



Unveiling mesophotic diversity in Hawai'i: two new species in the genera *Halopeltis* and *Leptofauchea* (Rhodymeniales, Rhodophyta)

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Two genera of the Rhodymeniales, *Halopeltis* and *Leptofauchea*, are here reported for the first time from the Hawaiian Islands and represent the deepest records for both genera. Molecular phylogenetic analyses of cytochrome oxidase subunit I (COI), *rbcL*, and large subunit ribosomal DNA (LSU) sequences for Hawaiian specimens of *Leptofauchea* revealed one well-supported clade of Hawaiian specimens and three additional lineages. One of these clades is described here as *Leptofauchea huawelau* sp. nov., and is thus far known only from mesophotic depths at Penguin Bank in the Main Hawaiian Islands. *L. huawelau* sp. nov. is up to 21 cm, and is the largest known species. An additional lineage identified in the LSU and *rbcL* analyses corresponds to the recently described *L. lucida* from Western Australia, and is a new record for Hawai'i. Hawaiian *Halopeltis* formed a well-supported clade along with *H. adnata* from Korea, the recently described *H. tanakae* from mesophotic depths in Japan, and *H. willisii* from North Carolina, and is here described as *Halopeltis nuahilihilia* sp. nov. *H. nuahilihilia* sp. nov. has a distinctive morphology of narrow vegetative axes that harbor constrictions along their length. The current distribution of *H. nuahilihilia* includes mesophotic depths around W. Maui, W. Moloka'i, and the island of Hawai'i in the Main Hawaiian Islands. Few reproductive characters were observed because of the small number of specimens available; however, both species are distinct based on phylogeny and morphology. These descriptions further emphasize the Hawaiian mesophotic zone as a location harboring many undescribed species of marine macroalgae.

Keywords: biodiversity; Faucheaceae; morphology; phylogeny; red algae; Rhodymeniaceae

Abbreviations: BOLD, Barcode of Life Database; COI, cytochrome oxidase subunit I; HPC, High Performance Computing; LSU, large subunit ribosomal DNA; MCE, Mesophotic Coral Ecosystem; MHI, Main Hawaiian Islands; NOAA, US National Oceanographic and Atmospheric Administration; NWHI, Northwestern Hawaiian Islands; PMNM, Papahānaumokuākea Marine National Monument; *rbcL*, ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit gene



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Received June 17, 2022, Accepted October 31, 2022

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INTRODUCTION

The red algal order Rhodymeniales is diverse in species richness and morphological complexity, and includes six families: Champiaceae, Faucheaceae, Fryeellaceae, Hymenocladaceae, Lomentariaceae, and Rhodymeniaceae (Filloramo and Saunders 2016, Guiry and Guiry 2022). The order has a complicated taxonomic history, including the addition or removal of multiple families, beginning shortly after its establishment by Schmitz (Schmitz 1889, Le Gall et al. 2008). Recent studies have clarified phylogenetic relationships in the order and have resulted in new taxonomic additions at multiple levels; for example, the proposal of the families Fryeellaceae and Hymenocladaceae, establishment of the genera *Neogastroclonium* L. Le Gall, Dalen and G. W. Saunders and *Pseudohalopeltis* G. W. Saunders, resurrection of the genus *Halopeltis* J. Agardh, and proposals for a number of new species (Gavio and Fredericq 2005, Afonso-Carrillo et al. 2006, Dalen and Saunders 2007, Le Gall et al. 2008, Schneider and Lane 2008, Rodríguez-Prieto and de Clerck 2009, Saunders and McDonald 2010, Schneider et al. 2012, Filloramo and Saunders 2015, Santiago et al. 2016).

The Rhodymeniales harbors substantial cryptic diversity (Saunders et al. 2006, Lozada-Troche and Ballantine 2010), with confusion existing even between non-sister families such as the Rhodymeniaceae and Faucheaceae (Le Gall et al. 2008). The Faucheaceae is the third largest family within the order and is characterized by three-celled carpogonial branches, terminal and cruciate tetrasporangia that are associated with adventitious cortical growth during nemathecium development, and well-developed *tela arachnoidea* (Saunders et al. 1999, Le Gall et al. 2008). In contrast, the Rhodymeniaceae (the largest family of the Rhodymeniales) is characterized by typically having four-celled carpogonial branches, mostly intercalary and cruciate tetrasporangia that develop in sori rather than in nemathecium, and *tela arachnoidea* either present or absent (Le Gall et al. 2008, Saunders and MacDonald 2010). The two families are nearly impossible to consistently tell apart at the gross morphological level because they can have very similar coloration, branching patterns, and habits.

Mesophotic coral ecosystems (MCEs) host substantial biodiversity, much of it unique, and many species have been recently described from this habitat (e.g., Ballantine et al. 2017, Schneider et al. 2019, Paiano et al. 2020, Cabrera et al. 2022). The depth range for MCEs differs based on locality but is typically defined as 30 to >150 m in tropical and subtropical areas (Lesser et al. 2009, Hinder-

stein et al. 2010, Baker et al. 2016, Pyle and Copus 2019). MCEs host a high level of organismal diversity (Rooney et al. 2010, Harris et al. 2013) and high levels of endemism (Kane et al. 2014, Baker et al. 2016, Pyle et al. 2016, Kosaki et al. 2017). This holds true for Hawaiian MCEs, where endemism levels are also high due to the isolation of the archipelago (Grigg 1988), and several new-to-science mesophotic algal species have recently been described (e.g., Spalding et al. 2016, Sherwood et al. 2020, Cabrera et al. 2022). Macroalgae are abundant in Hawaiian MCEs (Spalding et al. 2019), and algal beds have been found as deep as 160 m (Spalding 2012). Influxes of cold water in the Hawaiian mesophotic provide suitable habitat for algae typically found in temperate zones (Abbott and Huisman 2003), resulting in certain species and genera being found only in the mesophotic and absent from the better-studied adjacent shallow waters (Cabrera et al. 2022). In the Main Hawaiian Islands, mesophotic macroalgae may also be influenced by nearshore anthropogenically derived nitrogen via submarine groundwater discharge (Strait et al. 2022).

In Hawai'i, seven genera of the family Rhodymeniaceae have been previously reported (*Botryocladia*, *Chrysomenia*, *Coelarthrum*, *Drouetia*, *Erythrocolon*, *Halichrysis*, and *Rhodymenia*), and two from the Faucheaceae (*Gloiocladia* and *Gloioderma*) (Abbott and Littler 1969, Abbott 1999). Neither genus examined in this study (*Leptofauchea* and *Halopeltis*) has been previously documented in the Hawaiian Islands. *Leptofauchea* (Faucheaceae) contains 11 taxonomically accepted species, while *Halopeltis* (Rhodymeniaceae), a resurrected genus as of 2010 (Saunders and McDonald 2010), currently contains 10 species (Guiry and Guiry 2022). This study describes the phylogenetic relationships for Hawaiian representatives of these two genera, presents the description for one new species in each genus, and examines the biogeographic patterns for these new additions to the Hawaiian flora.

MATERIALS AND METHODS

Mesophotic algal specimens were collected by technical divers or the *Pisces IV* and *Pisces V* submersibles within the Main Hawaiian Islands and the Papahānaumokuākea Marine National Monument (PMNM). Most specimens were collected in 2006 off the west coast of Moloka'i (Penguin Bank). Two specimens were collected from the island of Hawai'i in 2015, and one was collected from PMNM in 2019. After collection, samples were mounted as herbarium specimens and preserved in silica gel for

molecular analyses. Specimen collection information is listed in Table 1.

