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Coral Venom Toxins

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The phylum Cnidaria contains a wide variety of unique organisms that possess interesting adaptations evolved over many years to help them survive in a competitive environment. One of these adaptations is the presence of venom, which has been of particular interest for studies aimed at identifying novel drug leads and for understanding the mechanisms involved in envenomation. The potency of the venom varies significantly amongst cnidarians, and although corals are often overshadowed by the jellyfish and sea anemone toxins, they also possess a range of interesting bioactive compounds. In this mini-review, we provide an overview of the toxins present in corals, highlighting the diverse structures and bioactivities.

Keywords: coral, sea anemone, toxin, nematocyst, venom

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INTRODUCTION

The organisms in the phylum Cnidaria represent some of the oldest living venomous creatures on the planet, including jellyfish, hydroids, sea anemones, and corals (Rachamim et al., 2015). The phylum is primarily defined by the presence of nematocytes, or stinging cells, in the tissue of these organisms (Ozbek, 2011). Nematocyte cells contain a tubule with a capsule of venom that, when stimulated, is everted, striking the prey/predator, penetrating the outer membrane and delivering the venom (Ozbek, 2011). These stinging cells have a variety of purposes but are mainly used for prey capture and defense (Greenwood, 2009).

Venoms often contain a complex mixture of compounds, including small molecules, peptides and proteins. These compounds can be highly potent and specific for biological targets, and the peptides in venoms are generally stable because of the presence of disulfide bonds making them of interest in drug design (Vetter et al., 2011; Utkin, 2015). Venomous creatures, such as spiders, scorpions, and cone snails have been well-studied and several databases have been established to collate the data on the toxins present (Kaas et al., 2008, 2012; Kuzmenkov et al., 2016; Pineda et al., 2018). Although cnidarians are generally less well-studied (Macrander et al., 2018), information about the toxins present is currently expanding.

Perhaps the most well-known of the cnidarian organisms are jellyfish. Their venom can be extremely potent and act, not only on small marine prey organisms, but can also have severe physiological effects on humans (Tibballs, 2006; Tibballs et al., 2011; Remigante et al., 2018). Although not as harmful as some jellyfish, other cnidarians, such as select sea anemones can elicit a stinging sensation in humans when the nematocytes in the tentacles are stimulated (Lubbock et al., 1981; Garcia-Arredondo et al., 2016). Several sea anemone toxins have been well-characterized, including an analog of a ShK toxin from *Stichodactyla helianthus*, which has entered Phase 2 trials for autoimmune diseases (Pennington et al., 2009; Chi et al., 2012; Prentis et al., 2018). There have been several recent reviews of sea anemone toxins regarding their bioactivity as well as their potential uses in the field of pharmaceutical development (Prentis et al., 2018; Thangaraj et al., 2018; Madio et al., 2019; Utkin et al., 2019). Corals are often overshadowed by the highly potent and potentially life-threatening toxins from jellyfish and clinically relevant sea anemones, but they too possess toxins of interest.

Much of the research on corals has focused on climate change impacts and secondary metabolites. In the past year alone there have been more than 3,750 journal articles published on "corals and climate change" (GoogleScholar) while there are around 388 articles published on "corals and toxins" (2 August 2019). The majority of research on corals and their secondary metabolites is not necessarily venom related. For example, metabolites from the gorgonian coral *Erythropodium caribaeorum* have been shown to act as a deterrent against reef fishes predating on the coral (Fenical and Pawlik, 1991). However, more recent studies have characterized venom derived coral toxins, with potential in the field of drug development (Radwan et al., 2002; Frazao et al., 2012; Rodriguez et al., 2012; Garcia-Arredondo et al., 2016). There is significant scope for future studies aimed at characterizing coral toxins, and in this *mini review* we highlight some of the structural and functional diversity that has already been uncovered.

