



UNIVERSITY of HAWAI'I®
MĀNOA

Chronicles of Deep Reef Flowers:
*A Phylogenetic Monograph of the Family Kallymeniaceae (Rhodophyta) Associated
with the Mesophotic Coral Ecosystems of Hawai'i*

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Ug sa pikas sa akong kasingkasing, si Moon ug si Wewyn, si Mama ug Papa, sa tanan.

Abstract

Cryptic diversity, alternating life histories, ecological plasticity, and evolutionary lability of the few morphological characters available for identification of expanded red blades in the Family Kallymeniaceae have steered multiple studies to focus on a molecular-assisted alpha taxonomy (MAAT) approach to resolving their systematics and taxonomy. Among previous Hawaiian work is the 2010 Rhodophyta Biodiversity Survey of the Hawaiian Islands, which included several specimens of these expanded red blades, and an in-progress assessment of Hawaiian mesophotic algal communities. As part of the current effort to resolve the taxonomic placement of these expanded red blades, we conducted a molecular survey using multiple gene regions followed by floristic taxonomic treatments to address the taxonomic crisis so prevalent in the group. At least 300 specimens of expanded red blades were collected from both shallow and mesophotic depths to contribute to the establishment of a more comprehensive biodiversity catalog of Hawaiian marine algae. These specimens also provided an opportunity to examine a largely unexplored community at depths as great as 162 m. In Chapter 2, I examined stipitate red blades in Hawai'i that we identified as *Psaromenia* and *Meredithia*, as models for investigating species delimitation in morphologically and molecularly diverse species. Our study resulted in the description of two new and presumed endemic Hawaiian species. In the subsequent supplementary chapter, I present the in-progress molecular survey that points to new species, new records and a number of range extensions for the expanded red blades of the Hawaiian Islands that still remain to be investigated in detail. The astounding species-level diversity unraveled and resolved in this study is prerequisite to investigating larger scale macroevolutionary patterns and highlights the importance of generating an accurate baseline dataset for future monitoring efforts.

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CHAPTER 1

“Perhaps the most systematically difficult group of red algae remaining to be studied on the Pacific coast ... is that known as “the foliose reds”.”

Isabella Abbott (1967, p. 139)

CHAPTER 1

INTRODUCTION

1.1 *Family Kallymeniaceae and its limited morphology keys*

The elusive yet simple morphologies of expanded red blades synonymously described as “foliose”, “blade-like”, “sheet-like”, and “expansive” are some of the most striking elements on most temperate and tropical reefs. These charismatic red blades represent some of the most inscrutable algal groups to identify at the genus and species levels (Saunders, 2008). Issues associated with understanding foliose red algal diversity, i.e., frequent misidentifications and extensive synonymy, are especially evident in the family Kallymeniaceae (Abbott, 1967; Saunders, 2005).

The Kallymeniaceae Kylin (Gigartinales, Rhodophyta) is a marine red algal family of approximately 46 genera united by their simple morphology of expanded red blades and reproductive traits (Guiry & Guiry, 2020). The names of the family, based on the genus *Kallymenia*, alludes to their most well-known attribute— “*kally*” (Latin) which directly translates to “beautiful membrane” (Gifford, 1853). Within the family, the genus *Kallymenia* J. Agardh *sensu lato* is the most widespread (Saunders et al., 2017). Members of the genus are known for their triphasic life history with isomorphic gametophytes and tetrasporophytes (Harper & Saunders, 2001; Searles, 1980). They are distinct from other expansive red blades by their vegetative morphology with medullary filaments having large branched stellate cells and layers of cortical cells outwardly diminishing in size (Coomans & Hommersand, 1990), and by their reproductive morphology with the carpogonial branch system consisting of a lobed supporting cell giving rise to multiple lobed subsidiary cells, and with one or more carpogonial branches arising from the subsidiary cell for the female reproductive structures (Hommersand & Fredericq, 1990).

Traditionally, identification at the level of genus for these marine red blades has been accomplished using the reproductive structures (Searles, 1980). However, these reproductive features are rarely observed and the added difficulty of sample preparation to detect these features put strong limits on the number of morphological characters available to distinguish species within the genus (Hommersand & Fredericq, 1990). Rampant phenotypic plasticity associated with environmental variability intensifies the uncertainty and misconstruction of the evolutionary relationships of *Kallymenia* with other genera in its family, and other red blades for which it is often mistaken, i.e., *Halymenia* C.Agardh (Collins & Howe, 1916; Hernández-Kantún et al., 2012; Le Gall & Saunders, 2010).

Two morphologically similar genera, *Kallymenia* and *Halymenia*, belonging in two completely different orders, have been a source of confusion for many years (Abbott & McDermid, 2002; D'Archino et al., 2014; Hernández-Kantún et al., 2012). Because of their different and sometimes conflicting sets of phenotypic characters, Abbott and McDermid (2002) suggested the use of stellate cell size and shape, located in the inner cortex, as a means of discerning *Kallymenia* and *Halymenia*. As such, morphologically identifying species through classic taxonomic approaches proves to be an arduous task, as circumscription of *Kallymenia* easily applies to expanded red blades outside of the genus and its family with convergent morphologies (Cianciola et al., 2010; Saunders, 2005). Evidently, disagreement continues regarding the taxonomic status of many genera and species within the family Kallymeniaceae (Harper & Saunders, 2001; Saunders, 2008). But on a more productive note, the most substantial and exhaustive monographs produced from these long-standing debate now serve as valuable baselines for taxonomic revisions (Kylin, 1956; Norris, 1964; Norris & Womersley, 1971).



Figure 1. 1 Herbarium specimens of Hawaiian expansive red blades that have been investigated in this study.

The Kallymeniaceae represents an interesting and important component of the reef ecosystem in the Hawaiian Islands, exhibiting unique adaptations in light-starved environments and expanding to as large as 75 cm (Spalding, 2012; Figure 1.1). The current study was motivated by curiosity stemming from the taxonomic work that needs to be completed before the Hawaiian marine macroflora are fully chronicled and can be readily identified, which will allow its ecological and evolutionary aspects to be studied.

1.2 *Taxonomic Markers*

The long history of systematics difficulties in the family Kallymeniaceae steered multiple studies to include molecular data in bolstering the limited diagnostic power of morphological taxonomic keys. This section examines the morphological and molecular data that can be used for Kallymeniaceae phylogenetic analyses.

1.2.1 *Prevailing Use of Morphology*

Despite the advent of molecular phylogenetics, the continuing use of morphology in algal phylogenetics is a prevailing standard. Very few studies have scrutinized the utility of morphological characters in phylogenetic analyses (De Clerck et al., 2013), as historically red algal systematics relied heavily on the rich variation in reproductive morphology (Kylin, 1928). Detailed morphological studies in red algae remain advantageous for the following reasons: (a.) These studies provide a reservoir of morphological character documentation that allows for the inclusion of rare taxa when there are collection limitations or when molecular data might be difficult to obtain. (b.) Morphological approaches allow already existing data to be integrated into contemporary taxonomic studies. (c.) Morphology in algal systematics, viewed in an evolutionary context, could further benefit future study of testing species-level hypotheses of biogeography and

adaptive character evolution. For these reasons, morpho-taxonomy and comparative morphology remain a cornerstone in red algal (including kallymeniacean) systematics.

In this study, morphological characters to be measured or determined for the Kallymeniaceae are listed in Table 1.1, following Vergés et al., 2006a, Vergés et al., 2006b and Vergés et al., 2014.

Table 1. 1 Summary of morphological and anatomical characters of Kallymenia species to be assessed in the proposed research.

<i>Overall Morphology</i>	<i>Vegetative Anatomy</i>	<i>Reproductive cell sizes</i>
Blade shape	Shape of cells in outer cortex	Tetrasporangia (µm)
Color of blade	Shape of cells in inner cortex (with reference to the cortex)	Carpospore (µm)
Orders of branching	Medulla features	Spermatangia (µm)
Axis texture	+/- Refractive ganglionic cells	
Type of margin	Thickness of the blade (µm)	
Spines or leaflets on margin	Thickness of cortex (µm)	
+/- Maculae on surface	Number of cells in cortex	
+/- Spines on surface	Inner cortex cell size (µm)	
Axis shape	Anticlinal filament diameter (µm)	
Axis width (µm)	Refractive ganglionic cell size (µm)	
Stipe shape		
Surface features		
Stipe length (µm long)		

1.2.2 *Advent of molecular tools*

In contrast to the prolonged saga of morphology as the primary data set for phylogenetics, inclusion of molecular data in algal phylogenetics took off rapidly. Use of multi-gene data and model-based species delimitation methods in algal studies currently stand as key techniques in determining realistic species boundaries (Harper & Saunders, 2001; Leliaert & De Clerck, 2017).

Phylogenetic reconstruction

Phylogenetic reconstruction, or the computer-based algorithmic analyses that provide resolution, support, and accuracy in the study of evolutionary reconstruction, are routinely conducted to overcome the limitations of relying on morphological data (Scotland et al., 2010; Oliveira et al., 2018). Taxonomists often still force-fit expanded red blades into existing taxonomic constructs, which can yield incorrect taxonomies and require further investigation and analysis with larger amounts of phylogenetic data from throughout the geographical and habitat ranges of algal species (Saunders et al., 2017; Yoon et al., 2006). On this note, multigene phylogenetic reconstruction, or the construction of richer character datasets by concatenating sample data from multiple gene markers, supersedes the level of confidence in single gene phylogenies (Harrison & Langdale, 2006).

DNA Barcoding

DNA barcoding, or the sequencing of short DNA fragments of selected molecular markers for species assignment, has developed as an effective starting point for molecular phylogenetic studies, as well as studies of geographical distribution and conservation of marine biodiversity (Trivedi et al., 2016). In the last 15 years, DNA barcoding has re-initiated a renaissance in taxonomy, leading to the establishment and/or revision of existing phylogenies in a number of organisms, including red macroalgae (Saunders, 2008; Sherwood et al., 2010b) and has grown progressively more effective as a molecular tool for species identification as comparative sequence data databases increase in coverage (Suriya et al., 2018). Streamlining the use of DNA barcoding into an integrative taxonomy by simultaneously employing ecological, genetic, and morphological data offers an

effective approach to accelerating discovery of new species and producing meaningful taxonomic information (Morrow et al., 2017).

The advent of MAAT (Molecular-Assisted Alpha-Taxonomy)

Molecular-Assisted Alpha Taxonomy (MAAT) emerged as an integrative approach for species delimitation, freeing the algal taxonomists in the 21st century from the exclusive use of morphology-based keys for simple or convergent morphologies (Cianciola et al., 2010). MAAT aims for concordance in the combination of barcoding data and morphological observations to solve taxonomic uncertainties among algal groups, particularly within red algae (Leliaert et al., 2014). MAAT became a recognized approach to clarifying boundaries within systemically difficult families, allowing for description of new genera and species in the field of phycology (Popolizio, 2015).