DNA was extracted with an OMEGA E.Z.N.A Plant DNA Kit (OMEGA Biotek, Norcross, GA, USA), following the manufacturer's protocols. Amplification of the 5' end of the mitochondrial cytochrome *c* oxidase I (COI-5P) barcode was accomplished using one of two sets of primers: GazF1 and GazR1 (Saunders 2005), or GWSFn and GWS-Rx (Saunders and Moore 2013). The plastidial *rbcL* gene was amplified in three fragments: F57 and R562 (Freshwater and Rueness 1994) or F8 and R646 for the first fragment, F481 and R1150 for the second, and F765 and R1381 for the third fragment (Wang et al. 2000). For the large subunit ribosomal DNA (LSU) marker, four pairs of primers and cycling conditions were used: T16 and T24, T25 and T20, T05, and T15 (Saunders and Moore 2013), and nu28SF and nu28SR (Sherwood et al. 2010). Successful polymerase chain reaction products were submitted for sequencing by GENEWIZ (South Plainfield, NJ, USA).

Editing and aligning of sequence data was conducted in Geneious Prime with downloaded reference sequence data from GenBank and BOLD (Barcode of Life Database) (Supplementary Table 1). Sequences of the three markers were aligned with MUSCLE v. 3.8.425 plug-in (Edgar 2004) using the default settings; sequences were aligned separately for each marker to check for inconsistencies, and phylogenetic analyses were conducted on the individual marker alignments as well as a concatenated alignment of taxa for which sequences of all three markers were available. The *Halopeltis* alignments included 26 *rbcL* sequences (1,383 bp), 33 COI sequences (664 bp), and 28 LSU sequences (2,717 bp), while the *Leptofaucha* alignments included 26 *rbcL* sequences (1,362 bp), 20 COI sequences (664 bp), and 25 LSU sequences (2,764 bp). A series of taxa were used as outgroups in the *Halopeltis* phylogeny, as per Schneider et al. (2012), and *Weberianbossea* was used as the outgroup for *Leptofaucha* (Filloramo and Saunders 2015). Alignments were then uploaded to CIPRES (Miller et al. 2010). JModelTest2 was used to determine the best fit model of evolution (in each case GTR + Γ + I) for individual marker alignments, and PartitionFinder 2.1.1 was used for the concatenated alignment (Lanfear et al. 2017) which was run with the greedy algorithm (Lanfear et al. 2012) with unlinked branch lengths and Bayesian information criterion as the selection criterion. Maximum likelihood phylogenies were constructed using RAxML (Stamatakis 2014) with 1,000 permutations and four threads on the University of Hawai'i High Performance Computing (HPC) cluster (<https://datascience.hawaii.edu/hpc>). Bayesian analy-

ses were conducted using MrBayes 3.2.7 (Ronquist et al. 2012) on HPC under 1,000,000 generations using four chains of Metropolis-coupled Markov Chain Monte Carlo with sampling every 1,000 generations. Sequences were submitted to GenBank and are available as accessions ON376332 and ON464700–ON464709 (COI), ON376333 and ON464710–ON464726 (*rbcL*), and ON689379–ON689391 and ON730775–ON730777 (LSU). Phylogenetic analyses are presented as concatenated marker trees for *Leptofaucha* (Fig. 1) and *Halopeltis* (Fig. 2), with analyses of individual markers included in Supplementary Figs S1–S6.

Anatomical features were observed by mounting specimens onto microscope slides and examining with a Zeiss AxioImager A1 compound light microscope (Pleasanton, CA) with an Infinity2-1RC digital camera (Lumenera Corporation, Ottawa, ON, Canada). Slides were made by hand-sectioning material using a razor blade and hydrating in water for 5 min followed by staining for 5 min with 0.5% aniline blue before mounting in 30% Karo. Digitization of herbarium sheets was performed in the Joseph F. Rock Herbarium (HAW) with a Canon EOS 5D Mark II Digital Camera (Tokyo, Japan) mounted on an MK Direct Photo-eBox PLUS 1419. Vouchers were accessioned at the Bernice P. Bishop Museum (BISH) under accession codes BISH785497–785512.

RESULTS

Concatenated COI + *rbcL* + LSU phylogenetic analyses of sequences of the genus *Leptofaucha* demonstrated that the mesophotic Hawaiian samples are distinct from all but two previously described species (*L. auricularis* E. Y. Dawson and *L. rhodymenioides* W. R. Taylor, which could not be ruled out due to a lack of confirmed molecular data for these species) (Fig. 1). Hawaiian mesophotic *Leptofaucha* samples were resolved in one clade in the concatenated analysis, with two additional specimens forming distinct lineages in other parts of the tree (*Leptofaucha* sp. 2 and sp. 3) (Fig. 1). A fourth lineage of Hawaiian specimens in the LSU (Supplementary Fig. S3) and *rbcL* phylogenies (Supplementary Fig. S1) corresponded to a recently described species from Australia, *L. lucida* Huisman and G. W. Saunders (Filloramo and Saunders 2015, Huisman and Saunders 2020). Specimens in this clade shared the key morphological features Huisman and Saunders (2020) described for this species, including a sprawling habit, abundant secondary anastomoses between branches, and a multi-layered cortex (e.g.,

Table 1. Collection data for *Halopeltis* and *Leptofaucha* specimens analyzed as part of the current study

