Gbx* and *Anthox7/8* have their expression associated to specific mesenteric compartments. Together with the results that suggested a patterning role for *Hox* genes in the secondary axis of *Nematostella*, these results give force to the idea that *Hox* genes may have a conserved patterning role associated to the mesenteric development at the Hexacorallia/Anthozoa level.

Contrarily of what was expected *Bmp5-8* is expressed differently in the proximal and distal sides of each polyp of *Antipathes caribbeana* and not along the secondary axis of the polyp. One possibility is that this gene was not indispensable for the secondary axis establishment and gained a function along the colonial axis of the polyp, an axis *N. vectensis* (and actiniarians in general) do not possess.

The growing quantity of publish data showing a conserved role of the BMP-pathway in the secondary axis of hexacorallians, together with the conserved bilaterality amongst octocorallians make that the hypothesis of homology between the secondary axis of bilaterians and anthozoans is becoming more and more reinforced. It is however important to study the expression of this pathway in octocorallians and ceriantharians in order to further test the idea. To our

knowledge the essays of ISH in both Octocorallia and Ceriantharia species have failed until the present day. Also, functional essays to test the role of the BMP-pathway in antipatharians are at this point not realistic due to all the constrains present in antipatharian manipulation referred along this document.

Finally we aimed to understand the symmetry difference between *Antipathes caribbeana* and *Plumapathes pennacea*. The limited results obtained in terms of differently expressed genes for *P. pennacea* and the late obtainance of the results did not allow a complete understanding of the problem but have the possible analysis has shown that there are molecular differences between the left (distal) and the right (proximal) sides of *A. caribbeana* in addition to a morphological difference between both sides. These **results confirm that the polyp of *A. caribbeana* possess 3 body axis that have implications in its morphology, the oral-aboral axis, the directive axis and the proximal-distal colonial axis. This is a unique case in cnidarians.**

In addition to the major questions of this work and as many aspects of the antipatharian biology are still understudied, there was a special attention to the observational aspect when dealing with our samples. From this comes that we could **confirm that simultaneous hermaphroditism does exist in Antipatharia** (as previous descriptions were dubious) and that we could **describe for the first time the existence of hermaphroditism at the polyp level**. Also it was possible to observe the settlement of tentacles after dissection which together with the observations we did on the swimming tentacles in terms of movement and fluorescence **pointed to the fact that the tentacles of *Antipathes caribbeana* seem to have the capacity of becoming asexual larvae that can settle and give rise to new colonies as an asexual manner of reproduction**.

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7 SUPPLEMENTARY DOCUMENTS

7.1 DETAILED PROTOCOL OF *IN SITU* HYBRIDIZATION FOR *ANTIPATHES CARIBBEANA* 255

7.2 TRANSCRIPTS UP-REGULATED IN THE DISTAL TENTACLE PAIR OF *ANTIPATHES CARIBBEANA* 257

7.3 TRANSCRIPTS DOWN-REGULATED IN THE DISTAL TENTACLE PAIR OF *ANTIPATHES CARIBBEANA* 262

7.4 TRANSCRIPTS UP-REGULATED IN THE DISTAL TENTACLE PAIR OF *PLUMAPATHES PENNACEA* 263

7.5 TRANSCRIPTS DOWN-REGULATED IN THE DISTAL TENTACLE PAIR OF *PLUMAPATHES PENNACEA* 264

7.1 Detailed protocol of *in situ* hybridization for *Antipathes caribbeana*

Immersions performed at room temperature were performed in a rocking platform shaker at a speed of 40 rpm.

Immersions performed at 58ºC were done in a water bath at a speed of 40 rpm.

