Ethelia hawaiiensis (Etheliaceae, Rhodophyta), a New Mesophotic Marine Alga from Manawai (Pearl and Hermes Atoll), Papahānaumokuākea Marine National Monument, Hawai'i[1]

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Abstract: A new species of mesophotic marine red algae, *Ethelia hawaiiensis* sp. nov., is illustrated and described. *Ethelia hawaiiensis* is distinguished from other members of the genus by its large diameter assurgent filament cells, and in having thallus cavities that are frequently inhabited by microalgae, as well as in DNA sequence. Analyses of mitochondrial COI, plastid *rbcL*, and nuclear SSU sequences demonstrated that *E. hawaiiensis* was distinct from other species of *Ethelia* and that it was not phylogenetically closely related to other known species. Both COI and *rbcL* analyses placed *E. hawaiiensis* within a clade of other *Ethelia* sequences, while the SSU analyses, which only included two previously described species of *Ethelia*, resolved *E. hawaiiensis* as sister to a clade comprising *Ethelia* plus the Peyssonneliales. Morphological differences between *E. hawaiiensis* and other members of the genus are discussed.

Keywords: coral reef, DNA sequencing, mesophotic, microscopy, Northwestern Hawaiian Islands, red algae, systematics

Ethelia Weber-van Bosse (1921: 297) is a crustose, gigartinalean marine algal genus that is distributed worldwide in tropical and warm-temperate waters, with occasional reports from temperate regions (Guiry and Guiry 2021). *Ethelia* was first described as a subgenus of *Peyssonnelia* by Weber-van Bosse (1913), and was later established as a separate genus (Weber-van Bosse 1921). Much confusion has arisen regarding the generitype over the course of subsequent taxonomic changes; this is summarized in Dixon et al. (2015), who also described a new family for the genus, Etheliaceae K. Dixon, C.W. Schneider and G.W. Saunders. *Ethelia* consists of 12 currently recognized species (Guiry and Guiry 2021), with six new species recently described by Dixon et al. (2015), and three by Ballantine et al. (2017).

Members of the genus *Ethelia* are crustose and cartilaginous, uncalcified, with uniaxial growth, and large-celled axial filaments within the thallus that produce smaller celled radiating cortical filaments to generate both dorsal and ventral cortices (Dixon et al. 2015). Gametangial reproduction has not been confirmed in any species, and only presumed, partially developed tetrasporangia have been reported (Dixon et al. 2015). Radial transverse sections of *Ethelia* are distinctive, with the large cells of the axial filaments dividing the thallus into upper and lower regions of cortical tissue.

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Recent surveys of Hawaiian mesophotic reefs have uncovered large numbers of undescribed species, and the crustose red algae are no exception. While studying peyssonnelioid specimens collected from mesophotic depths in the Main Hawaiian Islands and Papahānaumokuākea Marine National Monument (PMNM; Northwestern Hawaiian Islands), a single specimen of a noncalcified, crustose, gigartinalean alga was uncovered. Here, we demonstrate through analyses of the mitochondrial COI barcode region, the *rbcL* plastid gene, and the nuclear SSU gene, as well as light microscope observations, that the collection represents an undescribed species within the enigmatic genus *Ethelia*; the first report of the genus from the Hawaiian Islands.

MATERIALS AND METHODS

A single specimen of *Ethelia* was collected on 6 August 2019 at 75 m depth as part of a marine survey expedition to Manawai (Pearl and Hermes Atoll), PMNM, USA. The specimen was removed from a 3 to 4 m vertical ledge intermixed with encrusting invertebrates and turf algae. The specimen was preserved as several small pieces, detached from the substratum, and dried in silica gel desiccant. Morphological investigations of the sample were conducted by gently rehydrating small pieces in Modified Pohl's Solution (https://www.eeob.iastate.edu/research/bamboo/pdf/anatomy_protocols.pdf) for 30 min, hand sectioning with a double-edged razor blade, staining with 1% aniline blue, and mounting in 30–50% Karo™. Photomicrographs were taken on a Zeiss AxioImager A1 compound light microscope (Pleasanton, CA) with an Infinity2-1RC digital camera (Lumenera Corporation, Ottawa, Ontario, Canada). Both brightfield and differential interference contrast microscopy were employed.

