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Validation of Image-Based Species Identifications of Black Corals (Order Antipatharia) on Mesophotic Reefs

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Biodiversity, an important measure of ecosystem health, is challenging to ascertain using sampled specimens in remote deep-sea environments. As image-based identifications become a predominant method for deep-sea species characterizations, there is a need to evaluate the accuracy of species- and genus-level identifications from video and still images to provide a reliable measure of biodiversity. This study presents a validation of the ability to make accurate image-based identifications of black coral species in the northwestern Gulf of Mexico from standard-definition video collected by a remotely operated vehicle. Results indicate that the greatest number of misidentifications occurred at species-level groupings (42.2% error), whereas genus-level groupings possessed 12.0% error, and identifications to kappa groupings had no error. We recommend genus-level groupings to maintain accurate identifications while maximizing estimates of biodiversity.

INTRODUCTION

Ecosystem health is valuable in understanding the stressors, identifying causes, and potentially improving environmental management of an ecosystem (Rapport et al., 1998). A "healthy" ecosystem is considered stable and sustainable (Costanza, 1992). Although there are multiple measures that aim to assess ecosystem health, biodiversity is considered a valuable measure, as high biodiversity is considered to enhance the stability, resilience, and productivity of some ecosystems (Johnson et al., 1996; Folke et al., 2004). Species composition is also an important component in understanding ecosystem health, as a community comprised of multiple opportunistic species may be an indicator of stress. In remote environments, these measures can be challenging to ascertain.

Mesophotic coral ecosystems (MCEs) occur in the lower portion of the photic zone in tropical and subtropical regions, ranging from 40- to 150-m depth (Puglise et al., 2009). The upper depth limit of this zone represents the region in which a shift is observed in the biological assemblages (Kahng et al., 2010). Because of their unique depth range, MCEs are considered to be extensions of shallow-water coral ecosystems and deepwater assemblages, in addition to possessing their own unique biotic assemblages (Hinderstein et al., 2010). Like deep-sea habitats, MCEs have received relatively little attention in comparison with shallow-water habitats because of the challenges associated with accessing these environments. However, with the growing accessibility of resources needed to study these environments, such as remotely operated vehicles

(ROVs), further advancements in characterizing and understanding these habitats are being made every year. Historically, specimen collections and distribution information of mesophotic and deep-sea corals has primarily come from fisheries records, bycatch, and museum collections (Bryan and Metaxas 2007). Although current technologies allow for direct sampling of specimens, specimen sampling is still limited by the capacity of the sampling tool and by a conscious conservation as we understand the fragility of many of these organisms. However, these same sampling tools often have the ability to collect vast amounts of image-based data, with improving resolution, as technology improves, over time. The availability, ease of collection, and noninvasive nature of image-based data are helping to accelerate estimates of biodiversity in mesophotic and deep-sea habitats. However, before image-based identifications can be applied in research, their accuracy needs to be tested. A validation process is presented in this paper for black corals on mesophotic reefs in the northwestern Gulf of Mexico.

Black corals (Cnidaria: Anthozoa: Hexacorallia: Antipatharia) are slow-growing sessile species, potentially representing some of the oldest living animals on Earth, with colonies dated to over 4,000 yr old (Roark et al., 2009). Black corals are important components of the mesophotic and deep-sea benthic communities throughout the world. They are considered ecosystem engineers, providing valuable three-dimensional habitat for numerous associated fauna, fish, and invertebrate species (Jones et al., 1994; Hourigan et al., 2007). Because of their life history and complex morphology, these corals are particularly vulnerable
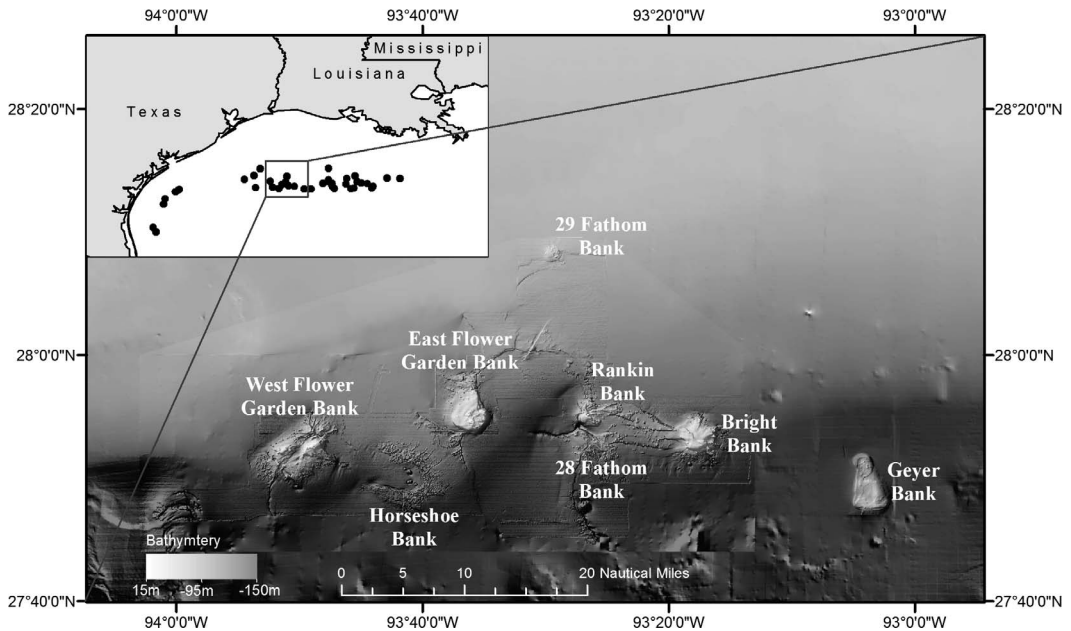
Fig. 1. Map showing the eight locations from which samples were collected in the northwestern Gulf of Mexico. Sites shown are 1. West FGBNMS, 2. Horseshoe Bank, 3. East FGBNMS, 4. 29 Fathom Bank, 5. 28 Fathom, 6. Rankin Bank, 7. Bright Bank, and 8. Geyer Bank.

