Evaluation of a Novel Nuclear Intron within the Deep-Sea Black Coral *Stauropathes arctica* (Cnidaria: Anthozoa: Hexacorallia: Antipatharia)

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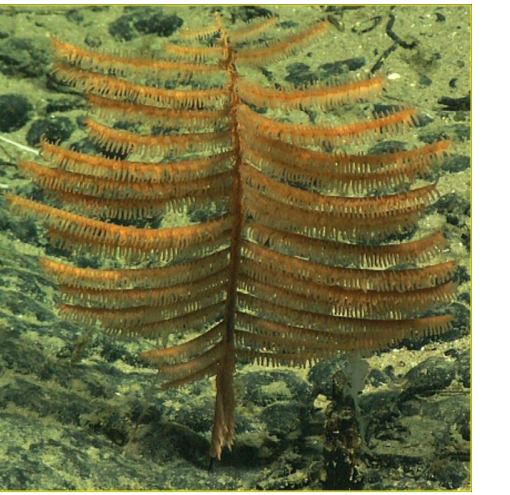
ABSTRACT

Black corals (antipatharians) are cosmopolitan in the world's oceans and occur between 4 and 8,600 meters depth. However, it wasn't until 2005 that the first black coral, *Stauropathes arctica*, was discovered among deep-sea fauna off eastern Canada. To elucidate there are additional unrecorded and undescribed species of black coral in both Canadian and bordering international waters, we screened 40 individuals of *S. arctica* (identification based on morphology) for cryptic species using mitochondrial DNA and nuclear ITS2. Three mitochondrial intergenic regions were analyzed, one of which (*nad5*-IGR-*nad1*) revealed five unique haplotypes. Nuclear ITS2 was unable to partition specimens into as many haplotype groups, and failed to reveal any additional cryptic species. To further examine genetic diversity within each of the unique haplotype groups, we sequenced a 532 bp segment of Signal Recognition Particle 54-kDa Subunit (SRP54) that contained a 384 bp intron. PCR products were cloned to determine the number of copies of SRP54 within each individual.

INTRODUCTION

- Screened 40 individuals of *S. arctica*
- The genus *Stauropathes* includes 3 species:
  - ⇨ *S. artica* (Arctic)
  - ⇨ *S. punctata* (eastern Atlantic)
  - ⇨ *S. staurocrada* (north central Pacific)

Fig. 2. *S. punctata* (Roule, 1905).

- *S. punctata* and *S. staurocrada* share identical haplotypes across 707 base pairs (bp) of mitochondrial *cox3*-IGR-*cox1*
- The inability to differentiate congeners from different oceans based on *cox3*-IGR-*cox1* led us to hypothesize that we would also be unable to differentiate *S. arctica*.
- Compared to other black coral genera , *S. arctica* has the lowest radial growth rates, which ranges from 33 ± 11 to 75 ± 11 μm/year (Sherwood et. al. 2008).
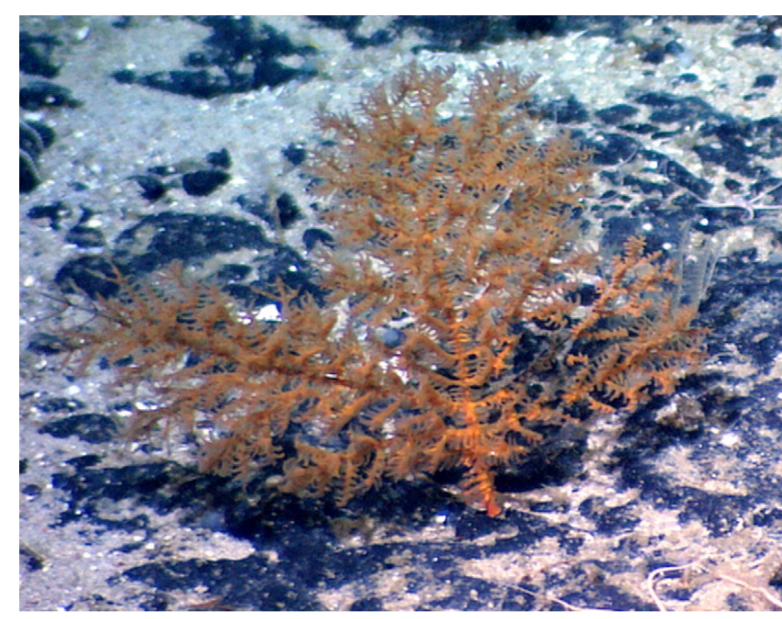- Type species of *S.arctica* was found in the stomach of a shark.

