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Discovery of *Aphanipathes verticillata* (Cnidaria: Anthozoa: Antipatharia) in the Hawaiian Islands

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Abstract

Mesophotic coral reef surveys conducted off Maui in 2008–2009 revealed several specimens superficially resembling the commercial black coral species *Antipathes griggi* Opresko 2009. After subsequent microscopic examination of the skeletal features, these colonies proved to be morphologically very similar to *Aphanipathes verticillata* Brook 1889, a species never before reported from the Hawaiian Islands. A comparison with samples of the type material of *A. verticillata* indicated that the specimens collected in Hawaiian waters differed from the type in having simplier and less dense tubercles on the skeletal spines, a character which merits the recognition of the Hawaiian population as a new subspecies, *A. verticillata mauiensis*. Colonies of the new subspecies exhibit considerable morphological variation; DNA analysis of fifteen specimens ruled out the possibility of the presence of a cryptic species. Further DNA investigations on specimens from various localities in the western Pacific and Indian Ocean are needed to better understand the genetic relationship between the two forms. The morphological similarity of *A. verticillata mauiensis* with *Antipathes griggi* raises questions concerning the validity of past field surveys evaluating the population size and structure of *A. griggi* since it is possible that the two species could easily be misidentified based on gross morphology alone. Additional studies are also needed to document the geographic and bathymetric distribution of the subspecies along the Hawaiian Island chain.

Key words: Aphanipathidae**,** *Aphanipathes verticillata mauiensis*, new subspecies, black coral, range extension.

Introduction

The shallow-water black coral fauna of the Hawaiian Islands is dominated by two species, *Antipathes grandis* Verrill 1928 (see Wagner *et al*. 2010, for detailed description) and *Antipathes griggi* Opresko 2009 (the latter formerly referred to as *A. dichotoma* Pallas 1766, see Opresko 2009, for redescription). Both species have been utilized in the commercial black coral industry (Grigg 2001, 2002; Wagner *et al*. 2010). Although the two species both reach a similar size of 1 meter or more, they can be differentiated by general features of the corallum with *A. grandis* having thinner, wispy terminal branchlets arising from all sides of the lower order branches, and *A. griggi* having thicker, mostly upright, often uniserially arranged, terminal branchlets. In addition, there can be color differences between the two species where *A. griggi* is typically red to reddish-orange while *A. grandis* can be red to pale to white. Recently, another species almost identical in color, size and general branching pattern to *A. griggi* was discovered in the Au‘au Channel at depths of 88 to 130 m. This species, identified as a new subspecies of *Aphanipathes verticillata* Brook 1889, was previously unknown in the Hawaiian Islands. Because it occurs in overlapping depths with *A. griggi*, it can easily be misidentified as that species. The purpose of this paper is to: 1) provide a description of the Hawaiian subspecies of *A. verticillata*, 2) describe how it differs from the typical form, and 3) illustrate the key features that differentiate it from *A. griggi*.

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Material and methods

Samples of the type specimen of *A. verticillata* Brook, consisting of several small branches, were obtained on loan from the Museum of Comparative Zoology (MCZ-68), Harvard University. The larger part of type, which is in the collections of the Zoological Museum of the University of Copenhagen (ZMUC ANT-208) was not available for examination; however, a photograph of this specimen is included here (Fig. 1A). About 20 colonies of *A. verticillata mauiensis*, all collected in the Au‘au Channel between the islands of Maui and Lāna‘i, were available for study. Living polyps were photographed shortly after collection. Photographs of the skeletal spines were made using scanning electron microscopes (SEM) housed at the Biological Electron Microscope Facility (BEMF) at the University of Hawai‘i at Mānoa, and at the U.S. National Museum of Natural History (NMNH), Smithsonian Institution. The Hawaiian specimens have been deposited in the collections of the NMNH (catalog numbers designated as USNM numbers) with subsamples of some specimens retained at the Bernice P. Bishop Museum (BPBM) in Honolulu, Hawai‘i. Analysis of the morphometrics of the skeletal spines was conducted from direct examination of the material using a low-power ocular microscope, by direct examination of photographs taken with the SEMs, or by using the imaging software ImageJ (W. Rasband, National Institute of Health) on SEM images.

DNA sequence data for *A. verticillata mauiensis* were obtained from 16 specimens (for a listing of specimens see “Material Examined“ under the description of the subspecies). GenBank numbers cited in the text refer specifically to specimen #7 collected on Pisces IV, dive 205 (USNM 1127629). Sequence information for this specimen has previously been discussed in Wagner *et al*. (2010) where it was listed as an “undescribed Aphanipathidae.“ All molecular protocols and primers used in this study are the same as those presented in Wagner *et al*. (2010). Total genomic DNA was extracted from material preserved in either ethanol or a DMSO (dimethyl sulfoxide) saturated salt buffer using a CTAB (2% hexadecyltrimethylammonium bromide) protocol as described in France *et al*. (1996), with slight modifications (100 µg Proteinase K; only a single organic, chloroform-based extraction). Primers were designed to flank two mitochondrial segments (*trnW*-IGR-*nad2* [IGR: intergenic region] and *cox3*-IGR-*cox1*, the latter including the 658 base-pair region corresponding to the COI ’Barcode of Life’ [Hebert *et al*. 2003]) and two nuclear regions (ITS1 and ITS2; ITS: internal transcribed spacer). Primer and polymerase chain reaction (PCR) profile information are the same as those presented in Table 2 of Wagner *et al*. (2010). Although anthozoan mtDNA has shown little-to-no intraspecific variation to date (Shearer *et al*. 2002; Hellberg 2006; Chen *et al*. 2008a, 2008b; Forsman *et al*. 2009; Thoma *et al*. 2009; McFadden *et al*. 2010), no nuclear DNA markers other than the ribosomal cistron (18S-ITS1-5.8S-ITS2-28S) are currently available for antipatharians. Thus, we targeted the longest IGR in the *Chrysopathes formosa* Opresko 2003 (GenBank accession no. DQ304771) mitochondrial genome (*trnW*-IGR-*nad2*: 448bp) as IGRs are typically more variable than other gene regions (e.g. McKnight and Shaffer 1997; but see Andolfatto 2005). The *cox1* barcoding region was also chosen as *cox1* provides additional genetic variation to differentiate taxa that is not provided by the *trnW*-IGR-*nad2* region (Brugler 2011). The nuclear ITS regions are currently the most variable markers available for anthozoan phylogenetics, but in some instances they have been shown to contain intra-individual variation (*e.g.*, Wei *et al*. 2006). PCR was performed using *Ex Taq*™ (TaKaRa BioUSA; final concentrations in a 25 µL reaction: 1X *Ex Taq*™buffer [Mg^{2+} free]; 1.5 mM $MgCl_2$; 0.4 mM dNTP mixture; 0.24 µM of each primer; 0.5 units *Ex Taq*™ polymerase; 2.5 µg acetylated bovine serum albumin [Promega BioSciences]; 40–45 ng template). PCR products were isolated using either a 1% low-melt agarose SF gel (Amresco, Inc.), followed by a two-hour to overnight agarase digestion (5 units per 100 µL agarose; Sigma-Aldrich Co.), or a Fermentas clean-up protocol utilizing exonuclease I and FastAP™ thermosensitive alkaline phosphatase (per manufacturer ’s specifications, except that shrimp alkaline phosphatase was replaced with FastAP™). Purified PCR product was cycle sequenced directly in an ABI BigDye® Terminator v1.1 (Applied Biosystems) cycle sequencing reaction following manufacturer’s protocols (except for one-fourth of the recommended ‘Ready Reaction Premix’ in 20 µL total volume reactions), and cleaned using Centri-Sep columns (Princeton Separations; following manufacturer’s protocols) containing DNA-grade Sephadex (G-50 Fine; GE Healthcare). Cycle sequencing products were electrophoresed on an ABI PRISM® 3100 or 3130*xl* Genetic Analyzer. All sequence traces were edited using Sequencher™ version 5.0 (Gene Codes Corporation) and subsequently transferred to Se-Al v2.0a11 Carbon (Rambaut 2002).

