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# The Complicated Evolutionary Diversification of the Mpeg-1/Perforin-2 Family in Cnidarians

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The invertebrate innate immune system is surprisingly complex, yet our knowledge is limited to a few select model systems. One understudied group is the phylum Cnidaria (corals, sea anemones, etc.). Cnidarians are the sister group to Bilateria and by studying their innate immunity repertoire, a better understanding of the ancestral state can be gained. Corals in particular have evolved a highly diverse innate immune system that can uncover evolutionarily basal functions of conserved genes and proteins. One rudimentary function of the innate immune system is defense against harmful bacteria using pore forming proteins. Macrophage expressed gene 1/Perforin-2 protein (Mpeg-1/P2) is a particularly important pore forming molecule as demonstrated by previous studies in humans and mice, and limited studies in non-bilaterians. However, in cnidarians, little is known about Mpeg-1/P2. In this perspective article, we will summarize the current state of knowledge of Mpeg-1/P2 in invertebrates, analyze identified Mpeg-1/P2 homologs in cnidarians, and demonstrate the evolutionary diversity of this gene family using phylogenetic analysis. We will also show that Mpeg-1 is upregulated in one species of stony coral in response to lipopolysaccharides and downregulated in another species of stony coral in response to white band disease. This data presents evidence that Mpeg-1/P2 is conserved in cnidarians and we hypothesize that it plays an important role in cnidarian innate immunity. We propose that future research focus on the function of Mpeg-1/P2 family in cnidarians to identify its primary role in innate immunity and beyond.

**Keywords: perforin, perforin-2, Mpeg-1, cnidarian, immunity, evolution, phylogenetics, macrophage expressed gene 1**

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## OVERVIEW OF CNIDARIA, INNATE IMMUNITY, AND MACROPHAGE EXPRESSED GENE 1/PERFORIN-2 PROTEIN

The phylum of Cnidaria possesses over 10,000 extant species which are united by the innovation of stinging cnidocyte cells and a polyp life stage (1). Cnidarians include stony corals, soft corals, sea anemones, and jellyfish and are known to be some of the most important organisms for promoting ocean biodiversity, as well as sources of novel compound discovery (2, 3). From an evolutionary perspective, Cnidaria is critical for our understanding of ancestral traits because they are the sister group with Bilateria, from which they split ∼604–748 million years ago [**Figure 1A**; (6, 7)].