Taxon	Sherwood Lab accession	Herbarium accession	Collection details	COI	<i>rbcL</i>	LSU
<i>H. nuahilihilia</i> Holotype	ARS 09898	BISH 785498	Hawai'i Island, Hawai'i, 20.01660° N, 155.84355° W, 90 m depth, Nov 11, 2015, coll. R. Kosaki & J. Leonard (MHI-20)	ON464701	ON464714	ON730776
<i>H. nuahilihilia</i> Paratype	ARS 09912	BISH 785497	'Au'au Channel (W. Maui), Hawai'i, 20.7585° N, 156.6658° W, 104 m depth, Nov 29, 2006, coll. T. Kerby (P4-190 #443)	ON464706	ON464720	ON689389
<i>H. nuahilihilia</i>	ARS 10478	BISH 785499	'Au'au Channel (W. Maui), Hawai'i, 20.7708° N, 156.6689° W, 87 m depth, Apr 5, 2009, coll. H. Spalding (P5-736 #50)	-	ON464724	-
	ARS 10479	BISH 785500	Penguin Bank (W. Moloka'i), Hawai'i, 21.0205° N, 157.3747° W, 76 m depth, Nov 17, 2006, coll. T. Kerby (P4-184 #14C)	ON464709	ON464725	ON730777
<i>L. huawelau</i> Holotype	ARS 09895	BISH 785501	Penguin Bank (W. Moloka'i), Hawai'i, 21.0205° N, 157.3747° W, 76 m, Nov 17, 2006, coll. T. Kerby (P4 184 #185-2)	-	ON464726	ON689381
<i>L. huawelau</i>	ARS 09896	BISH 785502	Penguin Bank (W. Moloka'i), Hawai'i, 21.0205° N, 157.3747° W, 76 m, Nov 17, 2006, coll. H. Spalding (P4 184 #141-2)	ON376332	ON464712	ON730775
<i>L. huawelau</i> Isotype	ARS 09900	BISH 785503	Penguin Bank (W. Moloka'i), Hawai'i, 21.0205° N, 157.3747° W, 76 m, Nov 17, 2006, coll. H. Spalding (P4 184 #171-4)	ON464702	ON376333	ON689384
<i>L. huawelau</i>	ARS 09905	BISH 785504	Penguin Bank (W. Moloka'i), Hawai'i, 21.0205° N, 157.3747° W, 78 m, Nov 17, 2006, coll. H. Spalding (P4 184 #217-2)	ON464703	ON464716	-
	ARS 09907	BISH 785505	Penguin Bank (W. Moloka'i), Hawai'i, 21.0205° N, 157.3747° W, 78 m, Nov 17, 2006, coll. T. Kerby (P4 184 #213)	ON464704	ON464717	ON689386
	ARS 09909	BISH 785506	Penguin Bank (W. Moloka'i), Hawai'i, 21.0205° N, 157.3747° W, 78 m, Nov 17, 2006, coll. T. Kerby (P4 184 #205)	ON464705	ON464719	ON689388
	ARS 10228	BISH 785507	Penguin Bank (W. Moloka'i), Hawai'i, 21.0205° N, 157.3747° W, 76 m, Nov 17, 2006, coll. T. Kerby (P4 184 #146)	ON464708	ON464723	ON689391
	ARS 09891	BISH 785508	Penguin Bank (W. Moloka'i), Hawai'i, 21.0455° N, 157.3524° W, 80 m, Nov 28, 2006, coll. M. Cremer (P4 189 #368)	-	ON464710	ON689379
<i>L. lucida</i>	ARS 09892	BISH 785509	Penguin Bank (W. Moloka'i), Hawai'i, 21.0455° N, 157.3524° W, 80 m, Nov 28, 2006, coll. M. Cremer (P4 189 #375)	-	ON464711	ON689380
	ARS 09901	BISH 785510	Penguin Bank (W. Moloka'i), Hawai'i, 21.0205° N, 157.3747° W, 76 m, Nov 17, 2006, coll. H. Spalding (P4 184 #192-2)	-	ON464715	ON689385
	ARS 09908	BISH 785511	Penguin Bank (W. Moloka'i), Hawai'i, 21.0205° N, 157.3747° W, 78 m, Nov 17, 2006, coll. T. Kerby (P4 184 #213-1)	-	ON464718	ON689387
	ARS 10068	BISH 785512	Penguin Bank (W. Moloka'i), Hawai'i, 21.0205° N, 157.3747° W, 76 m, Nov 17, 2006, coll. T. Kerby (P4 184 #188)	-	ON464722	-
<i>Leptofaucha</i> sp. 1	ARS 09899	-	Penguin Bank (W. Moloka'i), Hawai'i, 21.0205° N, 157.3747° W, 76 m, Nov 17, 2006, coll. H. Spalding (P4 184 #126-3)	-	-	ON689383
<i>Leptofaucha</i> sp. 2	ARS 09897	-	Hawai'i Island, Hawai'i, 20.01660° N, 155.84355° W, 90 m depth, Nov 11, 2015, coll. R. Kosaki & J. Leonard (MHI-21)	ON464700	ON464713	ON689382
<i>Leptofaucha</i> sp. 3	ARS 10013	-	Manawai (Pearl and Hermes Atoll), Hawai'i, 27.82025° N, 176.04132° W, 92 m, Aug 3, 2019 coll. J. Leonard & K. Lopes (NWHI-924)	ON464707	ON464721	ON689390

COI, cytochrome c oxidase I; LSU, large subunit ribosomal DNA.

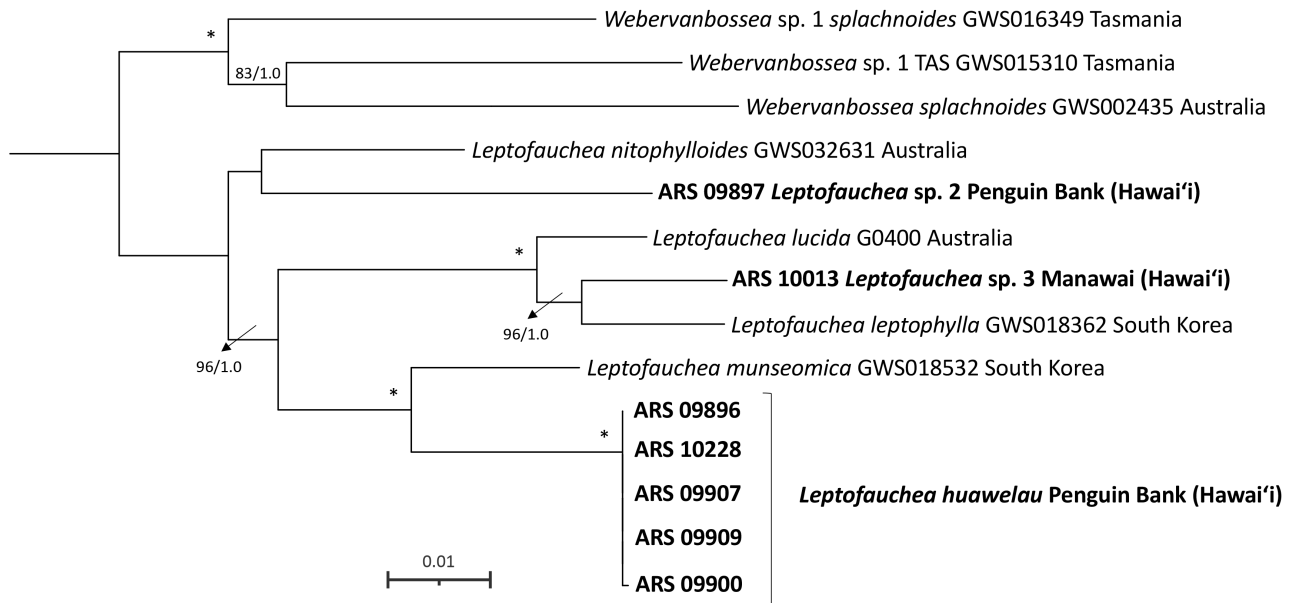


Fig. 1. Phylogenetic tree of the genus *Leptofaucha* using a concatenated alignment of 5' end of the mitochondrial cytochrome *c* oxidase I, large subunit ribosomal DNA, and *rbcl* sequences. Sequences generated in the current study are indicated in bold. Bayesian and maximum likelihood support values shown at the nodes, the first value represents the maximum likelihood bootstrap support (BP) value and the second is the Bayesian posterior probability (PP) support value. Full support is indicated with an asterisk (*) while support less than 70% BP / 0.70 PP is shown with a dash (-). Scale bar represents: substitutions per site.

