Morphological and molecular characterization of a new species of black coral from Elvers Bank, north-western Gulf of Mexico (Cnidaria: Anthozoa: Hexacorallia: Antipatharia: Aphanipathidae: Distichopathec)

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Abstract

The continental shelf edge of the NW Gulf of Mexico supports dozens of reefs and banks, including the West and East Flower Garden Banks (FGB) and Stetson Bank that comprise the Flower Garden Banks National Marine Sanctuary (FGBNMS). Discovered by fishermen in the early 1900s, the FGBs are named after the colourful corals, sponges and algae that dominate the region. The reefs and banks are the surface expression of underlying salt domes and provide important habitat for mesophotic coral ecosystems (MCE) and deep coral communities to 300 m depth. Since 2001, FGBNMS research teams have utilized remotely operated vehicles (e.g. ‘Phantom S2’, ‘Mohawk’, ‘Yogi’) to survey and characterize benthic habitats of this region. In 2016, a Draft Environmental Impact Statement proposed the expansion of the current sanctuary boundaries to incorporate an additional 15 reefs and banks, including Elvers Bank. Antipatharians (black corals) were collected within the proposed expansion sites and analysed using morphological and molecular methods. A new species, Distichopathes hickersoniae, collected at 172 m depth on Elvers Bank, is described within the family Aphanipathidae. This brings the total number of black coral species in and around the sanctuary to 14.

Introduction

The Flower Garden Banks National Marine Sanctuary (FGBNMS) is located off the Texas and Louisiana coasts on the continental shelf margin in the north-western Gulf of Mexico, and encompasses just three of the dozens of reefs and banks that occur in this area. Antipatharians are relatively common in some areas of the FGBNMS and 13 species (representing ∼5% of the 273 described species) have been recorded from the sanctuary (Opresko et al., 2016), occurring at depths ranging from 50–150 m. The reefs and banks of the north-western Gulf of Mexico, including Elvers Bank, harbour significant mesophotic coral ecosystems and deeper coral communities (ONMS, 2016; Boland et al., 2017; Semmler et al., 2017). Elvers Bank is located 65 km east of the East FGB (Figure 1) at the extreme edge of the continental shelf, extending to a depth of 205 m. Due to its shelf-edge location, surveys during the FGBNMS expeditions suggest a biological community somewhat different from other banks in the region, including assemblages of differing varieties of invertebrates not observed in other locations in the north-western Gulf of Mexico study area. While light measurements at this location are not available, it may be assumed that these deeper environments and associated biota require less light than those at shallower depths. During a National Oceanic and Atmospheric Administration (NOAA) survey cruise (Northwestern Gulf of Mexico Cruise 2016; Cruise DFH-30; Dive 382) conducted in September 2016, a large green, pinnately branched antipatharian colony was photographed at a depth of 172 m at Elvers Bank (Figure 2); however, samples of the colony were not collected at that time due to a malfunction of the manipulator arm on the remotely operated vehicle (ROV). The in-situ photos suggested that the colony represented an undescribed species. In September 2017, during a follow-up cruise to the same area (NW GOMEX Expansion Sites I; Cruise DFH-32; Dive 524), two colonies of what appeared to be the same species were collected at 172 m depth (Figure 3) and preserved in 100% ethanol and RNAlater for morphological and molecular analyses. These two specimens are the subject of this paper.
Materials and methods

This report is based on specimens collected during expeditions aboard the RV ‘Manta’ to reefs and banks of the north-western Gulf of Mexico, conducted by NOAA staff of the FGBNMS as well as students and staff of NYC College of Technology (CUNY). The ROV ‘Mohawk’, maintained and operated by the Undersea Vehicle Program at the University of North Carolina at Wilmington, was used to collect the specimens after in-situ photos were taken. Collected specimens were preserved in 100% EtOH and RNAlater and sent to the U.S. National Museum of Natural History (USNMNH), Smithsonian Institution, Washington, DC for further study. Photographs of the skeletal spines were made using a scanning electron microscope (SEM, Zeiss EVO MA 15) housed at the USNMNH. The specimens were coated with a 30–40 nm thick layer of 60% gold: 40% palladium. Analysis of the skeletal spines was conducted from direct examination of the material using a low-power binocular microscope or by examination of photographs taken with the SEM. The size of the polyps, referred to as the transverse diameter, was measured as the distance between the distal edge of the distal lateral tentacles and the proximal edge of the proximal lateral tentacles of the same polyp. The distance between spines was measured from the centre of the base of one spine to the centre of the base of an adjoining spine in the same axial row. The height of a spine was measured as the distance between the apex and the centre of the base of the same spine. Based on convention, the number of axial rows of spines was determined as the number of complete rows (those in which the bases of the spines are visible) that can be counted in one lateral view (also referred to as ‘one aspect’).