Results and Discussion

ANTIPATHARIA

Families in the order Antipatharia are differentiated by the number of mesenteries in the polyps (six in the Cladopathidae; ten in the Aphanipathidae, Myriopathidae, Schizopathidae and Stylopathidae; and 12 in the Leiopathidae), as well as by the size and morphology of the polyps and the morphology of the axial spines. Genera are differentiated mainly by the morphology of the corallum, and species within genera primarily by the size, shape and ornamentation of the axial spines and sometimes also by the size of the polyps (Opresko, 2003). Only recently has the morphology of living polyps been used to infer taxonomic relationships (i.e., the size and dimension of the polyp along both the transverse and sagittal axis, as well as the shape and relative length of the sagittal versus lateral tentacles, both of which are often obscured in preservation). Another character which may prove to be of special importance at the species and subspecies level, and which has only become available with the use of the scanning electron microscope, is the very fine ornamentation of the axial spines.

APHANIPATHIDAE Brook 1889

The species *Aphanipathes verticillata* Brook belongs to the family Aphanipathidae Opresko, 2004. This family is characterized by having skeletal spines with distinct conical tubercles and with the density of spines not increasing substantially on larger branches or stem. Other features of the family include: 1) polypar spines slightly to distinctly larger than the abpolypar spines; 2) maximum height of polypar spines usually more than two times the width at base; and 3) polypar spines either subequal in size, or with the circumpolypar spines slightly to significantly taller than other polypar spines and with the hypostomal spines similar in size to circumpolypar spines or greatly reduced or absent. The corallum of aphanipathids can be irregularly bushy or flabellate; the stem and branches can be simple or pinnulate. Pinnulated species do not have subpinnules. The polyps of aphanipathids are mostly 0.7–1.3 mm in transverse diameter (range 0.5 to about 2.5 mm).

Part of the original diagnosis of the family (Opresko, 2004) included a description of the tentacles of polyps as possibly being short, blunt, and subequal in size. Although the tentacles do have such an appearance after preservation and may have a similar appearance when the polyps are alive and partially contracted, there is some evidence that in fully expanded polyps the sagittal tentacles are longer than the laterals and the tips of the tentacles are pointed rather than rounded. In this regard the polyps would be similar to those found in the family Antipathidae.

Aphanipathes Brook 1889

Type species. *Aphanipathes sarothamnoides* Brook 1889 (see Opresko 2004 for discussion).

Diagnosis. Colony sparsely to densely, irregularly branched, bushy, sometimes broom-like, with short to long, straight or curved, often ascending branches. Branches not pinnulated.

Aphanipathes verticillata verticillata Brook 1889, emended

(Figures 1A, 2, 3)

Material examined. Schizoholotypes (three small branches) in the collections of the Museum of Comparative Zoology (MCZ 68) [NOTE: holotype in the University of Copenhagen Museum (ZMUC ANT 208) not examined]; USNM 99727, Japan, Ryukyu Islands, Okinawa Island, 26.50000° N, 127.84833°E, Horseshoe Cliffs, 1 km WNW of Onna Village, R. F. Bolland, 14, Nov. 1981, 79.2 m. (Coll. No. RFB 0996; 1 specimen, dry).

Type locality. Mauritius (no other data).

Diagnosis. Colony large (at least 80 cm in height), densely branched to the 10th order or more, with long, straight or slightly curved branches often disposed in single series on upper side of lower order branches (Fig. 1A). Distal branch angles variable, most frequently from about 45º to 60º. Polypar spines on branchlets 0.18−0.30 mm tall; abpolypar spines up to 0.2 mm; spines in longitudinal rows and in distinct verticils; spines mostly 0.25−0.36

mm apart in each row. Spines covered with minute conical tubercles extending from apex basally half the distance or more to the base. Tubercles present on both polypar and abpolypar spines. Tubercles on some spines becoming fused together laterally, and in some cases with fused apices forming wide shelf-like edge. Tubercles on distal edge of spines subequal or slightly larger than those on proximal edge. Height of tubercles along distal edge of polypar spines up to about 0.016 mm. Average density of tubercles on polypar spines 2.9/1000 μm^2. Polyps about 1.4 mm in transverse diameter, arranged in single series, with six to seven polyps per centimeter.

FIGURE 1. A. *Aphanipathes verticillata verticillata* Brook, holotype (ZMUC ANT-208); height ~80 cm (Brook, 1889) (Note: scale not available); B. *A. verticillata mauiensis* subsp. nov., holotype (USNM 1150095), scale bar = 10 cm.

Description of the holotype. Based on the description given by Brook (1889), the holotype is 80 cm tall and 70 cm wide. A photograph of the type (Fig. 1A, scale not available) indicates that the branching is moderately dense, with many branches arranged uniserially. Brook (1889) reported that as many as six branchlets arose along a 3 cm length of branch, and that many of the branches were 6–10 cm long. Spines of the MCZ schizotypes (Fig. 2) are conical, slightly inclined distally (abpolypar spines inclined more than polypar spines), and with numerous tubercles on their surface. On branchlets 0.3 mm to 0.4 mm in diameter, the polypar spines typically are 0.24 to 0.28 mm tall (as measured from middle of base to apex), and the abpolypar spines are generally 0.14 to 0.2 mm. On a branch 0.4 mm in diameter the polypar spines measure about 0.20 mm and the abpolypars 0.14 mm. Six or seven longitudinal rows of spines seen in one lateral view on branchlets (excluding rows where spines only partially visible). Distance between centers of bases of spines in same row 0.27 to 0.36 mm, with about four spines per millimeter in each row (equivalent to four verticils per millimeter). Tubercles present on both polypar and abpolypar spines; conical, simple or double or triple peaked, some with fused apices developing into broad, shelf-like edge; latter conditions resulting from fusions of adjacent tubercles. Size of tubercles quite variable; largest usually along distal edge; up to about 0.016 mm tall. Number of tubercles and number of fused tubercles vary from spine to spine and from branchlet to branchlet. Counts of tubercles made on three subsamples of schizotypes gave a mean density of 2.9 tubercles per 1000 μm^2 (N = 24). Spines having compound and fused tubercles occur more commonly on those parts of branchlets with a diameter of about 0.35 mm (excluding spines), whereas thinner and thicker parts of branchlets usually only have simple tubercles (NOTE: branchlets exceeding about 0.44 mm in thickness were not available for examination). On spines where only simple tubercles present, about 25 to 30 tubercles per spine visible in lateral view (including tubercles on distal and proximal edges). On spines with fused tubercles, estimated number of original single tubercles as high as 40 or more. Because only a few branchlets of the type were available for study, it cannot be concluded that fused tubercles are typical of the species, and in the original description of the species, fused tubercles are not mentioned, nor are they shown in Brook's illustration (Plate 12, fig. 25). Brook's illustration, however, is of a branchlet only 0.23 mm in diameter and, as noted above,

