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Biodiversity of Hawaiian Peyssonneliales (Peyssonneliaceae, Rhodophyta): new species in the genera *Incendia* and *Seiria*

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Abstract

Two new species, one in the genus *Incendia*, and one in *Seiria*, are illustrated and described here from mesophotic peyssonnelioid specimens collected in the Hawaiian Islands based on molecular and morphological analyses. Both genera are reported from Hawai'i for the first time. *Incendia lisianskiensis sp. nov.* differs from the other nine described members of the genus by its lack of hair cells, by the perithallial filaments arising at a more or less 90º angle from the hypothallus, while *Seiria mesophotica sp. nov.* is distinguished from the only other described species, *S. magnifusa*, by its lack of obvious and well-developed perithallial cell fusions. With the description of these two species the total number of recognized Hawaiian members of the Peyssonneliales rises to nine. Previously recorded species included *Peyssonnelia conchicola, P. inamoena*, *P. japonica*, *P. rubra*, *Ramicrusta hawaiiensis*, *R. lehuensis*, and *Sonderophycus copusii*.

Keywords: coral reef, DNA sequencing, mesophotic, microscopy, Northwestern Hawaiian Islands, Papahānaumokuākea Marine National Monument, red algae, systematics

Introduction

The last decade has seen a rapid increase in the systematic revision and description of new species within the crustose red algal order Peyssonneliales. In particular, several genera have been described, resurrected, or taxonomically clarified (e.g., *Incendia*, *Polystrata*, *Ramicrusta*, *Seiria*, *Sonderophycus*), with a number of species now recognized or described in these formerly more obscure genera (Zhang & Zhou 1981, Kato *et al*. 2006, Krayesky *et al*. 2009, Pueschel & Saunders 2009, Dixon & Saunders 2013, Ballantine *et al*. 2016).

The genus *Incendia* (2013: 84) was described in 2013 on the basis of a distinctive phylogenetic position and DNA barcode using COI, *rbcL*, and EF2 sequence data, and the presence of secondary pit connections in the perithallus (Dixon & Saunders 2013). Specimens also possessed multicellular rhizoids, distinguishing them from members of the closely related genus, *Ramicrusta* (1981: 538), which has unicellular rhizoids (Zhang & Zhou 1981, Dixon & Saunders 2013). Nine species are currently recognized within *Incendia*, with eight of these distributed in the Pacific (Australia, the Philippines, Vanuatu: *I. basilii* K.R.Dixon, *I. crenata* K.R.Dixon, *I. cryptica* K.R.Dixon, *I. cryptotricha* K.R.Dixon, *I. glabra* K.R.Dixon, *I. homosorora* K.R.Dixon, *I. regularis* K.R.Dixon, *I. undulata* K.R.Dixon) and one

recently described from Brazil (*I. yoneshigueana* Pestana, G.N.Santos, V.Cassano & J.M.C.Nunes) (Dixon & Saunders 2013, Dixon 2018, Pestana *et al*. 2020). The closely related genus, *Seiria* (2018: 239), was described in 2018 based on a morphology distinctive from *Incendia*, including extensive and directional cell fusions among lower and mid-perithallial cells, unicellular rhizoids, and the serial production and release of tetrasporangia (Dixon 2018). Molecular analyses of *Seiria* published by Dixon (2018) included only a COI barcoding analysis, but illustrated *Seiria* as distinct from other specimens of *Incendia*, although very similar in COI sequence.

Over the course of surveys of Hawaiian algal diversity, several specimens corresponding to *Incendia* and *Seiria* were collected from mesophotic depths (~30–150 m in tropical and subtropical waters) at Manawai (Pearl and Hermes Atoll), Kapou (Lisianski Island), and Moloka‘i. Here we provide molecular and morphological characterizations of these specimens supporting their description as two new species in the genera *Incendia* and *Seiria*.

Materials and methods

Three specimens of peyssonnelioid red algae were collected off west Moloka‘i in the Main Hawaiian Islands by the *Pisces IV* submersible through the Hawai‘i Undersea Research Laboratory (HURL), and at Kapou (Lisianski Island), and Manawai (Pearl and Hermes Atoll) in the Papahānaumokuākea Marine National Monument (PMNM), by National Oceanic and Atmospheric Administration (NOAA) divers (Table 1). Entire specimens were preserved in silica gel desiccant and/or pressed as herbarium specimens, and were assigned a Sherwood Lab accession number (a 5-digit number prefixed by “ARS”). Morphological and anatomical investigations of the samples were conducted by gently rehydrating small pieces of the specimens in a detergent (Modified Pohl’s Solution, Clark, unpubl.: https://www.eeob.iastate.edu/research/bamboo/pdf/anatomy_protocols.pdf) for 30 min, decalcifying in 5% HCl for 15 min to 2 hr, hand sectioning with a double-edged razor blade, staining with 1% aniline blue, and mounting in 30% 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) with brightfield or differential interference contrast optics. Specimens were deposited in the Bernice P. Bishop Museum (BISH) under accessions BISH 780872–780874.