1. Distribute between 5 to 8 branch fragments of around 8 polyps each for each condition in methanol.
2. Immerse the samples for 15 minutes in a solution (PBT) of 50% ethanol / 50% 0.1% (v/v) Tween 20 in 1x PBS.
3. Rinse 3 times with PBT
4. Immerse the samples for 15 minutes in PBT
5. Immerse the samples twice for 10 minutes in a solution of TEA 0.1M (triethanolamine in Dnase and Rnase free water) with a 7.8 pH.
6. Immerse the samples 5 minutes in 500µL of TEA 0.1M solution with 1.5µl of glacial acetic acid.
7. Add 1.5µl of glacial acetic acid to the solution of each sample and let in immersion for an extra 5 minutes.
8. Immerse 3 times for 15 minutes in PBT.
9. Postfix the samples in PAF4% for 60 minutes.
10. Immerse at least 3 times 5 minutes in PBT.
11. Immerse 10 minutes in a solution of 50% HB/50%PBT. HB (hybridization buffer: 50% formamide, 0.1% tween 20, 5x SSC, 0.1% DMSO, Heparine at 50µg/ml.
12. Immerse twice in HB for 10 minutes.
13. Immerse the samples at 58ºC for 3 hours in 800µl of HB with 100µg/ml of tRNA.
14. Denature 100ng of each probe (DIG labelled probes) at 95ºC for 5 minutes in 50µl of HB with 100µg/ml of tRNA. Put the denatured probes in ice immediately after denaturation.
15. Add the 50µl of solution with the denatured probe in each sample and immerse 72 hours at 58ºC.

16. Rinse with 58ºC HB solution.
17. Immerse at 58ºC with 58ºC for 60 minutes.
18. Rinse with 58ºC HB solution.
19. Immerse at 58ºC with 58ºC for 60 minutes.
20. Rinse with 58ºC HB solution.
21. Immerse at room temperature with 58ºC HB solution.
22. Immerse for 10 minutes in 50% HB/ 50% PBT.
23. Immerse twice for 5 minutes in PBT.
24. Immerse 60 minutes at room temperature in a blocking reagent 1X solution (diluted in MAB1X).
25. Immerse in Anti-Digoxigenin-AP (Roche) diluted 1/2000 with blocking reagent 1X for 4 hours.
26. Immerse 3 times for 15 minutes with PBT.
27. Immerse at 4ºC with PBT overnight.
28. Immerse 3 times in Dig wash 1X for 15 minutes.
29. Immerse overnight at 4ºC in TMN (100mM Tris-HCL pH 8.6, 50mM $MgCl_2$, 100mM NaCl, 2mM Levamisole, 0,1% tween 20).
30. Place the samples for staining in TMN solution with 0.8µL/mL of NBT (stock at 100mg/ml) and 2µl/ml of BCIP (stock at 50mg/ml) at room temperature.
31. Change the staining reaction medium every 24 hours.
32. Postfix the samples in PFA 4% for 60 minutes.
33. Rinse several times in PBT, perform DAPI staining and observe.

7.2 Transcripts up-regulated in the distal tentacle pair of *Antipathes caribbeana*

Sequence	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj	descrNematostella	descrHuman
Anspe_C516442*	629.9268729	-4.159024847	0.268095454	-15.51322406	2.82E-54	6.54E-50	no result with blastx	no result with blastx
Anspe_18980	277.7733412	-3.100862121	0.298099428	-10.40210691	2.43E-25	1.12E-21	no result with blastx	no result with blastx
Anspe_4761*	16691.48276	-2.149602056	0.211432104	-10.16686688	2.79E-24	1.08E-20	Predicted protein	no result with blastx
Anspe_9073	468.2732194	-2.19996605	0.268835487	-8.183317138	2.76E-16	9.14E-13	Predicted protein	tryptase alpha/beta 1
Anspe_16901	9861.25122	-1.767773633	0.217013578	-8.145912551	3.76E-16	1.09E-12	Predicted protein	meprin A, beta
Anspe_C279483	457.6325913	-2.025939161	0.261426385	-7.749558864	9.22E-15	2.14E-11	no result with blastx	no result with blastx
Anspe_C514096	510.7380941	-1.930253585	0.254159771	-7.594646389	3.09E-14	6.50E-11	Predicted protein	chymotrypsinogen B1
Anspe_13272	3130.897596	-1.765578596	0.24112353	-7.322299046	2.44E-13	4.35E-10	Predicted protein	chymotrypsin-like
Anspe_C521880	2060.882796	-1.856340621	0.25322181	-7.330887564	2.29E-13	4.35E-10	Predicted protein	no result with blastx
Anspe_C478195	229.5479307	-2.251769298	0.311401183	-7.231087815	4.79E-13	7.93E-10	no result with blastx	no result with blastx
Anspe_7758	529.2813179	-1.850150829	0.259510348	-7.12939134	1.01E-12	1.56E-09	Predicted protein	no result with blastx
Anspe_7755	714.4275849	-1.832855034	0.258116332	-7.100887504	1.24E-12	1.80E-09	Predicted protein	matrix metallopeptidase 24
Anspe_17409	979.5130355	-1.694567831	0.242085546	-6.999871976	2.56E-12	3.30E-09	Predicted protein	no result with blastx
Anspe_3650	1382.326364	-1.710684758	0.24779319	-6.903679458	5.07E-12	5.87E-09	Predicted protein	meprin A, beta
Anspe_19246	346.6749885	-2.084162373	0.309806382	-6.727306132	1.73E-11	1.82E-08	no result with blastx	no result with blastx
Anspe_C502182	3081.809789	-1.731072042	0.259396025	-6.673471745	2.50E-11	2.52E-08	no result with blastx	no result with blastx
Anspe_C524456	4396.459681	-1.402786807	0.212373197	-6.605291184	3.97E-11	3.83E-08	Predicted protein	no result with blastx
Anspe_C525142	3254.898379	-1.330417485	0.205156697	-6.484884518	8.88E-11	8.23E-08	Predicted protein	no result with blastx
Anspe_C511626	367.3828396	-1.761720338	0.274217264	-6.424542033	1.32E-10	1.18E-07	Predicted protein	transmembrane protease, serine 3
Anspe_17536	794.2517299	-1.480894587	0.237156164	-6.24438581	4.25E-10	3.52E-07	Predicted protein	['transmembrane protease, serine 2
Anspe_C512092	625.4790799	-1.775904153	0.285674794	-6.216523793	5.08E-10	4.06E-07	Predicted protein	no result with blastx
Anspe_13442	2031.194149	-	0.274201024	-	6.57E-10	5.08E-07	Predicted	meprin A, beta