CORAL TOXINS

Corals, primarily grouped into stony corals and soft corals, are members of the Anthozoa class of the phylum Cnidaria as shown in the phylogenetic tree in **Figure 1**. Toxins have been characterized from four of the Anthozoa order and examples of these toxins are given in **Table 1** to highlight the structural diversity, range of bioactivities and potential applications. The majority of research into toxins from organisms in the Anthozoa class has focused on sea anemones, because of the exciting therapeutic potential of some of the toxins, and several recent reviews have been published in this area (Prentis et al., 2018; Liao et al., 2019; Madio et al., 2019). Although there are distinct differences between sea anemones and corals, with stony corals having calcium carbonate skeletons in contrast to sea anemones (Shick, 1991; Stanley, 2003), the two organisms are in the same class and might have similar compounds to each other. We are only just beginning to appreciate the diversity of compounds present in the nematocysts of corals and potentially toxins present elsewhere in the tissue of these organisms. There is evidence that toxins can be delivered from anatomical structures other than nematocytes in sea anemones, with differences in localization between species (Moran et al., 2012; Bastos et al., 2016). It is possible a similar phenomenon also occurs in corals. While sea anemone toxins, such as the ShK toxins have not been found in corals to date, there are examples of other toxins and toxin families as outlined below for stony corals and soft corals and highlights the importance of characterizing coral toxins.

Scleractinia (Stony Coral)

Stony corals (Scleractinia) are reef building corals that absorb calcium carbonate from the water to form a hard skeleton, and occur in colonial or solitary aggregates (Stanley, 2003). The most well-characterized toxins from stony corals are a family of peptides termed small cysteine-rich peptides (SCRiPs) found in the ectoderm of a common stony coral, *Acropora millepora* (Sunagawa et al., 2009). SCRiPs contain a conserved eight cysteine framework, similar to the rattlesnake myotoxin domain in crotamine toxins (Jouiaei et al., 2015a) but there is limited sequence conservation beyond the cysteine residues. SCRiPs were originally thought to be found strictly in Scleractinia corals but recent studies have shown that homologs of SCRiPs are found in the sea anemones *Anemonia viridis* and *Metridium senile* (Jouiaei et al., 2015a). SCRiPS were also originally thought to be involved with biomineralization in reef building corals by playing a role in calcification of the skeleton of the coral but there is now evidence that they are a family of toxins rather than calcifying proteins (Jouiaei et al., 2015a). Two SCRiPs originally found in the ectoderm of the coral *A. millepora* were recombinantly expressed and incubated with zebrafish larvae. The larvae became paralyzed and insensitive to touch, consistent with neurotoxic action (Jouiaei et al., 2015a). The presence of SCRiPS in the ectoderm of *A. millepora* is also consistent with a role in prey envenomation as the ectoderm is lined by nematocytes (Grasso et al., 2011; Jouiaei et al., 2015a). Overall, SCRiPS remain of interest for further research because of their interesting framework and their presence in a variety of Anthozoa organisms.

It appears likely that SCRiPs are not the only toxins present in stony corals. Analysis of extracts from 11 different Scleractinian coral families collected from Heron Island on the Great Barrier Reef showed variable levels of toxicity in several assays including mice toxicity, haemolytic activity, and antimicrobial activity (Gunthorpe and Cameron, 1990). While the toxicity was variable, the majority of the species tested displayed some level of toxicity. Although the compounds responsible for the bioactivity were not characterized in this study, a recent study on the analysis of extracts from the nematocysts of three stony corals (Scleractinia corals), *Pseudodiploria strigosa*, *Porites astreoides*, and *Siderastrea sidereal* indicated the presence of a range of toxins and provided insight into the chemical composition. Extracts from all three corals were lethal to crickets, had haemolytic and nociceptive activity to varying extents, and exhibited PLA_2 and serine protease activities (Garcia-Arredondo et al., 2016). Interestingly, although these corals are not considered harmful to humans, the activity of these extracts is consistent with the physiological effects caused in humans by some hydroids, such as *Millepora alcicornis* and *Millepora complanate*, where the toxins work as lysins on erythrocytes (Garcia-Arredondo et al., 2016). Analysis of the extracts with SDS-PAGE indicated the presence of a broad range of proteins that differ under reducing conditions, and mass spectrometry analysis of a low molecular weight fraction indicated the presence of peptides with molecular weights in the range 3,000–6,000 Da. These peptide fractions were subsequently shown to be lethal to crickets and cause vasoconstriction. Further study is required to characterize these toxins, but it could be possible that some will have similarity to the SCRiPs family or the proteins with protease activity might have similarity to proteases present in other venoms, such as snake venom.

