Phylogenetic ecology of octocoral - gastropod associations

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Introduction and thesis outline

> “*There are some four million different kinds of animals and plants in the world. Four million different solutions to the problems of staying alive. This is the story of how a few of them came to be as they are.*”
> Sir David Attenborough – Life on Earth (1979)

Background

Symbiosis is amongst one of the most important evolutionary drivers on coral reefs. Corals cannot survive without dinoflagellates (zooxanthellae) living in their tissue because the dinoflagellates provide essential nutrients for the coral to survive and visually impaired alpheid shrimps dig out burrows and let goby fish live in the same burrow with them because the goby acts as a guard for the shrimp while it is digging the burrow. These are just two examples of many more, how species-specific interactions have evolved on coral reefs and how species have adapted. For this thesis the interactions between soft corals (Octocorallia) and marine snails of the family Ovulidae are studied. Soft corals form an important component of coral reefs because they are a host for many species such as: shrimps, brittle stars, copepods, nudibranchs, worms, fish, snails etc. Without octocorals the number of primarily marine invertebrates might be far less on coral reefs. Among the symbiotic snails are also the snails belonging to the family Ovulidae. These snails roam on the octocorals and in most cases perfectly blend in to the surrounding octocoral branches and polyps by using camouflage and mimicry.

Together these species groups are used as model taxa for research on the evolution of species associations in the marine environment and on the speciation and adaptation processes involved. Even though the octocorals and ovulid snails live in close association with each other, they both have their own evolutionary background and history of research therefore both species groups will be briefly introduced, separately, in the next two sections.

A brief introduction to Octocorallia

Octocorallia are an important component of coral reef ecosystems. Many species depend on Octocorallia as a host for unlimited food or as a refuge against potential predation. With almost 3,200 Octocorallia species described (Appeltans *et al.*, 2012; Ofwegen, 2015), their abundance in shallow and deep, and tropical and polar waters houses a magnitude of other marine species such as shrimps, snails, echinoderms, copepods, fish and worms (Whitley 1970; Mase 1989; Humes, 1990; Goh *et al.*, 1999; Buhl-Mortensen and Mortensen, 2004; Neves *et al.*, 2007; Reijnen *et al.*, 2010; Dias *et al.*, 2015;). Their contribution to the marine biodiversity in the oceans is therefore of great importance. Soft corals are like stony corals, also reef builders but to a lesser extent than the stony corals do (Jeng *et al.*, 2011). In addition, Gabay *et al.*, (2014) showed that some species of octocoral are less susceptible to ocean acidification; octocorals have a significant advantage over the less resilient scleractinians as host species. In addition, octocorals contain secondary metabolites that can be new sources for anti-biotics or anti-cancer medicines (Pawlik, 2012) and serve as a nursery for commercially important fish species (Poulos *et al.*, 2013).

The research on the ecological value of soft corals is not on par with other sessile invertebrates such as scleractinians. This is probably the result of difficulties in the identification of species which can be challenging and time consuming. Identification of Octocorallia is primarily based on the habitus and the minute calcareous skeletal parts referred to as sclerites. Each

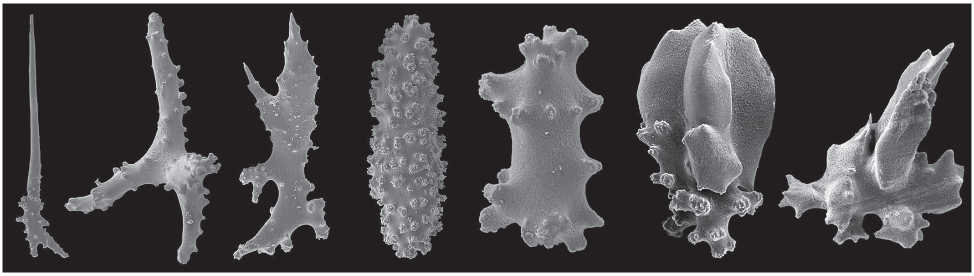

Fig. 1. Different types and shapes of sclerites found in Octocorallia photographed by Scanning Electron Microscopy (SEM). Images not to scale.

individual polyp and the tissue between the polyps, called coenenchyme, contain different types of sclerites. These sclerites showcase a plethora of shapes and forms, which are used to separate species and genera (Fig. 1).