		1.693489703		6.176088175			protein	
Anspe_C510560	402.619287	-1.685488713	0.273589106	-6.160657261	7.24E-10	5.42E-07	Predicted protein	no result with blastx
Anspe_882	2622.88027	-1.632033868	0.265648171	-6.14359158	8.07E-10	5.84E-07	Predicted protein	meprin A, beta
Anspe_19667	434.2736461	-1.680446441	0.273997463	-6.133072999	8.62E-10	5.88E-07	Predicted protein	lipase, member I
Anspe_C398628	160.5029791	-1.98805414	0.324060028	-6.134832962	8.52E-10	5.88E-07	no result with blastx	no result with blastx
Anspe_18723	1232.779209	-1.440955603	0.237366552	-6.070592466	1.27E-09	8.44E-07	no result with blastx	no result with blastx
Anspe_C506352	399.2527027	-1.597446866	0.263403881	-6.064629193	1.32E-09	8.51E-07	Predicted protein	meprin A
Anspe_15473	4203.001399	-1.47362631	0.244684971	-6.022545257	1.72E-09	1.08E-06	no result with blastx	no result with blastx
Anspe_C535500	1733.730599	-1.388558409	0.230770152	-6.017062429	1.78E-09	1.08E-06	Predicted protein	no result with blastx
Anspe_C357122	478.366264	-1.566283887	0.260611215	-6.01004024	1.85E-09	1.10E-06	no result with blastx	no result with blastx
Anspe_C400046	281.6223399	-1.907553623	0.317909142	-6.000310696	1.97E-09	1.14E-06	no result with blastx	no result with blastx
Anspe_C503958	7704.918123	-1.331979545	0.224461607	-5.934108564	2.95E-09	1.67E-06	no result with blastx	no result with blastx
Anspe_8767	823.2419431	-1.395253716	0.239475752	-5.826283879	5.67E-09	3.13E-06	Predicted protein	meprin A, beta
Anspe_C532532	368.2689259	-1.612031446	0.27834434	-5.791500725	6.98E-09	3.76E-06	Predicted protein	transmembrane protease, serine 9
Anspe_18885	1024.757962	-1.787613267	0.309101742	-5.783251997	7.33E-09	3.86E-06	Predicted protein	meprin A, beta
Anspe_3124	2309.326128	-1.600172247	0.278542935	-5.744795678	9.20E-09	4.74E-06	Predicted protein	no result with blastx
Anspe_1693	1720.065547	-1.260241154	0.221319998	-5.694203712	1.24E-08	6.25E-06	Predicted protein	neuronal pentraxin I
Anspe_C260961	268.904189	-1.66898219	0.295482958	-5.648319627	1.62E-08	7.99E-06	no result with blastx	no result with blastx
Anspe_C539122	10414.41651	-1.159697109	0.206214166	-5.623750929	1.87E-08	8.66E-06	Predicted protein	signal peptide, CUB domain, EGF-like 1
Anspe_C487311	122.9091132	-2.062253291	0.367443085	-5.612442781	1.99E-08	9.07E-06	no result with blastx	no result with blastx
Anspe_11171	2028.236455	-1.181354805	0.211549617	-5.584291862	2.35E-08	1.05E-05	Predicted protein	meprin A, beta
Anspe_C515572	2941.066303	-1.44030196	0.261171989	-5.514764284	3.49E-08	1.53E-05	no result with blastx	no result with blastx
Anspe_C464665	1757.156825	-1.20988277	0.2195483	-5.510781775	3.57E-08	1.53E-05	no result with blastx	no result with blastx
Anspe_C517740	619.817556	-1.405029685	0.255802264	-5.492639762	3.96E-08	1.67E-05	Predicted protein	mannose receptor, C type 2
Anspe_C524554	2369.997522	-1.17505407	0.214442399	-5.479579027	4.26E-08	1.76E-05	no result with blastx	no result with blastx
Anspe_C435221	217.1468067	-1.616567281	0.297670523	-5.430726789	5.61E-08	2.24E-05	no result with blastx	no result with blastx
Anspe_15662	704.9189013	-	0.318792799	-5.42604766	5.76E-08	2.26E-05	no result with	no result with blastx