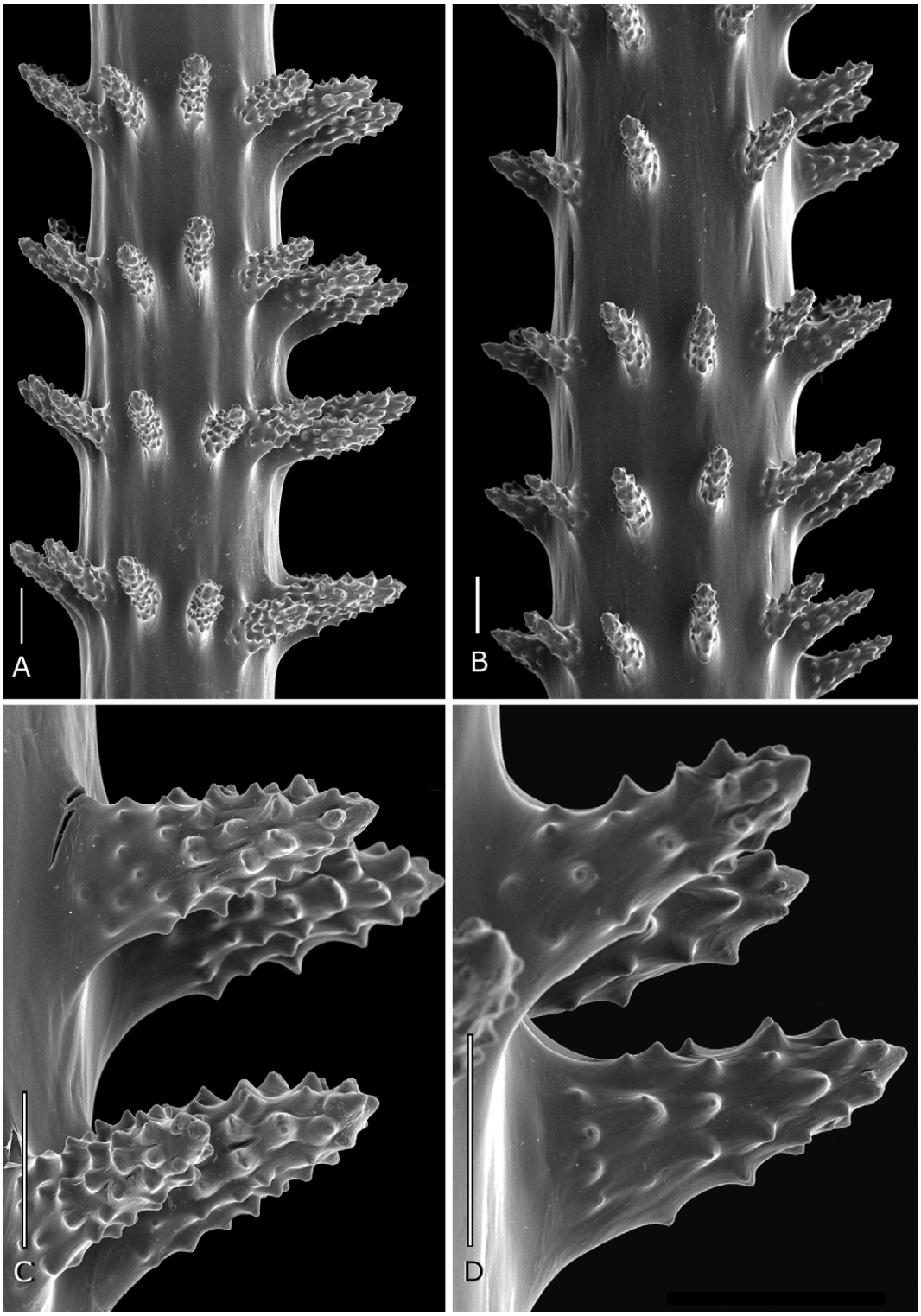

FIGURE 2. *Aphanipathes verticillata verticillata* Brook, schizoholotype (MCZ 68), spines on branchlets. Scale bars = 0.10 mm.

fused tubercles are not common on such thin branchlets. Additional subsamples from the type as well as additional specimens from the type locality are needed to determine how common fused tubercles are within *A. verticillata verticillata* (see also discussion of the specimen from Okinawa below).

Brook reported that the polyps in the type are arranged in a regular longitudinal series and that there were about six polyps per centimeter. On the MCZ schizotypes, three to four polyps occur over a branchlet distance of 5 mm, resulting in about seven polyps per centimeter. The transverse diameter of the individual polyps is about 1.4 mm.

Description of specimen from Okinawa. The specimen consists of two branched pieces that may have come from the same colony, one is 45 cm tall and the other 42 cm. A uniserial branching pattern is common in both. The

longest terminal branches are mostly 6−7 cm long (maximum 10 cm). A 6 cm branchlet is about 0.3 mm in basal diameter excluding spines and 0.6 mm including spines. Spines (Fig. 3) are quite variable in size; the polypar spines range from about 0.18 to 0.30 mm on terminal branchlets; the abpolypar spines are 0.12−0.20 mm. Spines 0.35 mm tall are found on branches 0.8 mm in diameter. Spacing of the spines in one row is generally 0.25 to 0.31 mm. Spines on branchlets are similar to those in the type in having some double and triple tubercles, and a few fused tubercles with shelf-like edges. Average density of tubercles is also similar to that of the type, 2.9 per 1000 μm^2 (range 1.1−4.2 per 1000 μm^2, N = 21). Polyps not present.

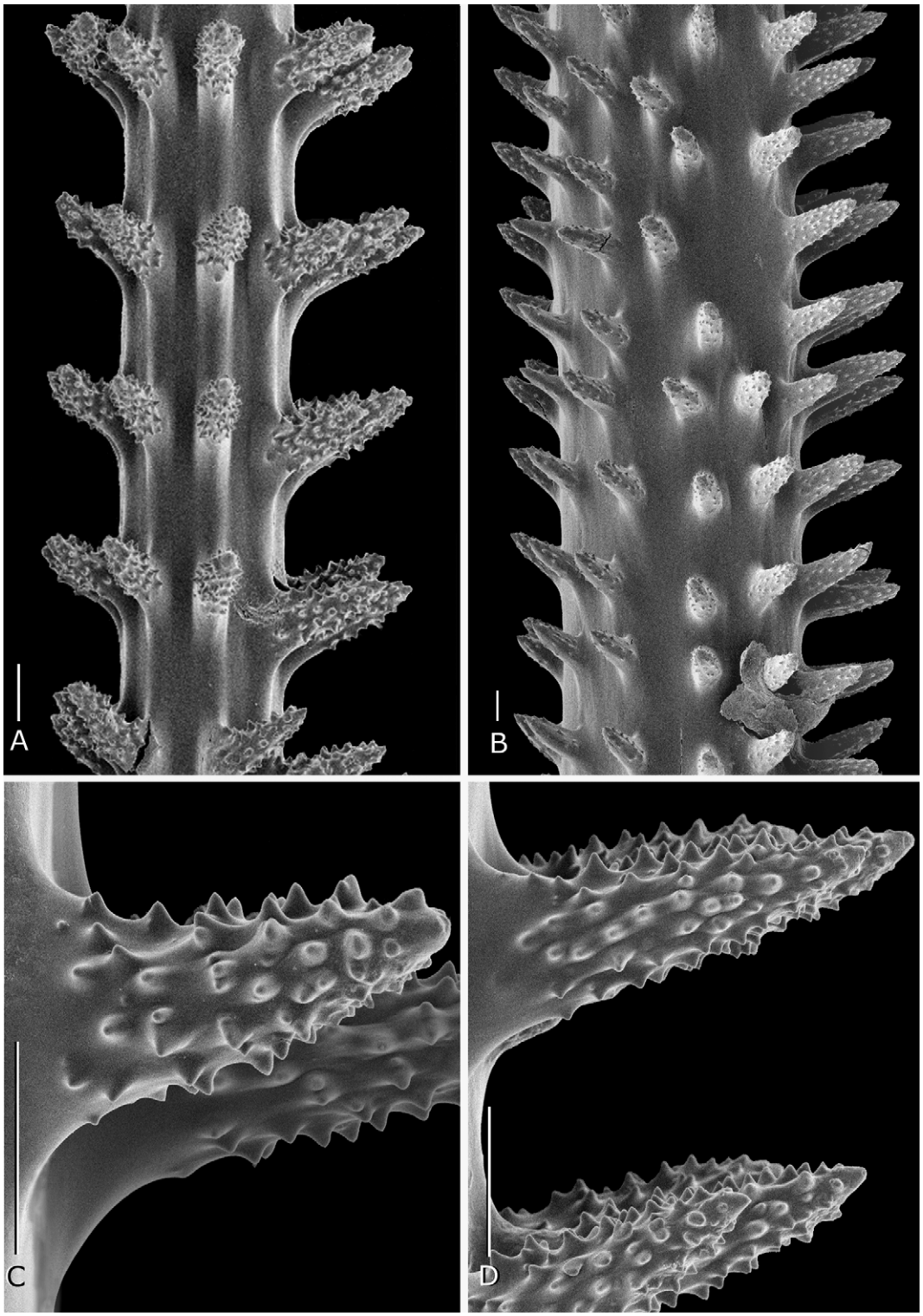

FIGURE 3. *Aphanipathes verticillata verticillata*, specimen from Okinawa (USNM 99727); A and C, spines on small branchlet; B and D, spines on branch. Scale bars = 0.1 mm.