1.2.3 General research approach

Using both morphological and molecular data as a framework to examine the traditional classification system and to test previously proposed relationships, this study will proceed following the flow chart in Figure 1.2, which summarizes the research approach.

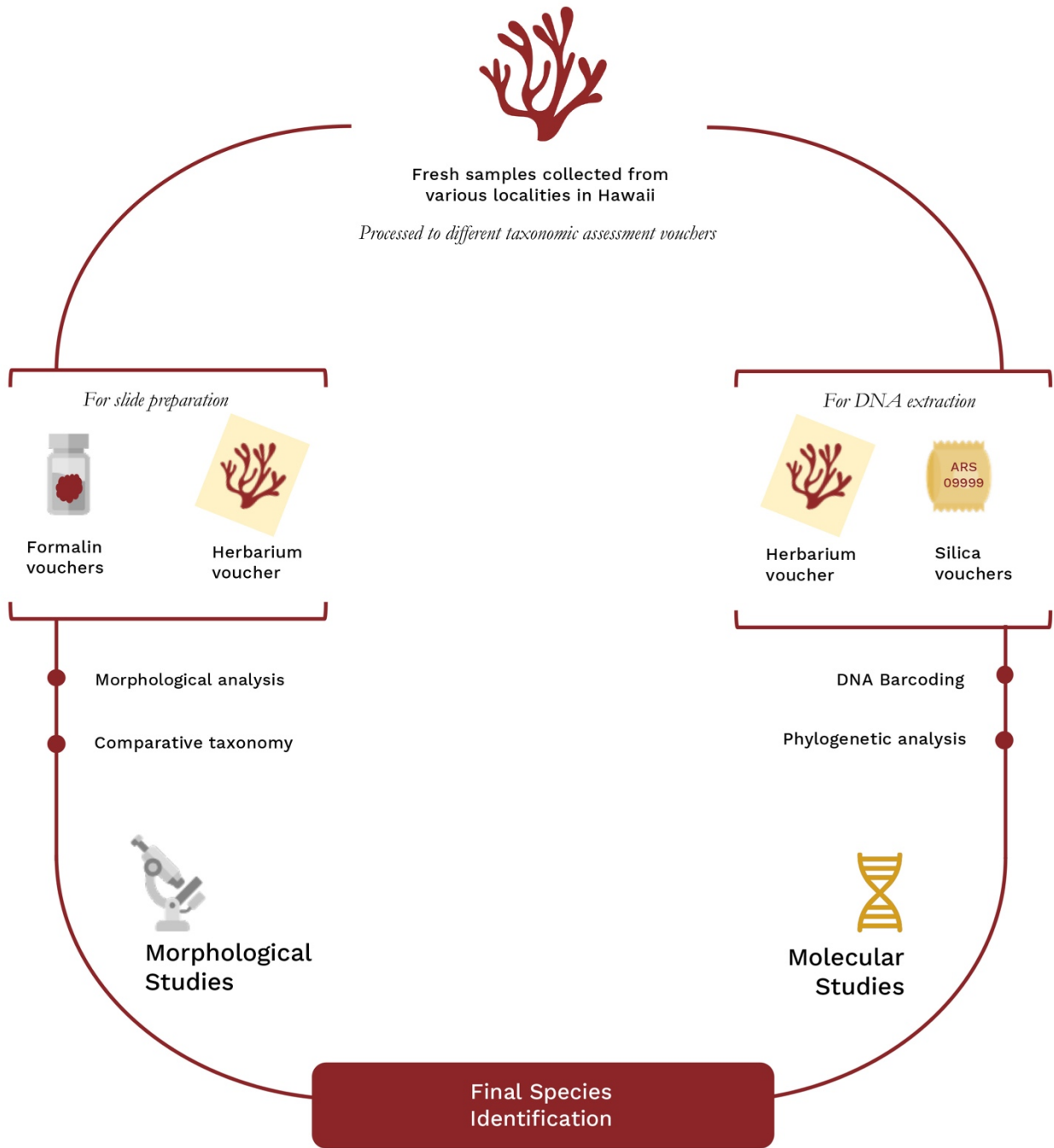


Figure 1. 2 Work flow-chart for taxonomic assessment of Hawaiian expanded red blades.

1.3 The modern family Kallymeniaceae

The genus *Kallymenia* as was erected by Agardh (1842) for the species *K. requienii* (J.Agardh) J.Agardh and *K. reniformis* (Turner) J.Agardh. However, it was only in the 1900s that surveys and research on these red blades gained much impetus, with Kylin (1928) being the first to formally elevate the group to the rank of family as the Kallymeniaceae. Womersley's (1994) monograph of kallymeniacean species in Southern Australia represents the latest treatment using the genus-level taxonomic framework based solely on morpho-anatomical observations.

With the emergence of various molecular tools, the circumscription of the members of the family has been in flux. Since that time, the taxonomy of these red blades has been carried out at a rapid pace. D'Archino et al. (2010; 2011; 2012; 2014; 2016; 2017; 2018), Saunders et al. (2017), Schneider et al. (2014; 2019), and Huisman et al. (2016) and their co-workers have pioneered major revisionary works for the Kallymeniaceae focused on the Atlantic, Indian and Australian regions, while the works of Vergés (2006a; 2006b; 2014) and colleagues have focused on the Mediterranean region. These phycologists laid the groundwork for future study of kallymeniacean systematics by producing a much-improved understanding of phylogenetic relationships within this notoriously difficult red algal family.

Compiling data from "A synoptic review of the classification of red algal genera a half century after Kylin's "Die Gattungen der Rhodophyceen" with four addenda by Wynne and Schneider Wynne (as listed below, 2010; 2016) or Schneider & Wynne (as listed below, 2007; 2013; 2019), the Family Kallymeniaceae now comprises 46 genera (see Table 1.2), altering our fundamental understanding of this family. Sixty-four years ago the family included 12 genera, which was but a quarter of what it is now (Kylin, 1958) — signifying the long, unfolding history that frames our present understanding of the Kallymeniaceae.

Table 1. 2 Currently recognized genera in the Family Kallymeniaceae.

FAMILY KALLYMENIACEAE

Austropugetia R.L.Moe, 2009
Austrophyllis Womersley & R.E.Norris, 1971
Austrokallymenia Huisman & G.W.Saunders, 2017
Beringia Perestenko, 1975
Blastophyllis D'Archino & W.A.Nelson, 2017
Callocolax F.Schmitz ex Batters, 1895
Callophyllis Kützing, 1843
Croisettea M.J.Wynne, 2018
Ectophora J.Agardh, 1876
Erythrophyllum J.Agardh, 1872
Euthora J.Agardh, 1847
Fulgeophyllis D'Archino, S.-M.Lin & Zuccarello, 2018
Glaphyrymenia J.Agardh, 1885
Glaphyrymeniopsis Kraft & G.W.Saunders, 2017
Hommersandia G.I.Hansen & S.C.Lindstrom, 1984
Hormophora J.Agardh, 1892
Huonia G.W.Saunders, 2017
Judithia D'Archino & S.-M.Lin, 2016
Kallymenia J.Agardh, 1842
Kallymeniopsis Perestenko, 1977
Leiomenia Huisman & G.W.Saunders, 2017
Leniea R.L.Moe, 2009
Meredithia J.Agardh, 1892
Metacallophyllis A.Vergés & L.Le Gall, 2017
Nereoginkgo Kylin, 1919
Nothokallymenia A.Vergés & L.Le Gall, 2017
Polycoelia J.Agardh, 1849
Psaromenia D'Archino, W.A.Nelson & Zuccarello, 2010
Pugetia Kylin, 1925
Rhipidomenia G.W.Saunders, 2017
Rhizopogonia Kylin, 1934
Rhytymenia Huisman & G.W.Saunders, 2016
Salishia Clarkston & G.W.Saunders, 2012
Stauromenia D'Archino & W.A.Nelson, 2012
Thalassiodianthus G.W.Saunders & Kraft, 2017
Thamnophyllis R.E.Norris, 1964
Tytthomenia G.W.Saunders, 2017
Varimania R.L.Moe, 2009
Verlaquea Le Gall & Vergés, 2017
Wendya D'Archino & S.-M.Lin, 2016
Zuccarelloa D'Archino & W.A.Nelson, 2017

1.4 Hawaiian *Kallymeniaceae*

Although cosmopolitan in distribution, most kallymeniacean diversity is concentrated in the temperate regions of the world (Saunders et al., 2017). Abbott (1999) reported her earliest encounters with *Kallymenia*, which she previously identified as *Pugetia* Kylin (Abbott, 1996), on Hawaiian reefs to be “unusual occurrences”. As it stands, the Family Kallymeniaceae is currently recorded as represented in the Hawaiian Islands solely by the genus *Kallymenia* with two species: *K. thompsonii* I.A. Abbott & McDermid and *K. sessilis* Okamura (Abbott & McDermid, 2002). Both species were identified based on morphological data alone, and were documented at depths of 5-20 m (Abbott & McDermid, 2002).

Taking Abbott’s (1999) and Abbott and McDermid’s (2002) systematics as a baseline for reassessment of Hawaiian *Kallymenia*, although most seminal, is not without problems. Abbott (1999) accounted for the likely misidentification of *K. sessilis* as it lacks the genus-level diagnostic feature of large ganglionic refractive cells and possession of a medullary structure that is similar to illustrations by Womersley for *K. cribogloea* (1971). She also primarily discriminated the Hawaiian endemic *K. thompsonii* from *K. sessilis* on the frequency of perforations in their blades, which is a feature extremely dependent on environmental factors (Abbott & McDermid, 2002). Nonetheless, Abbott (1999) very much suspected high morphological variability among the expanded red blades at the time, although she was unable to partition this diversity to her satisfaction. Her suspicions were later substantiated with the 2010 Rhodophyta Biodiversity Survey of the Main Hawaiian Islands (Sherwood et al., 2010a), which comprehensively sampled expanded red blades or *Kallymenia*-like members of the flora (see Wagner et al., 2016; Figure 1.4) in over two decades of collections from various localities from the Papahānaumokuākea Marine National Monument (PMNM) and the Main Hawaiian Islands (MHI) (Figure 3).

Recent and ongoing floristic surveys of Hawaiian Hawaiian Mesophotic Coral Ecosystems (MCEs), led by Sherwood, Spalding, and colleagues, provide two important insights about the Family Kallymeniaceae, (a.) that overlooked diversity, including numerous new taxa, as well as many rarely recorded members of the family are regular finds in MCEs, and (b.) that the phylogenetic placement of these red blades is only partially resolved based on existing data (Sherwood et al., 2010a; see Figure 1.3). It is therefore unsurprising that macrobenthic surveys have been underestimating the true diversity of these organisms by force-fitting these taxa as either *Kallymenia*, a widely applied name for foliose reds or, “unknown Gigartinales”, in published accounts of the Hawaiian macroflora.