to unsustainable harvesting and environmental threats (Bo et al., 2009). Worldwide, black corals are threatened by harvesting for the commercial jewelry trade and oriental medicinal purposes, deepwater fishing practices, dredging, and hydrocarbon exploration and dispersion chemicals (Fossa et al., 2002; Hall-Spencer et al., 2002; Mortensen and Buhl-Mortensen, 2004; Roark et al., 2009; Bai et al., 2011, Silva et al., 2015). Established black coral fisheries exist in the Caribbean, Hawai'i, and Asia (Grigg, 1975, 1976, 1993; Castorena and Metaca, 1979; Guitart et al., 1997; Huang and Ou, 2010). Although recent management strategies aim to make these fisheries sustainable, historically, they have been overexploited (Bruckner et al., 2008; Tsounis et al., 2010).

Multiple studies have noted the presence and distribution of black corals on reefs and banks throughout the northwestern Gulf of Mexico (Rezak et al., 1985; Opresko and Cairns, 1992; Cairns et al., 1994; Brooke and Schroeder, 2007; Robbart et al., 2009). However, no comprehensive study of this order has been conducted in the region to date. Of the 39 species of black coral reported from the western Atlantic, 30 of those have been documented in the Gulf of Mexico (Opresko 2009).

Black corals are noncalcareous corals with organic skeletons comprised of chitin and protein. Traditional species descriptions of black corals focus on morphological characters, including skeletal branching mode and pinnulation, polyp structure and size, and, in particular, spine morphology (Warner 1981; Perez et al., 2005; France et al., 2007). The ability to obtain high-resolution images through scanning electron microscopy (SEM) of the skeleton has enhanced the use of spine morphology as a taxonomic character (Wagner et al. 2010). However, this technique of species identification requires that a tissue sample of the colony be collected. For slow-growing, commercially valuable, and protected species, like black corals, the ability to make noninvasive image-based identifications is particularly valuable. As technology improves, the ability to capture high-resolution images of the seafloor is also improving, allowing researchers to obtain clear in situ images of organisms. National Oceanographic and Atmospheric Administration's (NOAA) Flower Garden Banks National Marine Sanctuary (FGBNMS) has been working to develop image-based identification catalogs for multiple mesophotic and deep-sea species for the northwestern Gulf of Mexico. This paper represents a validation process for FGBNMS black coral catalog (Hickerson et al., 2007, Opresko et al., 2016).

MATERIALS AND METHODS

Black coral specimens were collected using a Phantom S2 ROV on four research cruises to eight banks in the northwestern Gulf of Mexico (Fig. 1) between 2011 and 2012. Specimens were

TABLE 1. Comparison of image-based identification and laboratory identification. Table shows sample size, based on in situ identification, and the percent composition of image-based identifications by laboratory identification.

In situ identification	Sample size	Laboratory species identifications						
		Antipathes furcata	*Antipathes gracilis/atlantica*	*Elatopathes abietina*	*Acanthopathes thyoides*	*Acanthopathes* sp., cf. *A. thyoides*	*Aphanipathes pedata*	*Tanacetipathes tanacetum*
Antipathes furcata	11	100.0						
Antipathes gracilis/atlantica	11		72.7		9.1	9.1		
Elatopathes abietina	9			100.0				
Acanthopathes thyoides	8		12.5		50.0			
Aphanipathes pedata	7						100.0	
Tanacetipathes tanacetum	7							42.9
Stichopathes luetkeni	5							
Stichopathes sp.	5							
Stichopathes sp. (Opresko et al. In Review)	5							
Tanacetipathes barbadensis	5							20.0
Tanacetipathes sp.	4							50.0
Tanacetipathes hirta	3							33.3
Phanopathes expansa	2							
Plumapathes pennacea	1							