Gene Region	Length (Base Pairs)
cox3-IGR-cox1	888
nad5-IGR-nad1	438
trnW-IGR-nad2	597
SRP54	532 (intron=384)
ITS2	582

ASSUMPTIONS

- Black Corals have low levels of mitochondrial sequence evolution.
  - ⇨ 50-100 times slower than other multicellular animals
  - ⇨ 1 substitution between two individuals: a putative new species
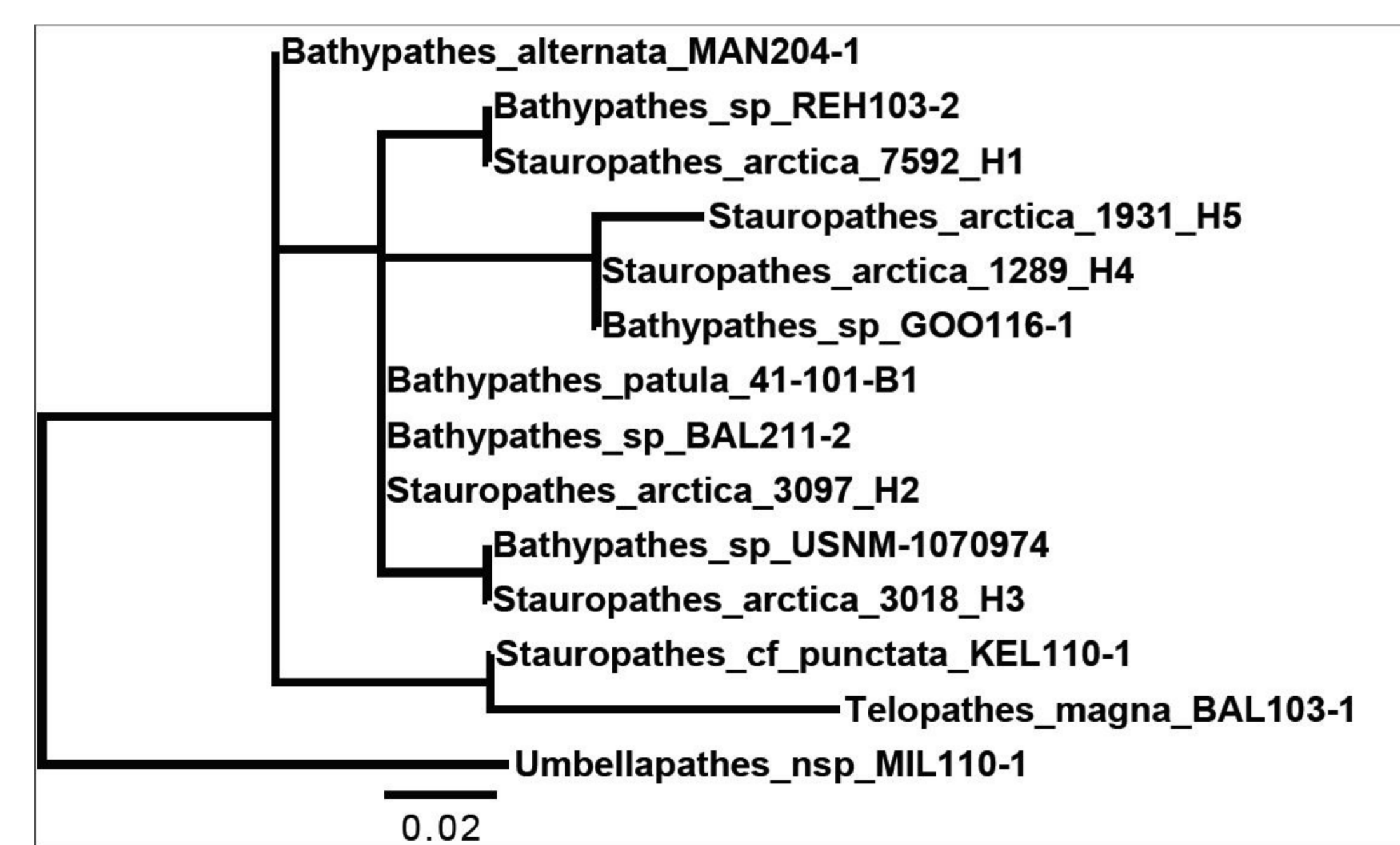
Fig. 7. Maximum likelihood-based phylogenetic tree built using the *nad5*-IGR-*nad1* mt gene region. The five *S. arctica* haplotypes do not form a monophyletic clade but rather are distributed among different species of *Bathypathes*. Within each clade containing an *S. arctica* haplotype, we find a species of *Bathypathes* sharing identical sequence. None of the five *S. arctica* haplotypes group with *S. cf punctata*. In fact, the latter groups with a different genus, i.e. *Telopathes magna*. Outgroup: *Umbellapathes n.sp.*