It has been clear for some time that there are undescribed taxa within the Kallymeniaceae in Hawai‘i and that the relationships of Hawaiian taxa need to be examined within a wider context. Despite this narrative, no subsequent studies have attempted to revise the taxonomy of the Hawaiian Kallymeniaceae until the present study.

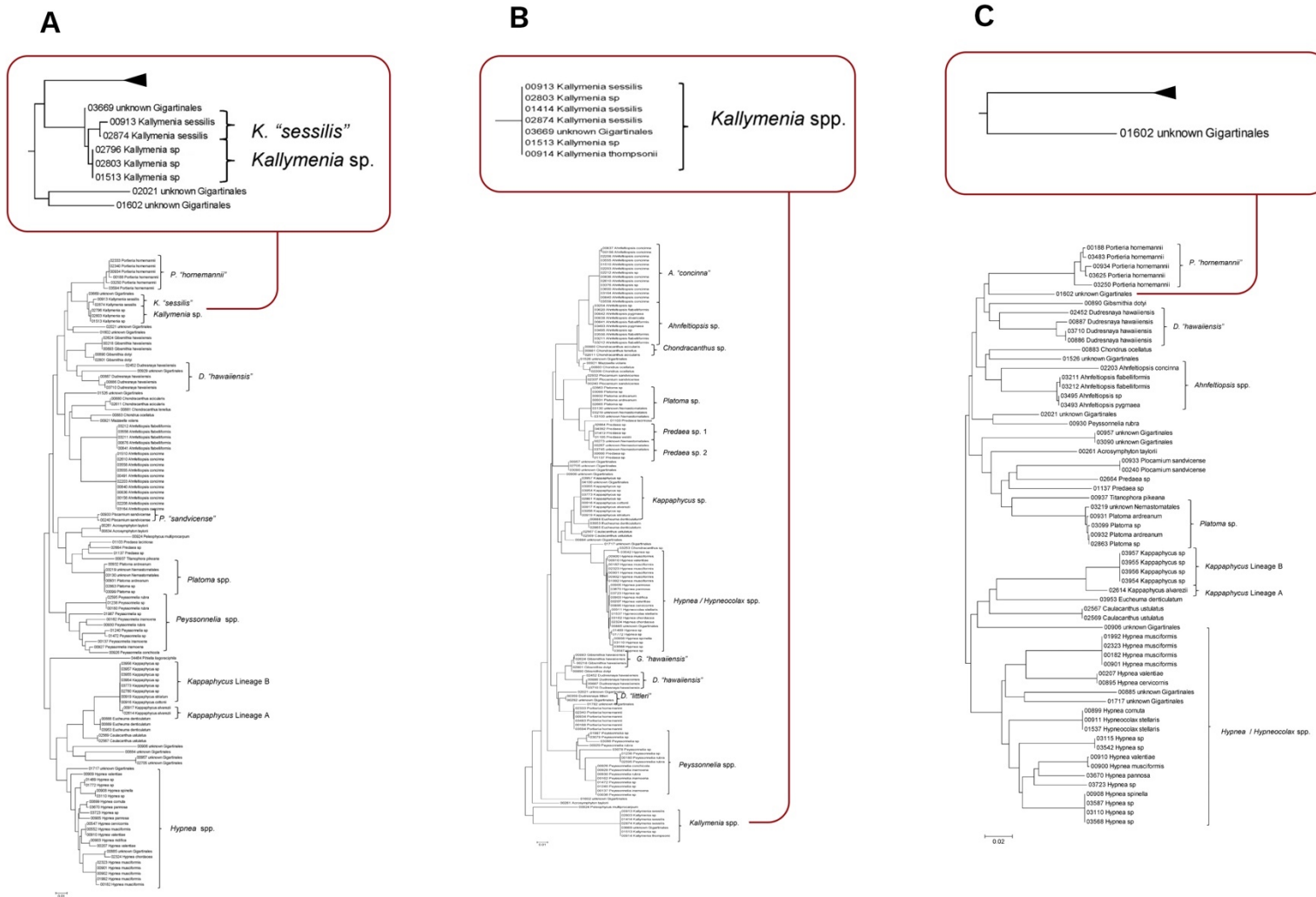


Figure 1. 3 Neighbor-joining tree based on (A) UPA, (B) LSU and (C) COI sequences of the Acrosymphytales, Gigartinales, Nemastomatales, Peyssonneliales and Plocamiales, modified from Sherwood et al. (2010a).

1.5 Uncharted flora

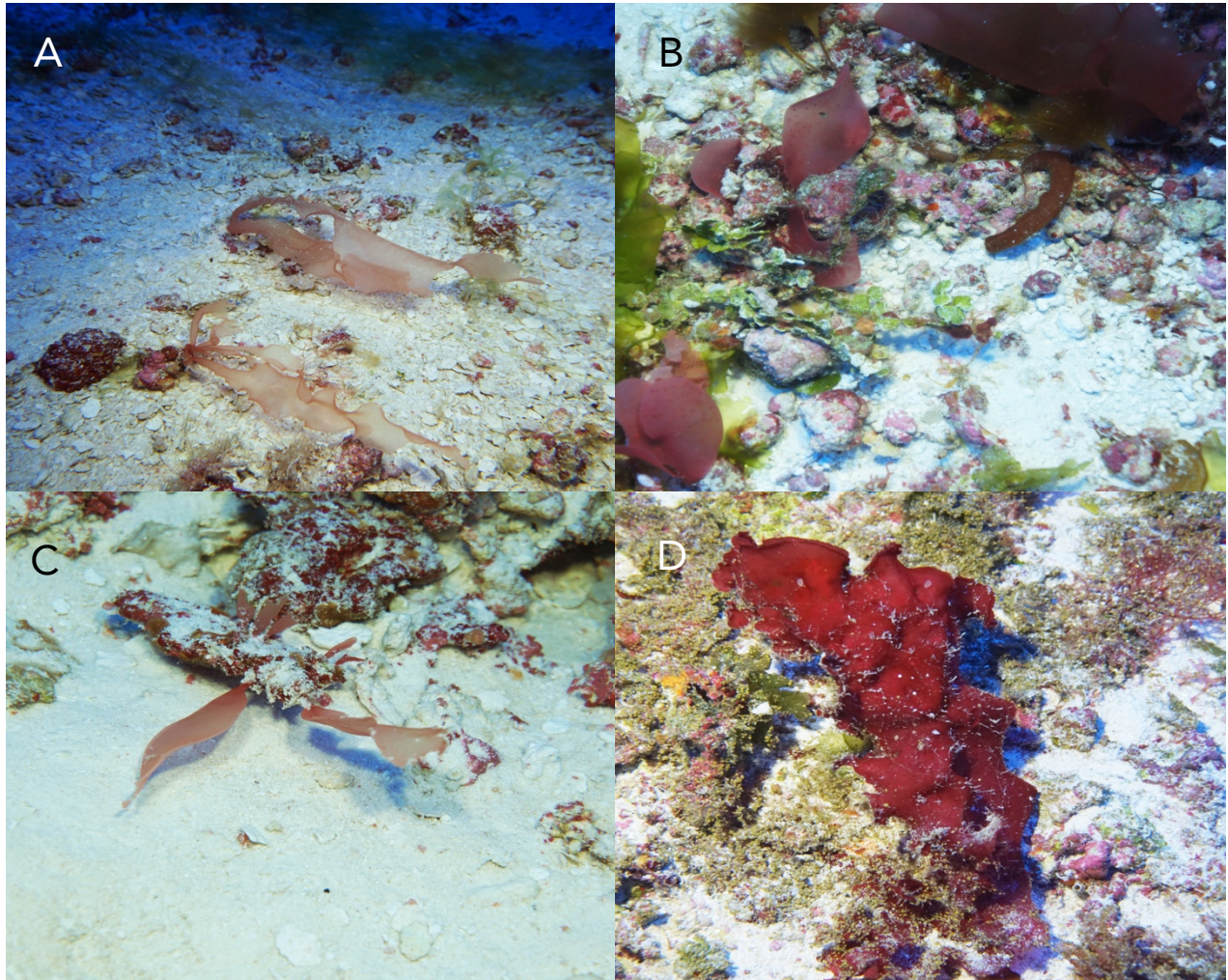


Figure 1. 4 Expanded red blades in situ. A. French Frigate Shoals at 79m B. Lisianski at 84m
C. French Frigate Shoals at 83m. D. Kure Atoll at 85m.

Beyond the easily accessible shallow water reefs exist thriving coral ecosystems at the limits of light penetration stretching from 30-150 m in the tropics, called Mesophotic Coral Ecosystems (MCEs) (Baker et al., 2016; Rossi et al., 2017). Most of these mesophotic habitats are dominated by macroalgae, which remain one of the most taxonomically understudied types of organisms in these environments (Kahng & Kelley, 2007; Rooney et al., 2010; Spalding, 2012; Spalding et al., 2019a). Hawaiian MCEs are enriched by the dominance of macroalgal

communities and their ability to create surfaces and local oxygen-rich environments supporting ammonia oxidation at depths with low dissolved oxygen levels (Rooney et al. 2010, Spalding, 2012; Trias et al., 2012).

One of the earliest attempts at assessing the composition of Hawaiian mesophotic macroalgal communities was conducted by Doty et al. (1974) using dredge hauls deployed between 10-165 m wherein he reported 21 species additions to Hawaiian flora and reported a total of 101 algal species occurring in the mesophotic, and by Abbott (1989), based on algae collected on deep lobster traps brought up from depths between 30 -100 m consisting of 205 algal taxa, 41 of which are new algal records for the Hawaiian Islands. Both of these studies hinted at the macroalgal diversity, particularly that of red blades. Lush foliose reds are commonly collected in these light-starved environments with discrete patches found as deep as 160 m (Spalding, 2012), and often entangled with *Sporochnus* C.Agardh, *Caulerpa* J.V.Lamouroux and *Halimeda* J.V.Lamouroux (McDermid & Abbott, 2006). Until recently, advances in diving technology, and use of submersibles and ROVs in MCE surveys (Kahng et al., 2019) with application of molecular systematics to dominant mesophotic macroalgal genera (Sherwood et al., 2010), an overwhelming number of putative new species were discovered expanding the known algal biodiversity in Hawai'i, majority of which, is in need of reexamination of its current taxonomic definitions (Abbott & Huisman, 2003; Agegian et al., 1985; Norris et al., 1995; Tsuda, 2014).