Subsamples of the specimens were subjected to molecular analysis which involved the sequencing of three mitochondrial gene regions (trnW-IGR-nad2, cox3-IGR-cox1 and nad5-IGR-nad1; IGR = intergenic region) and three nuclear gene regions (ITS2, 28S and SRP54). DNA extraction, DNA quantification, PCR primers and reagents, PCR thermocycling profiles, PCR cleanup, cycle sequencing, cycle sequencing cleanup, traditional Sanger sequencing on an ABI-3730xL, multiple sequence alignment,
model selection, and Maximum likelihood-based tree building followed the protocol detailed in MacIsaac et al. (2013). Brugler et al. (2013) showed that the nad5-IGR-nad1 region was the most variable region within the black coral mitogenome; however, the cox3-IGR-cox1 region revealed the greatest number of unique haplotypes (i.e. unique species). Given that the phylogenetic reconstructions presented in Brugler et al. (2013) remain the most comprehensive to date, newly obtained sequence data (corresponding to nad5-IGR-nad1 and cox3-IGR-cox1 only) were added to the multiple sequence alignments from Brugler et al. (2013). Maximum likelihood-based phylogenies were built to infer the evolutionary relationship of Distichopathes hickersonae to known taxa within the Order Antipatharia. Currently, datasets for the three nuclear gene regions (ITS2, 28S and SRP54) are not as comprehensive (in terms of representative species) as the mitochondrial gene regions; thus, these regions were simply used to look for genetic differences between the holotype and paratype.

Results

Systematics

Order ANTIPATHARIA Milne-Edwards & Haime, 1857
Family APHANIPATHIDAE Opresko, 2004

Genus Distichopathes Opresko, 2004


Diagnosis. Corallum monopodial, unbranched, or sparsely to densely branched; tending to be planar with overlapping

Remarks. The family was originally established with two subfamilies, the Aphanipathinae (including the genera Aphanipathes, Phanopathes, Tetrapathes, Pteridopathes and Asteriopathes) with subequal polypar spines, and the Acanthopathinae (including the genera Elatopathes, Distichopathes, Rhipidipathes and Acanthopathes) with very unequal (anisomorphic) polypar spines where the circumpolypar spines are very enlarged and the hypostomal spines are reduced or even absent (Opresko, 2004). Recent DNA sequencing studies (e.g. Brugler et al., 2013) using mitochondrial markers (cox3-IGR-cox1 and nad5-IGR-nad1) have not supported the recognition of these two subfamilies, and in fact the genera Elatopathes and Distichopathes have closer affiliations to the family Myriopathidae than to other genera in the Aphanipathidae (Figures 4 and 5). In addition, the genus Acanthopathes grouped with several different families depending on the molecular marker or tree-building algorithm employed, and the genus Rhipidipathes grouped with genera in the Antipathidae (not shown). These results, if confirmed by more extensive molecular studies using higher resolution markers (i.e. Ultra-Conserved Elements; see Quattrini et al., 2018), may necessitate a taxonomic revision of the family.
branches. Stem and branches pinnulate. Pinnules simple, not sub-pinnulate; arranged primarily in two lateral rows, but with simple short pinnules occurring very rarely on the abpolypar side of the axis. Pinnules also arranged alternately along the stem and branches.


Remarks. The genus *Distichopathes* is distinguished from the other genera in the family Aphanipathidae by the simple, bilateral pinnules and distinctly anisomorphic spines. Species of the genus resemble those of *Pteridopathes* which also have two rows of simple bilateral pinnules; however, in *Pteridopathes* the polypar spines are subequal in size or, in some cases, the circumpolypar spines are slightly taller than the other polypar spines. The anisomorphic spines of *Distichopathes* are similar to those of *Elatopathes*, the colonies of which also have simple pinnules, but in the latter case the pinnules are arranged in four to six axial rows.

Species assigned to *Distichopathes*. Three species are currently assigned to the genus, *D. filix* (de Pourtalès, 1867), *D. disticha* Opresko, 2004, and *D. hickersonae* sp. nov.

Distribution. Species of *Distichopathes* are known only from the North-western Atlantic.

![Fig. 4. Partial results of a Maximum likelihood-based phylogenetic reconstruction using mitochondrial nad5-IGR-nad1 sequence data focusing specifically on the families Aphanipathidae and Myriopathidae. The MAFFT L-INS-i v7 based alignment consisted of 49 sequences and 686 sites. jModelTest v2.1.1 selected the TPM3uf + G model of sequence evolution. PhyML v3.1 utilized a BioNJ starting tree, best of NNI and SPR tree topology search options, and 1000 non-parametric bootstrap replicates. The tree was rooted internally to the Leiopathidae. Node support for *Distichopathes hickersonae* grouping with *Elatopathes abietina* is 99.9. *Distichopathes hickersonae* and *Elatopathes abietina* are genetically identical across 318 comparable base pairs. Node support for the clade grouping sister to *Distichopathes + Elatopathes* (which includes *Stylopathes*, *Antipathes*, *Tancipathes*, *Plumipathes* and *Myriopathes*) is 78.](https://doi.org/10.1017/S002531542000051X)
diameter and are placed in a single series on one side of the pin-
nules, with 8–10 polyps per cm.