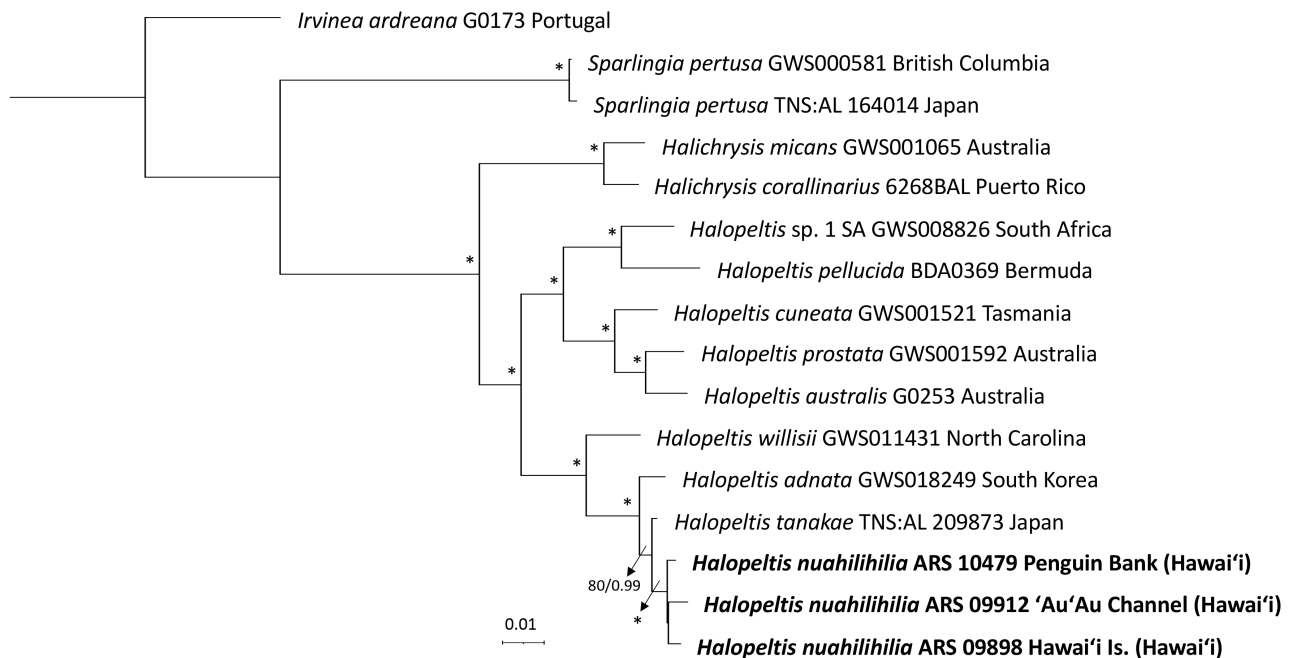


Fig. 2. Phylogenetic tree of the genus *Halopeltis* using a concatenated alignment of 5' end of the mitochondrial cytochrome *c* oxidase I, large subunit ribosomal DNA, and *rbcl* sequences. Sequences generated in the current study are indicated in bold. Bayesian and maximum likelihood support values shown at the nodes, the first value represents the maximum likelihood bootstrap support (BP) value and the second is the Bayesian posterior probability (PP) support value. Full support is indicated with an asterisk (*) while support less than 70% BP / 0.70 PP is shown with a dash (-). Scale bar represents: substitutions per site.

Supplementary Fig. S7D). A fifth lineage (*Leptofauchea* sp. 1) was recovered only in the LSU single-marker analysis (Supplementary Fig. S3). The clade containing most of the Hawaiian mesophotic specimens in the concatenated analysis, which is described in the next section as a new species, includes five Hawaiian specimens from Penguin Bank, MHI (ARS 09896, 09900, 09907, 09909, 10228), and belongs to a clade with *L. munseomica* Filloramo and G. W. Saunders, from South Korea, with full support (Filloramo and Saunders 2015).

All currently described species of *Halopeltis* are represented in the concatenated phylogeny and / or individual marker trees, as well as several additional lineages of undescribed species, labeled in GenBank as “GWS” collections (Fig. 2, Supplementary Figs S4–S6). The Hawaiian mesophotic samples sequenced in this study form a well-supported clade in the concatenated analysis with full support that is distinct from all other available sequences for the genus. Three MHI samples are included within this clade (ARS 09898, 09912, 10479), which belongs to a larger clade that also includes *H. adnata* (Okamura) G. W. Saunders and C. W. Schneider, *H. tanakae* Mas. Suzuki and R. Terada, and *H. willisii* Freshwater and G. W. Saunders with full support (Schneider et al. 2012, Suzuki and Terada 2021).

The formal descriptions for the one new species each of *Leptofauchea* and *Halopeltis* from the Hawaiian specimens are presented below, with comparisons of general thallus morphology among related species presented in Table 2 (*Leptofauchea*) and Table 3 (*Halopeltis*).

***Leptofauchea huawelau* E. A. Alvarado, F. P. Cabrera and A. R. Sherwood sp. nov. (Fig. 3A–I)**

Description. Thalli up to 21 cm in height × 18 cm in width, attached by a stipe, light pink to deep red-pink in color. Plants tall, with irregular to loosely dichotomous branching. Vegetative axes narrow, 2–4 mm broad with constrictions frequently present below or above branching points, constrictions ranging in breadth from <1–1.75 mm. Internodal lengths of axes between points of branching up to 6.4 cm. Axes flat and thin, ranging in thickness from 120–150 µm. Apices commonly spatulate, lighter in color than other sections of the thallus, occasionally attenuated. Cortical cells loosely arranged in surface view, darkly staining, with a single outer layer, cells spherical to slightly axially elongated, 7.4–17 µm in diameter. Inner cortex composed of 1–2 incomplete layers of cells, which grade into the medulla. Medulla composed of 2–3 layers of cells, irregularly arranged and visible from surface

view. Medullary cells large and axially elongated, 27–81 µm in length × 18–47 µm in width, lightly staining, and thin-walled. Tetrasporangial nemathecia slightly darker than the surrounding thallus, positioned near apices, round to elliptical in shape, and elongated towards the branch apex, 1,370 × 740 µm, with 4–7-celled paraphyses. Tetrasporangia cruciate or decussate, spherical to ovoid, 7.6–29.8 µm in diameter. Gametangial reproduction not observed.

Holotype. BISH 785501 (ARS 09895, West Moloka’i, Hawai’i, 21.022172° N, 157.216848° W, 72 m depth, Nov 17, 2006, leg. Terry Kerby, field code P4-184 #185).

Holotype DNA accession numbers. ON464726 (*rbcl*), ON689381 (LSU).

Isotype. BISH 785503 (ARS 09900).

Isotype DNA accession numbers. ON464702 (COI), ON376333 (*rbcl*), ON689384 (LSU).

Etymology. Specific epithet derived from the Hawaiian language, pronounced “hoo-ah-veh-la-oo.” “Hua” is often translated as “fruit, egg, seed.” “Wēlau” means “tip” or “end.” Simply put, this name describes the “bulbs” at the apices of the branches that reflect its growth potential. New branches bud from these hua, creating more bulbs and continued growth. The first part of the name defines the epithet as a noun, and is thus not declinable in Latinized form.

Distribution. Mesophotic depths of Penguin Bank, Main Hawaiian Islands, Hawai’i, USA.

Specimens examined. BISH 785501–785507 (ARS 09895, 09896, 09900, 09905, 09907, 09909, 10228).