The unique diversity of Hawaiian mesophotic flora is contributed to by the clarity of the oceanic waters, which allow light penetration to deeper depths (with enriched nutrients levels and lower temperatures) that are essentially comparable to conditions of shallow euphotic zones in temperate reefs (Santelices, 2007; Spalding, 2012). Pioneering studies that foray into Hawaiian

MCE research have been slowly piecing together knowledge on the endemism, connectivity and refuge role of Hawaiian MCEs (Pyle et al., 2016; Spalding et al., 2019b).

1.5.1 Endemism

Hawai‘i is at least 4000 km away from any continent, making it one of the most remote oceanic island chains of the world (Fleischer et al., 1998). As a consequence, a high degree of endemism abounds in the Hawaiian archipelago owing to the combination of the isolation of the islands and the subsequent evolution of endemic species (Friedlander & Brown, 2019). Island endemism in Hawai‘i has been well-studied in more recognizable organisms such as fishes and corals, but only scantily in macroalgae. Pyle et al. (2016) reported that the rate of endemism among fishes in the Hawaiian waters increases with depth. Comparably, hidden species among Hawai‘i’s dominant mesophotic coral genera *Leptoseris* and *Pavona* have been uncovered by Luck et al. (2013). Expeditions to Hawaiian MCEs have reported seaweed communities to be floristically rich (Pyle et al., 2016; Veazey, 2018) and unsatisfactorily explored (Kahng et al., 2010). Few species of expanded red blades inhabit upper MCEs (30–60 m) but are found to exhibit richer morphological diversity at the lower depth limits of MCEs (>60 m) (McDermid & Abbott, 2006; Lesser et al., 2019; Spalding, 2012). In-progress assessments to characterize Hawaiian mesophotic floras using both molecular and morphological analyses are yielding a wealth of species new to science or new records to the islands (Sauvage et al., 2019; Sherwood et al., 2019; Silva & Chacana, 2014; Spalding et al., 2016; Wade et al., 2018). Full characterization and identification of mesophotic macroalgae at the species level will allow for similar studies of endemism and exploration of cryptic diversity across broad gradients of depth and latitude in the Hawaiian archipelago.

1.5.2 Connectivity

Generating an accurate phylogenetic framework for the mesophotic flora will help rectify the existing lack of awareness of the importance and general understanding of the community dynamics within MCEs (Baker et al., 2016; Pyle et al., 2016). With collaboration, linkages between Hawaiian MCEs and other tropical ecosystems will mend a critical gap in understanding genetic connectivity between shallow and mesophotic algal populations and their role as dominant communities in coral reef ecosystems. Overall, a more integrative and complementary view of the expansive red blades will provide opportunities to gain new insights into fundamental ecological dynamics (Kahng et al., 2010) and enhance success of conservation strategies in the marine tropics (Slattery, 2012). One of the prime reasons for the interest in red blades is that on the one hand they represent an incomplete taxonomic circumscription, while on the other hand the interesting alliance documented between Atlantic (Ballantine et al., 2015; Schneider et al., 2019), Indian (Schneider et al., 2014) and Pacific (D'Archino et al., 2010, Saunders et al., 2017) species in the family Kallymeniaceae also makes them the best possible candidates for genetic connectivity and biogeographic studies of pan-tropico-temperate marine algae.

1.5.3 Refuge

Reef systems worldwide are facing increasing degradation due to climate change, which is influencing currents, increasing water temperatures, increasing sea levels, and contributing anthropogenic stressors driving eutrophication (Yan et al., 2016), ocean acidification (Nakano & Iida, 2018), and mortality of various marine organisms (Kealoha et al., 2020). These aforementioned pressures generated a surge of scientific attention on the lesser-studied extension of shallow-water reefs: MCEs as prospective refuge for organisms dwelling in the deteriorating

shallow reefs. MCEs are characterized by the presence of light-dependent organisms, usually found at depths extending from 30 m to over 150 m in tropical and subtropical regions (Holstein et al., 2016). The perceived stability of MCEs experiencing lower levels of disturbances, attributed to their depth, has been formalized as the “Deep Reef Refuge” Hypothesis (DRRH) (Bongaerts & Smith, 2019). The DRRH rests on the assumptions that (a) deep reefs are less susceptible to anthropogenic and natural impacts than their shallower counterparts, and therefore, (b) may serve as a source of species to replenish degraded shallow reef populations (Slattery, 2012; Holstein et al., 2016). The ‘Replenishing’ potential of mesophotic reefs largely depends on the extent of species overlap with their shallow-water counterparts (Bongaerts & Smith, 2019). Empirical support for the DRRH has been varied as most observations have been limited to the Caribbean, with few studies in the Indo-Pacific (Kahng et al., 2010). Current knowledge of the ‘replenishing’ potential among Hawaiian mesophotic macroalga has been, so far, varied among species and needs further investigation (Sauvage et al., 2019; Sherwood et al., 2019; Silva & Chacana, 2014; Spalding, 2012; Wade et al., 2018). With an uncertain climate future, charting life in the deep has never been a more timely endeavor.

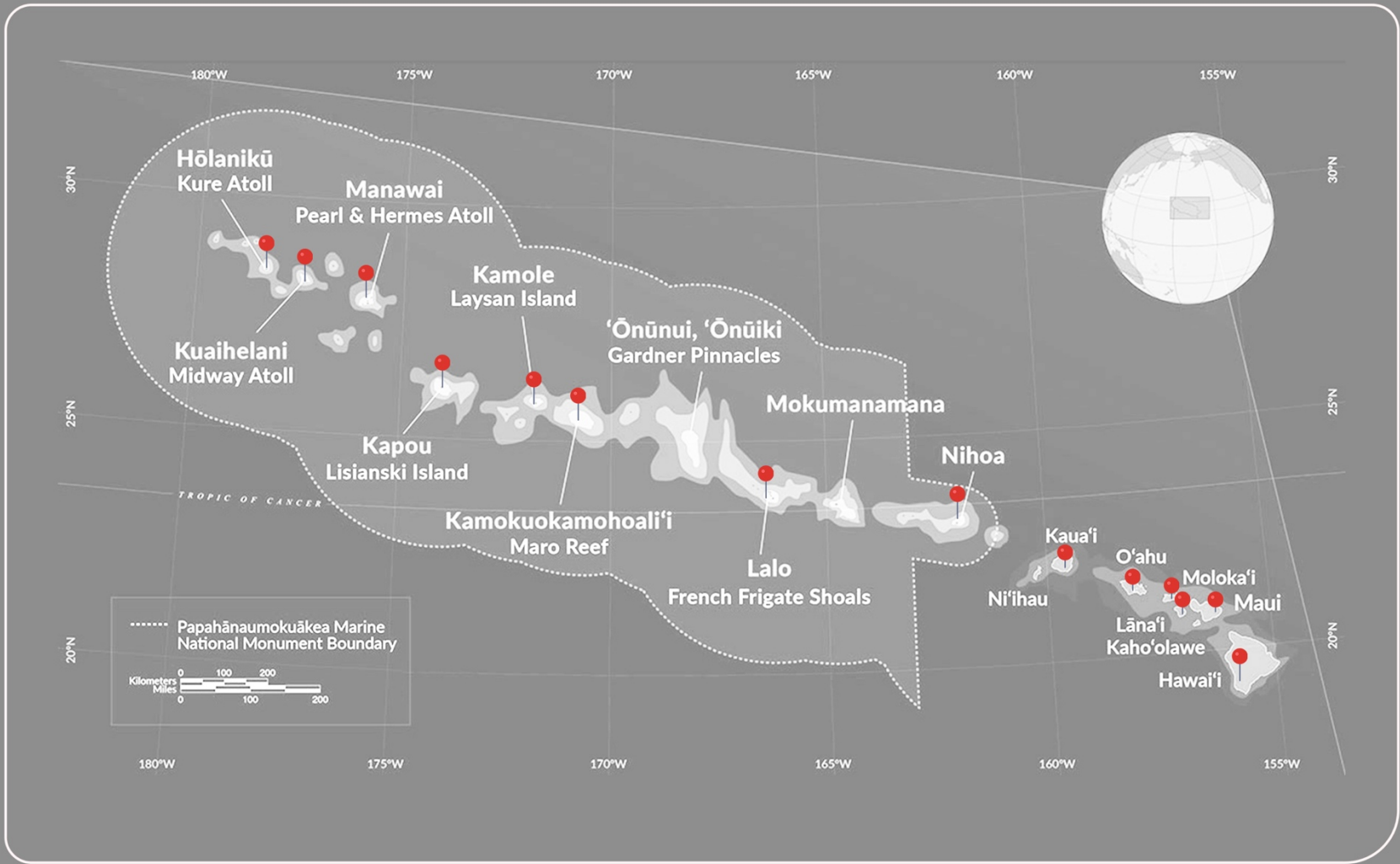


Figure 1. 9 Map of collection sites around the Main Hawaiian Islands (MHI) and Northwestern Hawaiian Islands (NWHI). Map by NOAA - <http://www.papahanaumokuakea.gov/>, Public Domain, <https://commons.wikimedia.org/w/index.php?curid=50934273>.

1.6 Research Questions

The goals of this study are to determine the number and identity of Hawaiian “red blade” seaweeds in the Family Kallymeniaceae from shallow and MCE habitats to allow a more comprehensive biodiversity catalog be created with opportunities to examine a largely unexplored community at depths as great as 118 m, and to reconstruct accurate phylogenetic relationships for these taxa through DNA barcoding and molecular phylogenetic analyses using multiple genetic markers, paired with morphological analysis of diagnostic characters, to establish an accurate taxonomic framework. In addition, this research also aims to provide insight into the expansive Hawaiian mesophotic red blades as members of the marine flora, and the following questions will be studied as part of this research:

- Do these species contain cryptic diversity?
- Are there species shared among shallow and deep communities?
- Are red blade species shared among biogeographic realms
in the Pacific and elsewhere?

