INVESTIGATION OF THE TUMOR NECROSIS FACTOR RECEPTOR ASSOCIATED FACTORS IN *POCILLOPORA DAMICORNIS*

Rachel Sandquist, Nikki Traylor-Knowles, Michael Connelly

University of Miami, Rosenstiel School of Marine and Atmospheric Science 4600 Rickenbacker Causeway, Miami, FL 33149

Abstract

- Coral reefs are vital marine ecosystems which are declining due to pressures such as elevated temperature, ocean acidification, nutrient loading, and increase incidence of disease.
- It is thought that these pressures have a negative effect on corals' innate immune systems driving a stress induced inflammatory immune response, as well as, bleaching.
- In this study, the coral *Pocillopora damicornis* was investigated for tumor necrosis factor receptor associated factors (TRAFs).
- Thirty-seven TRAF proteins were identified in *P. damicornis*. When compared to the TRAF proteins seen in other animals, *P. damicornis* seems to possess a very diverse and expansive repertoire of TRAF proteins. The large and diverse repertoire of TRAF like proteins seen in *P. damicornis* may be indicative of the diverse molecular mechanisms behind their ability to tolerate a high level of stress.

Introduction

- Humans are having an unprecedented impact on the health of coral reefs. (Hughes et al., 2003)..
- Current research suggests that stressors in the environment can harm coral health via the disruption of their immune systems and symbioses, thereby causing an increase in the incidences of coral disease and coral bleaching (C. V. Palmer, 2018). That being stated, the immunological and molecular mechanisms behind this vulnerable state require further research (Traylor-Knowles & Connelly, 2017).
- Corals have evolved very complex innate immune systems which allow them to combat the many pathogens and stressors they encounter in their sessile lifestyles. (Palmer & Traylor-Knowles, 2012)
- One of the protein families known to mediate immunological signaling pathways in higher metazoans are tumor necrosis factor receptor associated factors (TRAFs) (Xie, 2013). TRAFs have been shown in mammals to function as both an adaptor protein, as well as, E3 ubiquitin ligases in pathways usually resulting in inflammation and apoptosis (Xie, 2013).

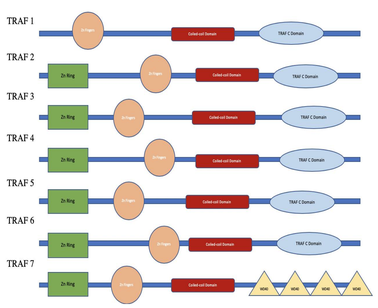


Figure 1. Schematic of mammalian TRAF proteins with the major domains. The design does not include the variation in the number of zinc fingers seen in the different TRAFs, nor the variability in the placement of the Zn RING domain or the coiled-coil region. Adopted from Xie *et al.* 2013

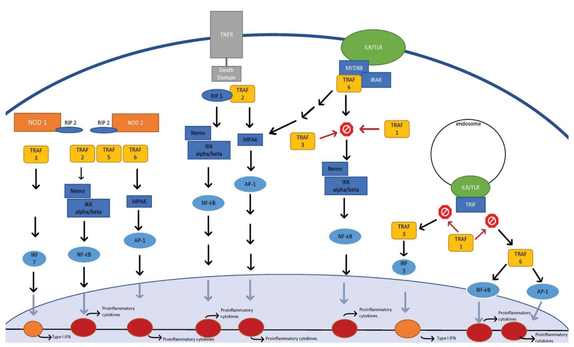


Figure 2. Mammalian immunological cascades which utilize TRAFs in the activation of transcription factors. The pathways pictured are relevant to known receptors in coral immunology. Signaling cascades included are toll-like receptors/ interleukin receptors, NOD- like receptors, and tumor necrosis factor receptors present in coral innate immunology. Black arrows represent initiation of cascade, red arrows represent inhibition, and blue arrows represent the movement of transcription factors into the nucleus. Adapted from Dhillon *et al. 2019,* O'neill *et al.* 2007, Chung *et al. 2002*, and Xie *et al.* 2013

Objectives

- In this thesis, I aimed to identify the repertoire of TRAF proteins in a known robust coral, *Pocillopora damicornis*, as well as, phylogenetically compare the observed *P. damicornis* TRAFs to TRAFs observed in another metazoan .
- I hypothesized that *Pocillopora damicornis* will have a complete repertoire of TRAF proteins, if not an evolutionarily expanded and diverse family of proteins.