***Aphanipathes verticillata mauiensis* subsp. nova**
(Figures 1B, 4–6, 8A–D)

Material examined. Holotype (USNM 1150095*; schizoholotype BPBM D-1877), Au‘au Channel, between the islands of Maui and Lāna‘i, Hawaiian Islands, 20.9409ºN, 156.7609ºW, 88 m, Pisces V, Dive 739, 7 April 2009, Tony Montgomery (large dry specimen labeled “bench”and small subsample with polyps preserved in alcohol for DNA analysis). Additional specimens all collected from the Au‘au Channel: four samples from Pisces V, Dive 739, 91–127 m [USNM 1150087* (BPBM D-1875), 1150092* (BPBM D-1876), 1150093* (BPBM D-1878), 1150094* (BPBM D-1879)]; six samples from Pisces V, Dive 716, 88–130 m (USNM 1157687; 1157500*, 1157501, 1157502*, 1157503, 1157504*), two of which with subsamples deposited at the BPBM (BPBM D-1873, D-1874); three specimens from Pisces IV, Dive 204, 90–113 m (USNM 1127629*, 1127632, 1157505); four specimens from Pisces IV, Dive 205, 97–111 m (USNM 1127630*, 1127631*, 1127633*, 1127635); and four samples from Pisces IV, Dive 206, 93–114 m (USNM 1128318*, 1128389*, 1128320*, 1157506*). [NOTE: Specimens whose USNM number is followed by an asterisk were included in the DNA analysis].

Diagnosis. Colony size and shape similar to that of *A. verticillata verticillata*; large, sparsely to densely branched to 12th order or more. Branches long, straight or slightly curved, often disposed in single series on upper or lower side of next lower order branches. In places, successive orders of branches arising on the same, usually outer side of lower order branches. Spines on branchlets conical to slightly compressed laterally, arranged in longitudinal rows and in verticils, and covered with minute conical tubercles. Maximum size of polypar spines on branchlets typically 0.19−0.27 mm (up to 0.30 mm); abpolypar spines mostly 0.09−0.15 mm tall. Six to nine longitudinal rows of spines visible in lateral view on branchlets. Spines mostly 0.28−0.36 mm apart within each row (maximum about 0.5 mm). Tubercles usually simple, conical, with an average density of 2.4 per 1000 μm^2 (range 1.62−3.50 per 1000 μm^2; N = 7). Largest tubercles (0.012−0.015 mm) on distal edge of spines; proximal edge sometimes with very few tubercles. Polyps mostly 1.2−1.5 mm in transverse diameter (range 0.72−1.81 mm; mean 1.35 mm; N = 421) with interpolypar space of 0.3−0.4 mm. Polyps arranged in single series on branchlets, with 5−8 polyps per centimeter (mean 5.6/cm; N = 90).

Description of holotype. Colony (USNM 1150095) about 80 cm tall (Fig. 1B). Branches spread out in all directions so that overall width of colony about 70 cm. Basal plate 6 cm in diameter. Multiple stems arise from basal plate; thickest of these is about 1 cm in diameter. Colony branched to 12th order; largest branches near base of corallum spread out somewhat laterally, with next highest order branches in many cases arranged uniserially on upper side, and extending vertically. Major branches are up to 35 cm in length; with 10 to 15 branches of next higher order occurring along 10 cm. Spacing of branches very variable, from 0.5 cm to 2 cm or more apart; generally, not more than two or three branches per 2 centimeters. On upper parts of corallum five or more orders of branches can arise successively on same side of each lower order branch, often on the convexly curved outer side. Most branches of corallum vertically directed although some on outer edges extend out somewhat horizontally. Distal branch angle very variable; mostly 30º to 60º; wider where lower order branches are upright, although in such cases higher order branches curved upward to become parallel to lower order branch from which they arose. Smallest terminal (unbranched) branchlets reaching maximum size of about 8 cm. Branch 7 cm long about 0.8 mm in diameter at proximal end, including spines, and about 0.4 mm in diameter excluding spines; diameter at midpoint 0.6 mm (total) and about 0.35 mm excluding spines. Average midpoint diameter of terminal branchlets 0.60 mm with spines included and 0.36 mm excluding spines (N = 10). Distal 3 cm of branchlets often collapsed when dried due to thinness of sclerenchyme.

Spines on branchlets generally conical, but slightly compressed laterally, with relatively acute apex and covered over much of their surface with small conical tubercles (Figs 4–5). Spines unequal in size around circumference of axis; polypar spines on terminal branchlets mostly 0.2 to 0.24 mm in height (from middle of base to apex), but up to 0.30 mm; abpolypar spines on branchlets mostly 0.14 to 0.18 mm in height (range 0.13−0.22 mm). On branchlets, six to nine longitudinal rows of spines visible in lateral view. Distance of adjacent spines in one row mostly 0.3−0.34 mm (range 0.22−0.38 mm). Polyps mostly 1.1−1.4 mm in transverse diameter, arranged on branchlets in a single series with 6−7 per centimeter.

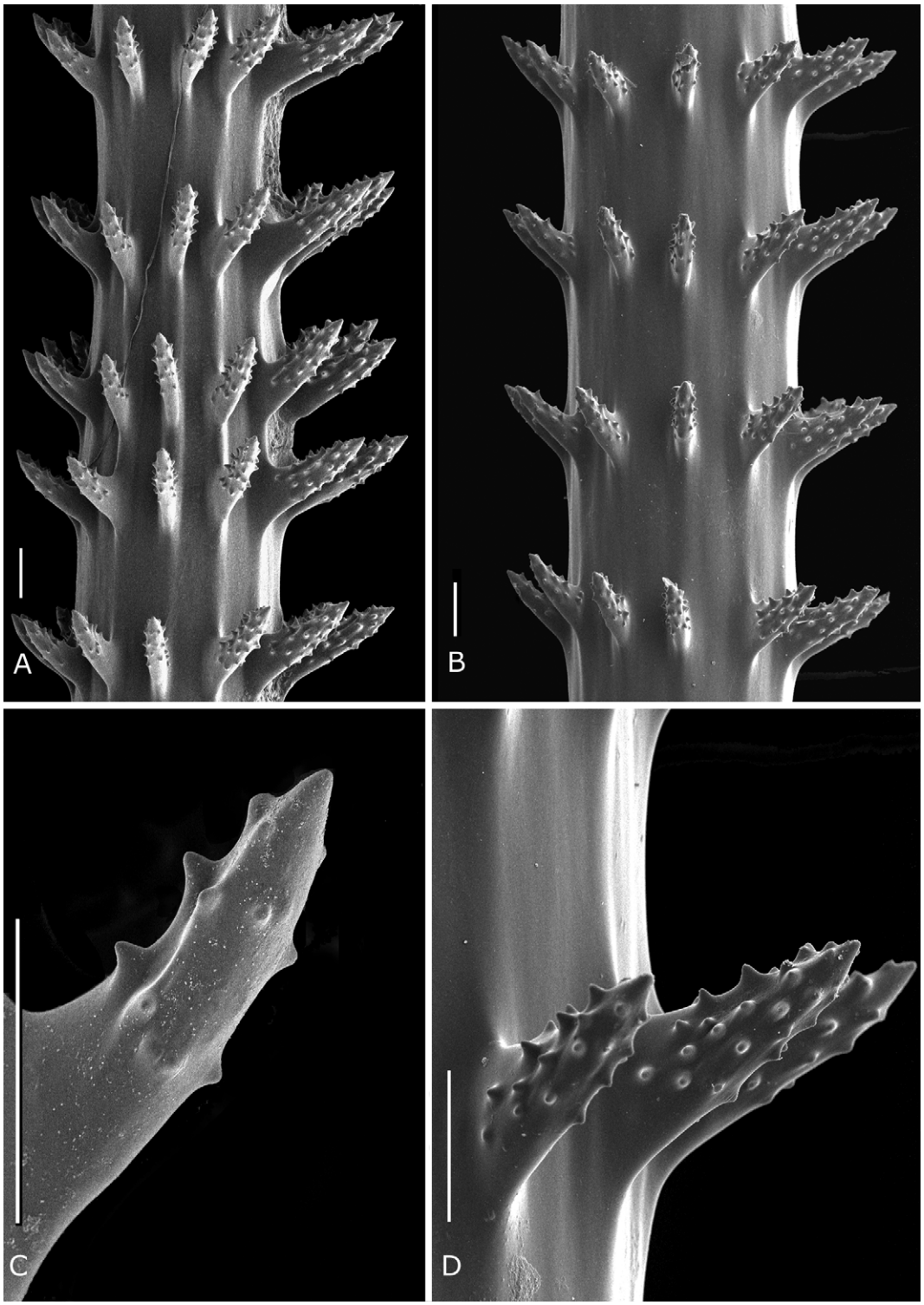

FIGURE 4. *Aphanipathes verticillata mauiensis*, holotype (USNM 1150095), A–D, spines on branchlets. Scale bars = 0.1 mm.