The specimens were extracted for genomic DNA using an OMEGA E.Z.N.A.® Plant DNA DS Kit (OMEGA Biotek, Norcross, GA, USA). The 5’ end of the COI gene (cytochrome oxidase subunit I) was amplified using the GWSFn (Le Gall & Saunders 2010) and GWSRx (Saunders & Moore 2013) primers. The *rbcL* (ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit) marker was amplified as three overlapping fragments using primer pairs from Freshwater & Rueness (1994), Gavio & Fredericq (2002), Kim *et al*. (2010), and Kang & Kim (2013). 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 2021.0.3 (http://www.geneious.com) with available sequences for related genera from GenBank and BOLD (Table 2). Distances between sequences are reported as p-distances, as calculated in Geneious Prime. DNA barcode analysis of the COI sequences was performed by constructing a neighbor-joining tree based on Kimura-2-parameter distances using MEGA (Stecher *et al*. 2020). This technique was employed only as a tool for visualizing potential species delimitation, and not for phylogenetic inference (DeSalle & Goldstein 2019). For the *rbcL* phylogenetic analyses, sequences were aligned with reference sequences representing the available diversity of genera in the Peyssonneliales in GenBank and analyzed with PartitionFinder 2 (Lanfear *et al.* 2017). Maximum Likelihood (ML) analyses were performed 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 MrBayes v. 3.2.7a (Huelsenbeck *et al*. 2003) through the CIPRES Science Gateway (Miller *et al*. 2010) using four chains of Metropolis-coupled Markov Chain Monte Carlo for 5,000,000 generations, sampling every 500 generations and other parameters set as default. Twenty-five percent of sampled trees were discarded as burn-in to determine posterior probabilities. Tracer v1.7.1 was used to estimate the burn-in cutoff and to check if further runs were required to reach convergence, with the average standard deviation of split frequencies value = 0.0097 (Rambaut *et al*. 2018).

TABLE 1. Specimens of *Incendia* and *Seiria* from the Hawaiian Islands characterized as part of the current study.

Species	Sherwood Lab accession	BISH accession	Field code	Collection information (latitude/longitude in decimal degrees)	GenBank accession (COI)	GenBank accession (*rbcL*)
Incendia lisianskiensis sp. nov.	ARS 09969	BISH 780872	NWHI- 846	Kapou (Lisianski Island), Papahānaumokuākea Marine National Monument, Hawai'i, USA, 26.025277°N, 174.15694°W, 55 m depth, 30 July 2019, leg. B. Hauk	MZ043101	MZ047757
Seiria mesophotica sp. nov.	ARS 09666	BISH 780873	P4-189-cont.8-#328	off West Moloka'i, Hawai'i, USA 21.045528°N, 157.3524°W, 80 m depth, 28 November 2006, leg. H. Spalding	MZ043099	MZ047754
Seiria mesophotica sp. nov.	ARS 09967	BISH 780874	NWHI- 833	Manawai (Pearl and Hermes Atoll), Papahānaumokuākea Marine National Monument, Hawai'i, USA, 27.9625°N, 175.8361°W, 67 m depth, 31 July 2019, leg. S. Matadobra	MZ043100	MZ047756

TABLE 2. Voucher and GenBank accession information for sequences used in comparative analyses of the COI and *rbcL* markers.

Species	Voucher number	GenBank accession (COI)	GenBank accession (*rbcL*)
Incendia basilii	VT030	JX969745	-
Incendia crenata	VT015	JX969689	-
Incendia crenata	VT095	JX969714	-
Incendia crenata	VT114	JX969723	-
Incendia cryptotricha	VT070	JX969738	-
Incendia glabra	VT141	JX969688	JX969774
Incendia regularis	VT039	JX969750	JX969803
Incendia undulata	VT005	JX969706	-
Incendia yoneshigueana	ALC132951_P19	-	MN990096
Incendia lisianskiensis sp.nov.	ARS09969	MZ043101	MZ047757
Metapeyssonnelia corallepida	K85	-	EU349214
Metapeyssonnelia sp.	DLB8375	-	JQ429756
Peyssonnelia armorica	SAP097585	-	AB325852
Peyssonnelia atropurpurea	SAP098399	-	AB325853
Peyssonnelia atropurpurea	CM04	-	JX969782
Peyssonnelia boergesenii	K135	-	EU349145
Peyssonnelia boergesenii	K156	-	EU349147
Peyssonnelia bourdouresquei	RH02031	-	KC998947
Peyssonnelia coriacea	LAF5357	-	KR732905
Peyssonnelia coriacea	LAF5463	-	KR732903
Peyssonnelia distenta	K167	-	EU349141
Peyssonnelia distenta	K235	-	EU349140
Peyssonnelia dubyi	CM03	-	JX969785
Peyssonnelia dubyi	SAP097598	-	AB325857
Peyssonnelia harveyana	SAP097591	-	AB325859
Peyssonnelia harveyana	SAP097593	-	AB325860
Peyssonnelia immersa	SAP098402	-	AB325861
Peyssonnelia inamoena	K113	-	EU349118
Peyssonnelia inamoena	K160	-	EU349127
Peyssonnelia japonica	SAP102032	-	AB325815
Peyssonnelia japonica	SAP102043	-	AB325818
Peyssonnelia japonica	SAP102049	-	AB325820
Peyssonnelia meridionalis	SAP098403	-	AB325864
Peyssonnelia nordstedtii	K208	-	EU349148
Peyssonnelia orientalis	SAP102071	-	AB325865
Peyssonnelia orientalis	ARS09966	-	
Peyssonnelia polymorpha	LAF5355	-	KR732911
Peyssonnelia polymorpha	LAF5361	-	KR732912
Peyssonnelia rubra	LAF5461	-	KR732915
Peyssonnelia rubra	LAF5462	-	KR732916
Peyssonnelia squamaria	K217	-	EU349180
Peyssonnelia squamaria	K218	-	EU349181
Polystrata dura	SAP098381	-	AB325868
Polystrata dura	SAP098388	-	AB325869
Polystrata dura	SAP102073	-	AB325870
Polystrata dura	SAP102074	-	AB325871