Toxins have also been predicted from the proteomics analysis of proteins discharged from nematocysts the stony coral *Acropora digitifera*, and the genome of this organism (Gacesa et al., 2015). A total of 55 potential toxins were predicted based on the genome but only 12

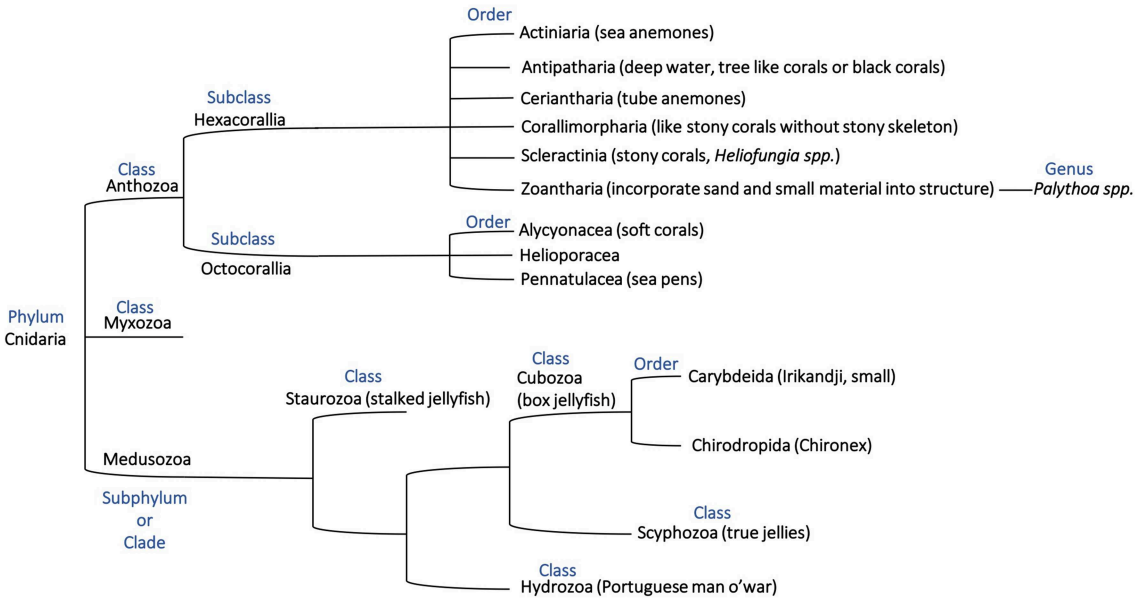

FIGURE 1 | Phylogenetic tree representing the phylum Cnidaria based on previous descriptions (Bridge et al., 1992, 1995; Berntson et al., 1999; Marques and Collins, 2004; Collins et al., 2006; Daly et al., 2007; Jimenez-Guri et al., 2007; Feng et al., 2014; Zapata et al., 2015) and showing the relationship of organisms discussed in this mini-review.

TABLE 1 | Selected toxins present in the class Anthozoa, phylum Cnidaria.

Toxin	Species	Target	MW (kDa)
HCRG21[a]	Sea anemone *Heteractis crispa*	TRPV1	6.1
APETx[b]	Sea anemone *Anthopleura elegantissima*	ASIC3, hERG K_V, $Na_V1.2$, 1.6, 1.8 channels	4.5–4.6
SCRiPs[c]	Scleractinia coral	–	4.3–5.8
ShK[d]	Sea anemone *Stichodactyla helianthus*	I_{DR} K_V, $K_V1.4$ channels	4.3–5.8
PcKuz3[e]	Zoantharia *Palythoa caribaeorum*	6-OHDA-induced neurotoxicity	5.7
Crude venom[f]	Zoantharia *Palythoa caribaeorum*	Nav1.7, $Ca_V2.2$, I_A, and I_{DR} channels	1.8–9

[a] *Monastyrnaya et al. (2016).*
[b] *Diochot et al. (2004), Moreels et al. (2017).*
[c] *Sunagawa et al. (2009).*
[d] *Castaneda et al. (1995), Prentis et al. (2018).*
[e] *Liao et al. (2018).*
[f] *Lazcano-Perez et al. (2016, 2018).*

were found based on the proteomic analysis of the nematocyst extracts (Gacesa et al., 2015). These toxins are suggested to be phospholipases and toxic peptidases based on their similarity to other known toxins found on Tox-Prot (Gacesa et al., 2015). Furthermore, an haemolytic toxin from the actinoporin family has recently been characterized in *Stylophora pistilata* and was suggested to be a non-nematocyst protein (Ben-Ari et al., 2018).