collected opportunistically at predetermined ROV dive sites. The ROV was equipped with a Sony National Television Standards Committee high-resolution video camera and two 250-W deep-sea power and light tungsten-halogen lights (Horn and Taylor, 2012).Using the live video feed from the ROV, species identifications were made and recorded, in situ, on the basis of FGBNMS identification keys (Hickerson et al., 2007; Opresko et al., 2016). Species identifications were made by highly experienced researchers familiar with the region and the FGBNMS field guide. Specimens were then collected using a single-function manipulator mounted on the ROV and brought to the surface. Samples were photographed on deck and preserved in 95% EtOH. Laboratory identifications were made using traditional morphological techniques and
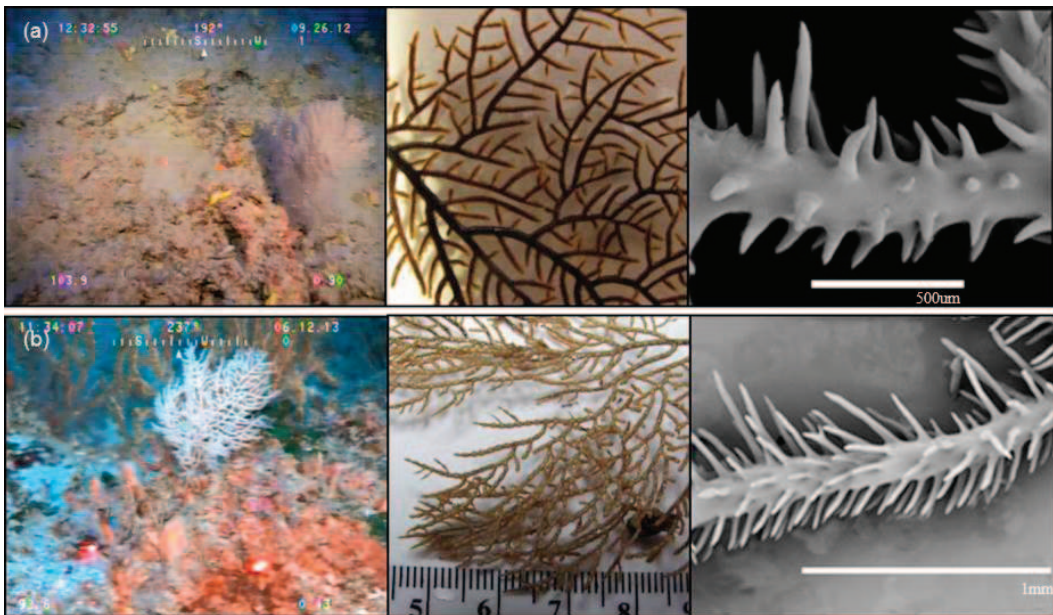

Fig. 2. In situ and SEM images of specimens that form the genus *Acanthopathes*. Images of (a) *A. thyoides* and (b) *A.* sp. cf. *thyoides*.

TABLE 1. Extended.

				Laboratory species identifications				
Stichopathes luetkeni	*Stichopthes sp.* (Opresko et al., 2016)	*Tanacetipathes hirta*	*Phanopathes expansa*	*Plumapathes pennacea*	Ellisellidae	*Stichopathes* cf. *pourtalesi*	*Tanacetipathes* sp., cf. *T. paula*	*Tanacetipathes thamnea*
			9.1					
			37.5					
		14.3					14.3	28.6
40.0					20.0	40.0		
40.0	20.0					40.0		
					40.0	60.0		
		80.0						
		25.0						25.0
		33.3						33.3
			100.0					
				100.0				