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TABLE 2 (Continued**)**

Species	Voucher number	GenBank accession (COI)	GenBank accession (*rbcL*)
Polystrata fosliei	K101	-	EU349211
Polystrata fosliei	K102	-	EU349212
Polystrata fosliei	K155	-	EU349213
Polystrata sp.	1BER	-	KT310709
Ramicrusta aranea	VT106	JX969701	JX969780
Ramicrusta appressa	VT108	JX969695	-
Ramicrusta appressa	GWS025723	JX969707	-
Ramicrusta australica	PLD004	JX969724	JX969787
Ramicrusta bonairenesis	CL-2016a	KX417374	-
Ramicrusta fujiiana	P10	-	MN990097
Ramicrusta fujiiana	P11	-	MN990098
Ramicrusta fujiiana	P12	MN990086	MN990099
Ramicrusta fujiiana	P13	MN990087	MN990100
Ramicrusta fujiiana	P15	-	MN990101
Ramicrusta fujiiana	P22	MN990088	MN990102
Ramicrusta fujiiana	P136	MN990089	-
Ramicrusta fujiiana	P156	MN990090	-
Ramicrusta fujiiana	P2	MN990085	-
Ramicrusta hawaiiensis	ARS09600	MN623629	MN623630
Ramicrusta lateralis	VT109	JX969721	-
Ramicrusta lehuensis	ARS09609	MN623631	MN623632
Ramicrusta monensis	CL-2016b	KX417375	-
Ramicrusta nanhaiensis	GWS002520	JX969713	-
Ramicrusta paradoxa	P1	MN990091	MN990103
Ramicrusta paradoxa	P7	MN990092	MN990104
Ramicrusta paradoxa	P2	MN990095	-
Ramicrusta paradoxa	P18	MN990093	-
Ramicrusta paradoxa	P21	MN990094	-
Ramicrusta textilis	GWS001755	JX969749	KC130226
Ramicrusta textilis	VT079	JX969690	JX969775
Ramicrusta textilis	VT160	JX969704	-
Ramicrusta textilis	DLB-7794	KX417373	-
Ramicrusta textilis	SD17056	MK616540	-
Ramicrusta textilis	SD17001	MK616539	-
Ramicrusta textilis	SD17079	MK616538	-
Ramicrusta trichaurea	VT105	JX969719	-
Riquetophycus polypus	K183	-	EU349170
Riquetophycus polypus	K185	-	EU349179
Riquetophycus sp.	K175	-	EU349172
Riquetophycus sp.	K236	-	EU349171
Seiria magnifusa	GWS016756/ABMMC8644-10	HM918341	-
Seiria magnifusa	GWS016757/ABMMC8645-10	HM918342	-
Seiria magnifusa	GWS016758/ABMMC8646-10	HM918343	-
Seiria magnifusa	GWS016727/ABMMC8615-10	HM918333	MW996699
Seiria magnifusa	GWS034602/OZSEQ3934-13	MW996536	-
Seiria mesophotica sp.nov.	ARS09666	MZ043099	MZ047754
Seiria mesophotica sp.nov.	ARS09967	MZ043100	MZ047756

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TABLE 2 (Continued**)**

Species	Voucher number	GenBank accession (COI)	GenBank accession (*rbcL*)
Sonderophycus capensis	K214	-	EU349186
Sonderophycus capensis	K216	-	EU349188
Sonderophycus capensis	PLD002	JX969697	-
Sonderophycus capensis	PLD009	JX969694	-
Sonderophycus capensis	JJ010	JX969743	-
Sonderophycus capensis	GWS025074	JX969733	-
Sonderophycus capensis	GWS025003	JX969748	-
Sonderophycus capensis	GWS024474	JX969739	-
Sonderophycus capensis	GWS024511	JX969708	-
Sonderophycus capensis	GWS024475	JX969696	-
Sonderophycus capensis	GWS024536	JX969710	-
Sonderophycus capensis	GWS024535	JX969752	-
Sonderophycus capensis	VT061	JX969700	-
Sonderophycus capensis	G0418	KC130203	-
Sonderophycus copusii	ARS09651	MT012464	MT012465
Sonderophycus copusii	ARS09653	MT012466	MT012467
Sonderophycus copusii	ARS09686	MT012468	MT012469
Sonderophycus coriacea	GWS001476	JX696711	-
Sonderophycus coriacea	LAG04	JX969705	-
Sonderophycus coriacea	PLD003	JX969693	-
Sonderophycus coriacea	GWS016389	JX969687	-
Sonderophycus coriacea	GWS015628	JX969698	-
Sonderophycus fervens	ABMMC188407	-	JX969779
Sonderophycus fervens	ABMMC246508	-	KC130225

Results

Incendia lisianskiensis A.R. Sherwood *sp. nov.* (Fig. 1A–J)

Type:—USA. Hawai'i, Papahānaumokuākea Marine National Monument, Kapou (Lisianski Island), 26.025277°N, 174.15694°W, 55 m depth, 30 July 2019, *B. Hauk* (holotype BISH 780872; ARS 09969; field code NWHI-846).