**Description of the holotype**
The holotype (USNM 1517703) is a moderately sized complete colony about 21 cm tall and 17 cm wide (Figures 3A, B & 6A).

The colony is branched to the fifth order, and the branching is planar with overlapping adjacent branches. The diameter at the base of the stem just above the holdfast is 3.7 mm. Several large first-order branches originate just above the holdfast, one of which extends to the top of the colony. The stem and branches possess simple, filiform pinnules up to about 2 cm long and 0.3 cm wide.
mm in diameter near the base. The pinnules vary slightly in size from branch to branch, and can also be shorter on the lower parts of the branch compared with the more distal segment. Newly forming pinnules at the branch tips gradually increase in size proximally until they reach a consistent length. The pinnules are arranged in two lateral rows with members of each row spaced
The two other nominal species in the genus are *D. filix* (de Pourtalès, 1867) and *D. disticha* Opresko, 2004. Specimens that conform to Pourtales’ original description of *D. filix* characteristically are monopodial, unbranched, and have very short pinnules 0.6–1 cm long (Figure 8A). Colonies of *D. disticha* are sparsely branched, with up to three orders of branching, and have pinnules that are mostly 3–7 cm long (maximum about 12 cm) (Figure 9A). In contrast, colonies of *D. hickersonae* can be densely branched (up to five orders of branching) and have pinnules that are up to 2 cm in length. The fact that the examined colonies of *D. disticha* and *D. hickersonae* are of comparable size would support the supposition that the differences in the length of pinnules and the density of the branching are not age-related intraspecific phenomena.

The pinnular spines of *D. hickersonae* are morphologically more similar to those of *D. disticha* (Figure 9B) than to those of *D. filix* (Figure 8B). In *D. filix* the spines usually have a clavate shape in that some are slightly swollen towards the apex, and the apolypar spines can be distinctly flattened and bent upwards at the apex. In contrast, the spines of *D. disticha* and *D. hickersonae* are more regularly cylindrical. In terms of the size of the spines, *D. hickersonae* is also more similar to *D. disticha*. The circumpolypar spines are mostly 0.26–0.30 mm tall in *D. hickersonae* (maximum about 0.35 mm); up to 0.4 mm in *D. disticha*, and up to 0.55 mm in *D. filix*. In the samples examined, the circumpolypar spines, hypostomal spines and abpolypar spines in *D. hickersonae* are similar to or slightly larger than the corresponding ones in the other two species (Table 1). Although the number of observations is limited, one difference seen between *D. hickersonae* and *D. disticha* is in the number of hypostomal spines that are reduced in size; in *D. hickersonae* only two such hypostomal spines are found in each polyp area, whereas in *D. disticha* three are present.

The maximum height of the spines, especially the polypar spines, has in the past been a key character in separating closely related antipatharian species. The differences seen here in the size of the circumpolypar spines between *D. disticha* and *D. hickersonae*, although not great, are suggestive that as larger suites of specimens are examined, the population distribution of this character will be different for each of these species.

**Remarks**

As in most antipatharian species complexes, the morphological boundaries between the three nominal species of *Distichopathes* are likely to be difficult to clearly define because of the inherent variability in most taxonomic characters. Overlaps in characters such as the length of the pinnules and size of the spines between closely related species is to be expected. The information presented in Table 1 is based on small sample sizes, especially for *D. hickersonae* and *D. disticha*; therefore, the values shown,
such as the typical length of the pinnules and height of the spines will most likely change as more colonies are analysed. In addition, some specimens previously assigned to *D. filix* (see Table 4 in Opresko, 1972), particularly those that have intermediate pinnule lengths of 1–2 cm, will have to be re-evaluated to determine if the morphology of the spines provides a better indication of whether they are more closely related to *D. filix* or to *D. hickersonae*.

### Molecular analysis
We compared the DNA sequence of the holotype (USNM 1517703) and paratype (USNM 1548274) using a combination of three mitochondrial and three nuclear gene regions. The holotype and paratype were genetically identical across all six gene regions (total number of base pairs compared: 2878 bp). We sequenced 1701 bp of mitochondrial DNA, which consisted of 492 bp from *trnW-IGR-nad2*, 880 bp from *cox3-IGR-cox1* and 329 bp from *nad5-IGR-nad1* (IGR: intergenic region). We also sequenced 1177 bp of nuclear DNA, which consisted of 603 bp from ITS2, 1701 bp of mitochondrial DNA, which consisted of 492 bp from three mitochondrial and three nuclear gene regions. The holotype was examined in the SEM lab at the USNMNH, of the ROV operators Lance Horn, Jason White and Eric Glidden are greatly appreciated. The specimens were examined in the SEM lab at the USNMNH, and assistance was generously provided by S. Whitaker. DMO and MRB are Research Associates at the USNMNH and gratefully acknowledge that affiliation. MRB is also a Research Associate at the American Museum of Natural History and gratefully acknowledges that affiliation as well.

### References