Methods

I. Identification of TRAF homologous proteins

- The TRAF 6 protein query from *Homo sapiens* (ascension number NP_004611.1) was ran in protein-protein BLASTP 2.10.0+ against the entire *P. damicornis* repertoire of proteins (genome assembly ASM370409v1) (Camacho et al., 2009; Cunning et al., 2018). Proteins which had an e-value cut-off of e-03 were included in a reciprocal BLASTP search against the *H. sapiens* protein repertoire (Camacho et al., 2009). Proteins which had a significant human TRAF homolog where then considered TRAF homologs.

II. Phylogenetic analysis of TRAF proteins

- A MUSCLE proteins alignment was performed on TRAF protein sequences for *Homo sapiens, Drosophila melanogaster, Hydra vulgaris, Suberites domuncula, Acropora digitfera*, and *Stylophora pistillata*. Next, the aligned sequences were phylogenetically mapped using a neighbor-joining tree with 1000 bootstrapping replicates in MEGAX version 10.1.7. (Stecher et al., 2020).

III. Domain characterization of *P. damicornis* TRAF proteins

- Using HMMSCAN the TRAF proteins were individually analyzed to determine protein length and identify characteristic protein domains.(Eddy, 1998).

Results

Table 1. Identified P. damicornis TRAF-like proteins. Protein homology was determined based upon BLASTP results and the hypothesized TRAF protein type was determined based on PHMMER domain matches. The following proteins have the complete homologues domains to those seen in H. sapien TRAF proteins.

Accession Number	Gene Symbol	Locus Tag	Hypothesized TRAF
XP_027051303.1	LOC113678603	pdam_00013439	TRAF 2-6
XP_027050717.1	LOC113678044	pdam_00017488	TRAF 2-6
XP_027037290.1	LOC113665766	not listed	TRAF 2-6
XP_027041659.1	LOC113669780	pdam_00024941	TRAF 1
XP_027037295.1	LOC113665771	pdam_00023582	TRAF 2-6
XP_027057730.1	LOC113684530	pdam_00000407	TRAF 2-6
XP_027049931.1	LOC113677326	pdam_00023872	TRAF 1
XP_027057639.1	LOC113684441	pdam_00000406	TRAF 2-6
XP_027057631.1	LOC113684441	pdam_00000406	TRAF 2-6
XP_027057622.1	LOC113684441	pdam_00000406	TRAF 1
XP_027037292.1	LOC113665768	pdam_00023581	TRAF 2-6
XP_027051971.1	LOC113679218	pdam_00017873	TRAF 2-6
XP_027058408.1	LOC113685127	pdam_00006317	TRAF 2-6
XP_027059528.1	LOC113686127	pdam_00025575	TRAF 1
XP_027059293.1	LOC113685909	not listed	TRAF 1
XP_027060193.1	LOC113686733	pdam_00004416	TRAF 2-6
XP_027049714.1	LOC113677155	pdam_00019184	TRAF 2-6
XP_027036280.1	LOC113664860	not listed	TRAF 2-6
XP_027055627.1	LOC113682660	pdam_00003859	TRAF 1
XP_027045772.1	LOC113673565	pdam_00018119	TRAF 1
XP_027045081.1	LOC113672934	pdam_00014026	TRAF 2-6
XP_027055610.1	LOC113682647	pdam_00003858	TRAF 2-6
XP_027040060.1	LOC113668371	pdam_00024076	TRAF 2-6
XP_027060102.1	LOC113686654	pdam_00004419	TRAF 2-6
XP_027060205.1	LOC113686741	pdam_00004418	TRAF 1
XP_027039303.1	LOC113677645	pdam_00025153	TRAF 1
XP_027055594.1	LOC113682628	pdam_00003854	TRAF 2-6
XP_027055592.1	LOC113682628	pdam_00003854	TRAF 2-6
XP_027055591.1	LOC113682628	pdam_00003854	TRAF 2-6
XP_027037293.1	LOC113665768	pdam_00023581	TRAF 1
XP_027055595.1	LOC113682628	pdam_00003854	TRAF 1
XP_027041482.1	LOC113669608	not listed	TRAF 2-6
XP_027059277.1	LOC113685897	pdam_00015870	TRAF 1
XP_027039304.1	LOC113667645	pdam_00025153	TRAF 1
XP_027043900.1	LOC113671840	pdam_00001995	TRAF 2-6
XP_027059278.1	LOC113685897	pdam_00015870	TRAF 7
XP_027047238.1	LOC113674939	pdam_00009334	