various identification keys and original species descriptions (Gray, 1857; Pourtales, 1874; Brook, 1889; Brook, 1965; Warner, 1981; Cairns et al., 1994; Opresko, 1996, 2001a,b, 2004; Warner and Opresko, 2004; Loiola and Castro, 2005; Opresko and Sanchez, 2005; Perez et al., 2005; Opresko et al., 2016). The species *Antipathes atlantica* and *Antipathes gracilis* were combined for identification purposes. These two species are very similar morphologically, and an unpublished genetic analysis suggests that these two species are genetically similar (on the basis of a single marker) and should potentially be considered the same species (C. G. Umaña, personal communication to D. Opresko, 15 Dec. 2012). Additionally, there appears to be considerable plasticity in skeletal morphology of the *Tanacetipathes* genera; therefore, genetic studies are needed to determine
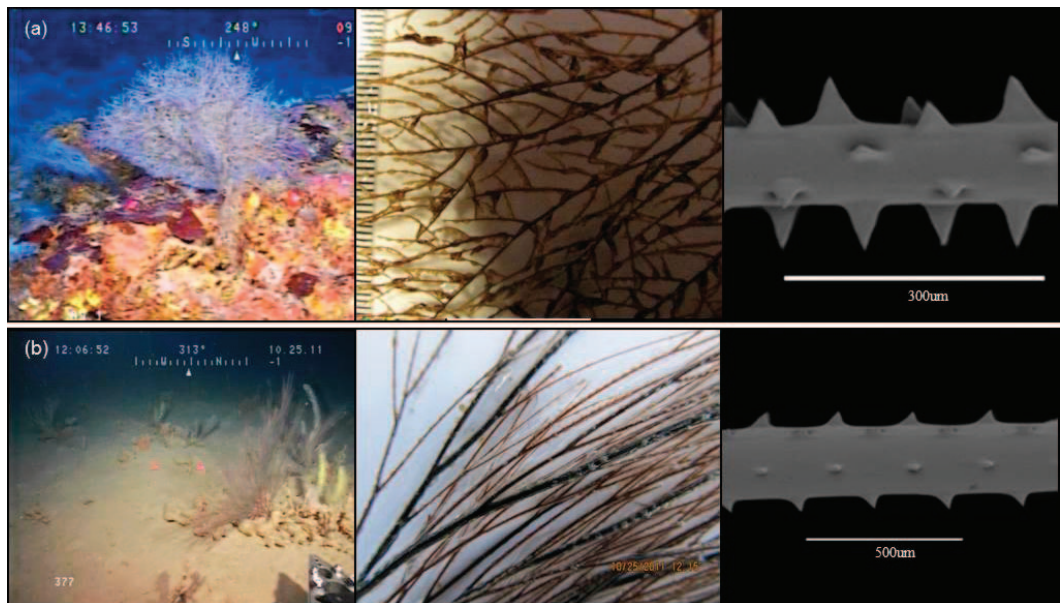

Fig. 3. In situ and SEM images of specimens that form the genus *Antipathes*. Image (a) *A. atlantica/gracilis* and (b) *A. furcata*.
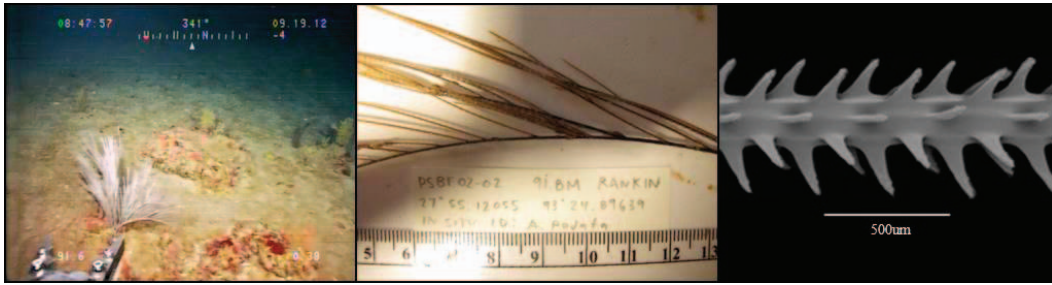
Fig. 4. In situ, prepreservation image, and SEM images of specimens that form the genus *Aphanipathes*. Images are of *A. pedata*.

the validity of currently recognized species and their morphological limits.

Morphological characteristics used for laboratory identification included: corallum branching mode, subpinnule branching patterns, spine morphology and size, and polyp size and density. Whole-colony morphology was obtained from ROV footage, and size was obtained using two lasers mounted on the ROV whose beams delineated a 15-cm span in the frame of the video. Polyp morphology was obtained from preserved samples using dissecting and light microscopes, with ocular micrometer for scaling. Tissue was removed from a small section of each colony to obtain a clean sample for analysis of skeletal morphology. A light microscope with ocular scale was used to obtain skeletal and spine measurements. For unusual, unique, or hard-to-identify samples, specimens were processed for SEM analysis. These samples were mounted on stubs with carbon tabs, and oriented to obtain a view of polypar and abpolypar spines. Samples were coated with gold–palladium using a sputter coater (70-mm target distance, with 30 mA for 30 sec). A table-top SEM (Hitachi TM3000) was used to obtain detailed images and measurements of the skeletal morphology and spines.

