Mitochondrial Genomes of Cauliflower Corals (Nephtheidae)

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Declaration

DNA sequencing data was collected with the help from Kevin Hopkins at Zoological Society of London. Data processing with help from the supervision of Dr. Chris Yesson who also provided the samples along with Barbara Neves at Department of Fisheries and Oceans, Canada.

Word limit: 2852

Abstract

Taxonomy of Nephtheidae has largely been a challenge with the genomes of few species sequenced while like many octocorals looping and repetitive sequences have provided problems in locating the correct gene order. Long range sequencing using the MinION sequencing device was used to sequence the DNA of *Drifa flavescens*, *Drifa glomerata*, *Drifa groenlandica* and *Duva florida*. The phylogeny of octocorals often uses the gene mtMutS this gene was not found in the species *Drifa flavescens*, *Drifa glomerata*, *Drifa groenlandica* and *Duva florida*. These four species display three novel genomes in which *Drifa groenlandica* and *Duva florida* display the same gene order with direction of transcription.

Introduction

Nephtheidae, a family of cold water corals can be found in aggregations forming what is considered coral gardens that create habitats supporting both benthic and epibenthic fauna. Cold water corals are typically found within deeper regions of the marine environment between 200-1000m (Hebbeln et al. 2019). Due to their location the cold water corals found in deep water locations are vulnerable to physical disturbance by trawling of which can lead to removal and the stirring of sediment (Herrera et al., 2012). Soft corals have been noted as the largest component of bycatch, areas with frequent trawling show a lower abundance (Long et al., 2020). Regions of the marine environment that are biodiversity and ecosystem functioning hotspots but have a high level of vulnerability to disturbance and a low recovery potential may be listed as a Vulnerable Marine Ecosystem (VME) by the FAO (2009) (Ashford et al., 2019).

Figure 1: Coral gardens in the Davis Strait, Greenland containing the Nephtheidae corals. Laser dots are 20cm apart to give an idea of scale (Long et al., 2020).

The soft coral family, Nephtheidae (Gray 1862) are used as a VME indicator taxa and are found in high densities in the Davis Strait, Greenland identified as a VME in a study by Long et al., (2020). This area is largely dominated by species within the genera *Gersemia*, *Duva*

and *Drifa* forming cauliflower coral gardens of which is additionally seen in Iceland (Buhl-Mortensen et al. 2019) and Canada (Devine et al. 2019).

Nephtheidae is comprised of 20 genera and around 500 currently described species (Hu et al. 2011). The taxonomy of Nephtheidae corals are shown to be challenging due to lack of morphological features. *Gersemia rubiformis* (Ehrenberg 1834) was originally placed in Nephtheidae but has been shown to display morphologically more similar to Alcyoniidae rather than Nephtheidae (Williams, 2013; Mcfadden and Hochberg 2003). The placement of *Gersemia rubiformis* within Alcyoniidae has been further suggested using the mtMutS and nad2 genes (McFadden et al. 2006). DNA barcoding will allow for molecular phylogenetics and further understand the evolutionary relationships between members of Nephtheidae. Preliminary studies using the mitochondrial genomes (Murphy 2014; Ayre 2016) suggested a missing mtMutS gene region in *Drifa flavescens, Drifa glomerata, Drifa groenlandica* and *Duva florida.* The mtMutS octocoral DNA barcode region is the most widely sequenced region for comparing Octocorals as is novel to this taxon (van der Ham et al. 2009). In contrast to the 'universal barcode' cox1 to show the levels of variation within the taxa. The mtMutS gene codes for a functional homolog of a non-eukaryotic mismatch repair protein of which was related to mtMutS (Brockman and McFadden 2012). Compared to other Anthrozoans, octocorals have slower rates of mitochondrial gene evolution of which is theorised to be explained by the mtMutS gene (Bilewitch and Degnan 2011). Due to this slow rate of evolution there is a lack of molecular markers available within Nephtheidae (McFadden et al. 2010).