Morphological variation. Twenty samples of *A. verticillata mauiensis* have been collected to date, including 10 complete colonies, ranging in height from 25 to 150 cm. A summary of the major morphometric features of these specimens is presented in Table 1. As has been described for other species of antipatharians, characters associated with the skeleton and polyps can be quite variable. Several of the specimens are sparsely branched with little evidence of a uniserial arrangement of higher order branches (Fig. 8C), whereas in others this feature is evident throughout the colony (Fig. 8A). In the samples examined, the height of the polypar spines (from middle of base to apex) ranged from 0.09 to 0.30 mm (mean 0.17 mm), that of the abpolypar spines from 0.05 to 0.20 mm (mean 0.12 mm); the spacing of the spines ranged from 0.20 to 0.51 mm (mean 0.32 mm); the transverse diameter of the polyps from 0.72 to 1.81 mm (mean 1.35 mm); and the density of the polyps from 4.49 to 7.79 per centimeter (mean 5.85 per centimeter). Wide variation in one

character can occur within the same colony. For example, the size of the polypar spines of the holotype (USNM 1150095) ranges from 0.19 to 0.30 mm, and the polyp density in specimen B2 from Pisces V, Dive 739 ranges from 5 per centimeter on some branchlets up to 8 per centimeter on others. However, there are also specimens where a character may be consistently above or below the average as in a specimen from Pisces V, Dive 716 (specimen No. 6) where the polypar spines consistently measure, at least in the subsamples examined, not more than 0.15 mm in height. To estimate the typical range in maximum size of the polypar spines, the maximum size for each of the 20 specimens was recorded and an evaluation was made of the size frequency distribution (Fig. 6). For ninety percent of the colonies the maximum size of the polypar spines falls in the range of 0.19−0.27 mm, with a mean and median of about 0.22 mm.

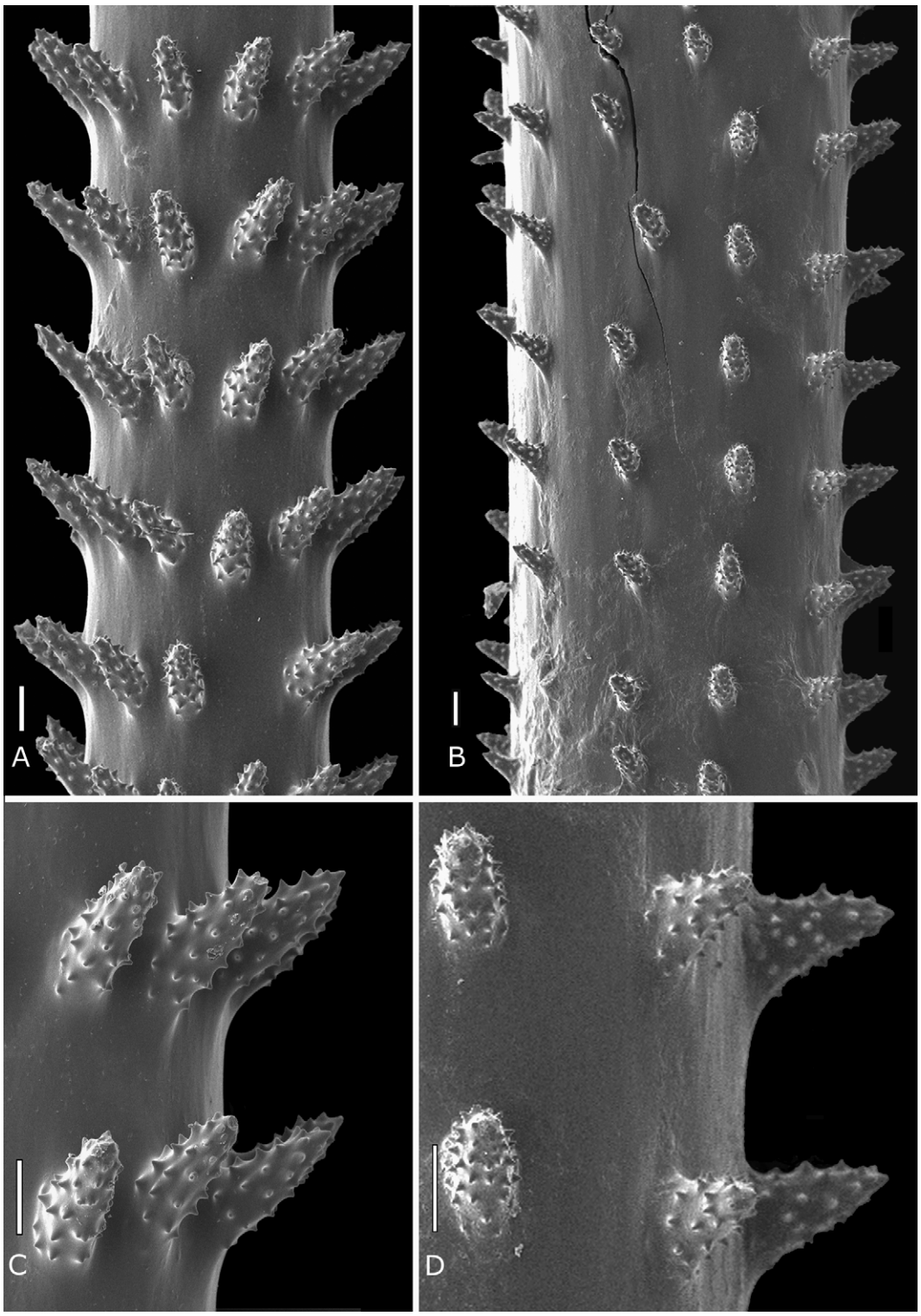

FIGURE 5. *Aphanipathes verticillata mauiensis*, holotype (USNM 1150095), A–D, spines on branches. Scale bars = 0.1 mm.

TABLE 1. Summary of Morphometrics for Specimens of *A. verticillata mauiensis*

Character	**Mean**	**Std. Dev.**	**Range**
Branch diameter at midpoint (mm)	0.90	0.13	0.60–1.23
Polyp transverse diameter (mm)	1.35	0.17	0.72–1.81
Polyp spacing (mm):	1.71	0.26	0.89–2.76
Polyp density (#/cm):	5.85	0.68	4.49–7.79
Branchlet diameter including spines (mm):	0.67	0.15	0.36–1.20
Branchlet diameter excluding spines (mm):	0.40	0.13	0.19–0.89
Polypar spine height (mm):	0.17	0.038	0.09–0.30
Abpolypar spine height (mm):	0.12	0.028	0.05–0.20
Spine spacing (mm):	0.32	0.042	0.20–0.51