1.7 References

- Abbott, I.A. (1967). Studies in some foliose red algae of the Pacific coast. I. Cryptonemiaceae. *Journal of Phycology*, **3**: 139–149.
- Abbott, I. A. (1996). New species and notes on marine algae from Hawai'i. *Pacific Science*, **5**: 142–156.
- Abbott, I.A. (1999). *Marine Red Algae of the Hawaiian Islands*. Bishop Museum Press, Honolulu.
- Abbott, I. A., & Huisman, J. M. (2003). New species, observations, and a list of new records of brown algae (Phaeophyceae) from the Hawaiian Islands. *Phycological Research*, **51**:173–185.
- Abbott, I. A., & McDermid, K. J. (2002). On two species of *Kallymenia* (Rhodophyta: Gigartinales: Kallymeniaceae) from the Hawaiian Islands, Central Pacific. *Pacific Science*, **56**: 149–162.
- Agardh, J. G. (1842). *Algae maris mediterranei et adriatici: observationes in diagnosin specierum et dispositionem generum*. Apud Fortin, Massin.
- Agegian, C., Abbott, I., Harmelin-Vivien, M., Salvat, B., Gabrie, C., & Toffart, J. (1985). Deep water macroalgal communities: a comparison between Penguin Bank (Hawaii) and Johnston Atoll. *Proceedings of the Fifth International Coral Reef Congress, Tahiti 27 May - 1 June 1985, Vol. 5: Miscellaneous Papers (A). Cinquieme Congres International Sur Les Recifs Coralliens, Tahiti, Polynesie Francaise, 27 Mai - 1er Juin 1985, Vol. 5: Miscellaneous Papers (A)*, **5**:47–50.
- Baker, E.K., Puglise, K.A. & Harris, P.T. (2016). *Mesophotic coral ecosystems — A lifeboat for coral reefs?* The United Nations Environment Programme and GRID-Arendal, Nairobi and Arendal.
- Ballantine, D. L., Ruíz, H., & Norris, J. N. (2015). Notes on the benthic marine algae of Puerto Rico, XI: new records including new *Meredithia* (Kallymeniaceae, Rhodophyta) species. *Botanica Marina*, **58**:355–365.
- Bongaerts, P., & Smith, T. B. (2019). Beyond the “Deep Reef Refuge” hypothesis: a conceptual framework to characterize persistence at depth. In *Mesophotic Coral Ecosystems* (881–895). Springer International Publishing, Cham.
- Cianciola, E. N., Popolizio, T. R., Schneider, C. W., & Lane, C. E. (2010). Using molecular-assisted alpha taxonomy to better understand red algal biodiversity in Bermuda. *Diversity*, **2**: 946–958.
- Collins, F. S., & Howe, M. A. (1916). Notes on species of *Halymenia*. *Bulletin of the Torrey Botanical Club*, **43**:169–182.
- Coomans, R. J., & Hommersand, M. H. (1990). Vegetative growth and organization. In *Biology of*

the Red Algae (275–304). Cambridge University Press, Cambridge.

D'Archino, R., Nelson, W. A., & Zuccarello, G. C. (2010). *Psaromenia* (Kallymeniaceae, Rhodophyta): a new genus for *Kallymenia berggrenii*. *Phycologia*, **49**:73–85.

D'Archino, R., Nelson, W. A., & Zuccarello, G. C. (2011). Diversity and complexity in New Zealand Kallymeniaceae (Rhodophyta): recognition of the genus *Ectophora* and description of *E. marginata* sp. nov. *Phycologia*, **50**:241–255.

D'Archino, R., Nelson, W. A., & Zuccarello, G. C. (2012). *Stauromenia australis*, a new genus and species in the family Kallymeniaceae (Rhodophyta) from southern New Zealand. *Phycologia*, **51**:451–460.

D'Archino, R., Nelson, W. A., & Zuccarello, G. C. (2014). *Amalthea* and *Galene*, two new genera of Halymeniaceae (Rhodophyta) from New Zealand. *Botanica Marina*, **57**:185–201.

D'Archino, R., Lin, S. M., Gabrielson, P. W., & Zuccarello, G. C. (2016). Why one species in New Zealand, *Pugetia delicatissima* (Kallymeniaceae, Rhodophyta), should become two new genera, *Judithia* gen. nov. and *Wendya* gen. nov. *European Journal of Phycology*, **51**:83–98.

D'Archino, R., Nelson, W. A., & Sutherland, J. E. (2017). Neither *Callophyllis* nor *Gelidium*: *Blastophyllis* gen. nov. and *Zuccarelloa* gen. nov. (Kallymeniaceae, Rhodophyta) for three New Zealand species. *Phycologia*, **56**:549–560.

D'Archino, R., Lin, S. M., & Zuccarello, G. C. (2018). *Fulgeophyllis* (Kallymeniaceae, Gigartinales), a new genus to accommodate two New Zealand species. *Phycologia*, **57**:422–431.

De Clerck, O., Guiry, M. D., Leliaert, F., Samyn, Y., & Verbruggen, H. (2013). Algal taxonomy: a road to nowhere?. *Journal of Phycology*, **49**:215–225.

Doty, M. S., Gilbert, W. J., & Abbott, I. A. (1974). Hawaiian marine algae from seaward of the algal ridge. *Phycologia*, **13**:345–357.

Fleischer, R. C., McIntosh, C. E., & Tarr, C. L. (1998). Evolution on a volcanic conveyor belt: using phylogeographic reconstructions and K–Ar-based ages of the Hawaiian Islands to estimate molecular evolutionary rates. *Molecular Ecology*, **7**:533–545.

Friedlander, A. M., & Brown, E. K. (2019). The Hawaiian Archipelago. In *World Seas: An Environmental Evaluation* (713–741). Academic Press, Cambridge.

Gifford, I. (1853). *The marine botanist: An introduction to the study of the British seaweeds: containing descriptions of all the species, and the best method of preserving them*. Nabu press, Charleston.

Guiry, M.D. & Guiry, G.M. (2020). AlgaeBase. World-wide electronic publication, National University of Ireland, Galway. <http://www.algaebase.org>.

- Harper, J. T., & Saunders, G. W. (2001). Molecular systematics of the Florideophyceae (Rhodophyta) using nuclear large and small subunit rDNA sequence data. *Journal of Phycology*, **37**:1073–1082.
- Harrison, J.C., & Langdale, J. A. (2006). A step by step guide to phylogeny reconstruction. *Plant Journal*, **45**:561–572.
- Hernández-Kantún, J. J., Sherwood, A. R., Riosmena-Rodriguez, R., Huisman, J. M., & De Clerck, O. (2012). Branched *Halymenia* species (Halymeniaceae, Rhodophyta) in the Indo-Pacific region, including descriptions of *Halymenia hawaiiiana* sp. nov. and *H. tondoana* sp. nov. *European Journal of Phycology*, **47**:421–432.
- Holstein, D. M., Smith, T. B., & Paris, C. B. (2016). Depth-independent reproduction in the reef coral *Porites astreoides* from shallow to mesophotic zones. *PLoS One*, **11**:e0146068.
- Hommersand, M., & Fredericq, S. (1990). Sexual reproduction and cystocarp development. In *Biology of the red algae* (305–345). Cambridge University Press, Cambridge.
- Huisman, J. M., Saunders, G. W., Le Gall, L., & Vergés, A. (2016). *Rhytymenia*, a new genus of red algae based on the rare *Kallymenia maculata* (Kallymeniaceae, Rhodophyta). *Phycologia*, **55**, 299–307.
- Kahng, S. E., Garcia-Sais, J. R., Spalding, H. L., Brokovich, E., Wagner, D., Weil, E., ... & Toonen, R. J. (2010). Community ecology of mesophotic coral reef ecosystems. *Coral Reefs*, **29**:255–275.
- Kahng, S. E., & Kelley, C. D. (2007). Vertical zonation of megabenthic taxa on a deep photosynthetic reef (50-140 m) in the Au‘au Channel, Hawaii. *Coral Reefs*, **26**:679–687.
- Kealoha, A. K., Doyle, S. M., Shamberger, K. E., Sylvan, J. B., Hetland, R. D., & DiMarco, S. F. (2020). Localized hypoxia may have caused coral reef mortality at the Flower Garden Banks. *Coral Reefs*, **39**:119–132.
- Kylin, H. (1928). Entwicklungsgeschichtliche Florideenstudien. *Lunds Universitets Årsskrift N.F. Avd. 2*, **84**(4): 1–127.
- Kylin, H. (1956). *Die Gattungen der Rhodophyceen*. pp. i-xv, 1–673, 458 figs. Lund: C.W.K. Gleerups.
- Le Gall, L., & Saunders, G. W. (2010). DNA barcoding is a powerful tool to uncover algal diversity: A case study of the Phyllophoraceae (Gigartinales, Rhodophyta) in the Canadian flora. *Journal of Phycology*, **42**: 374–389.
- Leliaert, F., & De Clerck, O. (2017). Refining species boundaries in algae. *Journal of Phycology*, **53**:12–16.

- Leliaert, F., Verbruggen, H., Vanormelingen, P., Steen, F., López-Bautista, J. M., Zuccarello, G. C., & De Clerck, O. (2014). DNA-based species delimitation in algae. *European Journal of Phycology*, **49**:179–196.
- Lesser, M. P., Slattery, M., Laverick, J. H., Macartney, K. J., & Bridge, T. C. (2019). Global community breaks at 60 m on mesophotic coral reefs. *Global Ecology and Biogeography*, **28**: 1403–1416.
- Luck, D. G., Forsman, Z. H., Toonen, R. J., Leicht, S. J., & Kahng, S. E. (2013). Polyphyly and hidden species among Hawai'i's dominant mesophotic coral genera, *Leptoseris* and *Pavona* (Scleractinia: Agariciidae). *PeerJ*, **1**:e132.
- McDermid, K. J., & Abbott, I. A. (2006). Deep subtidal marine plants from the Northwestern Hawaiian Islands: new perspectives on biogeography. *Atoll Research Bulletin*, **543**:525–532.
- Morrow, K. M., Bromhall, K., Motti, C. A., Munn, C. B., & Bourne, D. G. (2017). Allelochemicals produced by brown macroalgae of the *Lobophora* genus are active against coral larvae and associated bacteria, supporting pathogenic shifts to *Vibrio* dominance. *Applied Environmental Microbiology*, **83**:e02391–16.
- Nakano, T., & Iida, Y. (2018). Ocean acidification and carbon dioxide uptake in the global ocean. *PICES Press*, **26**:34–36.
- Norris, J. N., Abbott, I. A., & Agegian, C. R. (1995). *Callidictyon abyssorum*, gen. et sp. nov. (Rhodophyta), a new deep-water net-forming alga from Hawai'i. *Pacific Science*, **49**:192–201.
- Norris, R. E. (1964). The morphology and taxonomy of South African Kallymeniaceae. *Botanica Marina*, **7**:90–131.
- Oliveira, M. C., Repetti, S. I., Iha, C., Jackson, C. J., Díaz-Tapia, P., Lubiana, K. M. F., ... Verbruggen, H. (2018). High-throughput sequencing for algal systematics. *European Journal of Phycology*, **53**: 256–272.
- Popolizio, T. R. (2015). *Integrating molecular and traditional systematic techniques to redefine Red Algal (Rhodophyta) diversity in the Bermuda Islands*. Ph.D. Dissertation, University of Rhode Island, South Kingstown.
- Pyle, R. L., Boland, R., Bolick, H., Bowen, B. W., Bradley, C. J., Kane, C., ... & Parrish, F. A. (2016). A comprehensive investigation of mesophotic coral ecosystems in the Hawaiian Archipelago. *PeerJ*, **4**:e2475.
- Rooney, J., Donham, E., Montgomery, A., Spalding, H., Parrish, F., Boland, R., ... & Vetter, O. (2010). Mesophotic coral ecosystems in the Hawaiian Archipelago. *Coral Reefs*, **29**: 361–367.
- Rossi, S., Bramanti, L., Gori, A., & Orejas, C. (2017). *Marine animal forests: the ecology of benthic biodiversity hotspots*. Springer International Publishing, Cham.