Habit and morphology. Individuals are flattened, loosely dichotomously branched, attached by a stipe (Fig. 3A & B). Stipe 0.2–0.4 cm in length, 950–1,200 µm in width (Fig. 3A–D), with a multi-layered cortex and medulla. Dried specimens have a deep pink-red color that, in most cases, lightens to a very light pink near branch apices. Thalli with a lanky appearance, with long internodes between points of branching (Fig. 3B). Thalli thin in cross section, 120–150 µm (Fig. 3D) with large medullary cells in 2–3 layers and small pigmented cortical cells in 1–2 incomplete layers (Fig. 3G). In surface view cortical cells are loosely arranged, cortical cells small, mostly round, 7.4–17 µm in diameter (Fig. 3E). Medulla is 2–3 layers thick, with thin-walled cells, 27–81 µm long by 18–47 µm wide (Fig. 3D & G). Cruciate or decussate tetrasporangia are 7.6–29.8 µm in diameter, 11.5–26.0 µm in length (Fig. 3F). Tetrasporangia formed in nemathecia that are elongated towards branch apices, 1,370 × 740 µm in size. Nemathecial paraphyses composed of 4–7 cells (Fig. 3H & I). No gametangial reproduction has been confirmed in the

Table 2. Comparison of general morphology of *Leptofaucha huawelau* with related species of *Leptofaucha*

Taxon	Thallus height (cm)	Width of main axes (mm)	Thallus branching	Thallus constrictions	Anastomoses	Stipe	Medulla	Cortex
<i>L. chiloensis</i> Dalen and G. W. Saunders	to 9	to 20	Dichotomously branched	Slight, above points of branching	No	Absent	2–3 cell layers	Outer cortex of 2–4 cell layers, inner cortex a single layer
<i>L. cocosana</i> Filloramo and G. W. Saunders	to 2.8	to 4	ND	ND	Yes	Absent	2–3 cell layers	1–2 incomplete cell layers
<i>L. coralligena</i> Rodríguez-Prieto and De Clerck	to 5–9	4–6	Dichotomously to irregularly branched	Occasionally, above points of branching	No	Present, up to 1.5 mm in length	2–4 cell layers	1–2 cell layers
<i>L. huawelau</i> n. sp.	to 21	2–4	Primarily dichotomous	Above points of branching, and frequently at apices	No	Present, up to 2–4 mm in length	2–3 cell layers	1–2 incomplete cell layers
<i>L. leptophylla</i> (Segawa) Mas. Suzuki et al.	to 4	3–5	Dichotomously branched	Above points of branching, and frequently at apices	No	Present, 1.5–3 mm in length	3–4 cell layers	2 cell layers
<i>L. lucida</i> Huisman and G. W. Saunders	to 7	1.5–7	Subdichotomous to irregular	Above points of branching, and frequently at apices	Yes	Absent	2–3 cell layers	1–3 cell layers
<i>L. munseomica</i> Filloramo and G. W. Saunders	3–4.2	2–3	Dichotomously branched	Occasionally, above points of branching	No	Absent	3–4 cell layers	1–2 incomplete cell layers
<i>L. mitophylloides</i> (J. Agardh) Kylin	to 6	3–6	Dichotomously branched	Slight, above points of branching	No	Absent	1–2 cell layers	1–2 incomplete cell layers
<i>L. pacifica</i> E. Y. Dawson	to 7	4–5	Dichotomously branched	Slight, above points of branching	No	Present, 4–9 mm in length	2–3 cell layers	Outer cortex of 1–3 cell layers, inner cortex a single layer
<i>L. rhodymenioides</i> W. R. Taylor	to 6	4–9	Dichotomously branched at distinctively wide angles	Slight, above points of branching	No	Absent	2–3 cell layers	Outer cortex of 1–2 cell layers, inner cortex a single layer

As per Dalen and Saunders (2007), *L. auricularis* and *L. earleae* are not included in these comparisons.
 ND, not determined.

Table 3. Comparison of general morphology of *Halopeltis nuahilihilia* with related species of *Halopeltis*

Taxon	Thallus height (cm)	Width of main axes (mm)	Thallus branching	Thallus constrictions	Anastomoses	Stipe	Medulla	Intercalating cells	Cortex
<i>H. adnata</i> (Okamura) G. W. Saunders and C. W. Schneider	to 11	2–3	Irregularly to dichotomously branched	Slight, above points of branching	Present	Absent	2–3 cell layers	Present	1–2 cell layered inner cortex in addition to outer cortex
<i>H. australis</i> (J. Agardh) G. W. Saunders	4–20	2–10	Subdichotomously branched	Slight, above points of branching	Absent	Present (to 20 mm)	3–8 cell layers	Present	2–3 cell layers
<i>H. cuneata</i> (Harvey) G. W. Saunders	4–0	10–80	Foliose and usually divided into several lobes	Absent	Absent	Present (4–15 mm)	Typically 2 cell layers	Present	1–2 cell layers
<i>H. gracilis</i> G. W. Saunders	to 10	1.5–3	Loosely dichotomously branched, with abundant proliferous branches	Slight, above points of branching	Unclear	Unclear	2–4 cell layers	Present	Inner cortex of 1–2 cell layers and outer cortex of 1–2 cell layers
<i>H. nuahilihilia</i> sp. nov.	to 10.5	1–4	Subdichotomously branched	Present close to apices and sometimes above points of branching	Present	Absent	2–3 cell layers	Absent	1–2 cell layers
<i>H. pellucida</i> C. W. Schneider and G. W. Saunders	to 2	2–4	Pseudodichotomously branched	Present, but not prominent	Present	Present ("short")	1–2 cell layers	Absent	2–3 cell layers of pigmented cells
<i>H. prostrata</i> G. W. Saunders	to 10	4–9	Irregularly to dichotomously branched	Present, but not prominent	Unclear - grows in thick mats	Sometimes (to several cm's)	2–3 cell layers	Present	Inner cortex of 1–2 cell layers and outer cortex of 1–2 cell layers
<i>H. tanakae</i> Mas. Suzuki and R. Terada	to 5	to 5	Pseudodichotomously branched	Above points of branching	Present	Absent	2–3 cell layers	Present (sometimes)	2 cell layers
<i>H. verrucosa</i> (Womersley) G. W. Saunders	to 14	2–10	Subdichotomous to alternate	Present, but not prominent	Absent	Absent	2 cell layers	Present	Inner cortex of 1–2 cell layers and outer cortex of 1–2 cell layers
<i>H. willisii</i> Freshwater and G. W. Saunders	to 8	4–15	Pseudodichotomously branched	Slight, above points of branching	Present	Absent	2–3 cell layers	Present	2–3 cell layers of pigmented cells

Halopeltis austrina is omitted from the comparison as per comments by Saunders and McDonald (2010).