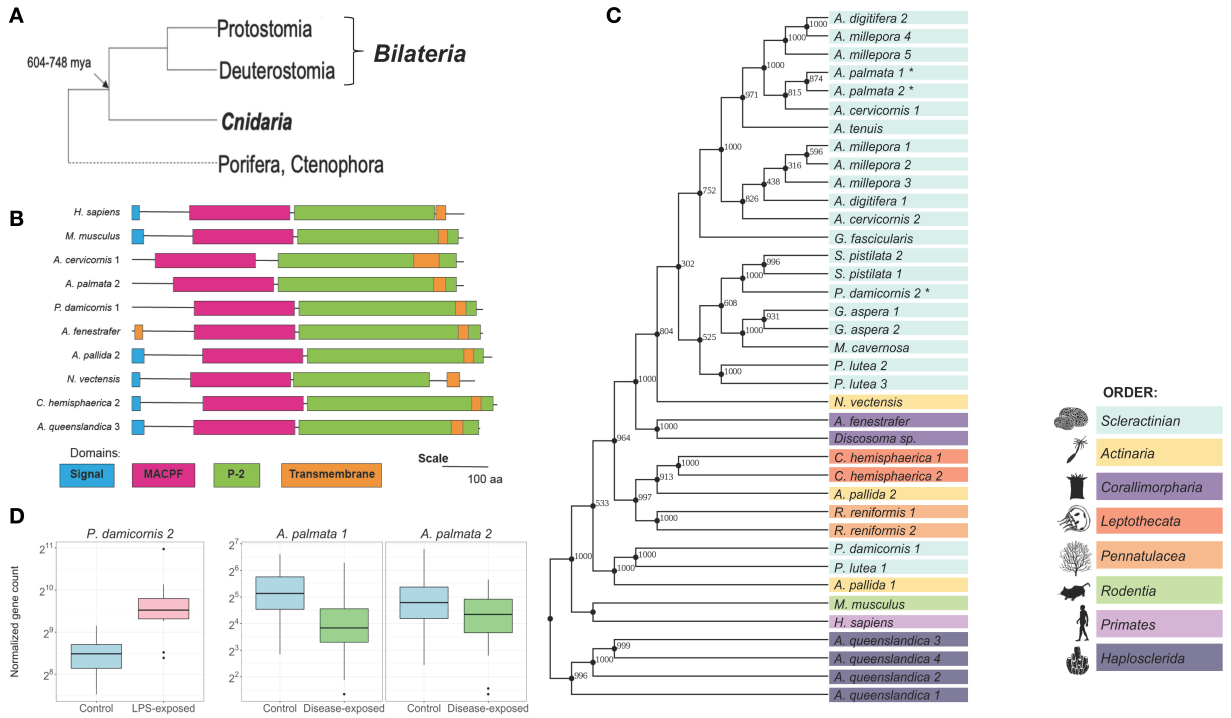


**FIGURE 1 |** Mpeg-1/P2 is highly diversified in Cnidarians. **(A)** Between 604–748 million years ago, bilaterians split from cnidarians. Cnidaria as sister group to Bilateria can inform our understanding of ancestral traits. **(B)** Domain architecture of P2. Amino acid sequence and domain lengths are drawn to scale. Signal peptide (blue), membrane attack complex/perforin (MACPF; pink), and transmembrane (orange) domains were identified using Hmmer. The P2 (green) domain was identified by aligning the P2 sequence of the human P2 protein. **(C)** A Maximum likelihood tree created in PhyML using the WAG +G +I model, as predicted by Smart Model Selection and ProtTest [Version 3.4.2; (4, 5)]. This tree shows the predicted evolutionary relationship of the whole P2 protein using 38 protein sequences. The percentage of replicate trees associated with the given taxa clustered together using 1000 bootstrap tests are shown at the nodes. All amino acid positions with <95% coverage were eliminated from the model. Tree is rooted taxonomically by *A. queenslandica*. **(D)** The response of Mpeg-1 to an immune stimulus and active white band disease infection in two coral species. In *P. damicornis* LPS exposure causes significant upregulation of a Mpeg-1 transcript (pdam_00017055, Swiss-prot E-value = 7.43E-143) compared with control. In *A. palmata,* exposure to white band disease identified two significantly differentially expressed downregulated Mpeg-1 transcripts (Apalm_v2_evm.model.Sc0a5M3_382_HRSCAF_692.335, Swiss-prot E = 3.55E-158; Apalm_v2_evm.model.Sc0a5M3_382_HRSCAF_692.340 Swiss-prot E-value = 1.69E-159).

The advent of next-generation sequencing technologies has revealed that stony corals possess a highly redundant and diverse innate immune system at the gene and protein levels (8–10). Stony corals maintain symbiotic associations with a diverse microbial community including bacteria, fungi, archaea, and dinoflagellates of the family Symbiodiniaceae (11). To maintain these relationships, they must possess a complex innate immune repertoire that can decipher between symbiont and pathogen (12). These intricate symbioses, paired with old evolutionary age are hypothesized to be the primary factors that have led to the complex diversity of stony coral innate immune proteins (13–15). We currently do not understand the function of many of these innate immune factors, but by examining their phylogenetics, protein domain architecture, and gene expression we can begin to better understand their possible significance.

Macrophage expressed gene 1/Perforin-2 protein (Mpeg-1/P2) is a pore forming effector molecule that is crucial for the innate immune response of both vertebrates and invertebrates (16). It has been identified in multiple organisms, including sponges, mollusks, zebrafish, ctenophores, sea anemones, and humans (16–27). Within invertebrates, limited studies have shown that both sponges and oysters upregulate Mpeg-1 in response to viral or bacterial infections (18, 28). In other invertebrates, including stony corals, little is known about the function or diversity of the Mpeg-1/P2 family. Previous studies have identified MACPF containing proteins in stony corals including within toxins from cnidocyte cells (29–34), however proteins containing both MACPF and P2 domains such as Mpeg-1/P2 have not been well-described.

In this perspective article, we will discuss the diversity of the Mpeg-1/P2 family within Cnidaria, with a focus on stony corals. Additionally, we describe the conserved protein domains of P2 in Cnidaria and show that Mpeg-1 homologs react to both a natural disease challenge, as well as a synthetic pathogen mimic. Lastly, we discuss the possible role of Mpeg-1/P2 in cnidarian innate immunity and future areas of investigation.

## CNIDARIA MPEG-1/P2 IS HIGHLY CONSERVED, DUPLICATED, AND COMPLICATED

To identify cnidarian homologs, BLASTp (Version 2.2.29+) searches using the *Mus musculus* P2 protein sequence were employed (35). To locate the P2 domain, each sequence was aligned against the isolated *Homo sapiens* P2 domain (amino acids 351–653) using Clustal Omega (36). From this, we identified cnidarian P2 protein homologs as highly conserved (**Figure 1B**, **Supplementary Table 1**). This is supported by both protein domain analysis and phylogenetic analysis (**Figures 1B,C**). There is a diversity of P2 homologs present indicating multiple duplication events within each cnidarian species and thus paralogs. One hypothesis for the retention of multiple P2 paralogs is that it may have evolved additional functions in cnidarians through the process of neofunctionalization. Alternatively, subfunctionalization could have occurred requiring multiple paralogs to perform the original ancestral function.

P2 proteins consist of an N-terminus regulatory signal peptide, the membrane attack complex/perforin (MACPF) domain, the perforin-2 (P2) domain, and the C-terminus transmembrane anchor (16). The MACPF domain generates pores in the lipid bilayer of bacteria cell membranes that leads to bacterial lysis (37). The defining P2 domain is important for Mpeg-1/P2 identification, but little is known about the functional mechanisms of this domain. A single missense or stop mutation in the P2 domain causes an inability to fight off bacterial infections indicating that it is important for the overall primary function of the P2 protein (38).