Description: Thallus crustose, prostrate, on coral rubble, reddish in color with orange markings (Fig. 1A). Thallus moderately calcified, with undulating margins, and raised ridges on the thallus surface Fig. 1A). Hypothallus filaments consisting of mostly unbranched, parallel files of rectangular cells, cells 5.0–7.0 µm in width × 16.3–19.0 µm in height, giving rise to perithallial assurgent filaments above and rhizoids below (Fig. 1B–E). Crusts thin, ranging from 120–190 µm. Perithallial assurgent filaments arising at more or less 90º angle from hypothallus (Fig. 1B–D). Cells of mid- to lower perithallus irregularly subisodiametric, 5.1–9.2 µm in width × 7.5–11.4 µm in height (Fig. 1B–D). Lower perithallial cells forming cellular projections and secondary pit connections (Fig. 1B–D). Upper perithallial cells smaller than those in the lower perithallus, isodiametric to slightly taller than broad; 3.4–7.2 µm width × 6.7–8.2 µm height (Fig. 1B,C). Rhizoids 4.8–10.0 µm diameter, medium to long, unbranched, and multicellular (Fig. 1F). Hair cells absent (Fig. 1G). Gametangial and tetrasporangial reproduction not observed.

Distribution and Habitat:—Known only from the type locality.

Etymology:—Named for the type locality.

Identification using DNA sequence data:—Neighbor-joining analysis of available COI sequences for currently recognized species of *Incendia* and *Seiria* (and other non-*Peyssonnelia* species in the Peyssonneliales) supported the recognition of *I. lisianskiensis* as a novel species (Fig. 2). The *Incendia lisianskiensis* COI sequence (MZ043101) differed by 7.4% (p-distance) from its most similar congener with COI representation, *I. regularis* K.R.Dixon, and by

>9.0% from all other available *Incendia* COI sequences. The *rbcL* phylogeny (ML topology is shown, with support values from both analyses included at nodes) also indicated a close relationship between *I. lisianskiensis* and *I. regularis*, supporting the inclusion of this specimen in the genus *Incendia*, and the distinction of *I. lisianskiensis* from other *Incendia* taxa included in the analysis (Fig. 3).

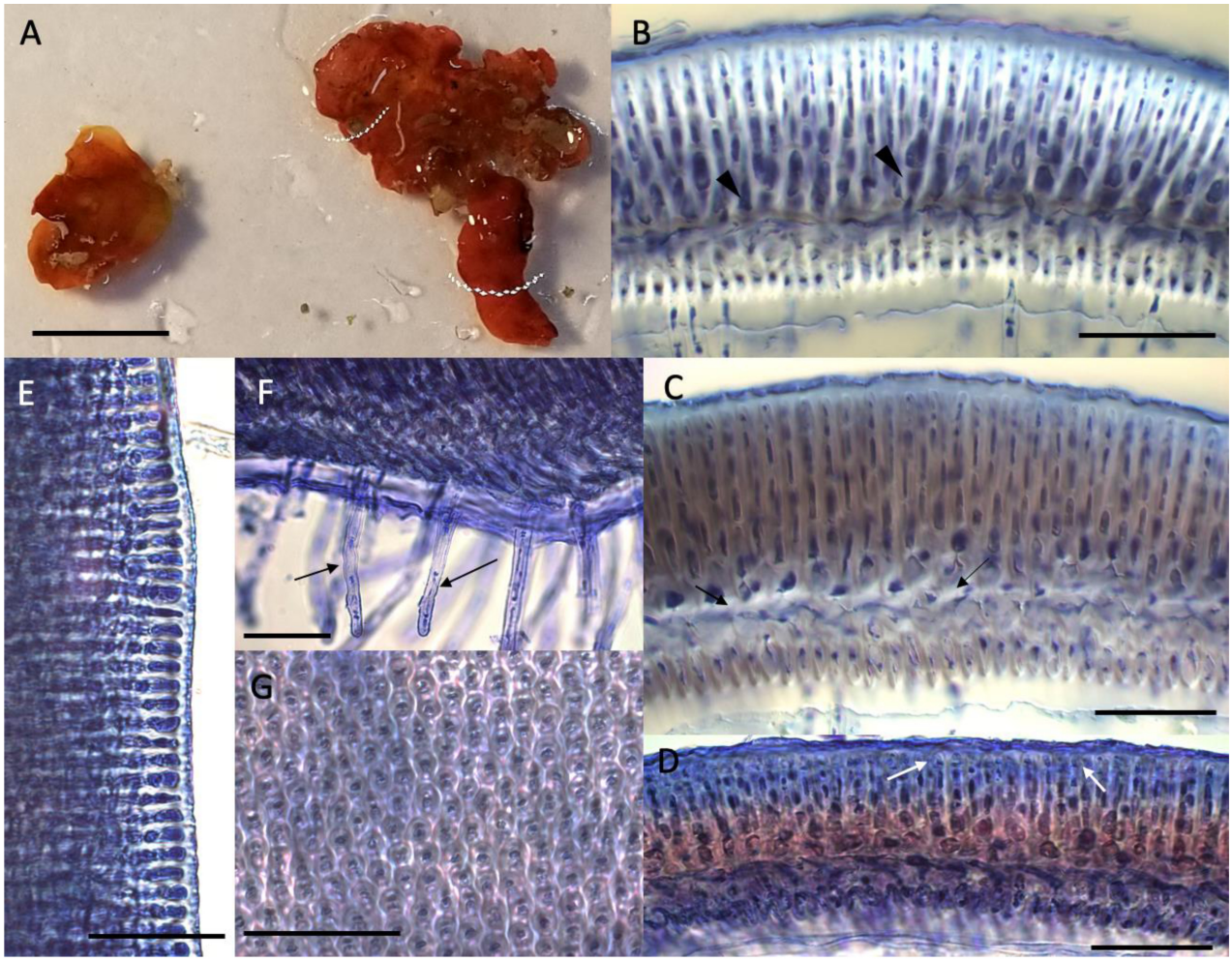

FIGURE 1. Habit and morphology of *Incendia lisianskiensis sp. nov.* (BISH 780872) (A). Image of specimen fragments immediately after collection. (B). Radial vertical section (RVS) through thallus illustrating perithallial assurgent filaments (arrowheads) arising at a more or less 90º angle from hypothallus. (C). RVS showing cellular projections in the lower perithallus (arrows). (D). RVS illustrating small, upper perithallial cells (arrows). (E). Marginal apical cells of the thallus. (F). Long, unbranched, multicellular rhizoids extending from the hypobasal cuticle (arrows). (G). View of the top surface of the crust, showing regularly sized cells, and a lack of hair cells. Scale bars: A = 1 cm; B-G = 50 µm.