Alcyonacea (Soft Coral)

Soft corals (Alcyonacea), formerly known as gorgonian corals, contrast stony corals in that they do no create a calcium carbonate skeleton (Alarif et al., 2019). In a similar study to the Scleractinia coral extract analyses, Radwan et. al demonstrated the effects of venom from three different soft corals on mice. The corals, *Nephthea* sp., *Dendronephthya* sp., and *Heteroxenia fuscescens*, were collected from the Red Sea and are known to cause a stinging effect in humans (Radwan et al., 2002). The data showed that extracts from nematocysts of all three corals resulted in fractions with bioactive effects including lethality to mice, haemolysis, vasopermeability, or dermonecrosis, with toxins from *H. fuscescens* the most lethal to mice (Radwan et al., 2002). Similar to the studies on stony corals, SDS-PAGE analysis of the venom extracts indicates the presence of a wide range of proteins ranging from ∼200 kDa to <6,000 Da. The bioactivity was not restricted to one class of protein, with two fractions from the *H. fuscescens* extract showing potent haemolytic activity with one fraction containing a protein of 116 kDa and the other containing a peptide of <6 kDa. The addition of a variety of lipid membrane components to the venom followed by addition of this mixture to human red blood cells instigated a protective response of the cells against the crude venom toxins (Radwan et al., 2002). The inhibition of a physiological response in the cells suggests that the binding site is occupied, with the most effective inhibition occurring by the addition of dihydrocholesterol (Radwan et al., 2002). Occupation of the binding site prevents the toxin binding and therefore eliciting a physiological response on the cells. Furthermore, this research also tested the mice for antibody production. Mice injected with a dose of venom, and provided with boosters throughout the study,

produced an immune response in 15 days with high levels of antibodies present in the blood (Radwan et al., 2002). Further studies are required to fully characterize the bioactive peptides and proteins present in the extracts.

Non-proteinaceous toxins are also present in soft corals. For example, the small molecule toxin sarcophine was isolated from the soft coral *Sarcophyton glaucum* and is toxic to fish as well as mice, rats and guinea pigs (Ne'eman et al., 1974). Ingestion by the animal led to a decrease in cardiac and pulmonary function as well as motor function and body temperature of the animals (Ne'eman et al., 1974). Using guinea pig ileum, it was shown that sarcophine acts as a competitive inhibitor of cholinesterase (Ne'eman et al., 1974). This coral was originally studied for its ecological characteristics when Ne'eman et al. observed that the fish in the area were not predating on this specific coral (Ne'eman et al., 1974) and subsequent studies lead to the characterization of sarcophine.

There have also been large scale studies on toxins of soft corals found on the Great Barrier Reef in which 136 different specimens from 15 different genera were analyzed (Coll et al., 1982). In this study two genera were found to be the most toxic and lethal to the fish species tested: *Lemnalia* and *Sarcophyton* (Coll et al., 1982). The different genera of coral tested exhibited a large range of affects, from no noticeable effect on the fish to causing death. This study and the studies on sarcophine involved extraction of coral tissue rather than nematocysts extracts, which makes it difficult to identify where the compound comes from in the coral, but these studies demonstrate the diversity of the compounds from corals and the potent activity they can possess.

EVOLUTION OF CORAL TOXINS

Insight into the evolution of coral toxins is primarily based on the SCRiP family of peptides, as these are the most well-characterized to date. As mentioned above, in contrast to the original suggestion, SCRiPS are not only found in corals but have been found in the sea anemones *Anemonia viridis* and *Metridium senile* (Jouiaei et al., 2015b). The toxin τ-AnmTx Ueq 12-1 isolated from the sea anemone *Urticina eques* also shows similarity to SCRiPs, in particular one cDNA matched the SCRiP *Anthopleura elegantissima* comp63456_c0_seq1 (Logashina et al., 2017). The presence of SCRiPs in corals and sea anemones suggests that these proteins evolved more than 500 million years ago, the estimated time when coral diverged from sea anemones (Shinzato et al., 2011). Furthermore, molecular evolutionary assessments indicate that coral SCRiPs have evolved under negative selection as no sites were found that were positively selected based on the Bayes Empirical Bayes approach (Jouiaei et al., 2015a). The role of negative selection in coral toxin evolution is supported by studies on the toxins of *Acropora digitifera* and *Stylophora* pistilata (Gacesa et al., 2015; Ben-Ari et al., 2018). Interestingly, this appears to be a general phenomenon for venoms of ancient lineages, whereas toxins from lineages that have evolved more recently appear to evolve under positive selection (Lynch, 2007; Casewell et al., 2011, 2012; Sunagar et al., 2012, 2013, 2014; Brust et al., 2013; Dutertre et al., 2014; Jouiaei et al., 2015a; Sunagar and Moran, 2015).