Figure 3. Neighbor-joining phylogenetic tree of investigated *Pocillopora damicornis* TRAF proteins compared to TRAF proteins identified in *Homo sapiens, Drosophila melanogaster, Hydra vulgaris, Suberites domuncula, Stylophora pistillata, and Acropora digitfera*. Neighbor joining tree computed with 1000 bootstrap replicates to show evolutionary divergence of different TRAF proteins.

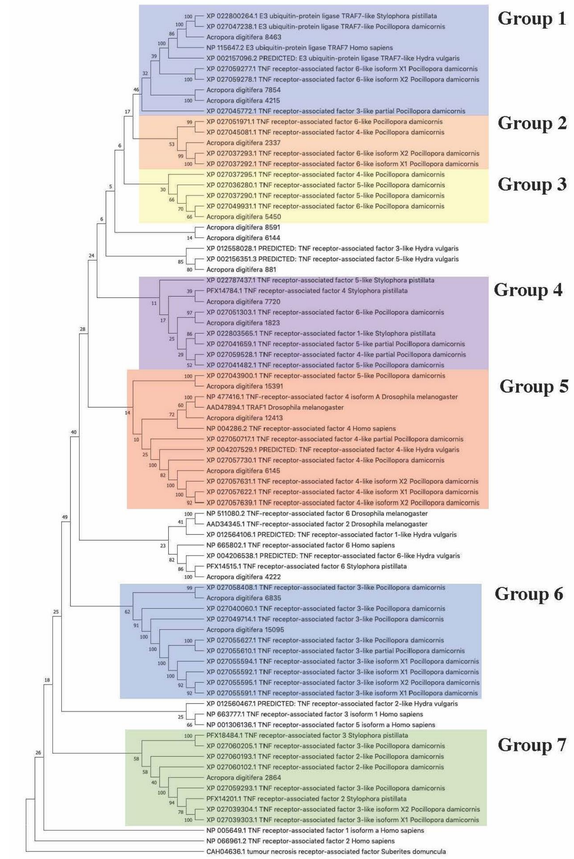


Figure 4. The predicted domains of the *P. damicornis* TRAF proteins groups 1- 4. The thirty-seven TRAF proteins were ran through HMMERSCAN against P.fam database to identify the protein domains and their amino acid coordinates within the protein. P.fam domain hits with cutoff e-03 were used as probable domains. The proteins are organized based on grouping in the phylogenetic tree (Figure 2)