ONGOING WORK

To explore potential connections across the North Atlantic Ocean, we recently collected 20 additional *S. arctica* colonies from the continental shelf and seamounts west of Scotland (NE Atlantic).

FUTURE WORK

We have located an intron within Vacuolar ATP Synthase Subunit B (VATPSβ). Preliminary sequencing resulted in multi-copy sequence traces. Currently cloning PCR products to separate different sequence types. Restriction site Associated DNA Sequencing (RAD-seq; Floragenex).

Fig. 1. *Stauropathes arctica* (Lutken, 1871). Photo courtesy of the WoRMS World Register of Marine Species.
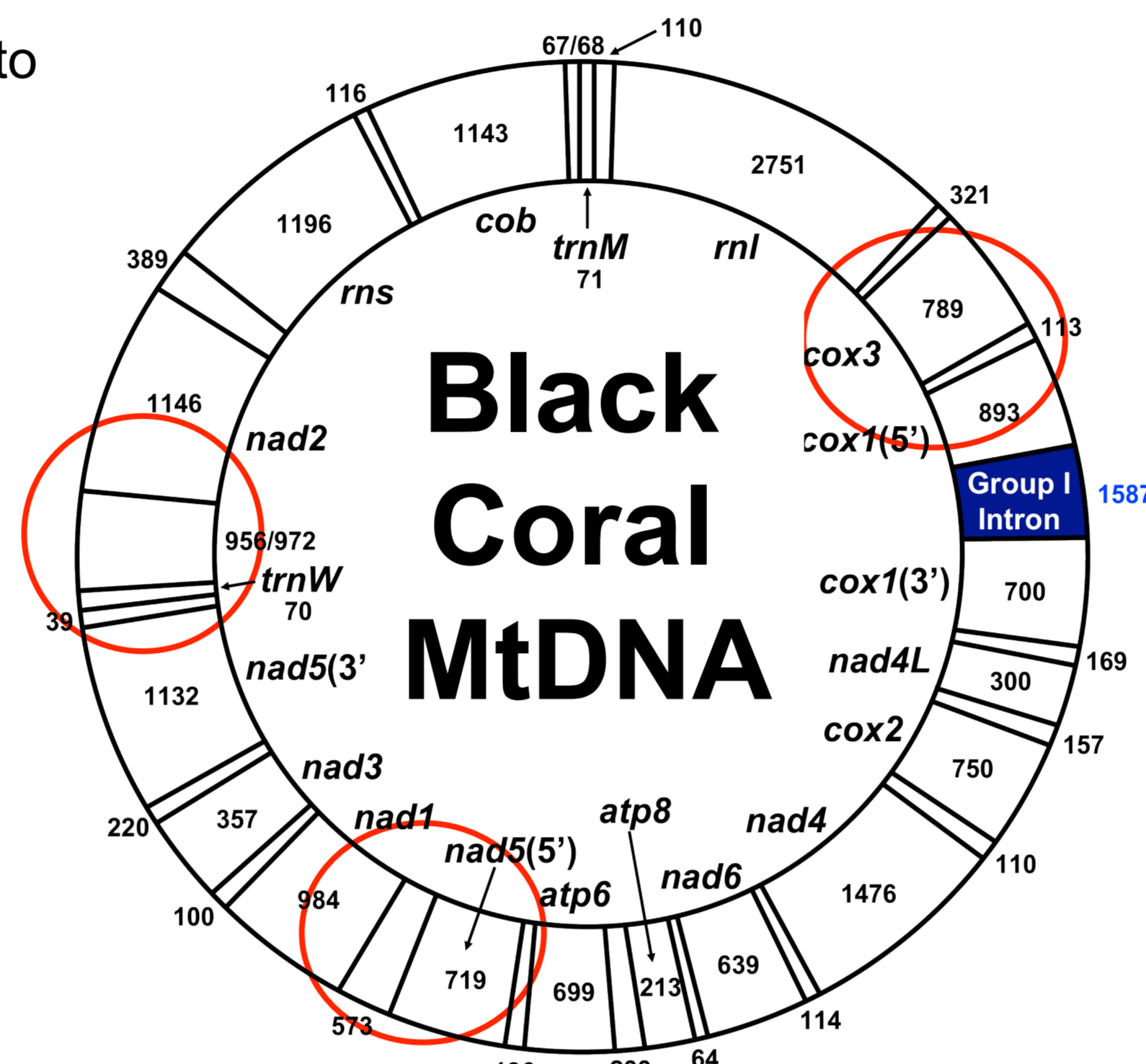