The importance of these characteristic sclerite shapes was not recognised until the late 1800s and early 1900s, mostly because microscopes were not advanced enough at the time. As a result species-specific sclerites were not or only poorly depicted and species descriptions were quite general and could fit most other species described in that specific genus or family. This hampers present-day taxonomic/systematic research on Octocorallia. Molecular approaches (McFadden *et al.*, 2006, 2014; Bilewitch *et al.*, 2014; Reijnen *et al.*, 2014; Zapata *et al.*, 2015) help in the identification of species and integrate these results with the morphological features to study the complex relationships between species.

The Octocorallia are subdivided in three different orders (Alcyonacea, Helioporacea (Blue Corals a.o), Pennatulacea (Sea Pens)). Each of these orders is retrieved as a monophyletic group but within the largest order, the Alcyonacea, none of the described suborders were retrieved as monophyletic clades (McFadden *et al.*, 2006). The systematics and taxonomy of the Alcyonacea remain inconclusive until today and can probably only be elucidated with large sampling effort of species across different oceanic bodies including the application of new molecular techniques such as RAD sequencing (Herrera and Shank, 2015) and full genome analyses (Zapata *et al.*, 2015). Until then, the traditional morphological characters will remain amongst the most important features in identifying Octocorallia.

A brief introduction to Ovulidae

Linnaeus (1758) was the first to binomially treat an ovulid gastropod in his *Systema Naturae* (*Bulla ovum*). Currently this species is known as *Ovula ovum* or as the ‘common egg cowry’. At the time the family Ovulidae was not yet erected and ovulid species (n=5) were placed in the genus *Bulla*. Fleming erected the family ‘Ovuladae’ in 1822, which was later changed in Ovulidae. Fleming did not provide an extensive description for the family, his remarks were: “Both extremities of the aperture canaliculated. The inhabitants of all the genera *Ovula*, *Calpurna*, and *Volva*, are unknown. The last genus includes the *Bulla patula* of Pennant.” As said, the soft tissue for all ovulid species was unknown and they had only little shells at hand. For the Cypraeidae described seven years earlier by Rafinesque, 1815, however, Fleming mentions that species have a large mantle that can cover the shell. It is not exactly clear when soft tissue be-came available for research, but Weinkauff (1881) compared Cypraeidae shells with those belonging to the Ovulidae and concluded that, based on the smoothness of the outer layer of the shell, and a line on the shell where both sides of the mantle meet, the Ovulidae must have a large mantle covering the shell like Cypraeidae. This is indeed one of the similarities between these two families, but it is not unique for these families and can, for example, be found in species belonging to the genera *Bulla*, *Lamellaria*, and *Trivia*.

The biggest shell morphological difference between the two is that Ovulidae lack dentation on the ventrum (ventral, axial side of aperture; and have a columellar depression instead) whereas all Cypraeidae species have dentation on both sides of the shell opening. Yet there are exceptions within the Ovulidae and some species have developed a dentated funiculum on the ventral, adapical side of the shell.

One other character that all Ovulidae have in common is that they are all associated with Anthozoa, with the largest number of species associated with Octocorallia. The first notions of association with Octocorallia were made in the late 1800s. Weinkauff (1881) mentions *Melithaea ochracea* as a host for *Ovula sempieri* (=*Prosimnia semperi*) and *Ovula spelta* (=*Neosimnia spelta*) was described as "*auf Korallen lebend*". *Neosimnia spelta* was also found by Schiaparelli *et al.* (2005), who observed different colour varieties of ovulid mantles when species were found on different hosts. Nonetheless, their molecular phylogeny showed that these morphological differences in the mantle pattern did not prove to represent different species. The