The specimen was extracted for genomic DNA using an OMEGA E.Z.N.A.® Plant DNA DS Kit (OMEGA Biotek, Norcross, GA, USA). A portion of the COI DNA barcode marker (cytochrome oxidase subunit I) was amplified using the GWSFn (Le Gall and Saunders 2010) and GWSRx (Saunders and Moore 2013) primers. The *rbcL* (ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit) marker was amplified as two overlapping fragments using the primer pairs rbcLF7 and rbcLJNR1 (Gavio and Fredericq 2002, Kang and Kim 2013) and rbcLF762 and rbcLR1442 (Kim et al. 2010). The SSU gene was amplified as two overlapping fragments using the primer pairs G01 and G14, and G04 and G07 (Harper and Saunders 2001). Successful PCR products were submitted for sequencing by GENEWIZ (South Plainfield, NJ, USA). Raw sequence reads for each gene were assembled, edited, and aligned using the MUSCLE v. 3.8.425 plug-in (Edgar 2004) in Geneious Prime 2019.1.3 (http://www.geneious.com) with other available sequences for *Ethelia* and related genera from GenBank and BOLD (Table S1). DNA barcode analysis of the COI sequences was performed by constructing a neighbor-joining framework based on Kimura-2-parameter distances using MEGA (Stecher et al. 2020). For the *rbcL* and SSU phylogenetic analyses, sequences were aligned with reference sequences and analyzed with PartitionFinder v. 1.1.1 (Lanfear et al. 2012). The *rbcL* alignment was constructed based on sequence representation from Dixon et al. (2015), and the SSU alignment was based on representation from Ballantine et al. (2017), but with omission of some distantly related orders (Rhodymeniales, Sebdeniales, Halymeniales). Maximum Likelihood (ML) analyses were performed on all alignments using RAxML-HPC2 on XSEDE v. 8.2.10 (Stamatakis 2014) via the CIPRES gateway (Miller et al. 2010) with 1,000 bootstrap replicates, and using the GTRCAT model. Bayesian inference was performed using the MrBayes plug-in v. 3.2.6 (Huelsenbeck and Ronquist 2001) through Geneious Prime 2020.2.3 (http://www.geneious.com) using four chains of Metropolis-coupled Markov Chain Monte Carlo for 1,000,000 generations and sampling every 500 generations; 100,000 chains were removed as burn-in prior to determining posterior probabilities.

RESULTS

Ethelia hawaiiensis *A. R. Sherwood, sp. nov.* (*Figure 1A–H*)

DESCRIPTION: Thallus maroon red, crustose, cartilaginous, noncalcified, attached to