Whole genome sequencing (WGS) allows for analysis of more sequences, therefore enhancing the current understanding of evolution. WGS also gives us many more regions that we can compare with other taxa that might not share the same genes or gene order. DNA sequences are often fragmented before sequencing, and fragments are built on each other based on similarity, this is more reliable with longer sequences as there is a reduced chance of an incorrect match. Short range sequencing can be problematic as only sequencing a few bases at a time (150-300bp) can cause issues where a sequence contains many repeat regions. Long-range sequencing is an alternative approach to the short reads as allows reads of up to a few hundred base pairs (Lu et al. 2016) to enable us to extract the full mitochondrial genome to confirm the novel gene orders to avoid the issues of looping and repetitive regions (Tyler et al. 2018), due to the reduced risk of mismatch of sequences (Goldstein et al. 2019). Mitochondrial genomes of Octocorallia in a paper by Figuroa and Baco (2015) were suggested to have variable gene orders therefore providing difficulty with assembly from short reads and comparisons between taxa would be tricky. Five species of Nephtheidae have had their mitochondrial genome extracted (Park et al., 2012; Park et al., 2010; Kwak et al., 2015) limiting molecular phylogenetics or revisions.

Preliminary studies using short read sequencing were conducted by Dr Chris Yesson along with Kevin Hopkins using *Drifa flavescens* (Molander 1915), *Drifa glomerata* (Verrill 1869), *Drifa groenlandica* (Molander 1915) and *Duva florida* (Rathke 1806). In the preliminary study displayed looping and repetitive regions within their DNA therefore the complete genome could not be extracted. The preliminary study suggested these species lacked the mtMutS gene. The fragmented assemblies recovered indicated novel gene orders that were not displayed in *Dendronephthya gigantea* (Park et al. 2010). D. gigantea shares the same gene order as *Dendronephthya* and *Scleronephthya*, the ancestral octocoral mitochondrial

gene order (Figueroa and Baco 2014). *Paraminabea aldersladei* displays a novel gene order but does contain the mtMutS coding region although displays a mtMutS-like protein of 3kb in length (Brockman and McFadden 2012). Although due to the nature of short read sequencing this could be a result of incorrect combining of the strands of DNA of which could be avoided with long range sequencing.

The projects aim is to provide the mitochondrial gene order for *Drifa flavescens*, *Drifa glomerata*, *Drifa groenlandica* and *Duva florida* using specimens collected from the Davis Strait and Baffin Bay in the Labrador Sea, between Greenland and Canada. Using long range sequencing to obtain the mitochondrial genome allowing for phylogenetic analyses.

Methods

Samples were collected from the Davis Strait within the Labrador Sea. Samples were collected as bycatch during routine stock assessment trawls around Greenland with the vessel Paamiut and species was confirmed using morphological features.

Sample	Species	Year	Location
Gr24_S12	***Drifa flavescens***	**2011**	**Greenland**
Gr166_S20	*Drifa flavescens*	2012	Greenland
ST-572-1_S10	*Drifa flavescens*	2016	Iceland
ST-129-1_S11	***Drifa glomerata***	**2017**	**Nares Strait**
AB-2015-SET-019_S1	*Drifa glomerata*	2015	Arctic Bay
Gr147_S18	*Drifa glomerata*	2012	Greenland
Gr171_S21	*Drifa groenlandica*	2012	Greenland
Gr103_S16	*Drifa groenlandica*	2010	Greenland
Gr161_S19	***Drifa groenlandica***	**2012**	**Greenland**
Gr58_S14	*Duva florida*	2009	Greenland
All-ST-572-2_S4	***Duva florida***	**2016**	**Iceland**

Table 1: Samples and species sampled including the year and location of collection. Those selected for the gene order to be mapped were highlighted in bold.

The Genomic DNA of samples (Table 1) was extracted from three samples, each from the species; *Drifa flavescens*, *Drifa glomerata* and *Drifa groenlandica* and two specimens from *Duva florida*; using the Bioline© ISOLATE II Genomic DNA Kit and following the manufactures instructions.

Samples were prepared for sequencing using the Ligation Sequencing Kit (Oxford Nanopore Technologies©) to prepare the libraries and Native Barcoding Expansion 1-12(Oxford Nanopore Technologies©) to index the libraries. The quantity of DNA extracted was frequently assessed through the production of the DNA sequence libraries using a Qubit 2.0 Fluorometer. The samples were sequenced using the using a MK 1 R9 Spot on Flow Cell (Oxford Nanopore Technologies©) on a MinION MK1B sequencing device. Samples were processed through MinKnow (Oxford Nanopore Technologies©) for slow base calling.