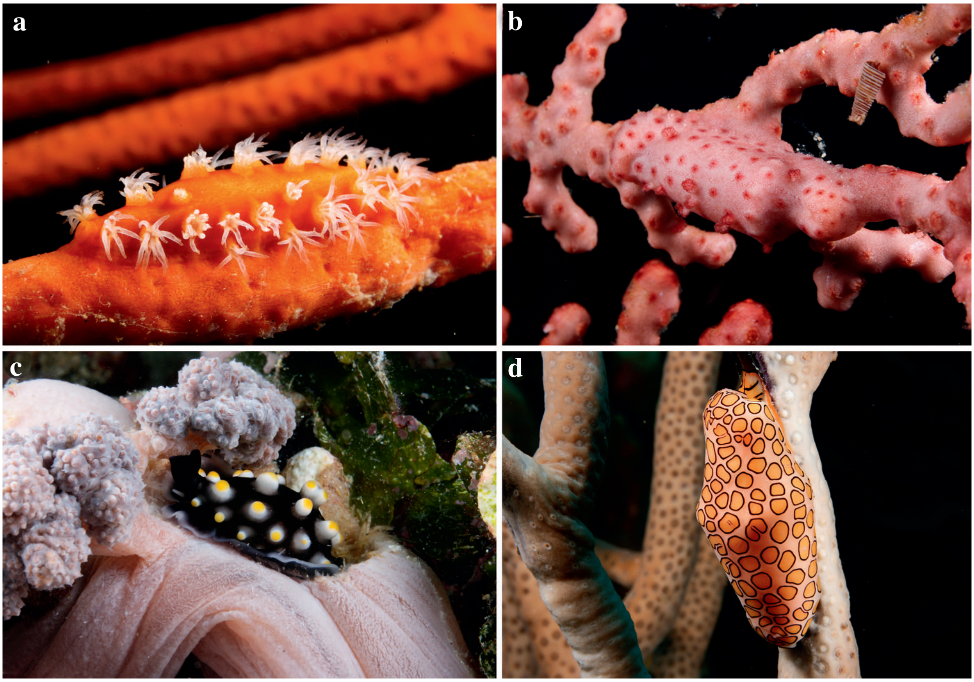

Fig. 2. a) Mimicked polyps on the mantle of an *Hiatavolva* sp. roaming on the branch of a Ellisellid b) *Prosimnia piriei* perfectly camouflaged on the branch of an *Euplexaura* sp. c) A juvenile *Ovula ovum* mimicking the habitus of a phyllidiid nudibranch d) One of the best known ovulids, *Cyphoma gibbosum*, with an aposematic mantle colouration and pattern.

mantles covering the shell in Ovulidae are to a certain degree variable in colour and are in most cases specially adapted to resemble the host coral the snails live on (camouflage) sometimes even mimicking host structures such as polyps (mimicry) or can be very unambiguous, advertising noxious properties (aposematism) (Fig. 2).

It is not exactly clear how ovulids obtain their colour but alimentary homochromy is thought to be one of the possibilities. In alimentary homochromy species sequester the pigments in their mantle by feeding on their host. This phenomenon is supported by data on European ovulid species, which shows that a species found on differently coloured hosts also showed similar differences in mantle colouration (Salvini-Plawen, 1972). Conversely, aposematic species are brightly coloured and do not match their host species, but probably use their colouration to advertise toxic properties (Rosenberg, 1992). The most common and popular aposematic species is *Cyphoma gibbosum* or better known under its vernacular name 'Flamingo tongue' which is a gregarious feeder on Caribbean octocorals (Gerhart, 1986).

Because soft tissue for ovulid species was not available for research purposes for a long time, only shell morphological features were used to systematically arrange the species in the Ovulidae. The first overview of ovulid species was commenced by Kiener (1843) and followed by Sowerby (1848), Reeve (1865), Weinkauff (1881), Tryon (1885), Schilder (1932) and Cate (1973). Each author discussed and rearranged the ovulid species according to their own studies. It was not until Schilder (1932), who had more specimens at hand by studying museum collections, that the first more modern attempt on re-classifying the family Ovulidae was conducted. The 'modern' classification attempt was later continued by Cate (1973) who was the first one to taxonomically and systematically deal with more than 100 nominal species and also provided data and images of type species. The most recent monograph on the Ovulidae deals with over 200 nominal species (Lorenz and Fehse, 2009) and also includes type localities and images of type species.

Where in the 19th century the locations for Ovulidae species often were not specified or not known at all, we now know that Ovulidae occur in all oceans except for the arctic seas. One ovulid species (*Simnia patula*) can even survive shallow cold-water environments and has been recorded from Norway (Høisæter *et al.*, 2011), which is, together with the Orkney Islands, probably the most northerly distribution for an ovulid. This is also the only ovulid species that can be found in Dutch waters (Schrieken *et al.*, 2011).

The majority of ovulid species is found on tropical coral reefs with their highest biodiversity in the "Coral Triangle". Most species occur on shallow coral reefs but some species have extended their depth distribution even below the mesophotic zone exceeding 1000 meter of depth (Lorenz and Fehse, 2009).