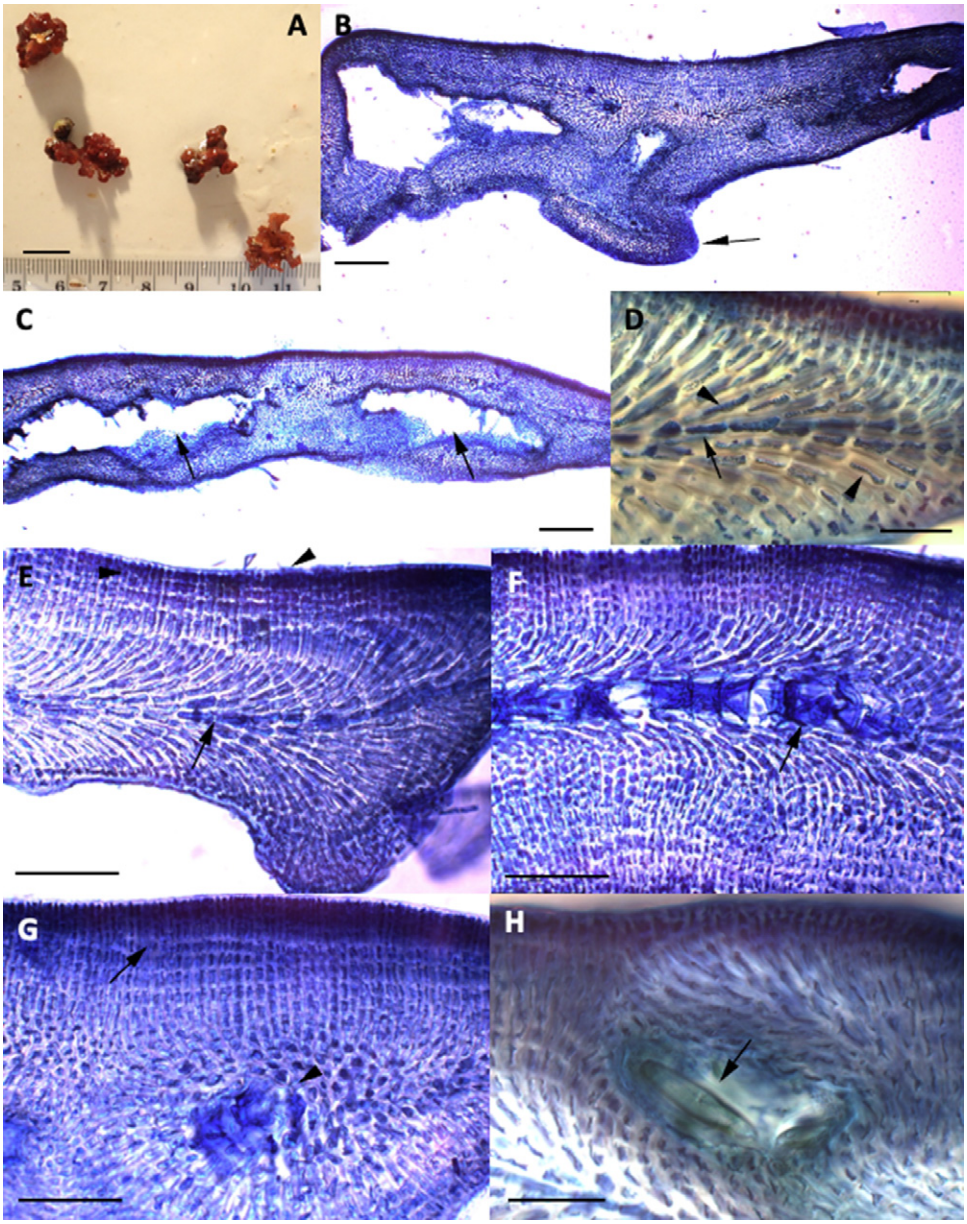

FIGURE 1. Habit and morphology of *Ethelia hawaiiensis* sp. nov. (all images from BISH 780620). (*A*) Image of specimen fragments immediately after collection. (*B*) Radial vertical section (RVS) through thallus illustrating haptera (arrow). (*C*) RVS through thallus illustrating multiple internal cavities (arrows). (*D*) RVS showing axial filament (arrow) and dorsal and ventral assurgent cortical filaments (arrowheads). (*E*) RVS illustrating the axial filament (arrow) and darkly staining terminal cortical cells of the dorsal cortex (arrowheads). (*F*) RVS showing section through large-diameter axial filament (arrow), with both dorsal and ventral assurgent cortical filaments. (*G*) Section illustrating regular, darkly staining terminal cortical cells of the dorsal cortex (arrow), and a section through the axial filament (arrowhead). (*H*) RVS illustrating a thallus cavity inhabited by diatoms (arrow). Scale bars: A, 1 cm; B, C, E–G, 100 μm; D, H, 50 μm.

TABLE 1

Comparative Morphological Features of *Ethelia hawaiiensis* (Table Organization and Terminology from Ballantine et al. (2017); Differences in Terminology to that Used in the Current Manuscript, where Present, are Explained in Footnotes)

Taxon	Crust Thickness (μm)	Primary Axial Cell Dimensions [Diameter × Length (μm)]	Proximal Cortical Filament Cells[a] [Diameter × Length (μm)]	Distal Perithallus Superior Cells[b]	Distal Perithallus Inferior Cells[c]	Ratio of Superior to Inferior Perithallus[d]	Peg-Like Attachments[e]	Distribution
E. hawaiiensis sp. nov.	340–600 typically, to 970	34–43 × 25–38	13–18 × 24–46, slightly enlarged at apical end, decreasing in size distally	Terminal cells subisodiametric, 5–8 × 6–12 μm	Less well-organized than upper portion of thallus, terminal cells subisodiametric, 9–14 × 7–14 μm	=	+	U.S.A. Hawai'i, Papahānaumokuākea Marine National Monument, Manawai (Pearl and Hermes Atoll); 75 m

[a] Assurgent filaments of the dorsal and ventral cortex.
[b] Terminal cells of the dorsal cortex.
[c] Terminal cells of the ventral cortex.
[d] Ratio of the dorsal to ventral cortex.
[e] Haptera.