Fig. 4. A black coral mitogenome. Targeted gene regions are circled in red.

Fig 5. Geographical distribution of *S. arctica* specimens analyzed in this study. The two most distant sampling sites are separated by 2981 km (1852 miles). Haplotypes do not appear to be partitioned based on geography (Haplotypes not shown).
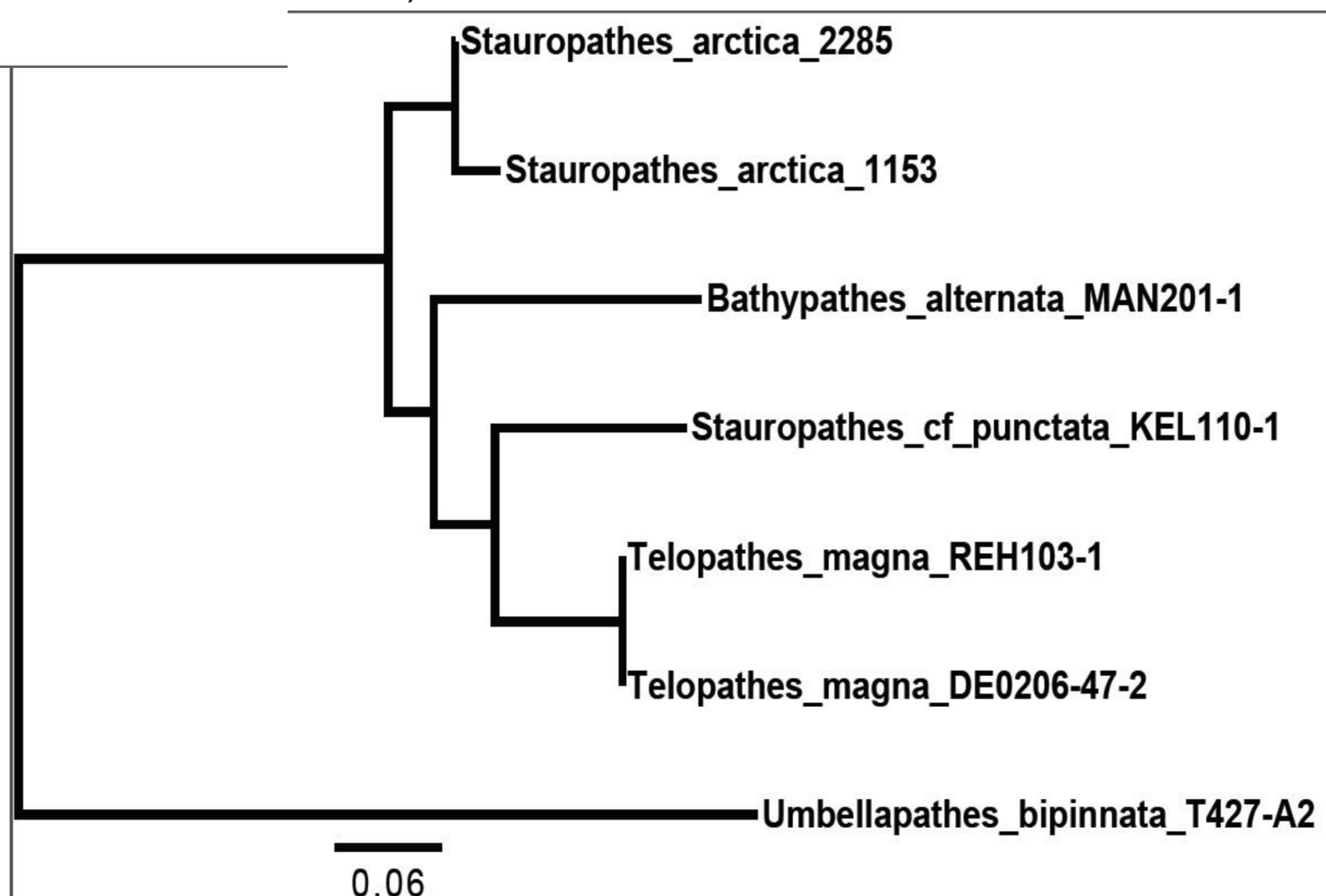