The percent error of identifications was calculated at the species and genus levels. In addition, groupings were made on the basis of Cohen's kappa coefficient (κ), combining species that were commonly mistaken for one another (referred to as kappa groupings). κ was calculated using JMP9$_{\circledR}$ to measure the agreement between the two identification methods: image based and laboratory based. κ is standardized between -1 and 1, where 1 indicates perfect agreement, 0 represents chance agreement, and a negative value indicates agreement less than chance (Viera
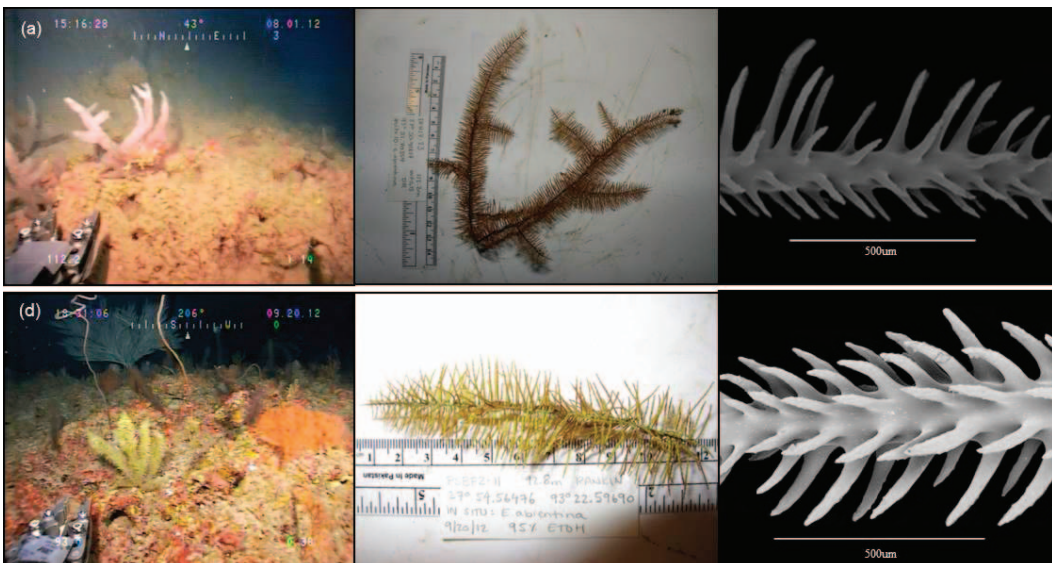

Fig. 5. In situ, prepreservation image, and SEM images of specimens that comprise the genus *Elatopathes*. All images represent the species *E. abietina*, with image (a) showing the white color-morph and (b) the green color-morph.
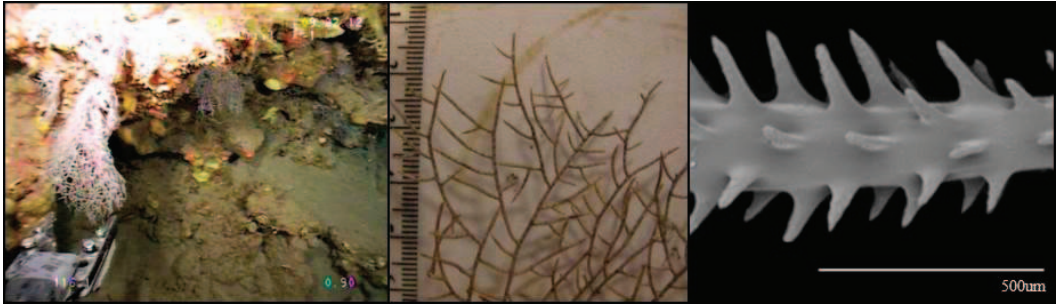
Fig. 6.   In situ, prepreservation image, and SEM images of specimens that form the genus *Phanopathes*. Images are of *P. expansa*.

and Garrett, 2005). Image-based identifications, where $\kappa = 1$ indicates perfect agreement, were considered to be accurately identified to species level, and recommended for species-level identification. Image-based identifications with $\kappa < 1$ indicate less than perfect agreement, and were grouped together on the basis of the species with which they were commonly misidentified.

Biodiversity was calculated for each of the identification levels. At the species level, this was species richness; at the genus level, this was genus richness; and at the kappa groupings level, this was group richness.

RESULTS

A total of 83 samples were collected from eight locations in the northwestern Gulf of Mexico. The total number of samples collected for each image-identified species ranged from 1 to 11. Samples were collected from 52 m to 141 m. A total of 14 species were identified in situ, and a total of 16 species were identified in the laboratory. Table 1 provides details on species that were correctly and incorrectly identified in situ. Figures 2–9 show imagery used to identify each of the species, including in situ and SEM images of each species. Samples from this study are stored in the collections of the Department of Invertebrate Zoology, National Museum of Natural History, Smithsonian Institution, in Suitland, MD, and in the FGBNMS Collections, in Galveston, TX.

Results from the validation of image-based species identifications indicate that, although some species can be consistently and accurately identified using images, other species were frequently misidentified. Species richness based on laboratory identifications was 16, with 15 representing antipatharians. Image-based richness at the species level was 11, at the genus level was 8, and at the kappa groupings level was 7. For the kappa groupings, four species were identified to species level, and 13 species were combined into three higher groupings. These organisms represent species that are difficult to distinguish in situ, and would require sampling to conduct traditional morphological identification to obtain species-level identifications. The three groups include the black coral sea fans (four black coral species), the *Stichopathes*-like sea whips (four black coral species and one gorgonian species), and the *Tanacetipathes* sp. (four black coral species). A complete list of the kappa groupings, with corresponding κ values, can be found in Table 2.

When identifications were reviewed at the species level, 42.2% error was recorded; identifications at the genus level possessed 12.0% error and identifications to the kappa groupings had 0.0% percent error (Fig. 10).

