

Title: Analysis of mitogenomes from the family Keratoisididae reveals mitonuclear discordance and the presence of unknown open reading frames

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Abstract

Historically, evolutionary studies have not been able to resolve relationships within Octocorallia but recent advances in genomic techniques have allowed large numbers of Ultra Conserved Elements and exons, collectively referred to as conserved elements, to be extracted from the nuclear genome. Few studies have compared evolutionary histories generated with these conserved elements and with those inferred from complete mitogenomes. We examined the characteristics of 26 complete mitogenomes and constructed both mitochondrial and nuclear (1418 conserved elements) phylogenies of the family Keratoisididae, a prolific group of exclusively deep-sea corals. Relationships among most keratoisidid groups were well supported by the conserved element phylogeny while many were unresolved from mitogenomic inferences. In addition, some incongruences were observed. The position of keratoisidid groups F1 and I4, and the observed polyphyly of D2 in the nuclear inference phylogeny, provide evidence for mitonuclear discordance within the group. Multiple novel Open Reading Frames (ORFs) of unknown function were found in the mitogenome between *cob* and *nad6*. These ORFs ranged in size from 84 – 537 bp and were present in all examined mitogenomes except those assigned to keratoisidid group A1 which only has a 10 bp gap between coding regions. Further investigation is required to determine whether these ORFs are expressed and, if so, what function they have. The presence of mitonuclear discordance within the family indicates that phylogenies constructed with concatenated mitochondrial and nuclear markers may be inappropriate.

Keywords: Mitonuclear discordance, comparative phylogenomics, Keratoisididae, Open Reading Frames, mitogenomics, Ultraconserved elements

Supplementary File 1. Code for trimming, assembling contigs and blastn.

Trimming of illumina raw reads

```
runtrimomatic PE INPUT_R1 INPUT_R2 OUTPUT_PE_R1 OUTPUT_UNPAIRED_R1 OUTPUT_PE_R2  
OUTPUT_UNPAIRED_R2 ILLUMINACLIP:TruSeq3-PE-2.fa:2:30:10:8:TRUE LEADING:3 TRAILING:3  
HEADCROP:5 SLIDINGWINDOW:5:15 MINLEN:30;
```

Spades assembly of contigs

```
spades.py -o output -1 INPUT_PE_R1 -2 INPUT_PE_R2 -s INPUT_SE_UNPAIRED --careful --cov-  
cutoff 2;
```

Blastn database creation and query

```
makeblastdb -in BAMBOO.contigs.fasta -out BAMBOO -dbtype 'nucl'
```

```
blastn -task blastn -query ON971064.fasta -db BAMBOO -evalue 1e-20 -num_threads 4 -out  
blastn.txt
```