Fig. 8. ML-based phylogenetic tree built using nuclear ITS2. Due to reduced taxonomic sampling, the relationship between *S. arctica* and *Bathypathes* is unclear. The two *S. arctica* sequences form a monophyletic clade and *S. cf punctata* continues to group with *Telopathes magna*. Similar to the mitochondrial phylogenies, *B. alternata* does not group with *S. arctica*.

METHODS

- Samples obtained from Canadian Department of Fisheries and Oceans
- Extracted whole genomic DNA
- Amplified three mitochondrial regions:
  - *cox3*-IGR-*cox1*, *nad5*-IGR-*nad1*, *trnW*-IGR-*nad2*
- Amplified nuclear 5.8S-ITS2-28S
- Using non-standard PCR profiling conditions we PCR amplified novel nuclear introns in *SRP54* and *VATPS-β*.
- Sequences were aligned using jModelTest v2.1.1 and phylogenetic trees were constructed using PhyML v3.0.

Fig. 3. *Bathypathes* (Book, 1889).

RESULTS

Gene Region	Number of Haplotypes/ Unique Sequences	Number of Colonies Examined
nad5-IGR-nad1	5	40
trnW-IGR-nad2*	1	14
cox3-IGR-cox1	1	38
ITS2	2	23
SRP54	2	3

*A minimum of three representatives from each nad5-IGR-nad1 haplotype were sequenced at trnW-IGR-nad2

- *trnW*-IGR-*nad2* and *cox3*-IGR-*cox1* only revealed a single haplotype, while nad5-IGR-nad1 revealed five haplotypes.

- Using mtDNA, we found that not only can *S. arctica* be differentiated from congeners, it is more closely related to a different genus within the family.