Seiria mesophotica A.R. Sherwood *sp. nov.* (Fig. 4A–J)

Type:—USA, Hawai'i, off West Moloka'i, 21.0455°N, 157.3524°W, 80 m depth, 28 November 2006, *H. Spalding* (holotype BISH 780873; ARS 09666; field code P4-189-cont.8-#328).

Description: Thallus crustose, prostrate, on coral rubble, brick red to dark, brownish maroon in color (Fig. 4A,B). Thallus moderately to heavily calcified, somewhat lobed or entire, mostly to completely adherent to substratum. Crusts thin, typically approximately 100 µm, ranging from 80–180 µm (Fig. 4C–E). Hypothallus filaments consisting of parallel files of cells (Fig. 4G), mostly unbranched, cells 4.6–8.8 µm in width and 18.2–27.7 µm in height, giving rise to perithallial assurgent filaments above and rhizoids below. Perithallial assurgent filaments arising at 60–90º angle from hypothallus (Fig. 4C–E). Perithallus thin, composed of short filaments that are mostly 4–8 cells long (Fig. 4C–E). Lower perithallial cells taller than broad, 14.5–24.8 µm in height × 5.9–8.0 µm in width (Fig. 4D). Perithallial cells becoming shorter towards upper portion of perithallus. Mid-perithallial fusion cells absent. Uppermost perithallial cells quasi-isodiametric, 7.8–15.8 µm width × 5.0–9.3 µm height (Fig. 4E). Cuticle on surface of perithallus 4–8

μm thick (Fig. 4C–E). Rhizoids 5.4–8.8 μm diameter, mostly short to medium length, unbranched, and unicellular (Fig. 4F). Hair cells absent (Fig. 4H). Gametangial reproduction not observed. Mature tetrasporangia 43–99 × 22–44 μm, cruciate decussate to irregularly cruciate (Fig. 4I), borne in raised, subcircular, scattered gelatinous nemathecia, nemathecial paraphyses up to 9 cells in length, 130 μm long (Fig. 4J).