- Santelices, B. (2007). The discovery of kelp forests in deep-water habitats of tropical regions. *Proceedings of the National Academy of Sciences*, **104**:19163–19164.
- Saunders, G. W. (2005). Applying DNA barcoding to red macroalgae: A preliminary appraisal holds promise for future applications. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **360**:1879–1888.
- Saunders, G. W. (2008). A DNA barcode examination of the red algal family Dumontiaceae in Canadian waters reveals substantial cryptic species diversity. 1. The foliose *Dilsea-Neodilsea* complex and *Weeksia*. *Botany*, **86**:773–789.
- Saunders, G. W., Huisman, J. M., Vergés, A., Kraft, G. T., & Gall, L. Le. (2017). Phylogenetic analyses support recognition of ten new genera, ten new species and 16 new combinations in the Family Kallymeniaceae (Gigartinales, Rhodophyta). *Cryptogamie, Algologie*, **38**:79–132.
- Sauvage, T., Ballantine, D. L., Peyton, K. A., Wade, R. M., Sherwood, A. R., Keeley, S., & Smith, C. (2019). Molecular confirmation and morphological reassessment of *Udotea geppiorum* (Bryopsidales, Chlorophyta) with ecological observations of mesophotic meadows in the Main Hawaiian Islands. *European Journal of Phycology*, **55**: 186–196.
- Schneider, C. W., Popolizio, T. R., & Saunders, G. W. (2019). Collections from the mesophytic zone off Bermuda reveal three species of Kallymeniaceae (Gigartinales, Rhodophyta) in genera with transoceanic distributions. *Journal of Phycology*, **55**: 415–424.
- Schneider, C. W., Saunders, G. W., & Lane, C. E. (2014). The monospecific genus *Meredithia* (Kallymeniaceae, Gigartinales) is species rich and geographically widespread with species from temperate Atlantic, Pacific, and Indian Oceans. *Journal of Phycology*, **50**:167–186.
- Schneider, C. W., & Wynne, M. J. (2007). A synoptic review of the classification of red algal genera a half century after Kylin's "Die Gattungen der Rhodophyceen". *Botanica Marina*, **50**:197–249.
- Schneider, C. W., & Wynne, M. J. (2013). Second addendum to the synoptic review of red algal genera. *Botanica Marina*, **56**:111–118.
- Schneider, C. W., & Wynne, M. J. (2019). Fourth addendum to the synoptic review of red algal genera. *Botanica Marina*, **62**:355–367.
- Scotland, R. W., Olmstead, R. G., & Bennett, J. R. (2010). Society of Systematic Biologists Phylogeny Reconstruction: The Role of Morphology. *Systematic Biology*, **52**:539–548.
- Searles, R. B. (1980). The strategy of the red algal life history. *The American Naturalist*, **115**: 113–120.

- Sherwood, A. R., Kurihara, A., Conklin, K. Y., Sauvage, T., & Presting, G. G. (2010a). The Hawaiian Rhodophyta Biodiversity Survey (2006-2010): a summary of principal findings. *BMC Plant Biology*, **10**:258.
- Sherwood, A., Sauvage, T., Kurihara, A., Conklin, K. Y., & Presting, G. G. (2010b). A comparative analysis of COI, LSU and UPA marker data for the Hawaiian florideophyte Rhodophyta: implications for DNA barcoding of red algae. *Cryptogamie, Algologie*, **31**:451–465.
- Sherwood, A. R., Lin, S. M., Wade, R. M., Spalding, H. L., Smith, C. M., & Kosaki, R. K. (2019). Characterization of *Martensia* (Delesseriaceae; Rhodophyta) from shallow and mesophotic habitats in the Hawaiian Islands: description of four new species. *European Journal of Phycology*, **55**:172–185.
- Silva, P. C., & Chacana, M. E. (2014). Validation of names of four Hawaiian species of *Codium* (Chlorophyta). *Nova Hedwigia*, **98**:253–256.
- Slattery, M. (2012). Mesophotic coral reefs: a global model of community structure and function. In *Proceedings of the 12th International Coral Reef Symposium*. ICRS, Queensland, Australia.
- Spalding, H.L. (2012). *Ecology of mesophotic macroalgae and Halimeda kanaloana meadows in the main Hawaiian Islands*. Ph.D. Dissertation, University of Hawai'i, Honolulu.
- Spalding, H. L., Amado-Filho, G. M., Bahia, R. G., Ballantine, D. L., Fredericq, S., Leichter, J. J., ... Tsuda, R. T. (2019a). Macroalgae. In *Mesophotic Coral Ecosystems* (507–536). Springer International Publishing, Cham.
- Spalding, H.L., Conklin, K.Y., Smith, C.M., O'Kelly, C.J. and Sherwood, A.R., 2016. New Ulvaceae (Ulvophyceae, Chlorophyta) from mesophotic ecosystems across the Hawaiian archipelago. *Journal of Phycology*, , **52**:40–53.
- Spalding, H. L., Copus, J. M., Bowen, B. W., Kosaki, R. K., Longenecker, K., Montgomery, A. D., ... & Toonen, R. J. (2019b). The Hawaiian Archipelago. In *Mesophotic Coral Ecosystems* (pp. 445-464). Springer International Publishing, Cham.
- Suriya, J., Krishnan, M., Bharathiraja, S., Sekar, V., & Sachithanandam, V. (2018). Implications and utility of DNA barcoding. In *DNA Barcoding and Molecular Phylogeny* (45–64). Springer International Publishing, Cham.
- Trias, R., García-Lledó, A., Sánchez, N., López-Jurado, J. L., Hallin, S., & Bañeras, L. (2012). Abundance and composition of epiphytic bacterial and archaeal ammonia oxidizers of marine red and brown macroalgae. *Applied Environmental Microbiology*, **78**:318–325.
- Trivedi, S., Aloufi, A. A., Ansari, A. A., & Ghosh, S. K. (2016). Role of DNA barcoding in marine biodiversity assessment and conservation: An update. *Saudi Journal of Biological Sciences*, **23**:161–171.

Tsuda, R. T. (2014). Bibliographic Catalogue of the Marine Benthic Algae in the Papahānaumokuākea Marine National Monument (Northwestern Hawaiian Islands). *Phytotaxa*, **167**:35–60.

Veazey, L. M. (2018). *Distribution modeling of mesophotic corals and algae to further ecological understanding and resource management of Hawaiian coral reefs*. Ph.D. Dissertation, University of Hawai‘i, Honolulu.

Vergés, A., Gey, D., Utgé, J., Cruaud, C., & Le Gall, L. (2014). Recognition of a new species of *Kallymenia* (Gigartinales, Rhodophyta) from Croatia (Mediterranean Sea) based on morphology and DNA barcode. *European Journal of Phycology*, **49**:332–344.

Vergés, A., & Rodríguez-Prieto, C. (2006a). Anatomical characteristics and reproductive structures of *Kallymenia lacerata* (Kallymeniaceae, Rhodophyta) from the Mediterranean Sea. *Cryptogamie, Algologie*, **27**:31–43.

Vergés, A., & Rodríguez-Prieto, C. (2006b). Vegetative and reproductive morphology of *Kallymenia patens* (Kallymeniaceae, Rhodophyta) in the Mediterranean Sea. *Botanica Marina*, **49**:310–314.

Wagner, D., Barkman, A., Spalding, H., Calcinai, B., & Godwin, L. S. (2016). *A photographic guide to the benthic flora and fauna from mesophotic coral ecosystems in the Papahānaumokuākea Marine National Monument*. Marine Sanctuaries Conservation Series ONMS-16-04. U.S. Department of Commerce, NOAA, Office of National Marine Sanctuaries, Silver Spring.

Wade, R., Spalding, H., Peyton, K., Foster, K., Sauvage, T., Ross, M., & Sherwood, A. (2018). A new record of *Avrainvillea* cf. *erecta* (Berkeley) A. Gepp & E. S. Gepp (Bryopsidales, Chlorophyta) from urbanized estuaries in the Hawaiian Islands. *Biodiversity Data Journal*, **6**:e21617.

Womersley, H. (1984). *The marine benthic flora of southern Australia*. South Australian Government Printing Division, Adelaide.

Womersley, H.B.S. (1994). *The Marine Benthic Flora of Southern Australia, Part IIIA, Bangiophyceae and Florideophyceae (Acrochaetiales, Nemaliales, Gelidiales, Hildenbrandiales and Gigartinales sensu lato)*. Australian Biological Resources Study, Canberra.

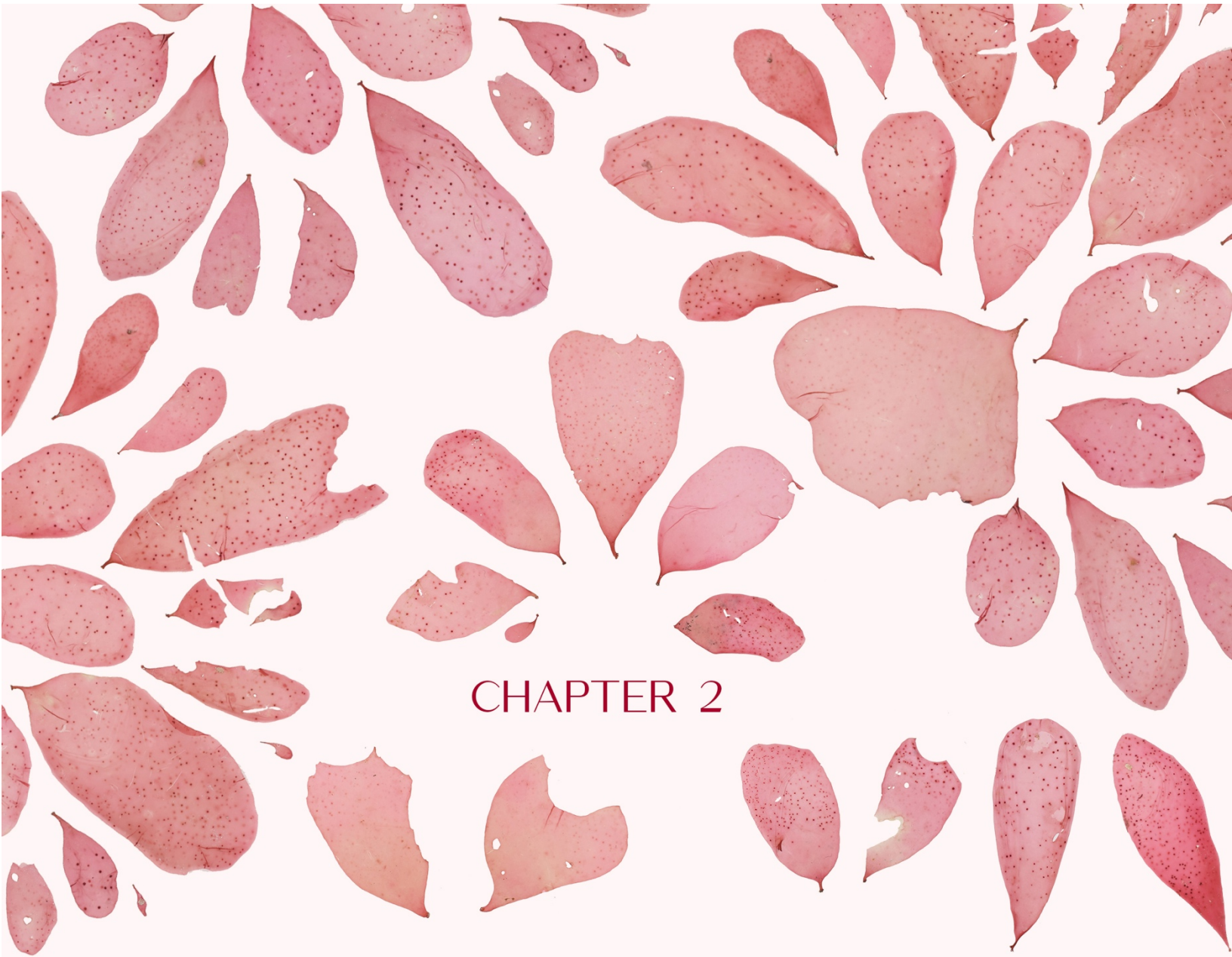
Womersley, H.B.S & Norris, R.E. (1971). The morphology and taxonomy of Australian Kallymeniaceae (Rhodophyta). *Australian Journal of Botany Supplementary Series*, **1**:1–62.