		1.729784923					blastx	
Anspe_C526048	234.2147368	-1.855839292	0.342897258	-5.41223136	6.22E-08	2.40E-05	no result with blastx	no result with blastx
Anspe_C511126	904.5961513	-1.290771036	0.241037873	-5.355054874	8.55E-08	3.25E-05	Predicted protein	ADAM metallopeptidase with thrombospondin type 1 motif, 18
Anspe_603	202.8483709	-1.824344014	0.343261516	-5.314735056	1.07E-07	3.99E-05	Predicted protein	no result with blastx
Anspe_C258134	226.9963828	-1.926510522	0.363416242	-5.301112877	1.15E-07	4.23E-05	no result with blastx	no result with blastx
Anspe_C526748	340.1283053	-1.450157207	0.275496432	-5.263796696	1.41E-07	5.11E-05	Predicted protein	meprin A, beta
Anspe_20301	6972.313449	-1.073312926	0.204675464	-5.243974556	1.57E-07	5.60E-05	Predicted protein	notch 3
Anspe_C428238	258.6856853	-1.51640563	0.289369016	-5.240387007	1.60E-07	5.63E-05	Predicted protein	meprin A, beta
Anspe_C527556	4311.988833	-1.208607688	0.230983141	-5.232449796	1.67E-07	5.79E-05	Predicted protein	no result with blastx
Anspe_3580	603.1081051	-1.557799113	0.301075664	-5.174111684	2.29E-07	7.69E-05	no result with blastx	no result with blastx
Anspe_C397280	407.2404111	-1.373134331	0.265322552	-5.17533968	2.27E-07	7.69E-05	no result with blastx	no result with blastx
Anspe_C534104	2306.352756	-1.420176546	0.274962698	-5.164978942	2.40E-07	7.96E-05	Predicted protein	no result with blastx
Anspe_C236028	193.0204535	-1.631224352	0.318277304	-5.125167055	2.97E-07	9.70E-05	no result with blastx	no result with blastx
Anspe_9775	323.2665039	-1.473475824	0.288405148	-5.109048271	3.24E-07	0.000104227	no result with blastx	no result with blastx
Anspe_C537724	1378.015181	-1.373429862	0.269294577	-5.100102198	3.39E-07	0.000107779	Predicted protein	ADAM metallopeptidase with thrombospondin type 1 motif, 18
Anspe_13811	1793.223085	-1.163842861	0.229404844	-5.073314232	3.91E-07	0.000122445	Predicted protein	lipase, member I
Anspe_14787	164.5501451	-1.65201527	0.327205571	-5.048860469	4.44E-07	0.000135541	Predicted protein	no result with blastx
Anspe_C288707	168.7104667	-1.623287859	0.323565094	-5.016881893	5.25E-07	0.000158076	no result with blastx	no result with blastx
Anspe_C299094	140.0580931	-1.690315055	0.337365954	-5.010330875	5.43E-07	0.000159412	no result with blastx	no result with blastx
Anspe_C507248	490.757343	-1.538816568	0.307072722	-5.011244754	5.41E-07	0.000159412	Predicted protein	meprin A, beta
Anspe_12117	462.0811912	-1.441239804	0.291127821	-4.950539584	7.40E-07	0.000209181	Predicted protein	transmembrane protease, serine 3
Anspe_C489802	214.41816	-1.522308202	0.307386637	-4.952421537	7.33E-07	0.000209181	no result with blastx	no result with blastx
Anspe_12757	368.8655988	-1.332047513	0.269922404	-4.934927577	8.02E-07	0.000223897	no result with blastx	no result with blastx
Anspe_3125	743.6984376	-1.254218659	0.254797657	-4.922410484	8.55E-07	0.000235866	Predicted protein	no result with blastx
Anspe_14090	616.5007454	-1.335514989	0.27171448	-4.915141025	8.87E-07	0.000241909	Predicted protein	no result with blastx
Anspe_C506634	6223.464357	-	0.257168504	-	1.01E-06	0.000271	no result with	no result with blastx