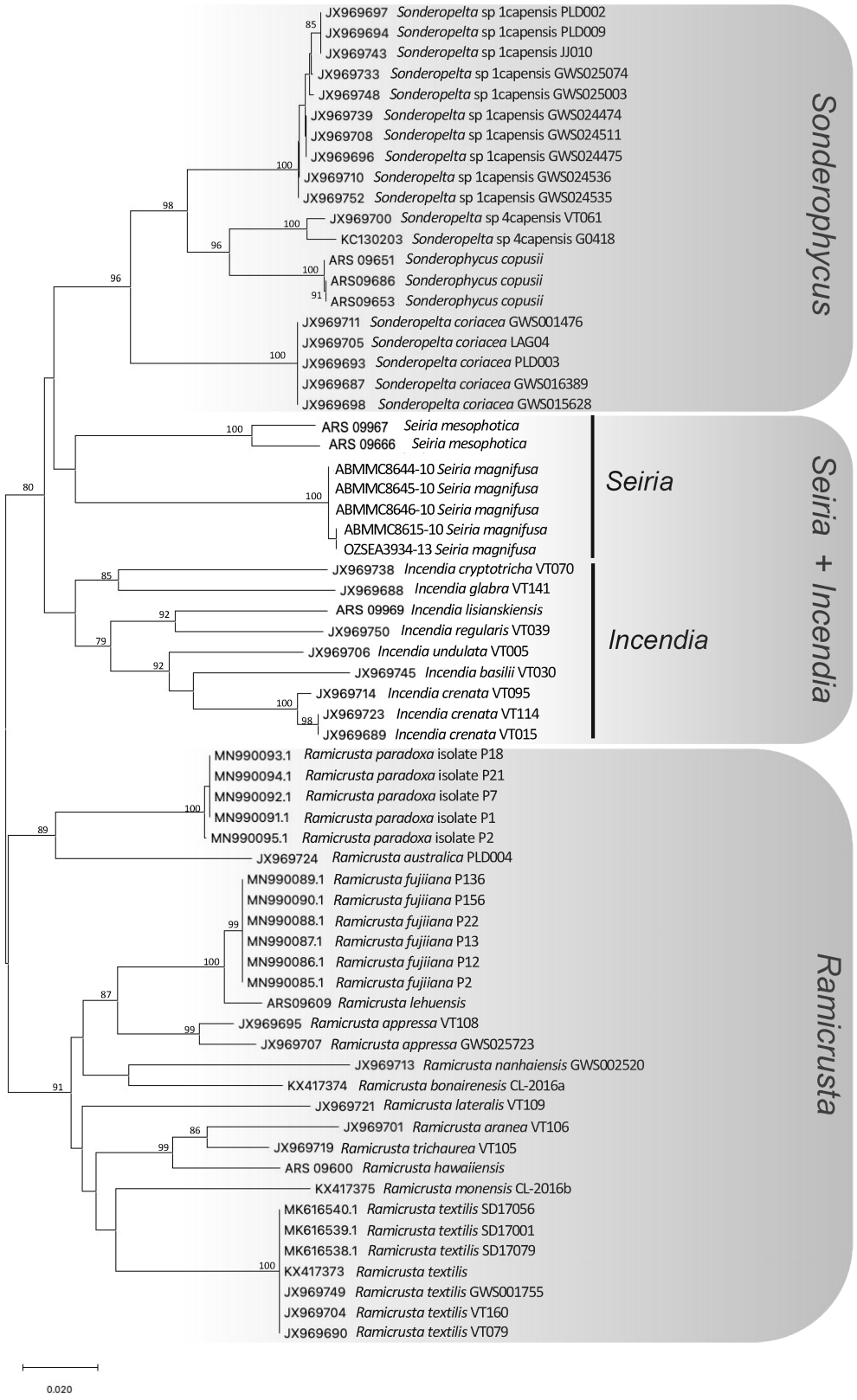

FIGURE 2. Neighbor-joining framework based on Kimura-2-parameter distances of COI sequences of specimens of the Peyssonneliales, including *Incendia* and *Seiria*. Scale bar = substitutions per site.

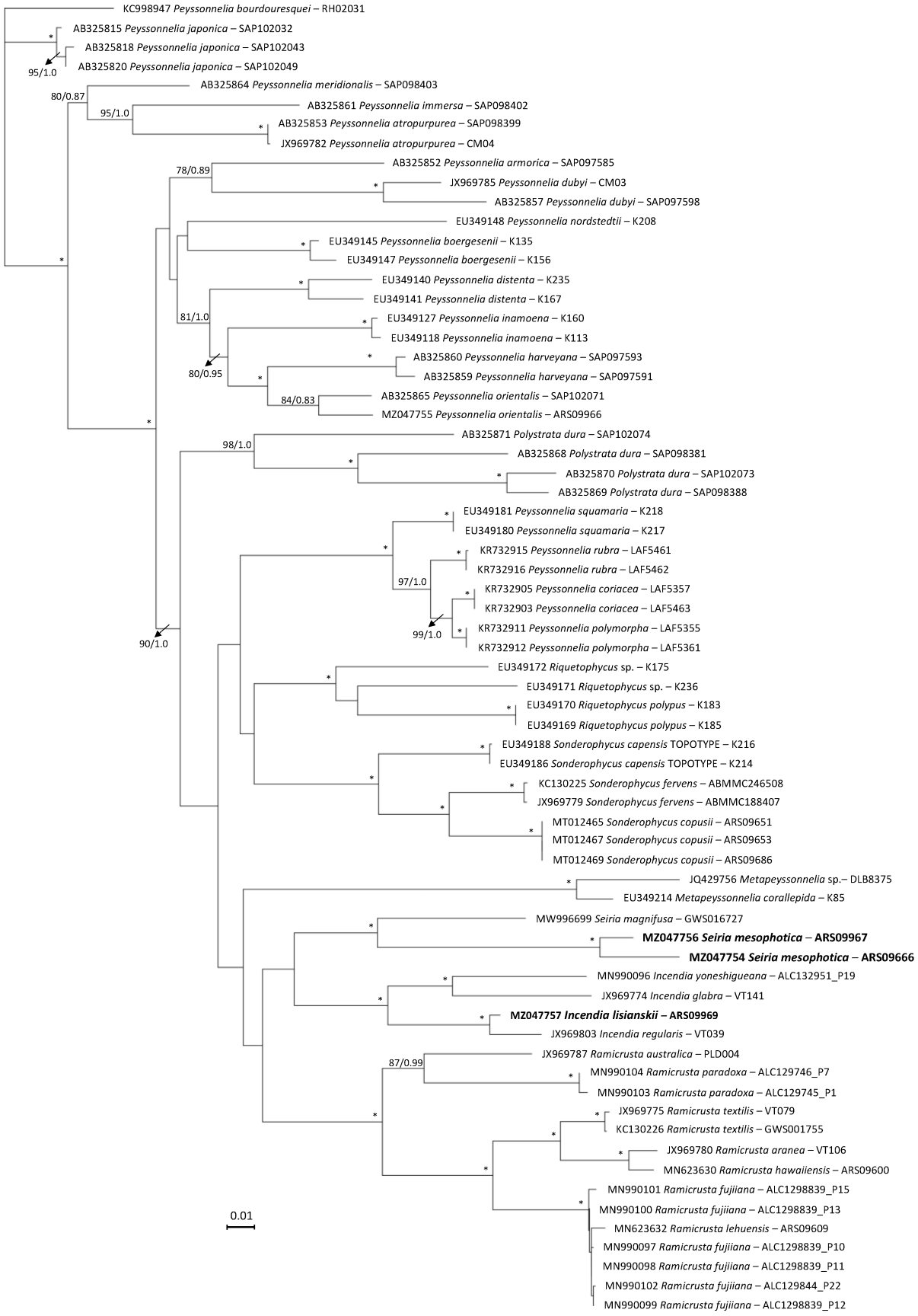

FIGURE 3. Maximum likelihood phylogenetic tree of *rbcL* sequences for *Incendia, Seiria*, and closely related genera in the Peyssonneliales. Numbers along branches indicate nodal support (first value = bootstrap support, second value = Bayesian posterior probabilities). Nodes with full support are indicated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