Species	Accession code
Dendronephthya castanea (Utinomi, 1952)	GU047877 (Park et al. 2012)
Dendronephthya gigantea	NC_013573 (Park et al. 2010)
Dendronephthya mollis (Holm, 1895)	HQ694725 (Park et al. 2012)
Dendronephthya puetteri (Kukenthal, 1905)	JQ886185 (Kwak et al. 2015)
Dendronephthya suensoni (Holm, 1895)	JQ290079 (Kwak et al. 2015)
Dendronephthya suensoni (Holm, 1895)	GU047878 (Park et al. 2012)
Eugorgia mutabilis (Williams & Guzman, 2013)	KY559405 (Poliseno et al. 2017)
Euplexaura crassa (Kukenthal, 1908)	HQ694728 (Park et al. 2012)
Junceella fragilis (Ridley, 1884)	KJ541509 (Wu et al. 2014)
Leptogorgia alba (Duchassaing &Michelotti, 1864)	KY559406 (Poliseno et al. 2017)
Leptogorgia sarmentosa (Esper, 1789)	KY559411 (Poliseno et al. 2017)
Protoptilum carpenterii (Kolliker, 1872)	NC_044089 (Hogan et al. 2019)
Sinularia ceramensis (Verseveldt, 1977)	NC_044122 (Chen et al, 2019)

Table 2: Octocoral genomes and their accession codes used to provide reference genes.

The DNA sequences were processed using Geneious© (Kearse et al., 2012) first by mapping the long reads to a preliminary sequence (Murphy 2014; Ayre 2016) for each species.

One sample for each species that showed the largest mean coverage across the preliminary sequences were selected (Table 1). The long sequences were mapped to the seed sequences previously produced in the preliminary experiments (Murphy 2014; Ayre 2016) suggested for each species, before the lengthened sequences were mapped to the short sequences to 'polish' the sequences on Geneious© (Kearse et al., 2012). This was completed until extension of the genome was no longer possible (Lannoy et al., 2017). Genes were located by applying annotations using already known genomes of octocorals (Table 2) based on similar sequences, this meant the gene order could be seen.

The assembled mitochondrial sequences were processed through the DNA Walker setting within the programme, GraphDNA (Thomas et al. 2007) to locate regions where there had been changes in the AT/CG composition, therefore.

A phylogenetic tree was produced using the assembled mitochondrial sequences and four reference samples from Nephtheidae, *Dendronephthya castanea*, *Dendronephthya mollis*, *Dendronephthya putteri* and *Dendronephthya suensoni* (Table 2) using MegaX (Molecular Evolutionary Genetics Analysis) (Kumar et al. 2018). *Sinularia ceramensis* was included in the phylogenetic assessment as the outgroup as a member of the family Alcyoniidae. The DNA sequences used from the species were from the genes cox1 to cob as consistently arranged in the same order throughout the mitogenomes therefore the simplest dataset alignment. Sequences were then aligned using the ClustalW (Higgins et al. 1994) system. The sequence lengths used was 2751 base pairs. The Maximum parsimony method was used to produce a phylogenetic tree, the bootstrap test to 500 replicates was used to look at the support for the nodes and the tree was obtained using the Subtree-Pruning-Regrafting

algorithm. The average pathway method was used to calculate the branch lengths (Nei and Kumar 2000).

Results

15 complete genes were found from the mitochondrial genomes of the four Nephtheidae species (Table 2) was extracted from the samples that were mapped.

Species	Sample	Length (bp)	Mean Coverage	Minimum Coverage
Drifa flavescens	Gr24_S12	26,648	86.6	10
Drifa glomerata	ST-129-1_S11	17,847	24.9	4
Drifa groenlandica	Gr161_S19	21,055	51.4	17
Duva florida	8773-1_S8	20,698	15.1	1

Table 3: The length in base pairs and mean coverage of samples Gr24_S12 (*Drifa flavescens*), ST-129_S11 (*Drifa glomerata*), Gr161_S19 (*Drifa groenlandica*) and 8773-1_S8 (*Duva florida*).

There is shown to be a range of lengths to obtain the full gene order and the coverage varied between species, *Drifa glomerata* displayed the largest coverage although displayed the shortest sequence length (Table 3). *Drifa flavescens* is shown to be the longest sequence although the mean coverage was not high (Table 3).