Molecular data on ovulids was first published by Meyer (2004) and followed by Schiaparelli *et al.* (2005). The close affiliation between Cypraeidae and Ovulidae was confirmed with the help of phylogenetic analyses by both authors. Yet, Meyer only analysed seventeen ovulids whereas Schiaparelli *et al.* (2005) reconstructed the first Ovulidae phylogeny based on 16S mtDNA sequences obtained from 33 nominal species. The authors also mapped shell morphological features on the phylogeny reconstruction and looked at the host species distribution of Ovulidae. Based on these molecular results some subfamilies were not retrieved as monophyletic entities and associations within nominal subfamilies remained problematic. The comparison with shell morphology showed that there is not much similarity in shell shape between closely affiliated clades of ovulid species. The differences in shell morphology, intrinsic associations with host species, perfect matching of colours and ornamentations between host and snails, or the occurrences of aposematic behaviour in the snails raises the questions how the obligate associations between Octocorallia and Ovulidae have originated. Due to the close ties between these species groups and the differences in morphology and appearances in the snails they lent themselves to be model organisms to study coevolution and the development of defence strategies in the marine environment.

Thesis outline

This thesis is built upon two pillars: the Octocorallia and the Ovulidae. To unravel the relationships and evolutionary history between these species, multidisciplinary approaches were used to study the relationships between and within both species groups. Molecular data, phylogenetic approaches, molecular dating, morphological analyses, bioactivity studies and SEM photography have all been used to shed light on the relationships within and between the Octocorallia and Ovulidae. What the chapters unifies are the locations where the research has been conducted. This thesis is divided in three sections that each more or less correspond with a geographically specified oceanic basin: the Atlantic (Chapter 1 to 3) and the Indo-Pacific (Chapter 4 to 6). The only exception is Chapter 7, which combines most of the data from the previous chapters and therefore has a global approach.

The first chapter in the 'Atlantic section' (**Chapter 1)** identifies the associations between Caribbean ovulids and octocorals and provides a provisional phylogeny reconstruction of Atlantic Ovulidae. However, in **Chapter 2**, a phylogeny reconstruction on a larger dataset with more specimens and additional molecular markers shows that synonymies are expected within Atlantic *Cyphoma*. In **Chapter 3** a more experimental approach is used to identify the bioactivity of the host corals of Ovulidae but also Cryptochiridae, which are solely associated with stony corals. With the help of a luminotester and light emitting *Aliivibrio fisheri* bacteria the bioactivity (EC_{50} values) of large numbers of Caribbean corals are assessed and plotted, together with host-symbiont data, onto phylogeny reconstructions of the respective host corals to assess if bioactivity of the hosts species is a possible driver in symbiont speciation.

In the second section, the chapters focus on octocoral and ovulid representatives from 'The Indo-Pacific'. **Chapter 4** investigates the morphological diversity and host preferences of species within the Ovulidae genus *Crenavolva*. One of these *Crenavolva* species is considered rare and confined to deep water. However there are only minor morphological differences when this species is compared to a congener from shallower depths. The systematic and taxonomic whereabouts of these *Crenavolva* species are assessed with a phylogeny reconstruction. In the next chapter, **Chapter 5**, the traditional subfamily division of the family Ovulidae is re-assessed with a large-scale molecular dataset. In particular species of the subfamily Aclyvolvinae have been troublesome in their identification. To shed light on the species identification, a four marker (mtDNA and nDNA) molecular approach, in combination with morphological analysis of the shell shapes of Aclyvolvinae species, is used. Systematic changes of genera and species in the subfamily Aclyvolvinae are proposed accordingly. In **Chapter 6** a similar approach to chapter 5 is used, on one of the most speciose gorgonian families and important hosts for Ovulidae, the Melithaeidae. The generic subdivision in the Melithaeidae has been questioned for decades by researchers. Morphological features are considered inconclusive and troublesome. To explicate on the generic divisions a combination of four molecular marker is used in combination with Scanning Electron Microscope (SEM) photographs of the morphological features of the sequenced specimens used as well as 44 type specimens of Melithaeidae species acting as a backbone for the molecular data. Since the type specimen of the type species for the family Melithaeidae is considered lost, a new type specimen for the type species of the family is assigned. By revising the genera in the family Melithaeidae six secondary homomyns are resolved. The junior secondary homonyms are provided with new species epithets.

The final chapter, **Chapter 7**, combines all data for the Ovulidae and the Octocorallia collected from various locations in the Caribbean, Mediterranean, Red Sea and Indo-West Pacific. First the species associations are plotted in a tanglegram identifying all the relations between Octocorallia as a host and Ovulidae as symbionts. Coevolutionary events between Octocorallia and Ovulidae are tested with cophylogenetic software and to determine the evolutionary speciation rates of both species groups molecular dating is used with the help of fossil records and sequence divergence estimates.