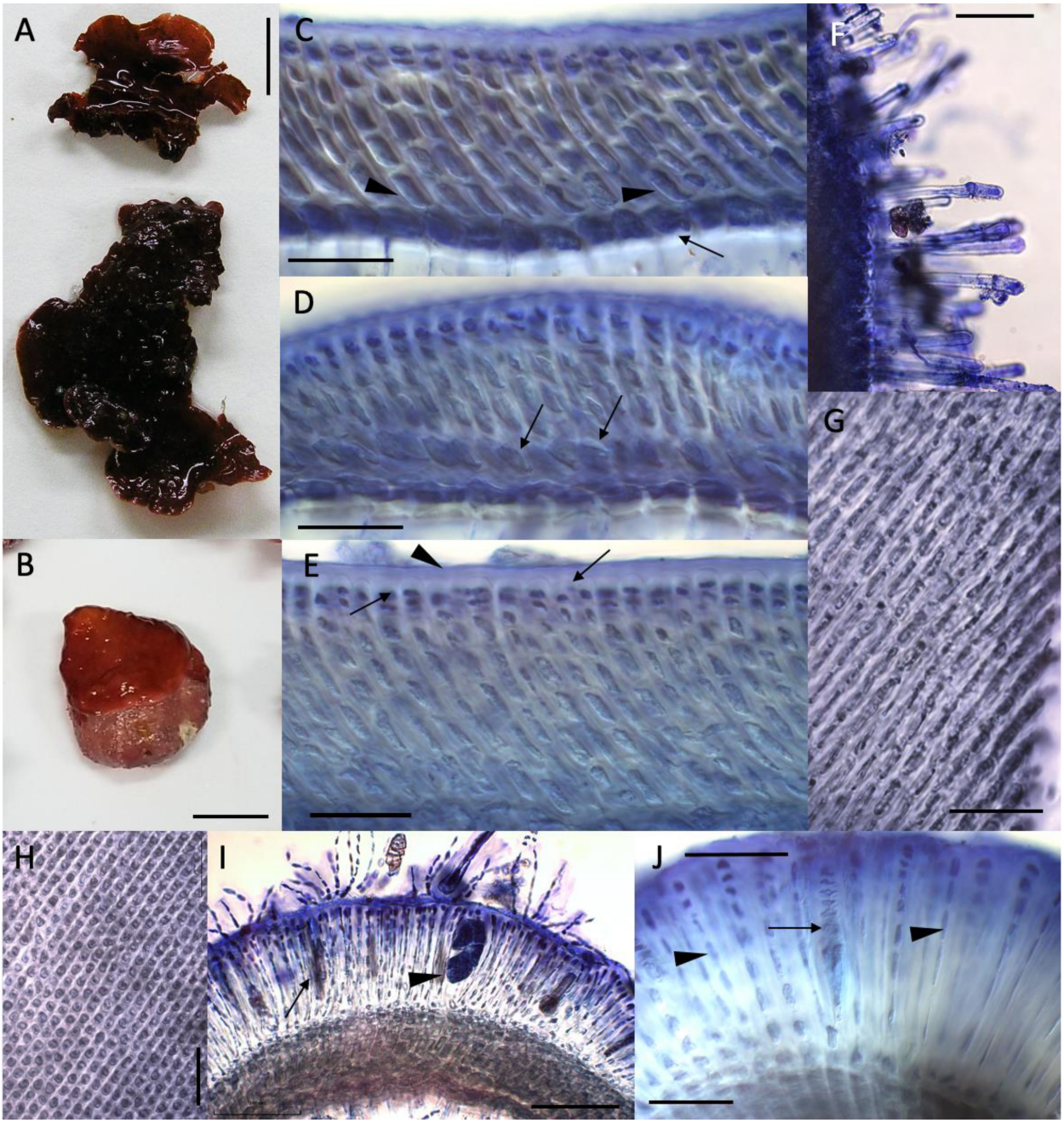

FIGURE 4. Habit and morphology of *Seiria mesophotica sp. nov*. (A). Image of specimen fragments (BISH 780873) immediately after collection. (B). Image of specimen (BISH 780874) immediately after collection. (C). Radial vertical section (RVS) through thallus illustrating the basal hypothallus (arrow), and perithallial assurgent filaments (arrowheads) (BISH 780873). (D). RVS showing large lower perithallial cells (arrows) (BISH 780873). (E). RVS illustrating small, quasi-isodiametric uppermost perithallial cells (arrows) and thick cuticle (arrowhead) (BISH 780874). (F). RVS showing unicellular rhizoids extending from the basal hypothallus (BISH 780873). (G). View of the underside of the crust, illustrating the parallel files of cells comprising the hypothallus (BISH 780873). (H). View of the top surface of the crust, showing regularly sized cells, and a lack of hair cells (BISH 780873). (I). RVS through a tetrasporangial nemathecium, with immature (arrow) and mature (arrowhead) tetrasporangia (BISH 780873). (J). Close up of a tetrasporangial nemathecium, illustrating a tetrasporangium (arrow) and paraphyses consisting of up to nine cells (arrowheads) (BISH 780873). Scale bars: A-B = 1 cm; C-H = 50 µm; I = 100 µm.

Other examined material:—USA, Hawai‘i: Papahānaumokuākea Marine National Monument, Manawai (Pearl and Hermes Atoll), 27.9625°N, 175.8361°W, 67 m depth, 31 July 2019, *S. Matadobra* (BISH 780874; ARS 09967; field code NWHI-833).