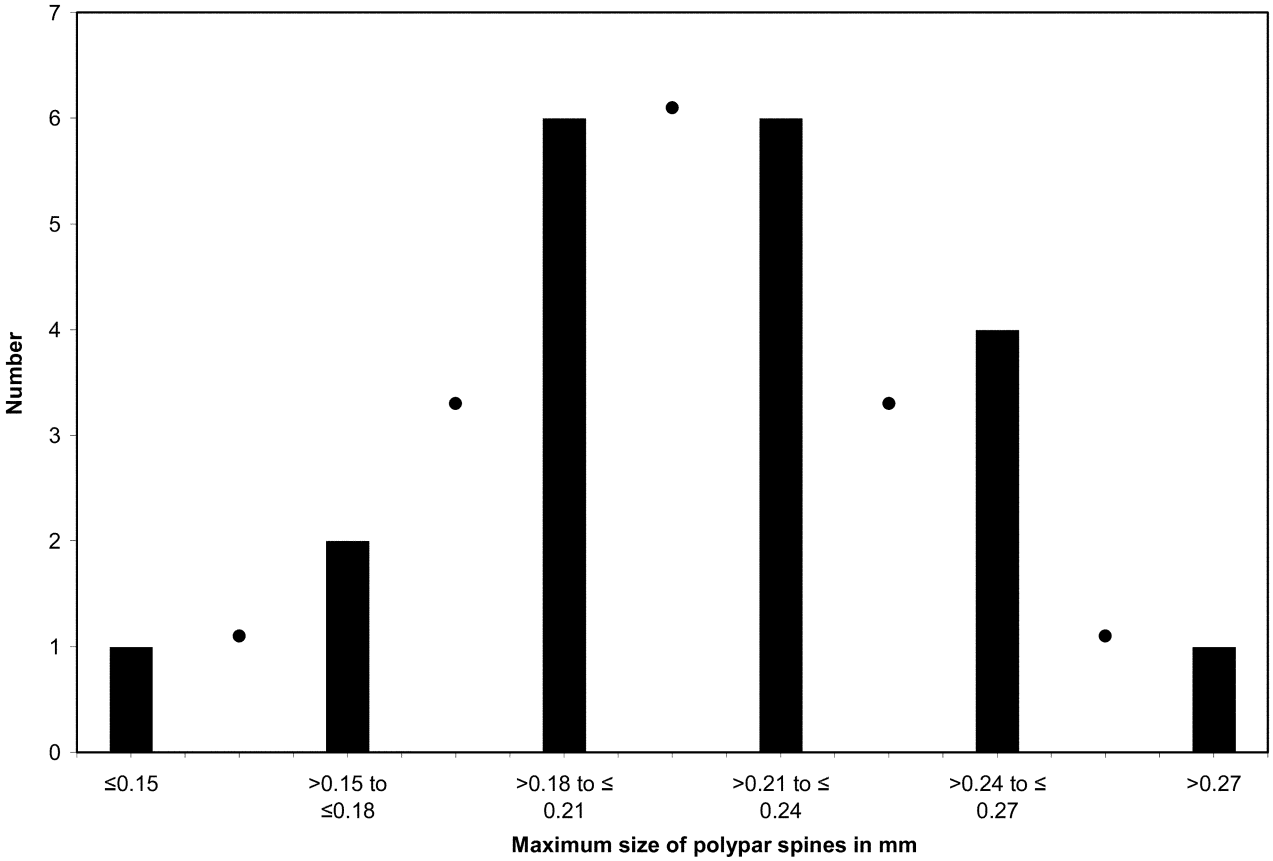

FIGURE 6. *Aphanipathes verticillata mauiensis*. Size frequency distribution of maximum size of polypar spines on branchlets (N = 20); dots indicate a normal distribution pattern.

Genetic Variation. Given the morphological variation in colony appearance and in characters such as the polyps and size of the spines, the possibility existed that a cryptic species was present among the suite of specimens examined. To address this issue, DNA studies of the new subspecies were carried out by coauthor M. Brugler.

Due to the low molecular weight of DNA obtained from several individuals, only a 16-member subset of the colonies analyzed morphologically were screened at the molecular level. Although sequence coverage was slightly reduced, the same four gene regions sequenced in Wagner *et al*. (2010) were analyzed (mitochondrial [mt] *cox3*-IGR-*cox1* [GenBank accession number HM060617], mt *trnW*-IGR-*nad2* [HM060612], nuclear ITS1 [HM060619], nuclear ITS2 [HM060627]). Four individuals screened at mt *cox3*-IGR-*cox1* shared identical haplotypes (alignment length: 1,168 bp; includes 124 bp *cox3*, 259 bp *cox3*-*cox1* IGR, and 785 bp *cox1*). The same held true for fifteen individuals screened at mt *trnW*-IGR-*nad2*: all individuals shared identical haplotypes (alignment

length: 614 bp; includes 448 bp *trnW-nad2* IGR and 166 bp *nad2*). Three segments of the nuclear ribosomal cistron were analyzed for two individuals, both of which shared identical sequences (noncontiguous alignment length: 791 bp; segment 1 includes 145 bp 18S and 156 bp ITS1; segment 2 includes 9 bp ITS1, 158 bp 5.8S, and 67 bp ITS2; segment 3 includes 256 bp 28S). Given that mt and nuclear DNA revealed no differences and that the internal transcribed spacers are currently the most variable markers within anthozoans, the hypothesis that can be advanced is that the specimens included in the description of *A. verticillata mauiensis* do not include any cryptic species.

Comparison of Genetic and Morphological Variation. There were fourteen specimens for which we had both morphological and genetic data. Across these fourteen specimens the diameter of the terminal branches ranged from 0.84 to 1.23 mm; the maximum transverse diameter of the polyps varied from 1.4 to 2.3 mm; the polyp density from 5.6 to 7.8 per centimeter; and the maximum size of the polypar spines from 0.15 to 0.30 mm. Thus, the genetic uniformity seen in these specimens is not reflected in morphological uniformity, and this is a clear indication of the morphological plasticity of this subspecies. The presence of such morphological plasticity necessitates the use of extreme care in describing new antipatharian taxa from a limited number of specimens as these may represent the extreme ranges of a single species. As in this case, future taxonomic studies of antipatharians would benefit greatly from using both morphological and genetic characters.

Genetic Comparisons. Unfortunately, soft tissue material suitable for DNA analysis was not available from the type specimen of *A. verticillata verticillata* or from any specimens that could be referred to the typical form; therefore, a genetic comparison of the two subspecies was not possible. However, a specimen of *A. verticillata mauiensis* was included in a large phylogenetic analysis of the Antipatharia conducted by Brugler (2011). In that study, representatives of the genus *Aphanipathes* did not form a monophyletic clade. *A. verticillata mauiensis* (referred to in that work as 'Aphanipathidae n.sp. 1, Pisces IV-205-7 [new ID – *Aphanipathes* cf. *verticillata*]'), grouped sister to the largest clade of antipathids (Antipathidae was polyphyletic). A specimen of *Aphanipathes pedata* from the Gulf of Mexico(DFH11-8A), a specimen identified as *Antipathes* sp. (USNM-1086470), and a specimen identified as *Aphanipathes* n.sp. from Hawaii (USNM-1010741) all grouped within the larger clade of aphanipathids (Aphanipathidae was also polyphyletic), but the first specimen was more closely related to *Phanopathes* spp. while the latter two were more closely allied with *Stichopathes spiessi* and *S. dissimilis*, two species currently assigned to the family Antipathidae, but with morphological affinities to the Aphanipathidae. These results suggest that the current toolbox of morphological characters used to differentiate taxa may need to be reevaluated in light of recent (Brugler 2011) and upcoming phylogenetic reconstructions of the order Antipatharia (i.e., Sánchez, J.A., Brugler, M.R., Miller, K., Umaña, C., Dueñas, L., Opresko, D., *in prep*. The evolutionary history of the order Antipatharia [black corals] as inferred from the predicted secondary structure of ITS2).

Distribution. Presently only known from the Keyhole Pinnacle in the Au'au Channel between the Islands of Maui and Lāna'i, in 88−130 m.

Comparison of *A. verticillata mauiensis* with *A. verticillata verticillata*. The only differences between the two subspecies are the density and morphology of the tubercles on the spines. On the schizotypes of *A. verticilllata verticillata*, as well as on the specimen from Okinawa, the tubercle density is 2.9 per 1000 μm^2, whereas measurements made on the holotype of *A. verticillata mauiensis* (USNM 1150095) from Hawai'i gave an average density of 2.4 per 1000 μm^2; a difference that is statistically significant at $p = 0.005$ (Mood's Median). Furthermore, there is a distinct difference between the two subspecies in the occurrence of fused tubercles; with the Hawaiian subspecies usually having only simple tubercles (very rarely a double tubercle may be found) and the typical form having, on some branchlets, fused tubercles with two or three apices, which in some cases form wide, shelf-like edges.