Wynne, M. J., & Schneider, C. W. (2010). Addendum to the synoptic review of red algal genera. *Biology Commons Botanica Marina*, **53**: 291–299.

Wynne, M. J., & Schneider, C. W. (2016). Third addendum to the synoptic review of red algal genera. *Botanica Marina*, **59**:397–404.

Yan, Z., Han, W., Peñuelas, J., Sardans, J., Elser, J. J., Du, E., ... & Fang, J. (2016). Phosphorus accumulates faster than nitrogen globally in freshwater ecosystems under anthropogenic impacts. *Ecology Letters*, **19**:1237–1246.

Yoon, H. S., Müller, K. M., Sheath, R. G., Ott, F. D., & Bhattacharya, D. (2006). Defining the major lineages of red algae (Rhodophyta). *Journal of Phycology*, **42**:482–492.



CHAPTER 2

Mai Poina 'Oe Ia'u.
Don't forget me

Queen Lili'uokalani

CHAPTER 2

Diversity of Kallymeniaceae from mesophotic reefs of Hawai‘i with description of *Psaromenia laulamaula* sp. nov. and *Meredithia [species epithet]* sp. nov.

2.1 Preface

Floristic surveys conducted in the last two decades throughout the Hawaiian Islands have resulted in the collection of many expanded red blades that cannot be placed in currently recognized taxa, signifying a breadth of diversity overlooked in published accounts of the Hawaiian marine macroflora. By undertaking a combination of molecular analyses and morphological observations to survey diversity in the Kallymeniaceae, a family that is particularly well represented in the mesophotic reefs of Hawai‘i, we identified two undescribed species belonging to *Psaromenia* and *Meredithia*, respectively. The outcome of the taxonomic study will be submitted for peer-review to the *European Journal of Phycology* (Cabrera et al. in prep.). The article is presented below as the main body of this chapter.

2.2 Publication

The following content is based on a manuscript prepared for submission to the *European Journal of Phycology*:

Cabrera, F., Huisman, J. M., Smith, C. M., Spalding, H., Kosaki, R., & Sherwood A. R.

Diversity of Kallymeniaceae (Gigartinales, Rhodophyta) from mesophotic reefs of Hawai‘i with description of *Psaromenia laulamaula* sp. nov. and *Meredithia [species epithet]* sp. nov.

**Diversity of Kallymeniaceae (Gigartinales, Rhodophyta) associated to the mesophotic reefs
of Hawai‘i with description of *Psaromenia laulamaula* sp. nov. and *Meredithia [species
epithet] sp. nov.***

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Abstract

Small stipitate red blades of varying morphology have been moderately well-collected from Hawaiian reefs, but poorly classified. Floristic surveys conducted over the last two decades throughout the Hawaiian Islands have yielded many expanded red blades, biased towards large macroalgal species that cannot be placed in currently recognized taxa, and signifying a breadth of diversity overlooked in published accounts. Little attention has been paid to smaller blades and their possible taxonomic significance. In targeted surveys of the Papahānaumokuākea Marine National Monument (PMNM) and Main Hawaiian Islands (MHI), we discovered two undescribed stipitate red algal blades that molecularly and morphologically matched *Psaromenia* and *Meredithia*, respectively; neither genus has been previously recorded in the Hawaiian Islands. Accordingly, these specimens are described here as new species within the family Kallymeniaceae and new records to the Hawaiian marine algal flora. *Psaromenia laulamaula* sp. nov., exclusively found at mesophotic depths (83–94 m) in NWHI, is easily distinguished from other members of the genus by its comparatively large, procarpic carpogonial branch system and solitary obovate pink to magenta blades that are unique to the species. Conversely, *Meredithia* [*species epithet under development*] sp. nov., occurring in both shallow (0–17 m) and mesophotic depths (55 m), has high morphological plasticity, with characters that overlap with other *Meredithia* species, and can only be unequivocally distinguished based on DNA sequences. These results confirm previously reported biogeographic affinities of Atlantic and Indo-Pacific blades in the *Psaromenia-Meredithia* clade. This study provides additional evidence of the extent of diversity in the Family Kallymeniaceae from mesophotic depths, which are largely uncharacterized, and provides further evidence that even small, inconspicuous members of the red blade macroalgal flora contain undescribed biodiversity. The findings of this study contribute to an improved

understanding of the community composition of macroalgae associated with mesophotic environments and the function of Hawaiian MCEs as refugia.

Keywords: biodiversity; Hawai'i; Kallymeniaceae; *Meredithia*; mesophotic; *Psaromenia*; Rhodophyta;

Introduction

Members of the florideophycean family Kallymeniaceae are among the most morphologically striking elements of coral reef communities and are easily recognizable by their elusive yet simple morphologies of expanded red blades (Saunders, 2008). Yet, these charismatic red blades remain one of the most inscrutable algal groups to identify at the genus level due to rampant phenotypic plasticity within the family (Evans & Saunders, 2017; Robuchon et al., 2014). In addition, many genera in the family have nebulous generic definitions and overlapping (and limited) diagnostic morphological characters that are often dependent on potentially variable attributes such as color, shape, or size of the blades (Hansen & Lindstrom, 1984; Tokida & Masaki, 1956). As a result, assigning species to the appropriate genus can be challenging when using morphological characters alone. Such is the case for two closely related genera in the family characterized by stipitate blades, *Psaromenia* D'Archino, W.A.Nelson & Zuccarello and *Meredithia* J.Agardh.

One of the earliest pulses of molecular-assisted alpha taxonomy (MAAT) effort in the family Kallymeniaceae resulted in the description of the New Zealand monotypic genus *Psaromenia*, represented by *P. berggrenii* (J. Agardh) D'Archino, W.A. Nelson & Zuccarello. Analyses of *rbcl* sequences showed that the taxon was more closely related to a strongly supported clade with members of novel, undescribed *Psaromenia* species from Australia and Korea and *Meredithia* than to members of the genus *Kallymenia*, to which it formerly belonged (D'Archino et al., 2010). *Meredithia* was first described by Agardh (1892) to include its type *M. microphylla* (J.Agardh) J.Agardh, *M. nana* J.Agardh and *M. polycoelioides* (J.Agardh) J.Agardh, the latter of which was later elevated to the monotypic genus *Rhipidomenia* Saunders et al. (2017) from Australia. *Meredithia microphylla* formerly belonged to the genus *Kallymenia* and was reported from England in the north-eastern Atlantic, with occurrence in the Mediterranean (Guiry & Maggs, 1982; 1984). Both *Psaromenia* and *Meredithia* share a long and convoluted taxonomic history of

being placed in and out of the genus *Kallymenia* (Agardh, 1899; Guiry & Maggs, 1984). Elevation of *Psaromenia* to the level of genus provided an impetus to investigate taxonomic placement of the closely related genus *Meredithia*, and other members of the family (Schneider et al., 2014).

The distinctiveness of *Meredithia* was readdressed in subsequent studies by Schneider et al. (2014); Ballantine et al. (2015) and Saunders et al. (2017), who reported the genus to be more speciose and cosmopolitan than previously recognized, with members described from the temperate Atlantic (*M. crenata* C.W.Schneider, G.W.Saunders & C.E.Lane, and *M. pulchella* Ballantine, Ruíz & Norris), the Pacific (*M. guiryorum* G.W.Saunders & C.W.Schneider, *M. kraftii* G.W.Saunders & C.W.Schneider, *M. norfolkensis* G.W.Saunders & C.W.Schneider, *M. nutleorum* G.W.Saunders & C.W.Schneider, and *M. opuntioides* G.W.Saunders & C.W.Schneider) and the Indian Ocean (*M. nana* J.Agardh, *M. pseudopeltata* G.W.Saunders & C.W.Schneider, and *M. compaginata* G.W.Saunders). Recent investigations have greatly increased the known diversity of *Meredithia* and suggested the presence of cryptic lineages, suggesting that systematics of this group still needs to be refined.

Similar to *Meredithia*, evidence of extensive cryptic diversity within the genus is also observed in *Psaromenia* (Schneider et al., 2014). Further study of the group by Schneider et al. accounted for the description of *Psaromenia septentrionalis* C.W.Schneider, Popolizio & G.W.Saunders, which increased the number of species to two, and broadened the distribution range of the genus to include both the Pacific and the Atlantic (2019). The *Psaromenia-Meredithia* clade has recently gained more attention due to its expanded geographical range and increased attention paid to the biodiversity of Mesophotic Coral Ecosystems (MCEs). MCEs are thriving coral reef ecosystems existing at the limits of light penetration, stretching from 30-150 m in the tropics and subtropics (Baker et al., 2016). Most of these MCEs are dominated by macroalgae, which remain one of the

most taxonomically understudied groups of organisms in these environments (Spalding et al., 2019). Curiously, Atlantic members of the clade are the few known thus far from mesophotic depths, with *P. septentrionalis* discovered at 90 m in Bermuda (Schneider et al., 2019) and *M. pulchella* collected at depths down to 70 m in Puerto Rico (Ballantine et al., 2015).