Distribution and Habitat:—Known from the off West Molokaʻi in the Main Hawaiian Islands and Manawai of the Papahānaumokuākea Marine National Monument at mesophotic depths of 67-80 m.

Etymology:—Named for the mesophotic depths at which the specimens were collected.

Identification using DNA sequence data:—A DNA barcoding analysis of COI sequences for the two specimens of Hawaiian *Seiria* (MZ043099 and MZ043100) and available COI data for related taxa demonstrated less than 4% sequence divergence between the two Hawaiian specimens (3.3% difference between ARS 09666 and ARS 09967), and much greater distance to the type species, *S. magnifusa* K.R.Dixon (12.0-12.2%; Fig. 2). The *rbcL* phylogeny supported *S. magnifusa* as the closest relative to *S. mesophotica*, with 12.1-13.0% difference in the sequences of the two taxa (Fig. 3).

Discussion

Collections of shallow and mesophotic specimens of crustose red algae in the Hawaiian Islands have recently yielded several species new to science, including species within the previously unreported genera *Ethelia*, *Ramicrusta*, and *Sonderophycus* (Sherwood *et al*. 2020, Sherwood *et al*. 2021a,b). The present study reports two new species of peyssonnelioid red algae from the Hawaiian Islands, representing two additional new genus records; neither *Incendia* nor *Seiria* have been previously recorded in the Hawaiian Islands. This study brings the number of confirmed peyssonnelioid genera in Hawaiʻi to five (now including *Incendia*, *Peyssonnelia*, *Ramicrusta*, *Seiria*, and *Sonderophycus*).

Incendia lisianskiensis is consistent with the morphological features defining the genus (e.g., bright orange markings on the thallus, perithallial assurgent filaments arising from a monostromatic hypothallus, secondary pit connections present in the lower hypothallus, and multicellular rhizoids), but differs from the nine other described species in several ways. Like *I. glabra* K.R. Dixon and *I. yoneshigueana* E.M.S. Pestana, G.N. Santos, Cassano & J.M.C. Nunes, *Incendia lisianskiensis* appears to lack hair cells (Dixon & Saunders 2013, Pestana *et al*. 2020); however, unlike the previous two species, *I. lisianskiensis* is not a member of the same *rbcL* clade, and instead forms a sister relationship with *I. regularis*, from Vanuatu (Fig. 3). *Incendia lisianskiensis* bears morphological similarity to *I. cryptica* K.R. Dixon, to which we infer a close relationship based on the COI barcode distance tree presented in Dixon (2018), but the sequences from that study do not appear to be publicly available, and thus could not be included in our analyses. However, *Incendia lisianskiensis* differs from *I. cryptica* in having perithallial filaments that arise at a more or less 90º angle from the hypothallus (in contrast to the more acute angle of *I. cryptica* and all other known *Incendia* species), and in lacking hair cells (Dixon & Saunders 2013, Dixon 2018, Pestana *et al*. 2020). Additionally, both the COI and *rbcL* analyses supported recognition of *I. lisianskiensis* as a distinct species (Figs 2–3). Although only three out of nine currently recognized species of *Incendia* have published *rbcL* sequences, the phylogenetic tree demonstrates that *I. lisianskiensis* is distinct from those other representatives, and that the phylogenetic position of *I. lisianskiensis* as sister to *I. regularis* is consistent with the sequence similarities of the COI analysis (Fig. 3). Dixon & Saunders (2013) reported 0–2.0% COI intraspecific divergence for members of this red algal group, and 4.1–12.7%, with a general cutoff of 4.0% or greater distance in COI as marking different species. Thus, the 7.4% difference in COI between *I. lisianskiensis* and its closest congener is consistent with its recognition as a distinct taxon from other sequenced members of the genus.

Seiria mesophotica sp. nov. lacks the remarkable perithallial cell fusions that characterize *S. magnifusa*, the generitype and only other described species in the genus. Other morphological differences between the two species include smaller hypothallial cell size in *S. mesophotica* (5–9 × 18–28 µm, versus 10–15 × 20–38 µm in *S. magnifusa*), and larger tetrasporangial sorus paraphysis size in *S. mesophotica* (up to 9 cells for a total length of 130 µm, versus up to 7 cells for a total length of 100 µm in *S. magnifusa*) (Dixon 2018). Thus, there are clear morphological differences to separate the two species. Thalli of both Hawaiian specimens of *S. mesophotica*, despite being geographically separated by approx. 2,000 miles, are relatively thin (approx. 100 µm), and have very similar ranges of measurements for vegetative morphological features, but only one of the two specimens was reproductive (ARS 09666; tetrasporic). The specimens share 96.7% identity (3.3% difference) in COI sequence and 95.8% identity (4.2% difference) in *rbcL* (although the corresponding *rbcL* sequences overlap by only 642 bp), but differ substantially from the sequences of *S. magnifusa* (12.0–12.2% for COI and 12.1–13.0% for *rbcL*).

Despite the obvious drawbacks of describing species based on single collections, the practice is not rare for this group of red algae (e.g., Dixon & Saunders 2013). Moreover, the difficulty with which mesophotic algal specimens are collected (i.e., the need for submersible or technical diving to access specimens at mesophotic depths) means that often

there are very few individuals to examine. This is all the more so for crustose species that lack the charisma of bladed specimens, and are usually subsampled from coral rubble brought to the surface, rather than targeted for collection. Nevertheless, these opportunistic collections are revealing substantial new Hawaiian mesophotic peyssonnelioid biodiversity. Similar work in tropical and subtropical regions worldwide will be critical to unveil this diversity at a global scale.

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