- *nad5*-IGR-*nad1* sequence data from a specimen of *S. arctica* (USNM 1167100: collected from the Donegal Shelf [NE Atlantic] at 1500 meters depth) grouped into haplotype 1.
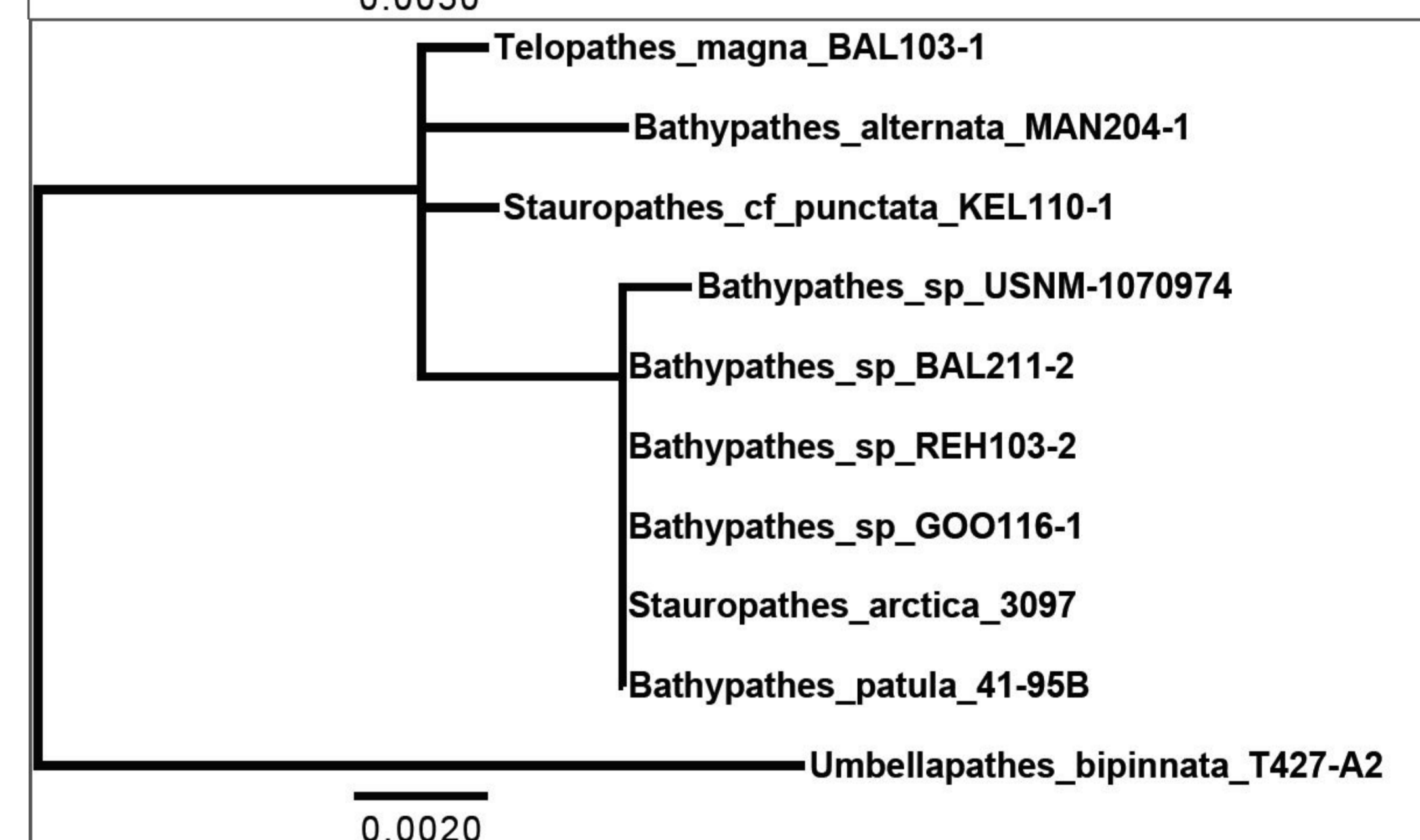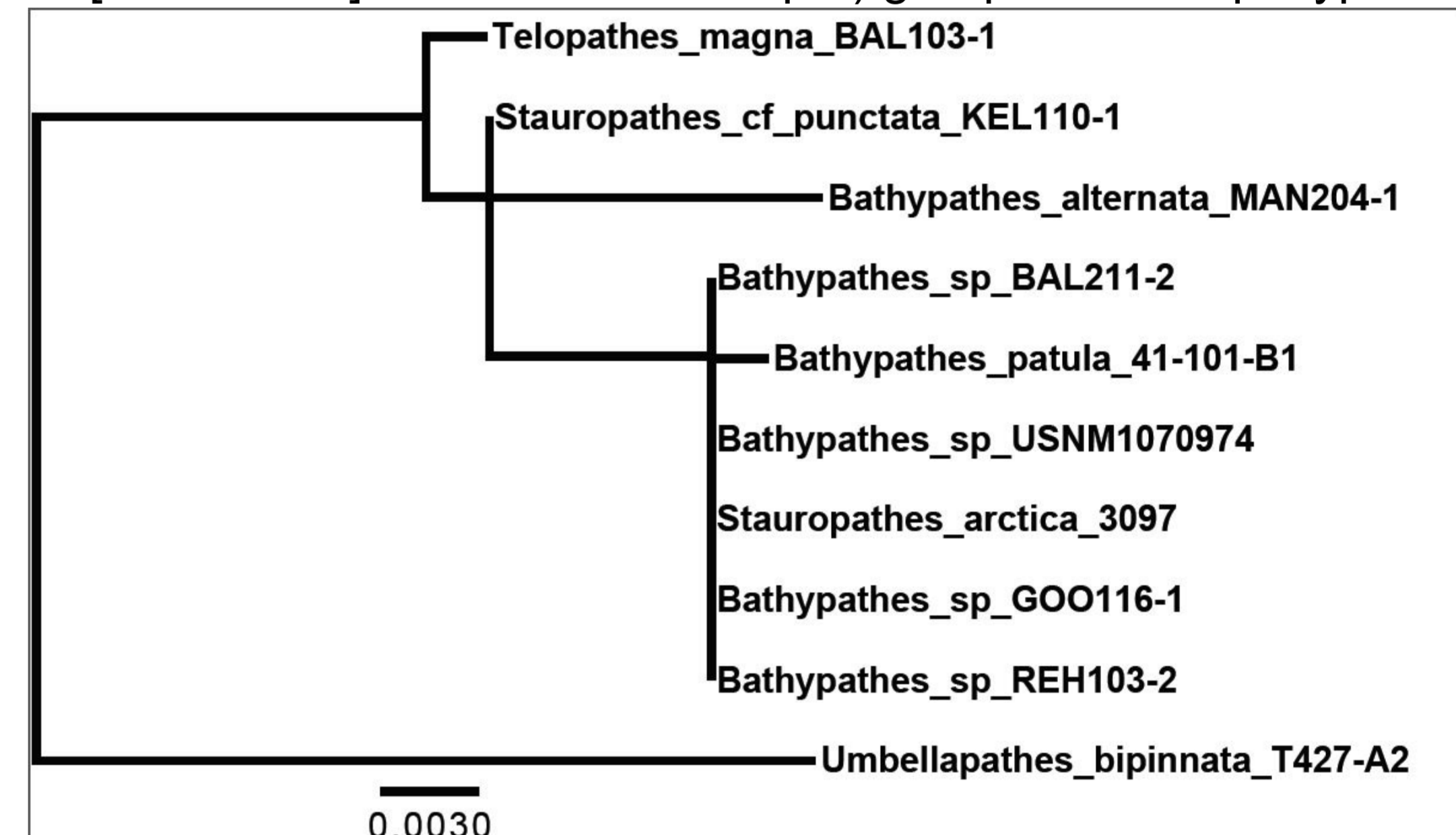