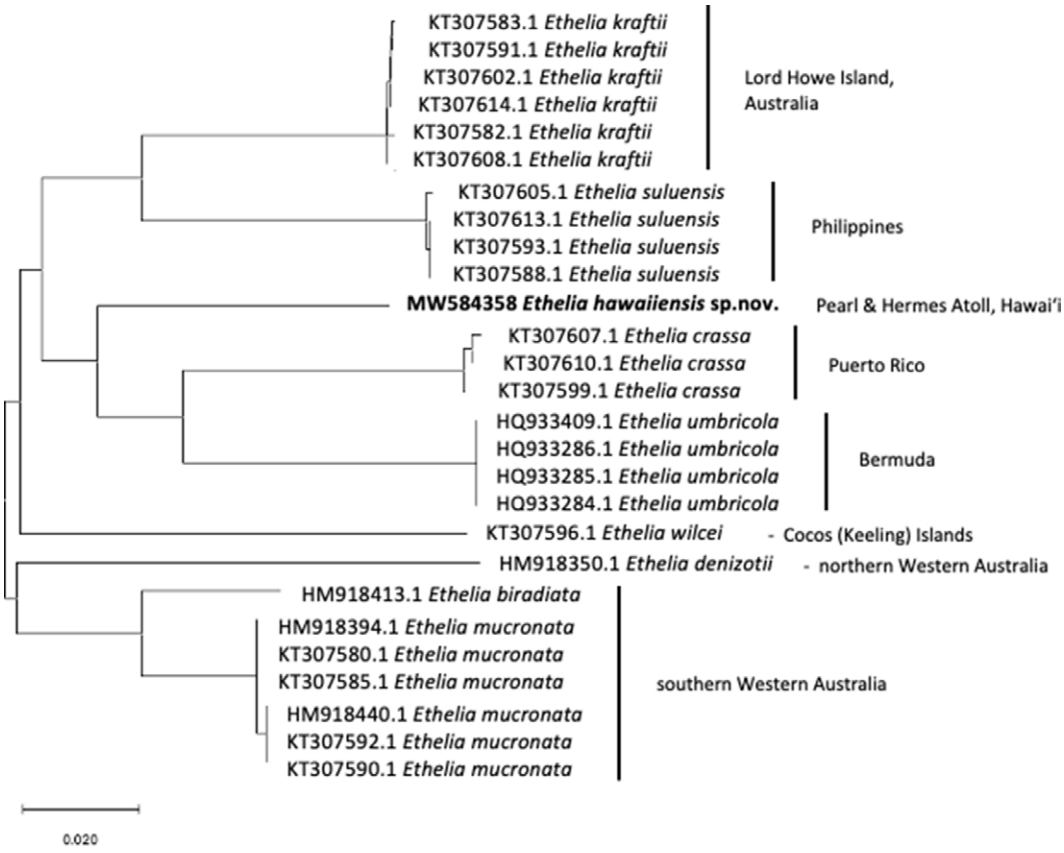

FIGURE 2. Neighbor-joining framework based on Kimura-2-parameter distances for the COI barcoding marker for specimens of *Ethelia*. *Ethelia hawaiiensis* is distinct from all other sequenced species of *Ethelia*. Scale bar = substitutions per site.