The recognition of the Hawaiian form as a subspecies is based on our current state of knowledge that the morphological differences described above are confined to geographically separated populations. In all other respects, and particularly in the verticillate arrangement of the spines, these two forms are more closely related to each other than to other members of the genus (see discussion below). The designation of infraspecific forms is not unusual in antipatharians (see Schultze 1902; van Pesch 1914), and is a reflection of the typical wide range of variation seen in most morphological characters, especially when dealing with large suites of specimens. Furthermore, it has been reported that geographic dispersion of antipatharians is likely to be limited due to the planulae being negative bouyant, non-feeding, and short-lived (Miller 1996); therefore, it would not be unexpected to find isolated populations forming unique morphotypes. Testing this hypothesis would require DNA markers for the analysis of intra-individual and inter-individual (population level) genetic variation; however, such markers are

not yet available for antipatharians. In the present case, such investigations would also be limited by the lack of fresh tissue samples of *A. verticillata verticillata*. We consider this a very important area for future investigation, not only for the species discussed here, but for antipatharian taxonomy in general.

Comparisons to other species of *Aphanipathes*. The genus as revised by Opresko (2004) consists of four nominal species: *Aphanipathes sarothamnoides* Brook 1889; *Antipathes salix* Pourtales 1880; *Aphanipathes verticillata* Brook 1889; and *Antipathes pedata* Gray 1857. All these species have an irregularly branched, bushy corallum, with relatively straight and usually elongated, upright branches. In all species except *A. salix*, the branches and branchlets tend to be arranged uniserially, at least on parts of the corallum. Morphometric comparisons of the four species are presented in Table 2. A lack of a sufficient number of specimens prevents a detailed statistical comparison of the taxa, and, in fact, the information presented for *A. verticillata verticillata*, *A. sarothamnoides*, and *A. salix* is based on just a few specimens. Therefore, differences seen in some of the values listed cannot be assumed to be definitive, since examination of additional specimens of each species is likely to increase the range of the values for each of the characters. There are, however, differences among the species in the morphology of the spines, particularly in the extent that the surface of the spines is covered with tubercles (Fig. 7). In general (exceptions are likely to occur), the tubercles on the spines of *A. salix* tend to be few in number and mostly near the apex, but with a few extending down to about half the distance to the base with an average tubercle density of 2.2 per 1000 μm^2 (range 1.8−2.4 per 1000 μm^2, N = 6); those of *A. sarothamnoides* cover about one-third to one-half the distance from the apex with an average tubercle density of 2.3 per 1000 μm^2 (range 1.6−3.0 per 1000 μm^2, N = 6); those of *A. pedata* cover one-half to three-quarters of spine surface with an average tubercle density of 3.2 per 1000 μm^2 (range 2.8−3.6 per 1000 μm^2, N = 7); whereas those of both forms of *A. verticillata* cover one-half to the entire surface with an average tubercle density of 2.9 per per 1000 μm^3 in the typical form and 2.4 per 1000 μm^2 in *A. verticillata mauiensis*. *Aphanipathes verticillata* is the only species in which the spines are arranged in verticils, although on some branches in some colonies the verticillate arrangement may not be very distinct.

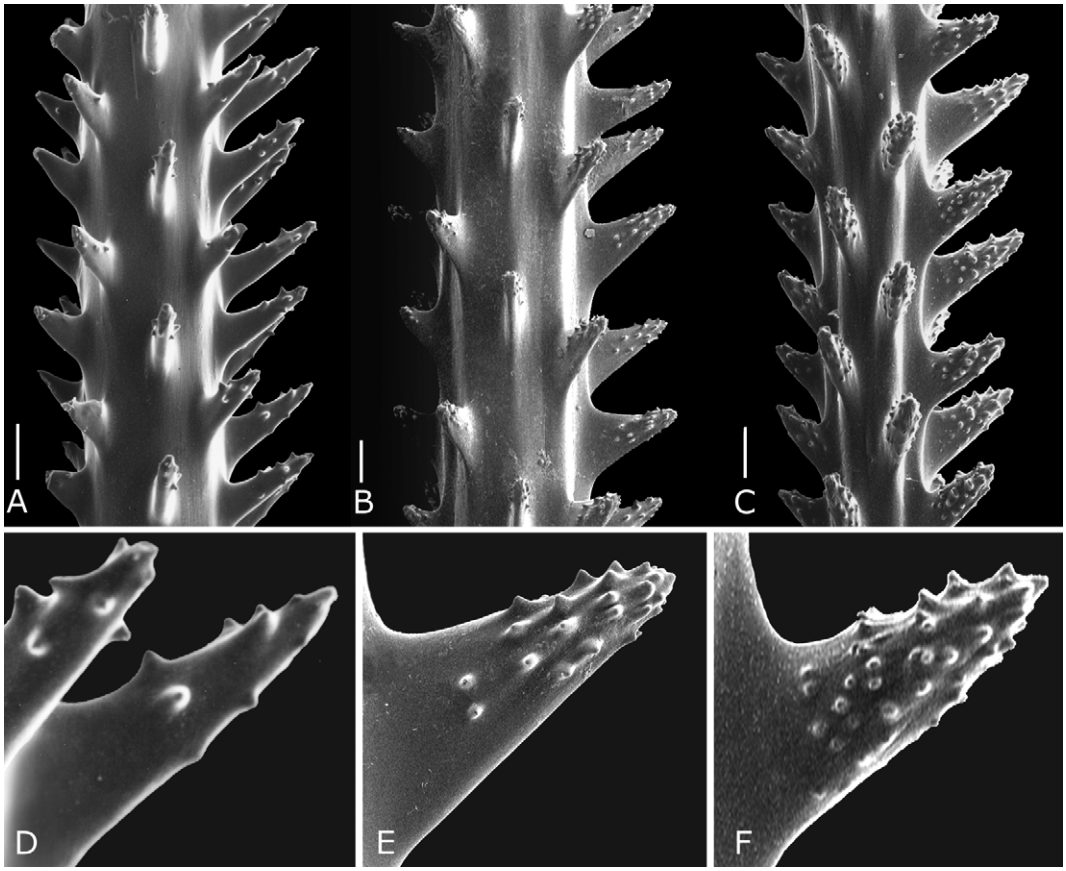

FIGURE 7. *Aphanipathes* species; spines on branchlets: A and D, *A. salix* (Pourtales); B and E, *A. sarothamnoides* Brook; C and F, *A. pedata* (Gray). Scale bars = 0.1 mm.

TABLE 2. Comparisons of Species of *Aphanipathes* and *A. verticillata mauiensis*

	A. verticillata *verticillata*[a]	*A. verticillata* mauiensis	*A. sarothamnoides*[b]	*A. salix*[c]	*A. pedata*[d]
Author	Brook, 1889	this report	Brook, 1889	Pourtales, 1880	Gray, 1857
Distribution	Mauritius and Okinawa	Hawai'i	New Hebrides, SW Pacific	Guadaloupe, Caribbean	West Indies
Depth range	79.2 m	88–130 m	115–238 m	335 m	60–309 m
Max. reported colony height (m)	0.8	1.5	0.3[e]	0.13[e]	0.5[e]
Max. terminal branchlet length (cm)	10	8	7	3	10
Transverse diameter of polyps (mm)	~1.4	1.2–1.5 (1.8)[f]	1.3	1.2	1.2–1.7
Polyp density (per cm)	6–7	5.2–6.5 (7.8)[f]	8	6	5–8
Height of polypar spines on branchlets (mm)	0.18–0.30	0.19–0.27 (0.30)[g]	0.20–0.26	0.20–0.22	0.22–0.29
Height of abpolypar spines on branchlets (mm)	0.12–0.20	0.09–0.15 (0.20)[f]	0.10–0.13	0.10–0.13	0.10–0.20
Spacing of spines in one row on branchlets (mm)	0.25–0.36	0.28–0.36 (0.51)[f]	0.29–0.51	0.26–0.31	0.25–0.33
Rows of spines on branchlet (one view)	6–7	6–9	6–8	5–7	7–9
Tubercle density (/1000 μm^3)	2.9	2.4	2.3[h]	2.2[h]	3.2[h]

[a]Data from Brook (1889) and direct observations made on type and specimen from Okinawa (USNM 99727).
[b]Data from Brook (1889), Opresko (2004) and direct observations made on type; no other specimens.
[c]Data from Pourtales (1880), Opresko (1972) and direct observations made on type; no other specimens.
[d]Data from Brook (1889), Opresko (1974) and direct observations made on type and 8 specimens in RSMAS collections.
[e]Incomplete colony; no holdfast.
[f]Range presented is ± one standard deviation from the mean (see Table 1); maximum shown in parentheses.
[g]Range represents 90% of the values around the mean maximum value of 0.22 mm (N = 20); maximum shown in parentheses.
[h]Data from spines on single branches illustrated in Fig. 7 of this report.