		1.257544072		4.889961451		806	blastx	
Anspe_C522334	540.9898893	-1.335788751	0.273420371	-4.885476326	1.03E-06	0.000274872	Predicted protein	transmembrane protease, serine 6
Anspe_6579	498.8392518	-1.433707429	0.295436526	-4.852844186	1.22E-06	0.000320536	no result with blastx	no result with blastx
Anspe_C529998	1189.285019	-1.232751402	0.255479967	-4.825237043	1.40E-06	0.000364157	Predicted protein	ADAM metallopeptidase with thrombospondin type 1 motif, 7
Anspe_C527824	622.2048284	-1.193870855	0.247818928	-4.817512798	1.45E-06	0.000374331	Predicted protein	no result with blastx
Anspe_C460534	358.2420494	-1.278374582	0.2682502	-4.765605322	1.88E-06	0.000469241	no result with blastx	no result with blastx
Anspe_C531492	454.846153	-1.486469166	0.313025653	-4.748713573	2.05E-06	0.000494237	no result with blastx	no result with blastx
Anspe_C533672	519.8021417	-1.312986658	0.276410934	-4.750125609	2.03E-06	0.000494237	Predicted protein	meprin A, beta
Anspe_C462877	331.943472	-1.316432817	0.277500767	-4.74388893	2.10E-06	0.000500943	no result with blastx	no result with blastx
Anspe_1462	156.4083129	-1.51464207	0.320126852	-4.731380888	2.23E-06	0.000527389	no result with blastx	no result with blastx
Anspe_C482925	440.7553108	-1.258292701	0.266471772	-4.722048757	2.33E-06	0.000546604	no result with blastx	no result with blastx
Anspe_C521782	630.5789273	-1.135102769	0.240825867	-4.713375612	2.44E-06	0.000564699	Predicted protein	lectin, galactoside-binding, soluble, 3 binding protein
Anspe_12753	188.9074006	-1.439351886	0.305854784	-4.705997621	2.53E-06	0.000579717	no result with blastx	carbonic anhydrase VII
Anspe_13756	5492.910627	-0.960317461	0.20429593	-4.700619632	2.59E-06	0.000589362	Predicted protein	fibulin 1
Anspe_C495284	521.4147831	-1.31879322	0.281894811	-4.67831676	2.89E-06	0.000650845	no result with blastx	no result with blastx
Anspe_C473816	1412.571784	-1.028369593	0.222553705	-4.620770496	3.82E-06	0.000843902	no result with blastx	no result with blastx
Anspe_C522948	2456.105975	-0.986401916	0.213753815	-4.614663437	3.94E-06	0.000852855	no result with blastx	no result with blastx
Anspe_7787	141.8912051	-1.542143826	0.336094266	-4.588426476	4.47E-06	0.000949618	no result with blastx	no result with blastx
Anspe_13002	190.9363528	-1.373159095	0.305171951	-4.499624194	6.81E-06	0.0014087	Predicted protein	meprin A, beta
Anspe_9181	238.4333217	-1.37621383	0.307015388	-4.482556526	7.38E-06	0.001499473	Predicted protein	no result with blastx
Anspe_C534254	814.6325706	-1.072081064	0.243362669	-4.405281504	1.06E-05	0.002110837	no result with blastx	notch 3
Anspe_C394298	531.8063028	-1.324874786	0.301462708	-4.394821484	1.11E-05	0.002196146	no result with blastx	no result with blastx
Anspe_4627	393.4183588	-1.164778032	0.265749267	-4.382996214	1.17E-05	0.002299201	Predicted protein	no result with blastx
Anspe_C517380	211.3721726	-1.355360818	0.309602051	-4.377751415	1.20E-05	0.002335424	no result with blastx	no result with blastx
Anspe_13491	465.6760261	-1.231745648	0.282497855	-4.360194688	1.30E-05	0.002509814	no result with blastx	no result with blastx
Anspe_C519766	497.5804445	-1.313769728	0.304447408	-4.315260017	1.59E-05	0.003053525	Predicted protein	lectin, galactoside-binding, soluble, 3 binding protein