substratum over entire lower surface, lacking rhizoids, attached by occasional, large, fleshy peg-like haptera (Figure 1*A*). Thallus medium to thick (340–600 μm typically, to 970 μm including haptera), with cavities of various sizes (Figure 1*B*,*C*,*H*). Axial filament cells 34–43 μm in diameter × 25–38 μm in length; branching (Figure 1*D*,*E*). Ratio of dorsal to ventral cortex approximately 1:1, with no consistent trend of one larger than the other (Figure 1*E*,*F*). Dorsal cortex composed of assurgent filaments, inner cells 13–18 μm diameter × 24–46 μm length, slightly enlarged at apical end, decreasing in size distally, terminal cells subisodiametric, 5–8 μm in diameter × 6–12 μm length (Figure 1*E–H*). Dorsal cortex terminates in regularly arranged files of cells (5–9) that become near-isodiametric, top 3–4 layers of cells densely stain. Inner ventral assurgent filament cells similar in dimensions to those of inner dorsal assurgent filaments, decreasing in size toward outer portion of thallus. Ventral cortex less well-organized than dorsal cortex, terminal cells subisodiametric, 9–14 μm diameter × 7–14 μm length (Figure 1*E–H*). Reproduction not observed.

HOLOTYPE: BISH 780620 (ARS 10008, Manawai (Pearl and Hermes Atoll), Papahānaumokuākea Marine National