Comparison of *Aphanipathes verticillata mauiensis* with *Antipathes griggi*. The two species are generally indistinguishable in the field; both reach a maximum size of 1 m or more, both are a reddish or reddish-orange when alive, and both tend to grow with upright branches which tend to be uniserially arranged on the lower order branches (Fig. 8). Specimens can occasionally be discerned in the field by a close evaluation of the polyp color. Polyps of *A. verticillata mauiensis* often have tentacles that are lighter in color at the tips and this can be a distinguishing character when present. The two species are similar in the size of the polyps, mostly 1.2−1.3 mm in *A. griggi* and 1.2−1.5 mm in *A. verticillata mauiensis*, and in the size of the polypar spines, generally 0.20−0.26 mm tall in *A. griggi* and 0.19−0.27 mm in *A. verticillata mauiensis*. The major differences in the two species occur in the arrangement and morphology of the spines. The spines of *A. verticillata mauiensis* are arranged in distinct verticils (Fig. 8D) whereas those of *A. griggi* are not (Fig. 8H). Furthermore, in *A. griggi* the spines are often bifurcated, multi-lobed, or jagged at the apex, with small, round to elongate papillae on the surface (Fig. 8H), whereas those in *A. verticillata mauiensis* are pointed or rounded at the tip, and covered with very distinct conical tubercles (Fig. 8D). In addition to the above described morphological differences, a recent comparison between *A. griggi* and *A. verticillata mauiensis* (identified only as an undescribed species of Aphanipathidae) using nuclear and mitochondrial DNA sequences also revealed substantial molecular differences between these two species (see Wagner *et al*. 2010, Fig. 10).

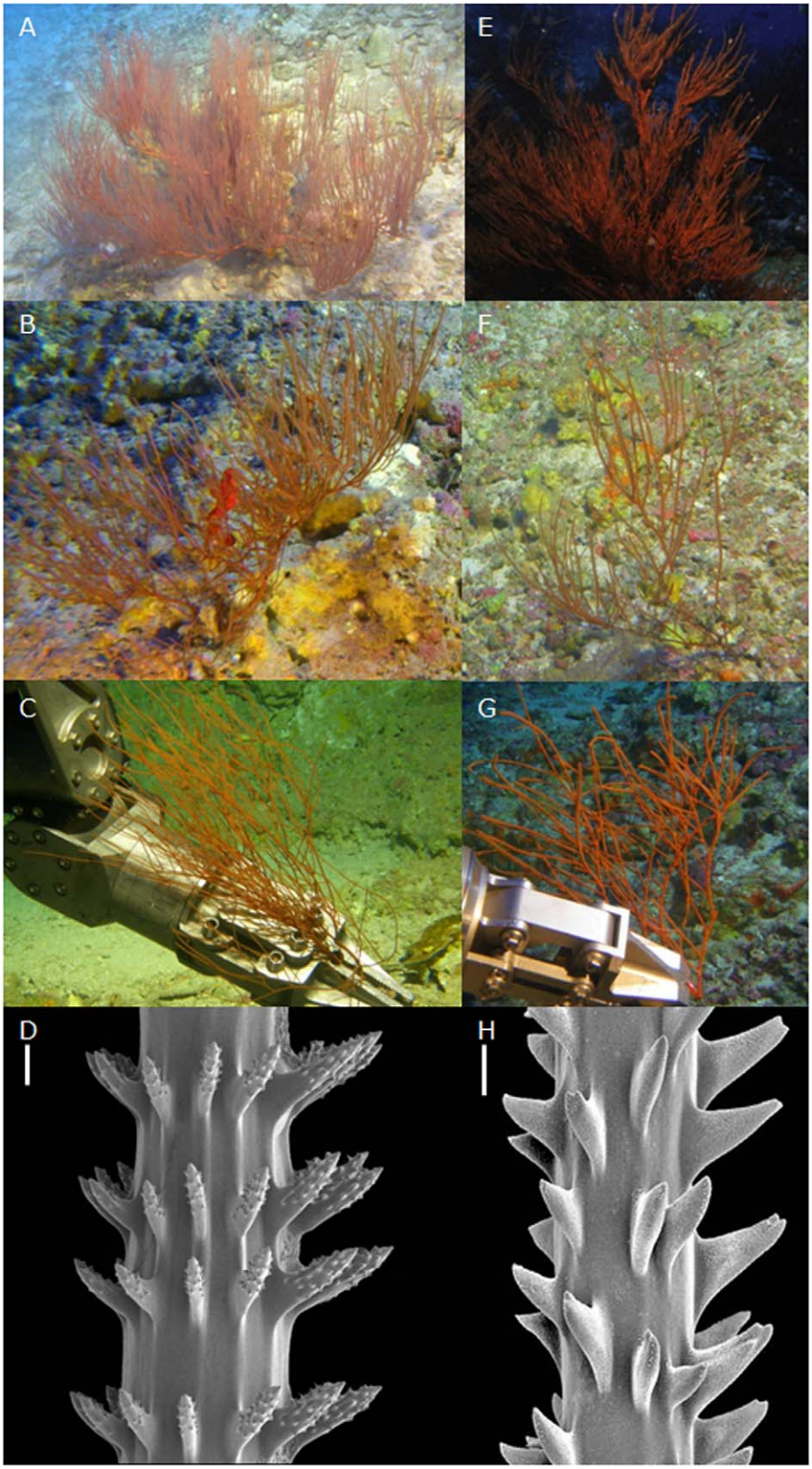

FIGURE 8. Morphological comparison between sympatric species *Aphanipathes verticillata mauiensis* (left; A–D) and *Antipathes griggi* (right; E–H). A. *A. verticillata mauiensis* holotype (USNM 1150095), *in situ*. B. USNM 1128320, *in situ*. C. USNM 1150087, *in situ*. D. spines on *A. verticillata mauiensis* holotype (scale bar = 0.1 mm). E–G. *Antipathes griggi*, colonies *in situ*. H. spines on *A. griggi* holotype (scale bar = 0.1 mm). (*In situ* photographs courtesy of the Hawai'i Undersea Research Laboratory).

There may be significant ecological differences as well. *A. griggi* has a wider reported depth range (<30–120 m) than *A. verticillata mauiensis*. *A. verticillata mauiensis* may extend deeper (88–130 m) than *A. griggi* and may have higher densities at the deeper depths. However, more surveys are needed to determine the ecological differences between these Hawaiian species, because *A. verticillata mauiensis* and *A. griggi* may well have been misidentified in previous surveys due to their similarities in gross morphology (Fig. 8).

Antipathes griggi is an important component of the commercial black coral trade in Hawaii. While it is not known if or to what extent specimens of *A. verticillata mauiensis* have been utilized commercially, the typical depth range of commercial harvesting is shallower than the shallowest reported specimen. No published data are available on the relative hardness of the skeletal axis of the two species, but gross observations of the samples used in this study would suggest that specimens of *A. verticillata mauiensis* are not substantially different in skeletal density from those of *A. griggi*.

Conclusion

Based on the microscopic examination of features of the skeletal spines, the antipatharian corals collected in the Au‘au Channel have been identified as a new subspecies of *A. verticillata* Brook, a species originally reported from Mauritius in the SW Indian Ocean. DNA sequence information from specimens of *A.verticillata* from various geographic localities in the western Pacific and Indian Ocean are needed to better understand the genetic relationship between these two forms.

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