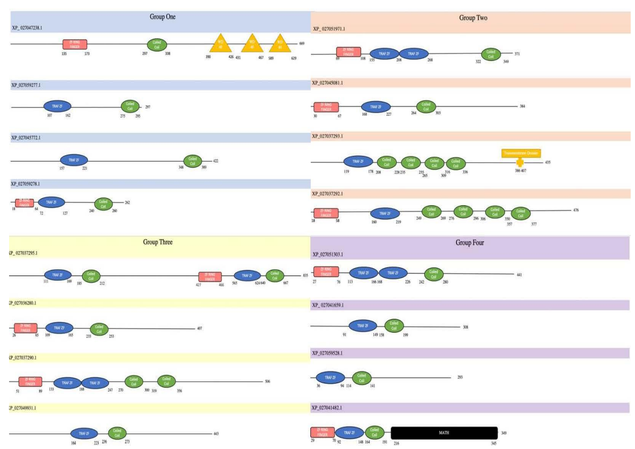
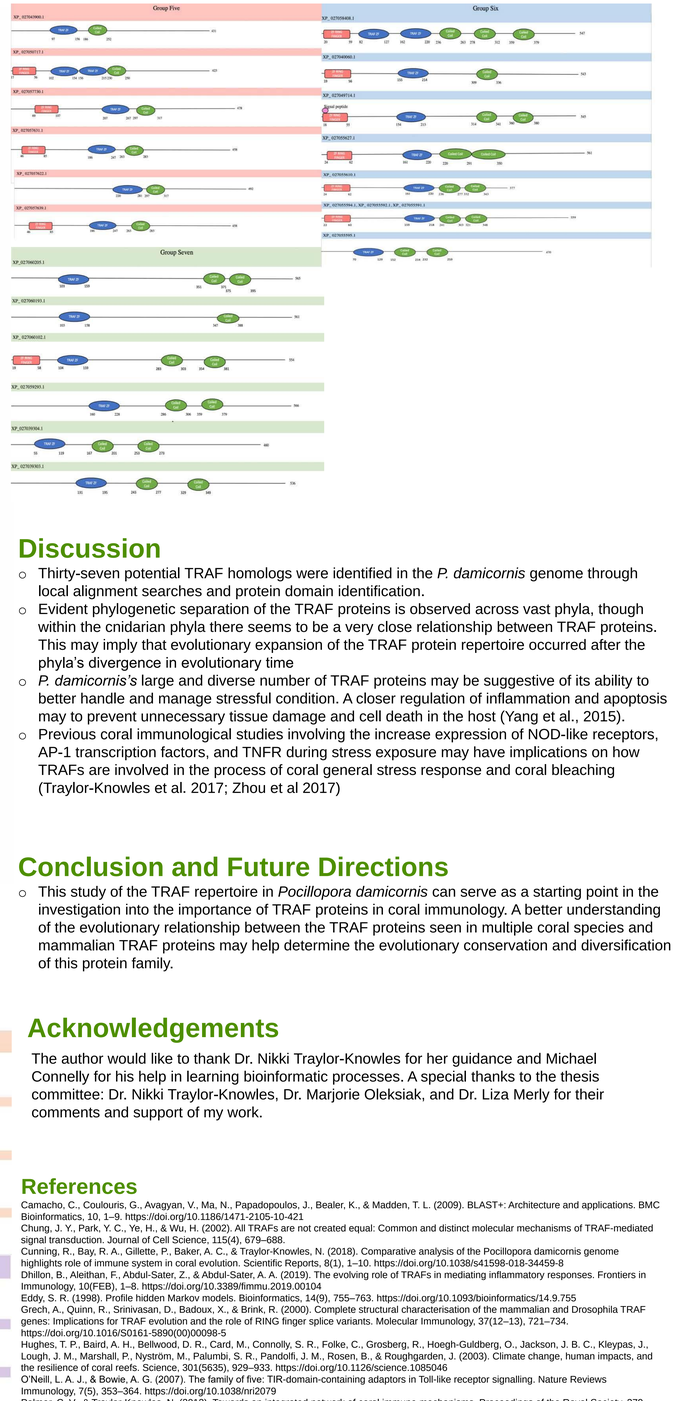


Figure 5. The predicted domains of the *P. damicornis* TRAF proteins groups 5-7
The thirty-seven TRAF proteins were ran through HMMERSCAN against P.fam database to identify the protein domains and their amino acid coordinates within the protein. P.fam domain hits with cutoff e-03 were used as probable domains. The proteins are organized based on grouping in the phylogenetic tree (Figure 2)



Discussion

- Thirty-seven potential TRAF homologs were identified in the *P. damicornis* genome through local alignment searches and protein domain identification.
- Evident phylogenetic separation of the TRAF proteins is observed across vast phyla, though within the cnidarian phyla there seems to be a very close relationship between TRAF proteins. This may imply that evolutionary expansion of the TRAF protein repertoire occurred after the phyla's divergence in evolutionary time
- *P. damicornis's* large and diverse number of TRAF proteins may be suggestive of its ability to better handle and manage stressful condition. A closer regulation of inflammation and apoptosis may to prevent unnecessary tissue damage and cell death in the host (Yang et al., 2015).
- Previous coral immunological studies involving the increase expression of NOD-like receptors, AP-1 transcription factors, and TNFR during stress exposure may have implications on how TRAFs are involved in the process of coral general stress response and coral bleaching (Traylor-Knowles et al. 2017; Zhou et al 2017)

Conclusion and Future Directions

- This study of the TRAF repertoire in *Pocillopora damicornis* can serve as a starting point in the investigation into the importance of TRAF proteins in coral immunology. A better understanding of the evolutionary relationship between the TRAF proteins seen in multiple coral species and mammalian TRAF proteins may help determine the evolutionary conservation and diversification of this protein family.