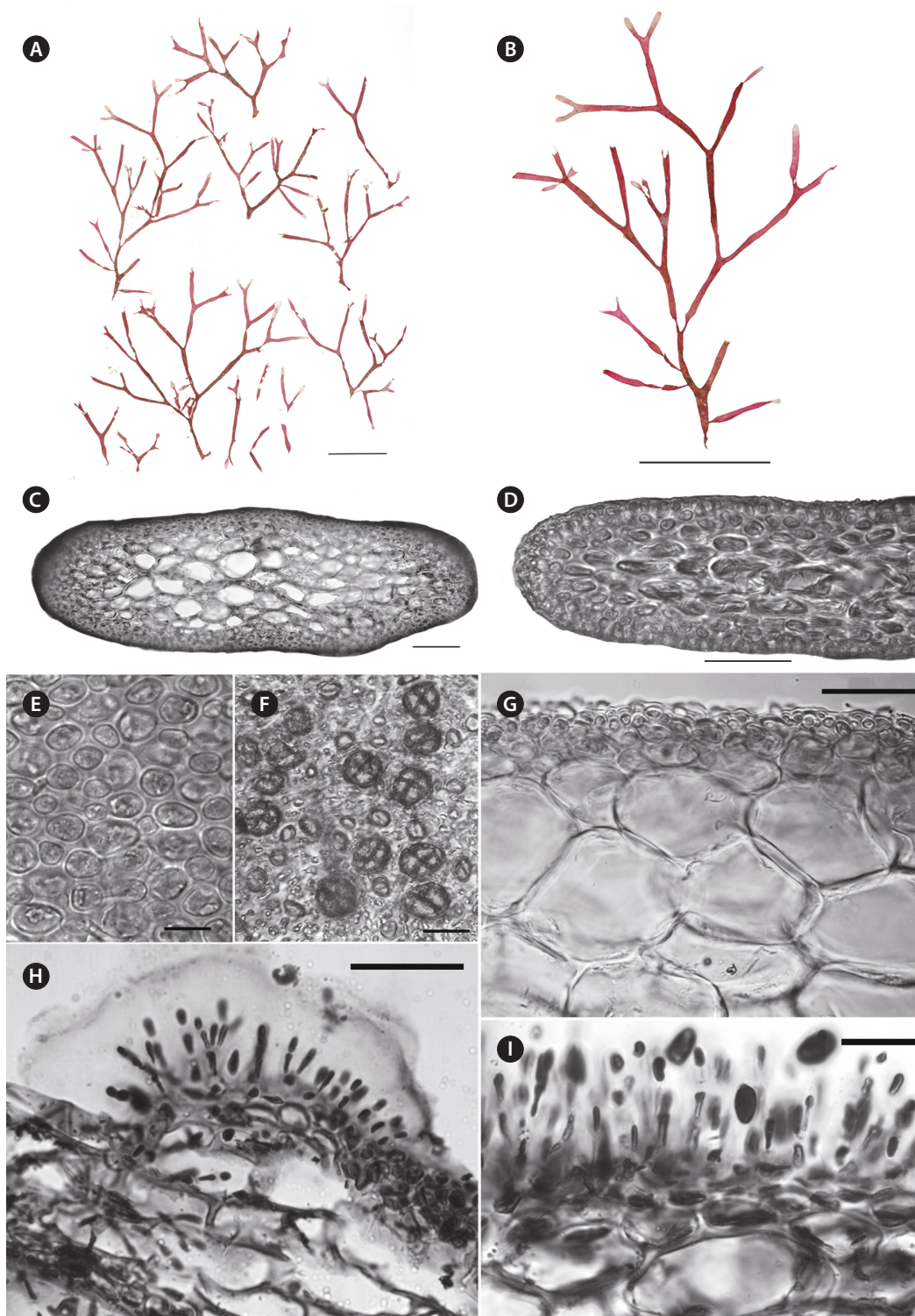


Fig. 3. Morphology of *Leptofaucha huawelau* sp. nov. (A) Image of the herbarium sheet containing the holotype (BISH 785501, ARS 09895). (B) Close up view of the holotype (BISH 785501, ARS 09895). (C) Cross section of the stipe (BISH 785501, ARS 09895). (D) Cross section of apical portion of the thallus, showing the cortex and medulla (BISH 785501, ARS 09895). (E) Surface view of the blade showing outer cortical cells (BISH 785501, ARS 09895). (F) Cruciate divided tetraspores (BISH 785505, ARS 09907). (G) Cross section of basal portion of the thallus, showing the cortex and medulla (BISH 785501, ARS 09895). (H) Tetrasporangia are laterally produced from paraphyseal cells or terminally on short filaments (BISH 785505, ARS 09907). (I) Close up of early developmental stages of tetrasporangia (BISH 785505, ARS 09907). Scale bars represent: A & B, 5 cm; C, D & H, 100 µm; E, 25 µm; F, G & I, 50 µm.