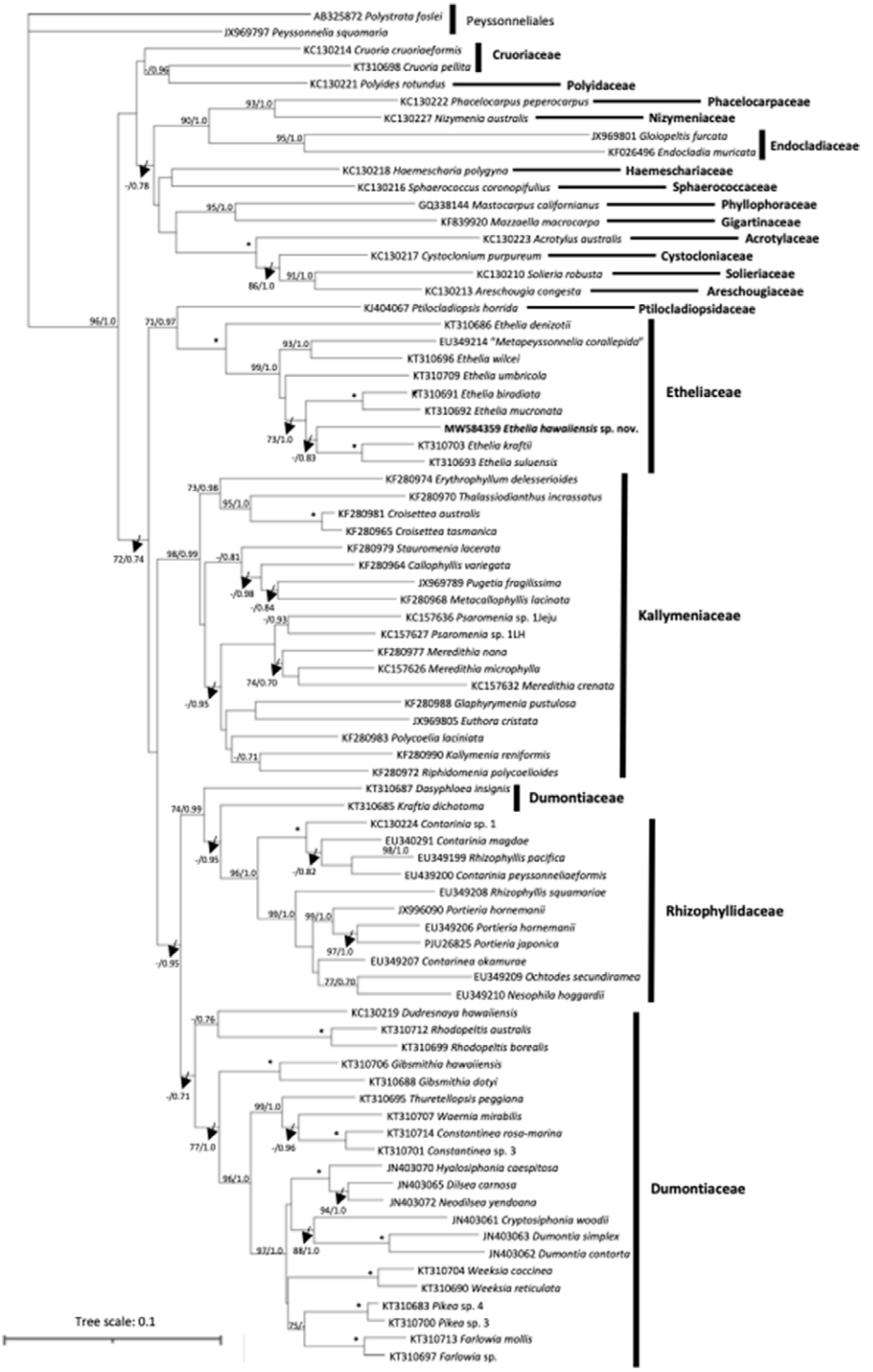

FIGURE 3. Maximum likelihood phylogenetic tree of *rbcL* sequences for *Ethelia* and closely related genera in the red algal orders Gigartinales and Peyssonneliales. Numbers along branches indicate nodal support (first value = bootstrap support; second value = Bayesian posterior probabilities). Nodes with full support are indicated with an asterisk. Scale bar = substitutions per site.

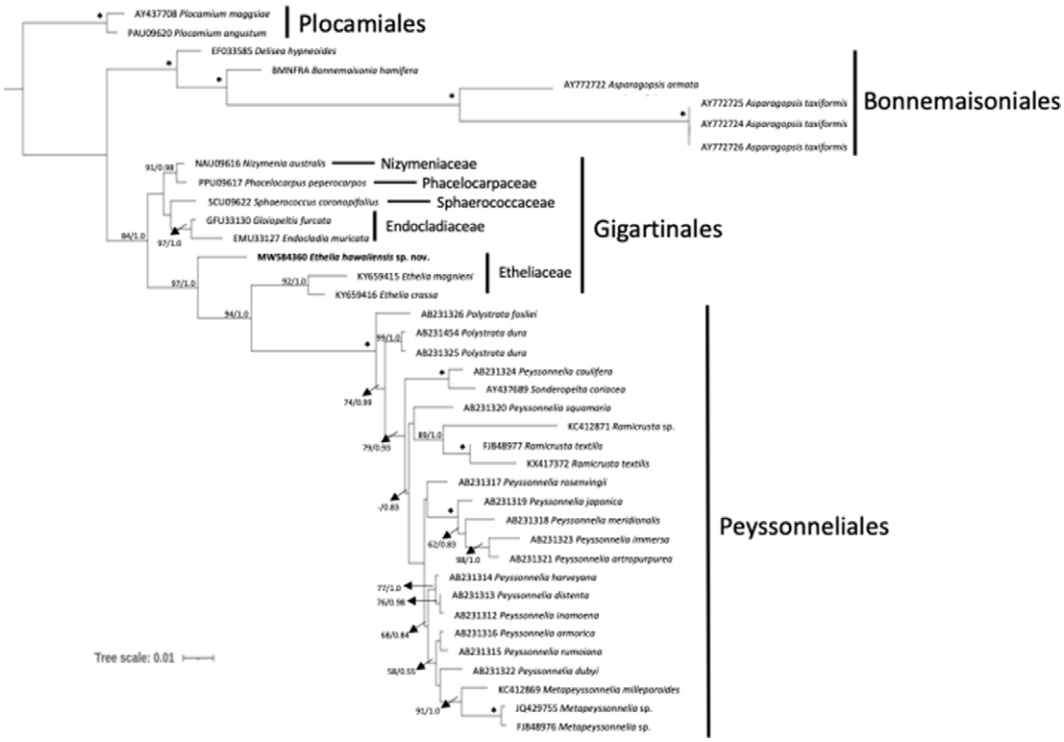

FIGURE 4. Maximum likelihood phylogenetic tree of SSU sequences for *Ethelia* and representative genera in the red algal orders Bonnemaisoniales, Gigartinales, Peyssonneliales, and Plocamiales. Numbers along branches indicate nodal support (first value = bootstrap support; second value = Bayesian posterior probabilities). Nodes with full support are indicated with an asterisk. Scale bar = substitutions per site.

Monument, Hawai'i, USA, 27.74129° N, 175.958° W, 75 m depth, 06.VIII.2019, leg. B. Hauk (NWHI-1041)).

Comparative morphological features of *Ethelia hawaiiensis* are shown in Table 1, which is presented as an extension of Table 1 from Ballantine et al. (2017; p. 648).