Fig. 6. Maximum likelihood-based phylogenetic tree built using *trnW*-IGR-*nad2* (top) and *cox3*-IGR-*cox1*(bottom). Both mitochondrial gene regions only revealed a single *S. arctica* haplotype. Similarly to the *nad5*-IGR-nad1 tree *S. arctica* does not group with *S. punctata* but is rather shares an identical sequence to *Bathypathes*.
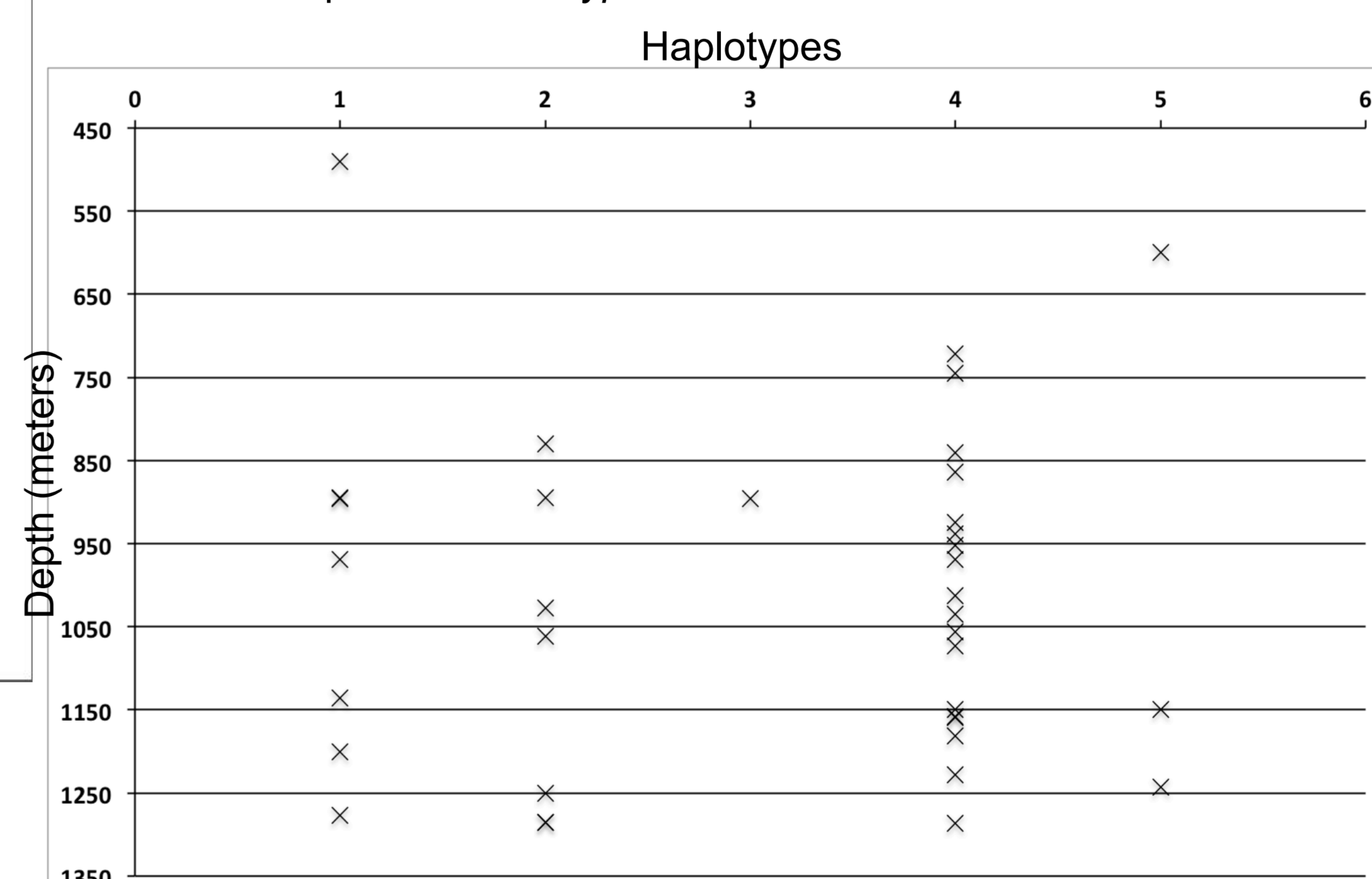

Fig. 9. Depth distribution of the five *nad5*-IGR-*nad1* haplotypes. Haplotypes do not appear to be partitioned by depth.

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