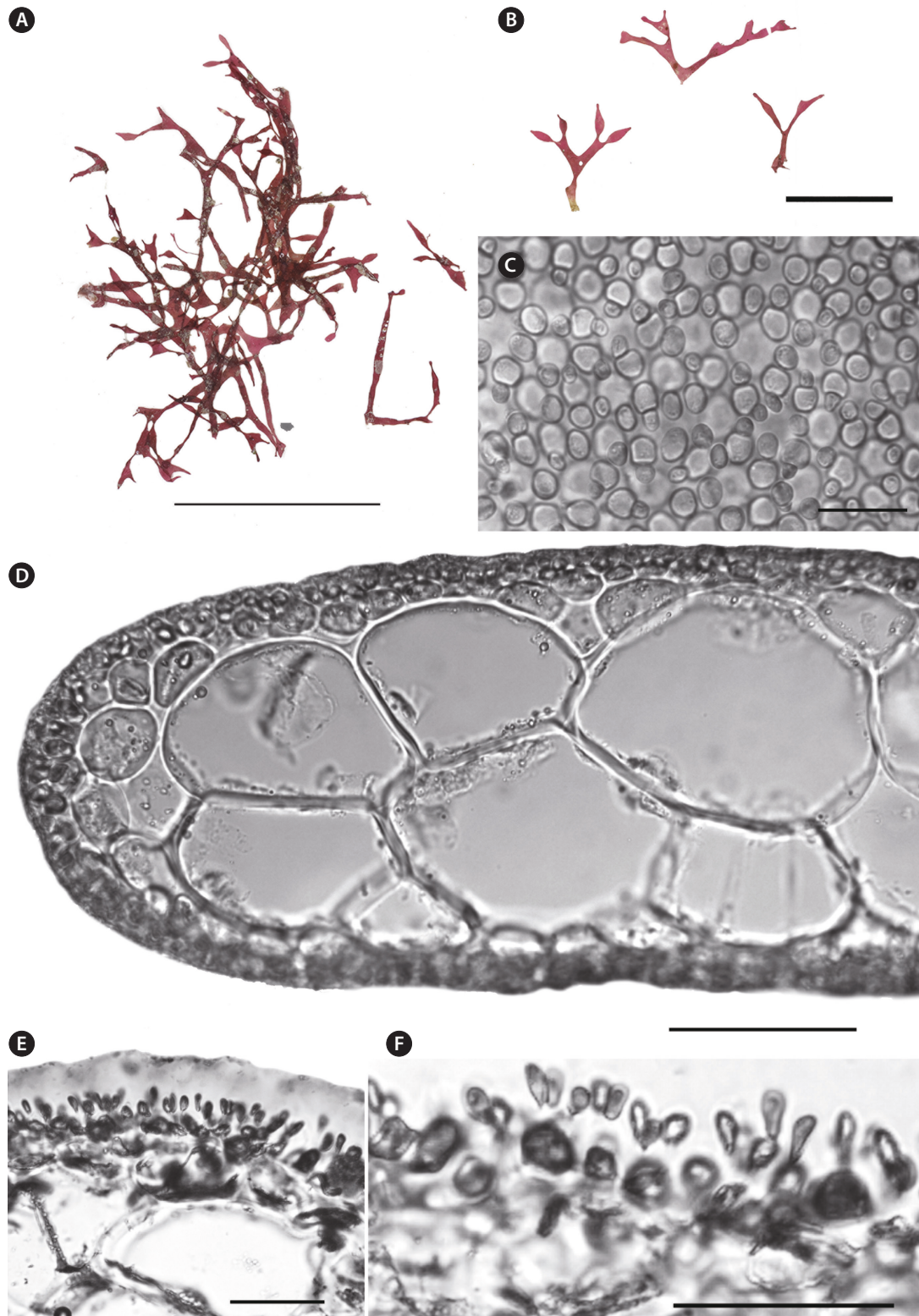


Fig. 4. Morphology of *Halopeltis nuahilihilia* sp. nov. (A) Image of the herbarium sheet of the holotype (BISH 785498, ARS 09898). (B) Image of the herbarium sheet of a paratype (BISH 785497, ARS 09912). (C) Surface view of the blade showing outer cortical cells (BISH 785498, ARS 09898). (D) Cross section of thallus, showing cortex and medulla (BISH 785498, ARS 09898). (E) Cross section of a developing spermatangial sorus (BISH 785498, ARS 09898). (F) Close up of spermatangia produced terminally on spermatangial mother cells (BISH 785498, ARS 09898). Scale bars represent: A & B, 5 cm; C, 25 µm; D, 100 µm; E & F, 50 µm.

specimens analyzed thus far for this species; however, all collections were made in the month of November, and it is possible that future collections representing other seasons will help to elucidate these characters.

***Halopeltis nuahilihilia* E. A. Alvarado, F. P. Cabrera and A. R. Sherwood sp. nov. (Fig. 4A–F)**

Description. Thalli up to 10.5 cm tall and 7.3 cm wide, including anastomosed portions. Plants layered on top of each other creating a tangled appearance *in situ*, spreading, with multiple anastomoses, attached to substratum by haptera. Thalli deep pink-purple to medium-pink in color. Vegetative axes narrow, 1–4 mm wide, subdichotomously branched. Constrictions along axes typically close to apices and sometimes close to points of branching. Vegetative axes flat and thin, up to 190 μm in cross section. Apices attenuate, spatulate, rounded, or ovular. Outer cortex composed of 1–2 layers, inner cortical layer incomplete. Cortical cells small and darkly staining, positioned irregularly, 11–17 μm by 8.5–13 μm . Medullary cells round to slightly elongated axially, 40–117 μm long by 40–79 μm wide, medullary cells in 2–3 layers, lightly staining. Small, intercolating cells absent. One or two elongate spermatangia measuring up to 4–15 μm long by 1.4–5 μm wide produced terminally by spermatangial mother cells 8.3–14 μm wide, formed in apical sori on the branches. Female gametangial and tetrasporangial reproduction not observed.

Holotype. BISH 785498 (ARS 09898, Hawai'i Island, Hawai'i, 20.016600° N, 155.843550° W, 90 m depth, Nov 11, 2015, leg. Randall Kosaki and Jason Leonard, field code MHI-20).

Holotype DNA accession numbers. ON464701 (COI), ON464714 (*rbcL*), ON730776 (LSU).

Paratype. BISH 785497 (ARS 09912, 'Au'au Channel, Maui, Hawai'i, 20.458500° N, 156.396000° W, 104 m depth, Nov 29, 2006, leg. Terry Kerby, field code P4-190 #443).

Paratype DNA accession numbers. ON464706 (COI), ON464720 (*rbcL*), ON689389 (LSU).

Etymology. Specific epithet derived from the Hawaiian language, pronounced “noo-ah-hee-lee-hee-lee-ah.” “Nu'a” describes the thick-growing nature of this alga. Additionally, “nu'a” refers to the way this alga grows, spreading out and appearing as a single mass of different branches connecting to each other. “Hilihili” is the action of braiding or plaiting. Its use in the name honors the twisted-like appearance of this alga, and its intertwining with other algal species. Moreover, “hili,” which translates as “dark red,” appropriately reflects the color of this

species.

Distribution. Mesophotic depths of Hawai'i Island, W. Moloka'i, and W. Maui, Hawai'i, USA.

Specimens examined. BISH 785497-785500 (ARS 09898, ARS 09912, ARS 10478, ARS 10479).

Habit and morphology. Specimens have a range of morphologies, from broadly branched, to less so (Fig. 4A & B). Specimens range in color from dark pink (Fig. 4A) to light pink (Fig. 4B). Apices are varied and present a variety of shapes: rounded, attenuate, and ovular. From a surface view, cortical cells are arranged loosely and irregularly, darkly staining (Fig. 4C). The outer cortex is composed of 1–2 layers with an incomplete inner layer, cells ranging in size from 11–17 μm by 8.5–13 μm (Fig. 4C & D). Medulla composed of 2–3 layers of these lightly staining cells (Fig. 4D). Medullary cells round to axially elongated, large and thin-walled, 40–117 μm long by 40–79 μm in width (Fig. 4D). Small, intercolating cells (which are reported for most species of *Halopeltis*) appear to be absent in the medulla of *H. nuahilihilia*. One or two spermatangia, 4–15 μm long by 1.4–5 μm wide in diameter, are grouped in surface sori situated in the apical parts of the branches, elongating from ovoid spermatangial mother cells 8.3–14 μm wide, that develop from the outer cortical cells (Fig. 4E & F). No other reproductive stages were observed.

DISCUSSION

Leptofauchea and *Halopeltis* are both new genus records for the Hawaiian Islands and contribute to the growing list of such additions to the Hawaiian flora (e.g., Huisman and Abbott 2003, Kraft et al. 2004, Sherwood and Carlile 2012, Conklin et al. 2014). Interestingly, specimens of both genera have very similar gross morphology and were collected from similar environments. Hawaiian *Leptofauchea* specimens were discovered growing in clumps among other macroalgal species, dispersed loosely in a sandy habitat, which resembles reports for other *Leptofauchea* species (Dalen and Saunders 2007). *Leptofauchea*, although largely reported from shallow waters, has been reported from depths greater than 30 m in other geographical regions (Gavio and Fredericq 2005, Dalen and Saunders 2007, Rodríguez-Prieto and de Clerck 2009), but *Halopeltis* had only been reported to 30 m (Saunders and McDonald 2010, Schneider et al. 2012) until the recent description of *H. tanakae* Mas. Suzuki and R. Terada from 50 m (Suzuki and Terada 2021). Thus, the samples studied here from the Hawaiian Islands are the deepest reported collections for both genera. In-

triguingly, however, *Leptofauchea* and *Halopeltis* are thus far only known from the mesophotic zone in Hawai‘i and have not been documented from depths shallower than 76 m in Hawai‘i. These genera add to our knowledge of algae present in MCEs of Hawai‘i, which have a high level of endemism for many groups of organisms (Kane et al. 2014, Baker et al. 2016, Pyle et al. 2016, Kosaki et al. 2017).