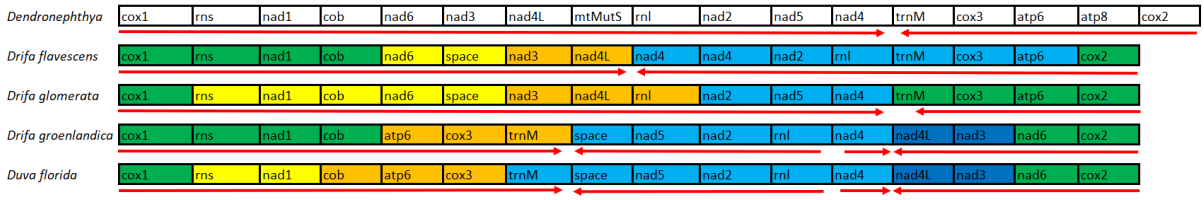

Figure 2: The order of genes within the mitochondrial genomes of *Drifa flavescens*, *Drifa glomerata*, *Drifa groenlandica*, *Duva florida* and *Dendronephthya*. Colours coordinate to region where there has been a change in AT/GC composition (Fig 3). Arrows indicate the direction of transcription. Space referring to where there was a gap in the gene order. The *Dendronephthya* gene order was obtained from Park et al. (2012).

The four sequences contained the same genes (Fig 2). Three different gene orders were produced. *Drifa groenlandica* and *Duva florida* share the same gene order. *Drifa flavescens* and *Drifa glomerata* show a reversed gene order between cob and cox2 in comparison to *Drifa groenlandica* and *Duva florida*. *Drifa flavescens* displays a reversed gene order between nad4L and trnM compared to *Drifa glomerata* (Fig 2). Neither *Duva florida*, *Drifa flavescens*, *Drifa glomerata* and *Drifa groenlandica* display the same gene order as *Dendronephthya* (Fig 2).

The direction of transcription is shown to be the same for *Drifa groenlandica* and *Duva florida*, containing four different directions of transcription. There is a change in direction of transcription between trnM and the gap in the DNA sequence, between rns and nad4, between nad4 and nad4L and finally, a change in direction of transcription between cox1 and cox2 (Fig 2).

Drifa flavescens and *Drifa glomerata* both display two directions of transcription between genes. There is a change in direction between transcribing away towards nad4L and nad4 in *Drifa flavescens* and; both transcribing towards nad4 and trnM in *Drifa glomerata* (Fig 2).

mtMutS is not shown to be expressed in either of the four species, in addition there is suggested to be a lack of the atp8 gene, a mitochondrial gene that encodes for ATP synthase (Fig 2). The possible location of the missing mtMutS region in *Drifa groenlandica* and *Duva florida* between trnM and nad5 in which the space in the DNA sequence (Fig 2) is around 1,123 base pairs long with an open read frame within of 891-591. In *Drifa flavescens* and *Drifa glomerata* there is a space in the DNA between nad6 to nad3 with two possible open read frames at around 1,021bp in *Drifa glomerata* and 2,711bp in *Drifa flavescens* with a possible open read frame.