Using PhyML (Version 3.0) phylogenetic trees were constructed to identify the relationships between cnidarian, mammalian, and sponge P2 proteins (**Figure 1C**) (39). A maximum-likelihood tree was created using the WAG +G +I model as recommended by both Smart Model Selection and ProtTest [Version 3.4.2; (4, 5)]. The resulting tree shows P2 homologs partitioning into groups based on the major clades of stony corals with Corallimorpharia forming a paraphyletic group sister to the stony corals in accordance with their known evolutionary relationship (40, 41). The 60% of the bootstrap support are over 90% indicating high confidence in our model (**Figure 1C**).

Taken together these results show that P2 proteins are highly conserved and diverse within Cnidaria, a pattern which has also been observed in other innate immunity genes (13, 14). The phylogenetic relationship of P2 clearly shows that diversification of this protein occurred within species which resulted in many unique paralogs for P2. Given this protein domain analysis and phylogenetic information, understanding if the genes associated with Mpeg-1 are expressed in response to an active infection or a synthetic immune stimulus would further bolster our hypothesis that Cnidaria possesses functional Mpeg-1/P2.

## STONY CORAL MPEG-1 GENES EXHIBIT ALTERNATE REACTIONS TO IMMUNE STIMULUS AND ACTIVE INFECTION

Two scleractinian coral transcriptomic datasets were mined for homologs of P2; one from *Pocillopora damicornis* exposed to the synthetic immune stimulus lipopolysaccharide (LPS), NCBI SRA BioProject PRJNA587509, (34) and the second from *Acropora palmata* exposed to the naturally occurring white band disease (WBD); NCBI SRA BioProject PRJNA529682, (42); **Figure 1D**. In *P. damicornis*, one Mpeg-1 homolog was found to be significantly upregulated (pdam_00017055, LFC = 1.21), while in *A. palmata* two paralogs of Mpeg-1 were found to be significantly downregulated in response to WBD (Apalm_v2_evm.model.Sc0a5M3_382_HRSCAF_692.340, LFC = −1.38; Apalm_v2_evm.model.Sc0a5M3_382_HRSCAF_692.335, LFC = −0.94). The presence of Mpeg-1 significant differential gene expression in these different coral species is evidence that it is responding to bacteria much like what has been previously seen in other organisms, however, unlike these other organisms, the response is more complicated and variable (16–24, 26, 43). With the presence of multiple paralogs, it is possible that some of these genes are not involved in innate immunity. Additionally, the variation in gene expression in *A. palmata* could be due to environmental challenges, as these were nursery reared corals (42). Further investigation into the function of these genes in multiple species of coral will be valuable for our understanding of the functional repertoire of this gene family in cnidarians, as well as, the effects of environmental stress.

## FUTURE DIRECTIONS FOR CNIDARIAN MPEG-1/P2

The conservation of Mpeg-1/P2, across both the cnidarian lineage and throughout evolutionary history provides evidence that it is an ancient immune factor important for survival. Specifically, within cnidarians, there is much we do not understand: (1) What is the function of Mpeg-1/P2? (2) What is the protein structure? (3) Why are there abundant gene duplications? (4) What other proteins do cnidarian Mpeg-1/P2 associate with? (5) Why do different Mpeg-1/P2 respond differently in distinct cnidarians? (6) In what cell lineage is Mpeg-1/P2 expressed? Investigating Mpeg-1/P2 within non-traditional model systems such as cnidarians will shed light on its full functional capabilities and lead to novel discoveries on the function of this family that could have medically relevant applications.

## DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. This data can be found here: https://github.com/brianwalters7/Cnidarian-Mpeg1/tree/v1.3.3.20. *Pocillopora damicornis* sequence data is available through NCBI: SRA BioProject PRJNA587509. *Acropora palmata* sequence data is available through NCBI: SRA BioProject PRJNA529682.

## AUTHOR CONTRIBUTIONS

BW and NT-K conceived the project and performed the phylogenetic analysis and protein analysis. MC and BY performed the transcriptomic analysis and figure production. All authors were involved in editing and writing of this paper.

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## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2020.01690/full#supplementary-material

**Supplementary Table 1 |** Summary of P2 homologs found in Cnidarians. P2 homologs were identified through BLASTp (Version 2.2.29+) using the Mus musculus protein sequence (NP_001361597.1). Twenty cnidarians genomes, one cnidarian transcript shotgun assembly, and one sponge genome were searched. This included 12 scleractinian corals, one soft coral, two coral-like anemones, three anemones, one hydra, one jellyfish, and one parasitic myxozoa. Candidate homologs from the BLAST searches were limited to E-value cut off (E-6). Sequences were further culled by removing candidates which lacked MACPF or P2 domain. Only three cnidarians (Anemonia viridis, Hydra vulgaris, and Thelohanellus kitauei) did not have homologs present. Lastly, an Orbicella faveolata homolog, was identified, but removed from analysis due to truncation.

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**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.