Fig. 7.   In situ and SEM images of specimens that form the genus *Plumapathes*. Images of *P. pennacea*.
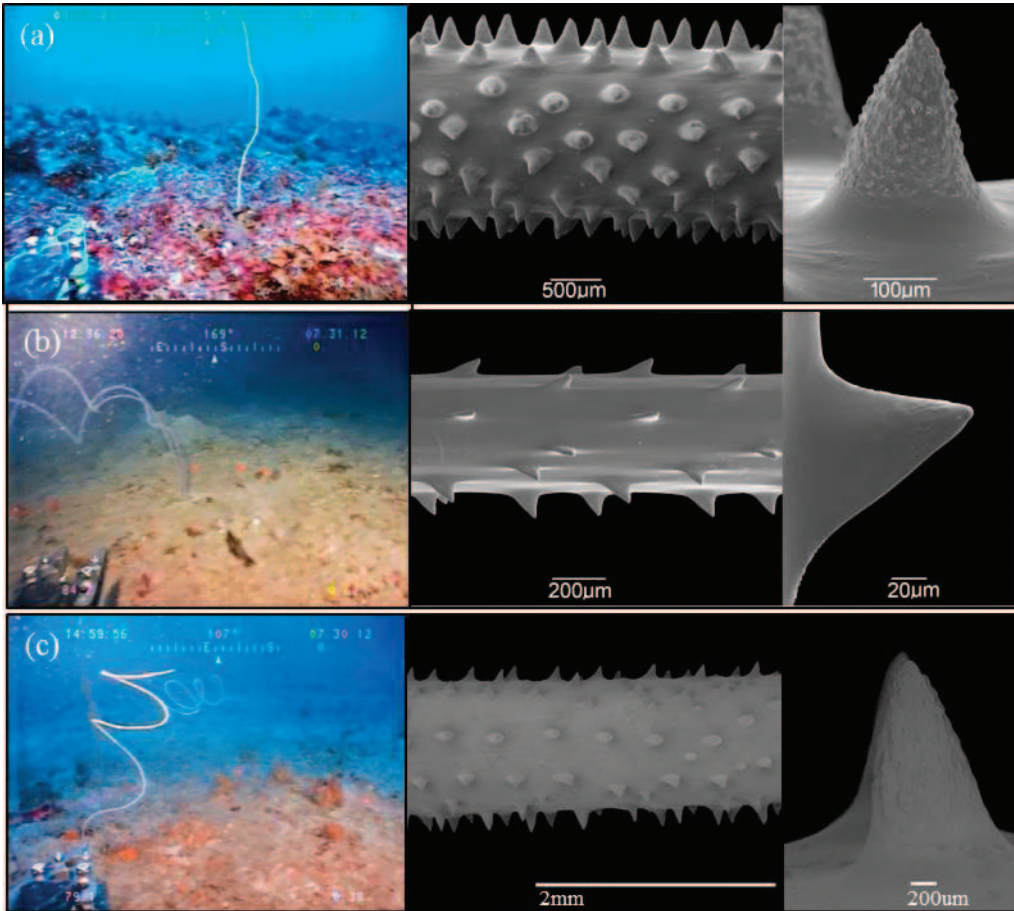
Fig. 8. In situ and SEM images of specimens that form the genus *Stichopathes*. Images are (a) *S. luetkeni*, (b) *S.* cf. *pourtalesi*, and (c) *Stichopathes* sp. (Opresko et al., in review).

DISCUSSION

Accurate identifications are critical to all types of experimental research and species management decisions, including making inferences of ecosystem biodiversity and health. With the comparative ease of image-based data collection, in comparison with specimen sampling, in remote environments, researchers and resource managers are relying more and more on the use of image-based data. Understanding the limitations of image-based data and interpreting biodiversity is essential. This study presents the first validation of image-based identifications of the black coral species from this region, highlighting validation assessment methods, in addition to providing a local guide of species that can be identified accurately and consistently in situ, and those that require grouping to higher levels. Although grouping organisms diminishes species resolution and makes accurate species distri-

bution modeling more challenging because of grouping of multiple habitat niches, the same issue is created by misidentifications. Identification to species level is ideal; however, balancing accuracy and maintaining maximum species resolution is possible.

Although this study supports the ability to make accurate image-based identifications of four black coral species, 13 species were misidentified to varying degrees. Evaluation of species-level identifications from image-based data, for these species, resulted in a biodiversity estimate close to the actual species richness derived after laboratory analysis, but with large percent errors for some species. At the genus level, both biodiversity and percent error were reduced. At the kappa groupings level, biodiversity was reduced even more and percent error was zero.