Anspe_C529832	12965.30928	-0.992477043	0.231887172	-4.279999773	1.87E-05	0.00345777	Predicted protein	no result with blastx
Anspe_C511992	300.6472467	-1.310597443	0.307363095	-4.264003923	2.01E-05	0.003664446	no result with blastx	no result with blastx
Anspe_C491286	198.8483049	-1.331690722	0.312766263	-4.257782498	2.06E-05	0.003738462	Predicted protein	mannose receptor, C type 2
Anspe_1441	181.1016704	-1.359448719	0.319428627	-4.255876283	2.08E-05	0.003741232	Predicted protein	transmembrane protease, serine 9
Anspe_C447537	151.5984066	-1.4831566	0.349634628	-4.24201861	2.22E-05	0.003949331	no result with blastx	no result with blastx
Anspe_6221	153.9925495	-1.381810948	0.325924798	-4.239661897	2.24E-05	0.003960555	no result with blastx	no result with blastx
Anspe_C466123	233.2680373	-1.395539872	0.330247152	-4.225743845	2.38E-05	0.004181566	no result with blastx	no result with blastx
Anspe_C531610	511.5683615	-1.097706511	0.260987496	-4.205973575	2.60E-05	0.004530156	Predicted protein	no result with blastx
Anspe_17202	239.4255793	-1.362214862	0.32966881	-4.132070792	3.60E-05	0.006126732	Predicted protein	no result with blastx
Anspe_C497874	403.0847531	-1.093378717	0.265462899	-4.118762814	3.81E-05	0.006444085	no result with blastx	no result with blastx
Anspe_5742	474.3194555	-1.072901005	0.26352921	-4.071279247	4.68E-05	0.007740399	no result with blastx	no result with blastx
Anspe_C449141	152.6194122	-1.442802838	0.354305248	-4.072202844	4.66E-05	0.007740399	no result with blastx	no result with blastx
Anspe_C518244	915.1766826	-1.048016201	0.259328838	-4.041263632	5.32E-05	0.008633826	no result with blastx	no result with blastx
Anspe_C310985	666.3344478	-1.021973939	0.253182727	-4.036507349	5.43E-05	0.008732068	no result with blastx	no result with blastx

* Transcripts for which an *ISH* pattern is presented in chapter 4.