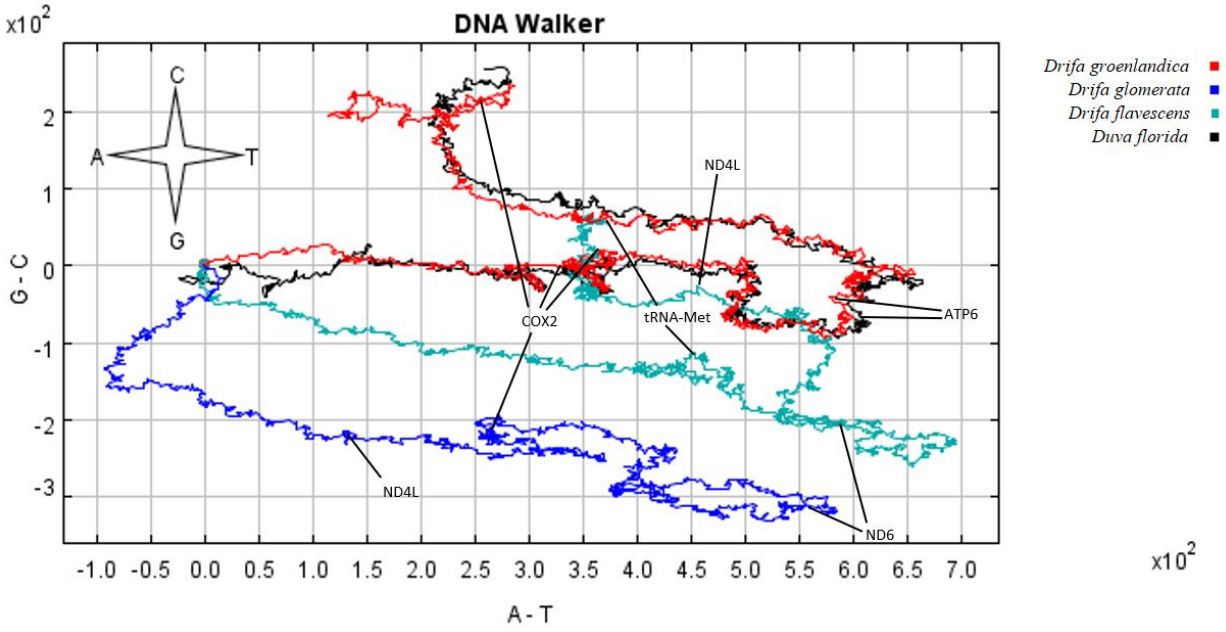

Figure 3: The A-T in comparison to G-C composition of mitochondrial DNA the Neptheidae species *Drifa groenlandica*, *Drifa glomerata*, *Drifa flavescens* and *Drifa florida* using the setting DNAWalker on the programme Graph DNA (Thomas et al. 2007).

Drifa groenlandica and *Duva florida* display the same gene order, direction of transcription (Fig 2) and the GC/AT composition is shown to be very similar throughout the genome sequence (Fig3). *Drifa glomerata* and *Drifa flavescens* displays the same gene order from cox1 to cob where it changes until trnM to cox2 (Fig 2), the GC/AT composition between the two species is vastly different throughout the DNA sequence (Fig 3).

Regions of the DNA sequence where the AT/GC composition changes is displayed where the cox2 gene ends, or before the beginning of atp6 in *Drifa glomerata* and *Drifa flavescens* and; nad6 *Drifa groenlandica* and *Duva florida* (Fig 3) these regions are where there is a reversal in gene order between the pairs of species (Fig 2). The GC/AT composition around cox2 is different between the four species. atp6 in *Drifa groenlandica* and *Duva florida* are close in composition and are located close to areas where there is a change in composition.

nad4L and trnM in *Drifa flavescens* and *Drifa glomerata* is shown to have a very different GC/AT composition (Fig3) between these two genes is where there is a reversal in the gene order between the two species (Fig 2).

Changes in direction of transcription does occur in some regions where there is a large change in CG/AT composition (Fig 3) such as between nad4 and nad4L and; between trnM and a gap in the gene order in *Drifa groenlandica* and *Duva florida*. This is also shown nad4 and nad4L in *Drifa flavescens* and between trnM and nad4 in *Drifa glomerata* (Fig 2).

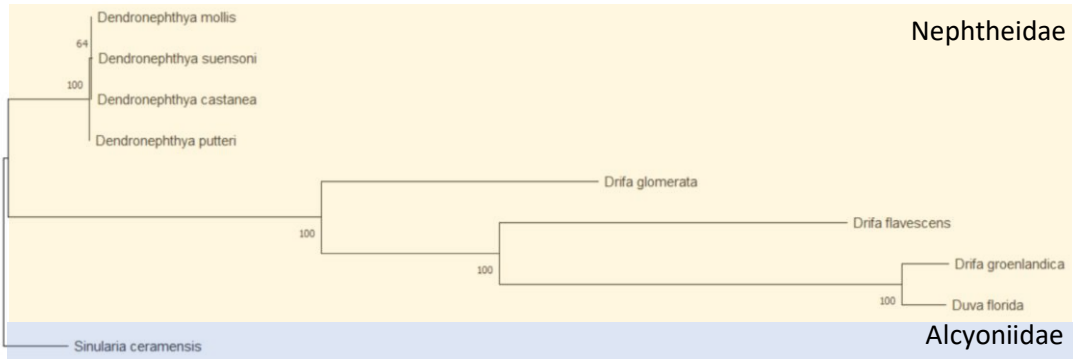

Figure 4: Phylogenetic tree using the Maximum Parsimony method, using bootstrapping at 500 replicates *shown at each node* and the subtree-pruning-self grafting method (Nei and Kumar, 2000). *Sinularia ceramensis* was selected as the outgroup as a member of the family Alcyoniidae. The phylogenetic tree was produced by MegaX (Molecular Evolutionary Genetics Analysis) (Kumar et al. 2018).