The high percent error in species-level identifications highlights that, for these specimens,
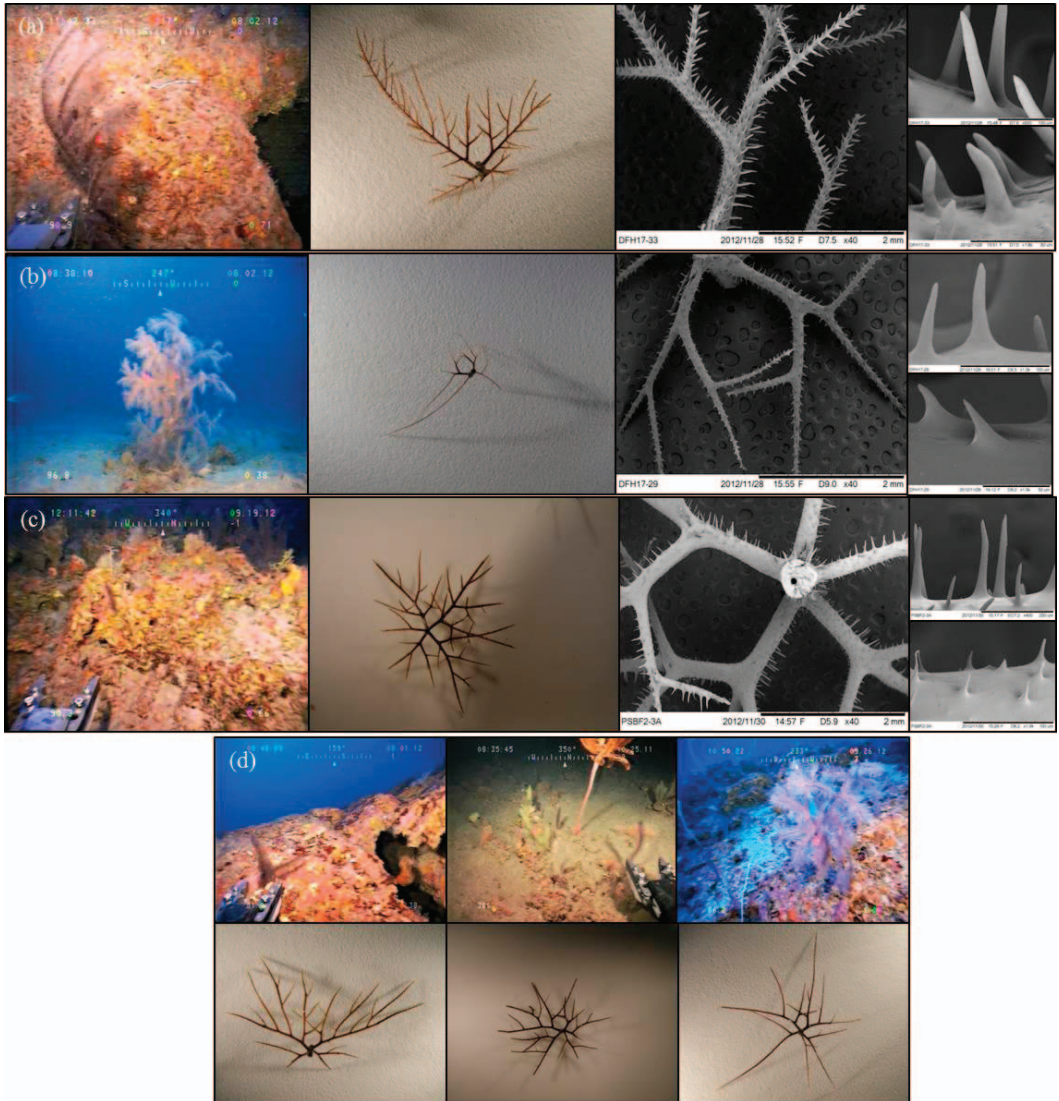
Fig. 9.   In situ, cross-section, and SEM images of specimens that form the genus *Tanacetipathes*. Images are (a) *T. thamnea*, (b) *T. hirta*, (c) *T.* cf. *paula*, (d) three forms of *T. tanactum*.

image-based species-level identifications should be used with caution. Conversely, although kappa groupings produced the lowest percent error in identifications, they also produced the lowest biodiversity and created groups of species that combine multiple genera and families in one group. We suggest that the optimal image-based identification level was at the genus level, where error was reduced and biodiversity maximized. Additionally, genus-level groupings are a more accurate ecological lumping of species than the lumping of species in the kappa groupings.