7.3 Transcripts down-regulated in the distal tentacle pair of *Antipathes caribbeana*

Sequence	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj	descrNematostella	descrHuman
Anspe_C518128	3332.670559	2.970253811	0.220706578	13.45793062	2.77E-41	3.20E-37	no result with blastx	no result with blastx
Anspe_C425139	1538822.527	2.543113293	0.19322623	13.16132539	1.46E-39	1.13E-35	no result with blastx	no result with blastx
Anspe_C483357	18706.59038	2.623980872	0.241086296	10.88399014	1.37E-27	7.96E-24	no result with blastx	no result with blastx
Anspe_C513190	1778.069185	1.680513927	0.213561219	7.869003239	3.57E-15	9.21E-12	no result with blastx	no result with blastx
Anspe_20113	9962.055864	1.443649738	0.203560505	7.091993319	1.32E-12	1.80E-09	no result with blastx	mesoderm development candidate 2
Anspe_C287837	1989.473164	1.914443327	0.277119854	6.908358614	4.90E-12	5.87E-09	no result with blastx	no result with blastx
Anspe_C450991	50661.16131	1.701827772	0.251917572	6.755494503	1.42E-11	1.57E-08	no result with blastx	no result with blastx
Anspe_C490534	12656.07051	1.372220299	0.214144656	6.40791289	1.48E-10	1.27E-07	no result with blastx	no result with blastx
Anspe_17266	5812.336567	1.166415823	0.206998426	5.634901883	1.75E-08	8.46E-06	no result with blastx	no result with blastx
Anspe_C483807	42954.25392	1.231740763	0.218734645	5.631210203	1.79E-08	8.46E-06	no result with blastx	no result with blastx
Anspe_C454869	11527.01399	1.075039333	0.197859409	5.433349559	5.53E-08	2.24E-05	no result with blastx	no result with blastx
Anspe_C482353	364.9023972	1.355572544	0.26830086	5.052434582	4.36E-07	0.000134802	no result with blastx	no result with blastx
Anspe_18491	902.5156401	1.15753678	0.231179745	5.007085631	5.53E-07	0.000160096	Predicted protein	myosin, light chain 9, regulatory
Anspe_C462489	3270.615308	1.132287092	0.236307969	4.791573887	1.65E-06	0.00042146	no result with blastx	no result with blastx
Anspe_17019	3471.596045	1.020588747	0.213822599	4.773063062	1.81E-06	0.000457103	no result with blastx	no result with blastx
Anspe_15806	44843.43984	1.027149387	0.215937885	4.756689119	1.97E-06	0.000485223	no result with blastx	no result with blastx
Anspe_15408	5709.638442	0.971345612	0.210166083	4.621800039	3.80E-06	0.000843902	no result with blastx	no result with blastx
Anspe_C509678	469.9875331	1.208372579	0.261653789	4.618211663	3.87E-06	0.000846313	Predicted protein	no result with blastx
Anspe_18396	20137.13975	0.938370698	0.203616862	4.608511735	4.06E-06	0.000870342	no result with blastx	mitochondrially encoded cytochrome c oxidase I
Anspe_C491290	6821.717595	0.942260478	0.208229947	4.525095906	6.04E-06	0.001271956	Predicted protein	no result with blastx
Anspe_C491456	608.0974427	1.206380165	0.267058066	4.517295364	6.26E-06	0.00130782	no result with blastx	no result with blastx
Anspe_C526134	2945.152919	0.999224362	0.222423148	4.49244772	7.04E-06	0.001444136	Predicted protein	charged multivesicular body protein 6
Anspe_392	82999.62011	0.934076867	0.211393042	4.41867367	9.93E-06	0.002001454	Predicted protein	no result with blastx
Anspe_C517140	906.0006974	1.086852193	0.252500798	4.304351517	1.67E-05	0.003181624	no result with blastx	no result with blastx
Anspe_19767	836.7132634	1.073035267	0.249604756	4.298937584	1.72E-05	0.003233828	Predicted protein	no result with blastx
Anspe_C516586	3539.962109	1.096361743	0.255256852	4.295131485	1.75E-05	0.003263281	Predicted protein	interferon, gamma-inducible protein 30
Anspe_6992	2153.211093	0.93088405	0.217561815	4.278710621	1.88E-05	0.00345777	no result with blastx	thioredoxin 2
Anspe_C502126	7419.563544	0.833297461	0.198202969	4.204263253	2.62E-05	0.00453048	no result with blastx	no result with blastx
Anspe_C506380	18973.24435	0.952252643	0.22670906	4.200329022	2.67E-05	0.004575781	no result with blastx	no result with blastx
Anspe_C480743	2561.039956	0.879670373	0.213847483	4.113540931	3.90E-05	0.006543907	no result with blastx	no result with blastx
Anspe_C510664	1039.388408	1.11608888	0.274364175	4.067910403	4.74E-05	0.007797435	Predicted protein	no result with blastx
Anspe_C485553	1322.894404	0.909570859	0.225096913	4.040796684	5.33E-05	0.008633826	Predicted protein	no result with blastx
Anspe_C495348	1735.511902	0.987879415	0.246224202	4.012113384	6.02E-05	0.009618868	no result with blastx	no result with blastx