Of the Hawaiian *Leptofauchea* lineages revealed in the phylogenetic analyses, two can be assigned taxonomic names (either from previously described species or newly named here). Three specimens of *Leptofauchea* collected in Hawai‘i (ARS 09897, ARS 09899, and ARS 10013) are included in the molecular phylogenetic analyses but are positioned with low-to-no support. Due to the poor phylogenetic resolution of these specimens, and the small number of specimens available per lineage, recognition of these as new species will await collection of additional specimens to include in the analyses. Analyses yielded full support for *L. huawelau* sp. nov., which is a member of a larger clade that includes *L. munseomica*. Morphologically, *L. huawelau* is distinct from other species based on overall thallus shape: plants are remarkably long (reaching 21 cm in height) and narrow (typically 2–4 mm), giving them a “leggy” appearance (Table 2) (Gavio and Fredericq 2005, Dalen and Saunders 2007, Rodríguez-Prieto and de Clerck 2009, Suzuki et al. 2012, Filloramo and Saunders 2015). The spatulate apices, resulting from constrictions below the apices, are similar to those reported for *L. leptophylla* (Suzuki et al. 2012). The second Hawaiian species corresponded to the recently described *L. lucida* from Western Australia (Huisman and Saunders 2020). The Hawaiian specimens of *L. lucida* share diagnostic characters with specimens from Australia, including the sprawling and anastomosed habit with frequent secondary anastomoses between branches and a multi-layered cortex (Huisman and Saunders 2020).

Hawaiian specimens of *Halopeltis nuahilihilia* sp. nov. exhibited different degrees of maturation and consequently some variation in overall morphology, which provides insight into the plasticity of these features in the species. The most distinctive differences between *H. nuahilihilia* and other described species are the combination of narrow vegetative axes, the presence of constrictions along these axes in the Hawaiian specimens, and the apparent lack of small, intercolating cells in the medulla (Table 3). While constrictions are present in *H. pellucida* (Schneider et al. 2012), *H. verrucosa*, and *H. prostrata* (Saunders and McDonald 2010), they are more prominent in the mesophotic Hawaiian specimens.

While *Leptofauchea huawelau* sp. nov. is thus far known

only from Penguin Bank in the MHI, Hawaiian *Leptofauchea* specimens were collected from a broad geographical range that extends from Hawai‘i Island in the south-east of the archipelago to PMNM in the northwest. The *L. lucida* specimens from Hawai‘i extend the known range of this species from shallower habitats (5–37.1 m) in Western Australia (Huisman and Saunders 2020) to 80 m depth in Hawaiian MCEs. In addition, *L. huawelau* is sister to *L. munseomica*, known only from South Korea (Filloramo and Saunders 2015). Many marine algal species from Hawai‘i have been demonstrated to be most closely related to taxa from the Northwestern Pacific or Australia (Abbott and Huisman 2003, McDermid and Abbott 2006, Spalding et al. 2016, Paiano et al. 2020, Sherwood et al. 2020, Cabrera et al. 2022), which suggests an interesting biogeographic connection between the two regions, but more information on the mesophotic flora of the Pacific Islands is needed to know the true range and biogeographic processes that account for this distribution. In contrast to the broad distributional range of *Leptofauchea* in Hawai‘i, Hawaiian *Halopeltis* specimens were collected from a much narrower range, which includes only Maui to Hawai‘i Island in the MHI (the southeasternmost part of the archipelago). *H. nuahilihilia* sp. nov. is most closely allied with *H. adnata* from Korea and *H. tanakae* from Japan (Schneider et al. 2012, Suzuki and Terada 2021), suggesting biogeographical affinities between Hawaiian species and species from eastern Asia.

While about 80% of all coral reef habitat is included in MCEs (Pyle and Copus 2019), most marine biodiversity studies focus on shallow reefs, yielding a biased impression of marine diversity, function, and ecology. Uncovering hidden diversity in MCEs can help understand evolutionary and biogeographic patterns by adding to the current body of knowledge. Similarly, having adequate representation of mesophotic species in phylogenetic analyses can illuminate the links between shallow and deep-water species. However, there is still much to be done to categorize macroalgal diversity of mesophotic habitats. Multiple marine macroalgal species have been newly described from the Hawaiian mesophotic (e.g., Spalding et al. 2016, Sherwood et al. 2019, 2020, Paiano et al. 2020, Cabrera et al. 2022) and these species represent just a small fraction of the diversity that has yet to be categorized (Sherwood et al. 2010). The two new red algal genera reported here from the Hawaiian mesophotic, *Leptofauchea* and *Halopeltis*, demonstrate the need for a better understanding of the biodiversity of deep reefs.

ACKNOWLEDGEMENTS

We gratefully acknowledge the NOAA Papahānaumokuākea Native Hawaiian Cultural Working Group for their invaluable contributions in developing the specific epithets for the Hawaiian species of *Halopeltis* and *Leptofauchea*. G. McFall, S. Matadobra, B. Hauk, T. Efird, A. Fukunaga, D. Wagner, and H. Owen assisted with diving and small boat operations. Our gratitude goes to Terry Kerby and the Hawai'i Undersea Research Laboratory (HURL) *Pisces IV* and *V* submersible and *RCV-150* pilots and crew, as well as the crew of the R/V *Ka'imikai-o-Kanaloa*, for access to mesophotic habitats around the Main Hawaiian Islands. Field work and specimen collections in the Papahānaumokuākea Marine National Monument were authorized under Papahānaumokuākea Marine National Monument research permit PMNM-2019-001 issued to R. Kosaki. This work was supported by the U.S. National Science Foundation (DEB-1754117), the U.S. National Fish and Wildlife Foundation (NFWF 0810.18.059023), the National Oceanic and Atmospheric Administration (NOAA) Papahānaumokuākea Marine National Monument, NOAA Coastal Ocean Program (NA07NOS4780187 and NA07NOS478190 to the University of Hawai'i), NOAA's Undersea Research Program and Coral Reef Conservation Program through the Hawai'i Undersea Research Laboratory (NA09OAR4300219 and NA05OAR4301108), and NOAA's Office of Ocean Exploration. The scientific views and conclusions, as well as any views or opinions expressed herein, are those of the authors and do not necessarily reflect the views of the above organizations.

CONFLICTS OF INTEREST

The authors declare that they have no potential conflicts of interest.

SUPPLEMENTARY MATERIALS

Supplementary Table S1. Voucher and GenBank accession information for sequences used in phylogenetic analyses in the current study (<https://www.e-algae.org>).

Supplementary Fig. S1. Phylogenetic tree of the genus *Leptofauchea* based on 5' end of the mitochondrial cytochrome *c* oxidase I (COI-5P) sequences (<https://www.e-algae.org>).

Supplementary Fig. S2. Phylogenetic tree of the genus *Leptofauchea* based on *rbcL* sequences ([https://www.e-](https://www.e-algae.org)

[algae.org](https://www.e-algae.org)).

Supplementary Fig. S3. Phylogenetic tree of the genus *Leptofauchea* based on large subunit ribosomal DNA (LSU) sequences (<https://www.e-algae.org>).

Supplementary Fig. S4. Phylogenetic tree of the genus *Halopeltis* based on 5' end of the mitochondrial cytochrome *c* oxidase I (COI-5P) sequences (<https://www.e-algae.org>).

Supplementary Fig. S5. Phylogenetic tree of the genus *Halopeltis* based on *rbcL* sequences (<https://www.e-algae.org>).

Supplementary Fig. S6. Phylogenetic tree of the genus *Halopeltis* based on large subunit ribosomal DNA (LSU) sequences (<https://www.e-algae.org>).

Supplementary Fig. S7. Morphology of *Leptofauchea lucida* (<https://www.e-algae.org>).

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