The majority of the species identified in this study have been previously observed in the northwestern Gulf of Mexico. However, during the course of this study three morphotypes were collected, including *Stichopathes* sp. (Opresko et al., 2016), *Tanacetipathes* sp., cf. *T. paula* and *Acanthopathes* sp., cf *A. thyoides*, that could not be assigned to any nominal species. Each of these morphotypes, represented by a single colony, possessed or lacked features that would allow for definitive species identification and will require further taxonomic study. Depths were recorded for each of the specimens, providing depth ranges for each of the species sampled. When reviewing published depths ranges for these species, two of the species that were found in

TABLE 2. Kappa statistics, sample depth ranges, and published depth ranges, grouped by recommended species groupings to maintain accurate image-based identifications. Boldface highlights specimens with depth range extensions from published records.

| Identification grouping	κ	Depth range of samples (m)	Published depth ranges (m)	References
Black coral sea fans	0.6396	104–129	129–207	Opresko, 1972
Acanthopathes thyoides				
Acanthopathes sp., cf. A. thyoides	−0.049	92	—	Cairns et al., 1994
Antipathes gracilis/atlantica	0.7809	62–99	20–91	Cairns et al., 1994
Phanopathes expansa	0.5135	82–116	129–144	Opresko and Cairns, 1992
Stichopathes-like Sea Whips				
Stichopathes cf. pourtalesi	0.1367	73–110	82	Cairns, 1979
Stichopathes luetkeni	0.5253	52–76	50–70	Cairns et al., 1994
Stichopathes sp. (Opresko et al. 2016)	0.3579	78	—	
Ellisellidae	0.0732	90–103	—	
Tanacetipathes spp.				
Tanacetipathes sp., cf. T. paula	−0.02	89	—	
Tanacetipathes hirta	0.3565	87–100	51–179	Cairns et al., 1994
Tanacetipathes tanacetum	0.4811	81–117	60–106	Cairns et al., 1994
Tanacetipathes thamnea	0.0425	77–91	70–106	Opresko, 2009
Antipathes furcata	1	97–124	15–70	Gray, 1857; Opresko and Sanchez, 2005; Lutz and Ginsburg, 2007
Aphanipathes pedata	1	92–112	60–310	Cairns et al., 1994; Opresko, 1974; Lutz and Ginsburg, 2007
Elatopathes abietina	1	93–141	31–310	Opresko, 1972; Rezak et al., 1985; Reyes et al., 2005; Lutz and Ginsburg, 2007
Plumapathes pennacea	1	57	3–229	Opresko, 1974; Colin, 1978; Lutz and Ginsburg, 2007
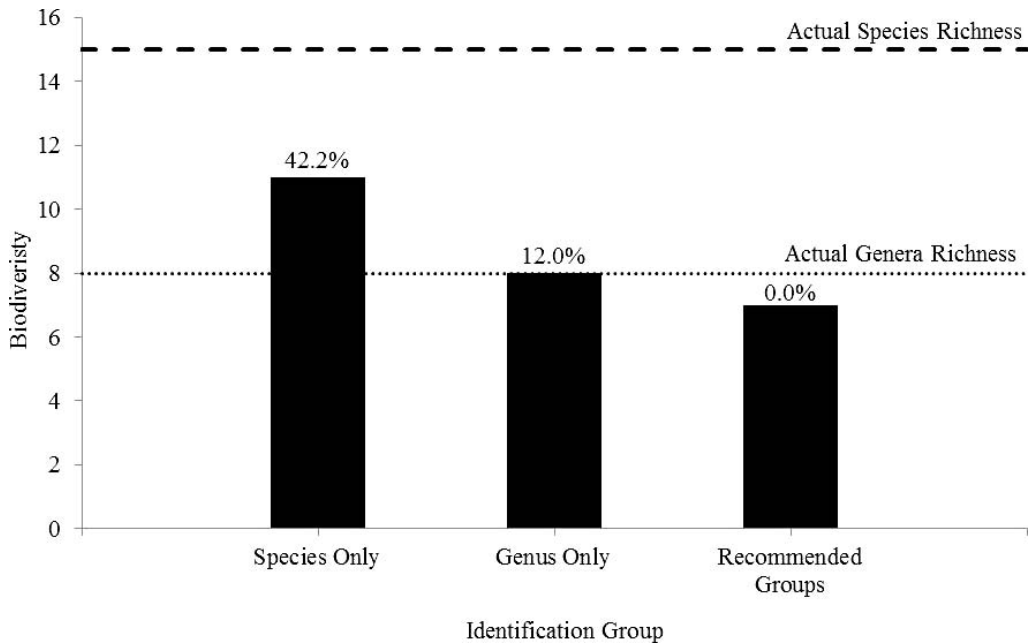
Fig. 10. Biodiversity measures for each of the identification groupings. Bars represent the number of species, genera, or groups accurately identified in image-based identifications and the value above each bar represents the percent error of the identifications.

this study occurred in shallower water than previously recorded (*A. thyoides* and *S. pourtalesi*) and five occurred deeper (*A. gracilis/atlantica*, *S. pourtalesi*, *S. luetkeni*, *T. tanacetum*, and *A. furcata*) (Table 2).

This study discusses the need to validate image-based species identifications and supports that genus-level identifications may represent a balance between identification accuracy and maintaining biodiversity for black corals in the northwestern Gulf of Mexico. However, it should be recognized that the characterization of a previously unstudied location for biodiversity should first be sampled for species, making species identifications using traditional morphological techniques, and then go through a validation process like that used here, for locale grouping protocols.

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