Acknowledgements

The author would like to thank Dr. Nikki Traylor-Knowles for her guidance and Michael Connelly for his help in learning bioinformatic processes. A special thanks to the thesis committee: Dr. Nikki Traylor-Knowles, Dr. Marjorie Oleksiak, and Dr. Liza Merly for their comments and support of my work.

References

Camacho, C., Coulouris, G., Avagyan, V., Ma, N., Papadopoulos, J., Bealer, K., & Madden, T. L. (2009). BLAST+: Architecture and applications. BMC Bioinformatics, 10, 1–9. https://doi.org/10.1186/1471-2105-10-421
Chung, J. Y., Park, Y. C., Ye, H., & Wu, H. (2002). All TRAFs are not created equal: Common and distinct molecular mechanisms of TRAF-mediated signal transduction. Journal of Cell Science, 115(4), 679–688.
Cunning, R., Bay, R. A., Gillette, P., Baker, A. C., & Traylor-Knowles, N. (2018). Comparative analysis of the Pocillopora damicornis genome highlights role of immune system in coral evolution. Scientific Reports, 8(1), 1–10. https://doi.org/10.1038/s41598-018-34459-8
Dhillon, B., Aleithan, F., Abdul-Sater, Z., & Abdul-Sater, A. A. (2019). The evolving role of TRAFs in mediating inflammatory responses. Frontiers in Immunology, 10(FEB), 1–8. https://doi.org/10.3389/fimmu.2019.00104
Eddy, S. R. (1998). Profile hidden Markov models. Bioinformatics, 14(9), 755–763. https://doi.org/10.1093/bioinformatics/14.9.755
Grech, A., Quinn, R., Srinivasan, D., Badoux, X., & Brink, R. (2000). Complete structural characterisation of the mammalian and Drosophila TRAF genes: Implications for TRAF evolution and the role of RING finger splice variants. Molecular Immunology, 37(12–13), 721–734. https://doi.org/10.1016/S0161-5890(00)00098-5
Hughes, T. P., Baird, A. H., Bellwood, D. R., Card, M., Connolly, S. R., Folke, C., Grosberg, R., Hoegh-Guldberg, O., Jackson, J. B. C., Kleypas, J., Lough, J. M., Marshall, P., Nyström, M., Palumbi, S. R., Pandolfi, J. M., Rosen, B., & Roughgarden, J. (2003). Climate change, human impacts, and the resilience of coral reefs. Science, 301(5635), 929–933. https://doi.org/10.1126/science.1085046
O'Neill, L. A. J., & Bowie, A. G. (2007). The family of five: TIR-domain-containing adaptors in Toll-like receptor signalling. Nature Reviews Immunology, 7(5), 353–364. https://doi.org/10.1038/nri2079
Palmer, C. V., & Traylor-Knowles, N. (2012). Towards an integrated network of coral immune mechanisms. Proceedings of the Royal Society, 279, 4106–4114.
Palmer, C. V. (2018). Immunity and the coral crisis. Communications Biology, 1(1), 1–7. https://doi.org/10.1038/s42003-018-0097-4
Stecher, G., Tamura, K., & Kumar, S. (2020). Molecular Evolutionary Genetics Analysis (MEGA) for macOS. Molecular Biology and Evolution, 1–7.
Traylor-knowles, N., & Connelly, M. T. (2017). What Is Currently Known About the Effects of Climate Change on the Coral Immune Response.
Traylor-Knowles, N., Rose, N. H., & Palumbi, S. R. (2017). The cell specificity of gene expression in the response to heat stress in corals. Journal of Experimental Biology, 220(10), 1837–1845. https://doi.org/10.1242/jeb.155275
Xie, P. (2013). TRAF molecules in cell signaling and in human diseases. Journal of Molecular Signaling, 8(1), 1. https://doi.org/10.1186/1750-2187-8-7
Zhou, Z., Wu, Y., Zhang, C., Li, C., Chen, G., Yu, X., Shi, X., Xu, Y., Wang, L., & Huang, B. (2017). Suppression of NF-κB signal pathway by NLRC3-like protein in stony coral Acropora aculeus under heat stress. Fish and Shellfish Immunology, 67, 322–330. https://doi.org/10.1016/j.fsi.2017.06.027