The phylogenetic tree shows a high support for the topography given, with the exclusion of the node for *Dendronephthya mollis* and *Dendronephthya suesoni. Duva florida* and *Drifa groenlandica* are suggested to be sister taxa (Fig 4) that correlates with the same gene order and order of transcription shown in Figure 2. *Drifa* is shown to be paraphyletic due to the nesting of *Duva florida* within *Drifa*. *Dendronephthya* is shown to be monophyletic in the results, and a separate branch with high support to the other taxa of Nephtheidae. The branch lengths between the species of *Dendronephthya* is suggested to be relatively short in comparison to *Drifa* and *Duva florida* (Fig 4).

Discussion

Using the long range sequencing we were able to obtain all the known genes from the genomes of *Drifa flavescens*, *Drifa glomerata*, *Drifa groenlandica* and *Duva florida* excluding mtMutS and atp8 (Fig 2) this would have been expected from the preliminary studies (Murphy 2014; Ayre 2016). There are large gaps listed as space on Figure 2 in the gene order in all four species although between nad6 and nad3 in *Drifa glomerata* and *Drifa flavescens* while between *Drifa groenlandica* and *Duva florida* the space is shown between trnM and nad5, seeing as the space in the DNA sequence although does occur with an open read frame that does not match with any sequences on the NCBI Database. This area does not correlate with mtMutS in *Dendronephthya* (Fig 2), order of transcription (Fig 2) or a change in GC/AT composition (Fig 2/3) so it is unlikely mtMutS is present there.

Duva florida and *Drifa groenlandica* are shown to be most closely related in both the gene order, transcription directions and phylogenetics (Fig 2; Fig 4). *Drifa flavescens* has been synonymised with *Drifa glomerata* according to the World Register of Marine Species (2020), due to the difference in gene order (Fig 2) suggests otherwise, therefore proposing the resurrection of *Drifa flavescens* that is also supported by morphological data (Jensen, 2003)This suggests that *Drifa* is paraphyletic containing *Duva florida* within although other species within the genus Duva have not been assessed. This is reflected in the gene order in which *Duva florida* and *Drifa groenlandica* have the same gene order and direction of transcription (Fig 2). Due to the similarity of *Duva florida* and *Drifa groenlandica* (Fig 2) it could suggest the possibility of a revision of the taxonomy of both species, with the use of multiple species from either genus.

Drifa flavescens, *Drifa glomerata*, *Drifa groenlandica* and *Duva florida* show a novel gene order (Fig 2) with three different gene orders that are not shown in other taxa elsewhere in the literature (Figueroa and Baco 2015; Park et al. 2010; Figueroa et al. 2015). *Drifa glomerata* is shown to be closest to the ancestral gene order while *Drifa glomerata* and *Drifa flavescens* display the ancestral order of transcription (Figueroa et al. 2015). The novel gene orders within *Duva florida*, *Drifa flavescens*, *Drifa glomerata* and *Drifa groenlandica* could improve the knowledge of the evolution of *Nephtheidae* and infer a basal species. The long branch lengths for the branch containing *Drifa groenlandica* and *Duva florida* and; the branches containing *Drifa glomerata* and *Drifa flavescens* (Fig 4) suggests that there is a higher level of genetic variability from each other and *Dendronephthya*. The short branch lengths between *Drifa groenlandica* and *Duva florida* (Fig 4) suggests lower genetic variability between the two species (Herrera et al. 2012). If more species of both *Duva* and *Drifa* were sequenced, then a more detailed phylogenetic tree can be built and increase the reliability of the phylogeny. Using nuclear genomes might help improve clarity of the phylogeny and could comparison to the mitochondrial genomes would allow for increased understanding and reliability.

By studying the four species of Nephtheidae it has allowed for further knowledge of the gene orders within the mitochondria of the family along with providing hints to their evolution and where changes possibly occurred. The mitochondrial genomes of octocorals have provided a challenge due to their repetitive regions and looping (Hogan et al. 2019) but using long range sequencing might have helped reduce these challenges.

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