7.4 Transcripts up-regulated in the distal tentacle pair of *Plumapathes pennacea*

Sequence	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj	descrNematostella	descrHuman
Anpen_19601	189.5375105	-2.801525686	0.239225815	-11.71080002	1.12E-31	3.20E-27	no result with blastx	ribosomal protein S15a
Anpen_C720642	1741.173425	-0.925905814	0.161630199	-5.728544675	1.01E-08	3.96E-05	no result with blastx	no result with blastx
Anpen_3722	76.37975263	-1.46056554	0.263724779	-5.538218841	3.06E-08	8.71E-05	no result with blastx	no result with blastx
Anpen_14929	267.8605088	-1.01277674	0.204494629	-4.952583558	7.32E-07	0.001491056	no result with blastx	no result with blastx
Anpen_C554355	88.1424767	-1.253273686	0.260838763	-4.804783128	1.55E-06	0.002759888	no result with blastx	no result with blastx
Anpen_C722860	634.1423025	-0.869956329	0.18168861	-4.788172089	1.68E-06	0.002822018	no result with blastx	no result with blastx
Anpen_C584648	153.5229931	-1.115883955	0.237324577	-4.701931716	2.58E-06	0.003672907	no result with blastx	no result with blastx
Anpen_C705086	411.78709	-0.858031799	0.187129328	-4.585234222	4.53E-06	0.005875423	no result with blastx	no result with blastx
Anpen_18293	2494.796326	-0.663537447	0.147115098	-4.510328675	6.47E-06	0.008021677	Predicted protein	no result with blastx
Anpen_17752	1369.159809	-0.68882669	0.153902615	-4.475730909	7.62E-06	0.008348421	Predicted protein	no result with blastx
Anpen_3873	1056.678175	-0.708787927	0.157808376	-4.491446813	7.07E-06	0.008348421	Predicted protein	transmembrane protease, serine 6

7.5 Transcripts down-regulated in the distal tentacle pair of *Plumapathes pennacea*

Sequence	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj	descrNematostella	descrHuman
Anpen_10856	68.01398649	2.74099484	0.267641746	10.2412829	1.30E-24	1.85E-20	Predicted protein	flotillin 2
Anpen_28668	93.48933461	1.915375824	0.258760012	7.402132229	1.34E-13	1.27E-09	no result with blastx	no result with blastx
Anpen_C720158	257.6267821	1.583337097	0.237846542	6.65696916	2.80E-11	1.99E-07	no result with blastx	no result with blastx
Anpen_C696376	82.64949159	1.693152222	0.258952766	6.538459684	6.22E-11	3.54E-07	no result with blastx	no result with blastx
Anpen_C554289	17597.88369	0.96207477	0.156842218	6.134029377	8.57E-10	4.07E-06	no result with blastx	no result with blastx
Anpen_C712390	162.9491322	1.337547853	0.234138406	5.712637565	1.11E-08	3.96E-05	no result with blastx	no result with blastx
Anpen_C730086	4508.650841	0.914169032	0.161759892	5.651394907	1.59E-08	5.04E-05	Predicted protein	eosinophil peroxidase
Anpen_C580244	306.7137608	1.091111818	0.207784214	5.251177645	1.51E-07	0.000391619	no result with blastx	no result with blastx
Anpen_14248	1707.467501	0.761282774	0.147191638	5.172051779	2.32E-07	0.000549979	no result with blastx	no result with blastx
Anpen_C402271	210.2302698	1.12154754	0.222655099	5.037151837	4.73E-07	0.001036033	no result with blastx	no result with blastx
Anpen_30461	202.6479078	1.0855475	0.219913317	4.93625176	7.96E-07	0.001513 34	no result with blastx	no result with blastx
Anpen_C624465	1773.304782	0.706810347	0.148325658	4.765260157	1.89E-06	0.002986746	no result with blastx	no result with blastx
Anpen_872	4012.061507	0.679031112	0.143910412	4.718429356	2.38E-06	0.003565587	no result with blastx	no result with blastx
Anpen_24809	190.7321691	1.14014801	0.243667262	4.679118557	2.88E-06	0.003910624	Predicted protein	no result with blastx
Anpen_C588121	2234.031849	0.736264849	0.164498096	4.475825966	7.61E-06	0.008348421	no result with blastx	no result with blastx
Anpen_C700784	184.2080451	0.988033714	0.222275814	4.445079729	8.79E-06	0.009275337	no result with blastx	no result with blastx