ETYMOLOGY: The specific epithet recognizes the only known location of the species (Hawaiian Islands).

DISTRIBUTION: Currently known only from Manawai (Pearl and Hermes Atoll), Papahānaumokuākea Marine National Monument, Hawai'i, USA, from mesophotic depths.

DNA SEQUENCE DATA: GenBank accessions MW584358 (COI), MW584359 (*rbcL*), MW584360 (SSU).

IDENTIFICATION USING DNA SEQUENCE DATA: Neighbor-joining analysis of available COI sequences for currently recognized species of *Ethelia* and the new Hawaiian specimen indicated that the new specimen is distinctive in sequence from all other related, available sequences (Figure 2). Eight of the 12 currently recognized species are represented in the COI analysis, with only *E. excrescens* D.L. Ballantine & N.E. Aponte, *E. magnieni* D.L. Ballantine, H. Ruíz, Lozada-Troche & J.N. Norris, *E. pacifica* Børgesen, and *E. vanbosseae* Feldmann lacking COI sequences. The Hawaiian specimen had only 89.9% COI identity to its most similar congeners, and differed by 67–95 bp from other sequences. Analyses of the *rbcL* marker, for which sequence data are available for seven other described *Ethelia* species, placed the Hawaiian specimen within the *Ethelia* clade, but demonstrated that it is distinct from other

species in the analysis (Figure 3); the same species are lacking *rbcL* sequence data as for COI, with the addition of *E. crassa* D.L. Ballantine, H. Ruíz, Lozada-Troche & J.N. Norris. The Hawaiian *Ethelia* was sister to an *rbcL* clade consisting of *E. kraftii* K.R. Dixon and *E. suluensis* K.R. Dixon (Figure 3). As also shown by Dixon et al. (2015), EU349214 ("*Metapeyssonnelia corallepida*") appears to be a species of *Ethelia*, and likely represents an undescribed taxon. Phylogenetic analyses of the SSU marker (for which only sequences of *E. crassa* and *E. magnieni* were available) not only indicated that the new Hawaiian specimen was distinct from these other two species but also resolved the new specimen as sister to the clade containing Etheliaceae and the Peyssonneliales (Figure 4).

DISCUSSION

DNA sequence analysis of the COI, *rbcL*, and SSU markers demonstrated that *E. hawaiiensis* is distinct from almost all other recognized species in the genus. However, three currently recognized *Ethelia* species lack DNA sequence data for any marker: *E. excrescens*, *E. pacifica*, and *E. vanbosseae*. *Ethelia hawaiiensis* differs from *E. excrescens* in having thallus cavities, in thallus color (dark maroon rather than pink), thallus thickness (to 600 μm rather than to 400 μm), and in geographic distribution (Hawai'i versus Bahamas) (Ballantine et al. 2017). *Ethelia pacifica* is a poorly known taxon, but differs from *E. hawaiiensis* in being thinner, lacking thallus cavities, and in possessing rhizoids (Børgesen 1924, Ballantine et al. 2017). *Ethelia vanbosseae*, another poorly known taxon, differs from *E. hawaiiensis* in being much thicker (800–1000 μm) and in lacking thallus cavities (Feldmann 1935, Ballantine et al. 2017). Comparative features of all recognized *Ethelia* species, except the new Hawaiian taxon, are shown in Ballantine et al. (2017), to which we add the comparative features of *E. hawaiiensis* (Table 1). More generally, *E. hawaiiensis* is distinctive from other known species of *Ethelia* by its large diameter assurgent filament cells (13–18 μm), and also in possessing thallus cavities of various sizes, that are often occupied by other algae, including diatoms, cyanobacteria, and filamentous algae (Figure 1*B*–*H*).

This study describes the first new species of *Ethelia* from the Hawaiian Islands, and also reports the genus from this location for the first time. Numerous new species of red algae have been discovered and described from the Hawaiian Islands, and a recent focus on specimens from mesophotic depths has only increased the rate of species discovery (e.g., Sherwood et al. 2019, 2020, Paiano et al. 2020). The genus *Ethelia* is updated to include 13 species worldwide, a number that is likely to increase with further exploration and characterization of crustose red algae from tropical and warm-temperate waters.

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