Given the large number of toxins that appear to be present in coral venom, further molecular characterization is likely to provide further insight into the evolution of this ancient lineage. In particular, characterization of some of the larger toxins might provide insight into origins of a range of toxins, as analysis of the venom from the sea anemone *Stichodactyla haddoni* showed that some venom peptides have similar sequences to housekeeping proteins involved in regulatory biological functions (Madio et al., 2017). This is a common trend across many venomous taxa because it is suggested that the main ways that toxins are recruited is via gene modification of regulatory proteins, such as sequence duplications (Fry et al., 2009). It has been shown that cnidarian organisms rely on similar structural frameworks of their toxins and then modify these toxins for activity on specific targets (Honma and Shiomi, 2006; Prentis et al., 2018). Because of this evolution from non-toxin related proteins, we see variability in the size of peptides found in nematocyte venom as the evolution of each peptide differs greatly (**Table 1**).

Despite the similarities between cnidarian organisms, such as coral and sea anemones, it is also likely that significant differences in the evolution of toxins will be found based on analysis of proteins found in the nematocysts of organisms from three different classes of Cnidaria, namely Anthozoa, Scyphozoa, and Hydrozoa (Rachamim et al., 2015). The organisms analyzed from these three classes were the sea anemone *Anemonia viridis* (Anthozoa), the jellyfish *Aurelia aurita* (Scyphozoa) and the hydrozoan *Hydra magnipapillata* (Hydrozoa). Although this analysis led to the identification of hundreds of proteins, only six proteins were common in all three species (Rachamim et al., 2015). Of these, most were structural proteins and only one of the six proteins common to all three species, the dickkopf protein, is predicted to function as a toxin (Rachamim et al., 2015). The *A. aurita* and *H. magnipapillata* venom showed the most similarities, mainly composed of cytotoxins and enzymes, while the *A. viridis* venom proteome composition was predominantly related to peptide neurotoxins (Rachamim et al., 2015). The general lack of conservation across these cnidarians might point to significant evolutionary differences and further cnidarian toxins promises to provide interesting insights into toxin evolution in general.

CHALLENGES IN CNIDARIAN TOXIN ANALYSES

In the study of venomous creatures, such as spiders and cone snails it can often be quite straightforward to isolate the venom with limited contamination from the environment or other tissues. Indeed, Australian funnel-web spiders (Atracidae) can release microlitres of venom onto their fangs that can be "easily" recovered (Wilson and Alewood, 2004). However, for corals and cnidarian organisms in general this is not always the case, and can complicate the toxin extraction process

(Garcia-Arredondo et al., 2016). A range of extraction methods have been used in the analysis of corals and sea anemones, including extraction of the toxins from the nematocyte in the tissue of the coral (Garcia-Arredondo et al., 2016) and homogenization of the whole tentacle of the sea anemone (Prentis et al., 2018). Homogenization of the whole tentacle will clearly yield more than just venom toxins, but even extraction of the toxins from the nematocyst can be complicated, given the small size of the nematocysts. Furthermore, it can be difficult to separate out the calcareous skeleton of the coral from the tissue itself (Garcia-Arredondo et al., 2016). The cellular contents can have implications for interpretation of the results because some components, such as minicollagens share characteristics similar to the cysteine-rich toxins of interest (Madio et al., 2017).

The difficulties in defining the origin of compounds, either from the nematocyst or other tissue, has significant implications for elucidating evolutionary relationships for these toxins. In particular, it is difficult to determine a common ancestor (Kayal et al., 2018). Genome and transcriptomic analyses are likely to provide further insight into the evolution of cnidarian toxins. Using an integrative approach of both genomic and transcriptomic analyses allows for a better understanding of the active toxins found in organisms compared to the potential toxins seen in the genome and will also allow comparison with other toxins from a range of venomous creatures.

CONCLUSION

Coral venoms are a source of interesting novel bioactive molecules with significant scope for further characterization of novel toxins. Further application of analysis technologies (e.g., genomics, transcriptomics, and proteomics) is likely to significantly enhance the knowledge in this field and identify novel classes of peptides/proteins. The well-characterized coral toxins, SCRiPs, have now been identified as likely neurotoxins, but given the highly microbial environment in which corals exist, further analysis of the nematocyst components of corals is likely to provide a new and unique source of antimicrobial molecules. Studies on coral extracts have indicated that such compounds exist. In addition, determining the composition of peptides that make up the venom from corals may provide insight into the overlap and differences between cnidarian groups.

AUTHOR CONTRIBUTIONS

CS, ND, and DW wrote the manuscript.

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