Floristic surveys conducted in the last two decades throughout the Hawaiian Islands have resulted in the collection of many expanded red blades that cannot be identified as currently recognized taxa, signifying a breadth of overlooked diversity in published accounts of the macroflora. In targeted surveys of the Papahānaumokuākea Marine National Monument (PMNM) and Main Hawaiian Islands (MHI), specimens were collected of two undescribed stipitate red algal blades that molecularly and morphologically matched *Psaromenia* and *Meredithia*, respectively; neither genus has been previously recorded in the Hawaiian Islands. Accordingly, these specimens are described here as new species within the family Kallymeniaceae and new records to Hawaiian marine algal flora.

Materials and Methods

Specimens were sampled during surveys of the intertidal in 2007 in Maui and from 2014-2019 in the PMNM by NOAA divers using rebreather diving technologies. The approximate locations of the sampling sites are shown on the map in Figure S1 and the specimen collection details are presented in Table 1. Specimens from which sequences were obtained are listed in Table S1, along with collection details, voucher numbers and GenBank accession numbers for *rbcL* and COI-5P sequences.

Morphological characterization

Anatomical and reproductive features were observed in material that was hand-sectioned with a double-edged razor blade. Sections were rehydrated in modified Pohl's solution (Clark, unpubl.: https://www.eeob.iastate.edu/research/bamboo/pdf/anatomy_protocols.pdf) for approximately 5 minutes, stained with 0.5% aniline blue for approximately 5 minutes, and then mounted in 30% Karo™. Sections of stipe and basal regions, which are generally thicker than apical cross sections, were rehydrated and stained for longer periods although rehydration and staining longer than 20 minutes caused the blades to disintegrate into a viscous mass of cells. Photomicrographs were taken on a Zeiss AxioImager A1 compound light microscope (Pleasanton, CA) with an Infinity2-1RC digital camera (Lumenera Corporation, Ottawa, Ontario, Canada). To illustrate the full view of the sections, several successive images from individual sections were combined using Autostitch free software (Ma et al., 2007). Herbarium sheets were digitized in the Joseph F. Rock Herbarium (HAW) using a Canon EOS 5D Mark II Digital Camera and a MK Direct Photo-eBox PLUS 1419.

Molecular sequencing

Total genomic DNA was extracted from silica gel-preserved or herbarium specimens using the OMEGA E.Z.N.A Plant DNA Kit (OMEGA Biotek, Norcross, GA, USA) following the manufacturer's protocol. The mitochondrial COI-5P region was amplified using the primer pairs GazF1 and GazR1 from Saunders (2005) following the recommended PCR amplification profile while the plastid *rbcL* gene was amplified using the following primer pairs: F7 and R753, F577 and R1381, and F993 and RrbcS start (Freshwater & Rueness, 1994), and the PCR amplification profile of Gavio & Fredericq (2002). Bidirectional DNA sequencing was performed at the Advanced Studies in Genomics, Proteomics and Bioinformatics (ASGPB) sequencing facility of

the University of Hawai‘i at Manoa (<http://asgpb.mhpcc.hawaii.edu/>). Sequence data were edited and aligned with additional sequences downloaded from GenBank (Table S1) in Geneious Prime (<http://www.geneious.com>).

Phylogenetic reconstruction

Sequence alignment was performed using the MUSCLE plug-in (Edgar, 2004) with default settings in Geneious Prime to construct sequence alignments for each gene: COI-5P with 25 sequences of 664 bp (base pairs) and *rbcL* with 26 sequences of 1358 bp, which were subsequently checked by eye. This alignment includes a representative of Dumontiaceae, *Dudresnaya hawaiiensis* as outgroup taxa. We analyzed the *rbcL* and COI datasets separately and concatenated sequence data from these markers to give a dual COI-*rbcL* dataset in PartitionFinder v.1.1.1 (Lanfear et al., 2012) to determine best partitioning strategy. Analyses suggested the selection of the General Time Reversible model with a gamma distributed rate variation among sites and a proportion of invariant sites (GTR+I+G) involving four partitions: (1) COI-5P and (3) codon positions of *rbcL*. The concatenated dataset, partitioned by gene and codon position, was used in phylogenetic reconstruction performed with (1) Maximum Likelihood (ML) (GTR+I+G) using RAxML (<https://www.geneious.com/plugins/raxml-plugin/>; Stamatakis, 2014) with 1000 bootstrap replicates, and (2) Bayesian Inference (BI) using MrBayes v. 3.2.6 (<https://www.geneious.com/plugins/mrbayes-plugin/>; Ronquist et al., 2012) based on the nucleotide substitution models as determined by the Akaike Information Criteria (AIC) in MrModeltest 2.3 (Nylander, 2008) through tree builder plugins in Geneious Prime. The Bayesian analysis was run with 2,000,000 generations of Markov Chain Monte Carlo iterations until the standard deviation of split frequencies was below 0.01. The first 10% of trees of each run were discarded as burn-in. Visualization of the tree was performed via the interactive Tree of Life

(<https://itol.embl.de/>) (Letunic & Bork, 2019). All new sequences generated in this study have been submitted to GenBank (accession numbers xxxxxxxx– xxxxxxxx [to be obtained]).

Results

Phylogenetic analyses

The COI+*rbcL* concatenated alignment was 2,022 base pairs (bp) in length and included both newly determined sequences and reference sequences from GenBank. The ML and BI analyses produced identical topologies; only the ML tree, with support values from both types of analyses superimposed, is shown (Fig. 1). Phylogenetic analyses confirmed the placement of Hawaiian specimens in two clades, one representing the genus *Meredithia*, and one representing *Psaromenia*, with full support. The concatenated COI+*rbcL* sequence analyses demonstrated the distinction of Hawaiian *Psaromenia* from the other two taxonomically accepted species in *Psaromenia*: *P. berggrenii*, the type species, has 8.83% divergence from the lineage of Hawaiian *Psaromenia*, and *P. septentrionalis* has 7.96% divergence from the Hawaiian lineage. The closest relative of the Hawaiian *Psaromenia* was shown to be an undescribed *Psaromenia* collected from Jeju Island, Korea, with 4.15% divergence. Hawaiian *Meredithia* was demonstrated to have 9.73% sequence divergence from *M. microphylla*, the generitype, and it was resolved as a close ally to *M. norfolkensis* from Australia with 1.88% sequence divergence. Together, these two species have been ascertained to be phylogenetically distinct taxonomic units within the *Meredithia*-*Psaromenia* clade and are proposed below as new species.

Taxonomic Treatment

***Psaromenia laulamaula* F.P.Cabrera, J.M.Huisman & A.R.Sherwood, sp. nov.**

(Figs 2–24)

Description: Thallus red, blade-like, solitary, stipitate, simple, lobed or rounded with smooth to undulate margins, becoming spatulate with maturity, from 1–11 cm in height, 0.3–8 cm in width. Blades rose-pink, progressing to a rich magenta at margins and basal regions of blade. Stipe present, 1–5 mm in length with a small discoidal holdfast. Blades multiaxial in structure, composed of a mostly filamentous medulla with abundant, lightly staining stellate ganglia with a diameter of 450–880 μm throughout the blade. Blades 250–300 μm thick near the margins, 200–225 μm thick through apical part of the blade, and 270–300 μm thick through the basal regions. Cortex composed of 1-3 cell layers decreasing in size towards the surface with the largest inner cortical cells measuring to 3–7 x 8–14 μm . Polycarpogonial branch systems borne on supporting cells produce mature cystocarps (up to 2 mm in diameter). Cystocarps scattered singly throughout the blades extending at least 500 μm above and below the thallus surface. Spermatangial mother cells (3–7 μm length x 5–10 μm breadth) present in sori on one side of the blade. Tetrasporangia not observed.

Holotype: BISH 776061 (ARS 09483; Lisianski Island (Papa'āpoho), Hawai'i (25.92698, -173.05490), 84 m, 14. IX.2014, leg. R. Kosaki & B. Hauk (NWHI-253)). GenBank accessions xxxxxxxx.

Isotype: BISH 776062 (ARS 09485; Lisianski Island (Papa'āpoho), Hawai'i (25.92698, -173.05490), 84 m, 14. IX.2014, leg. R. Kosaki & B. Hauk (NWHI-350)). GenBank accession xxxxxxxx.

Etymology: The specific epithet for *Psaromenia laulamaula* is derived from the Hawaiian language and was developed by Kalani Quiocho of the Cultural Working Group of the PMNM. The naming methodology used for *P. laulamaula* derives partly from the Hawaiian creation chant, the Kumulipo. During much of the Kumulipo, creation is articulated through a series of births which, for part of the chant, occur in pairs, with one living thing birthed in the sea and its counterpart on land (Beckwith, 1951). The species epithet, *laulamaula*, represents a Latinized combination of three descriptive terms. *Lau* (noun; literally, leaf) represents the leaf-like character of the blade. Following the Kumulipo, *lama* (noun; Hawaiian endemic ebony trees, or a light or torch), the ‘birthed on land’ counterpart, refers to the Hawaiian endemic ebony trees thriving in the highlands of Hawai‘i up to elevations of 1,200 m, which contrast with the blade, or the ‘birthed in the sea’ counterpart flourishing in lower mesophotic depths. *Ula* (adjective; red) describes the lush regal red color of the blades, which bestows honor on the Hawaiian royal family that bears this color in their culture and traditions. Translations provided by Wehewehe (<http://wehewehe.org/>).

Distribution: *P. laulamaula* is known throughout the PMNM from Kure Atoll (Hōlanikū), Midway Atoll (Kuaihelani), Pearl and Hermes Atoll (Manawai), Lisianski (Papa‘āpoho) and French Frigate Shoals (Lalo), and was exclusively observed at a mesophotic depth range of 83–94 m.

Specimens Examined: BISH 776063 (ARS 09489), BISH 776064 (ARS 09727), BISH 776065 (ARS 09730), BISH 776066 (ARS 09732), BISH xxxxxx (ARS 09959)

DNA sequence data: GenBank accessions xxxxxxxx– xxxxxxxx

Habit and vegetative morphology

Thalli are simple, almost leaf-like blades that are rounded to spatulate in shape with smooth margins, and are more or less undulate. Rather than upright, blades are curled, almost sprawling or lying prostrately on the substrate *in situ* (Figs 2-3, S1). The rose pink to red magenta blade color is retained even when dried. The solitary blades are 4–23 cm in height and 8–29 cm in width, attached by a stipe, 5–8 mm long (Figs 4–5). Blades are 250–300 μm thick along the margins (Fig. 6), 200–225 μm thick at the apex of the blade (Fig. 7) and thicken in the basal region to 270–300 μm thick (Fig. 8). Surface cortical cells are polygonal to sub-rounded in shape, 14–25 μm in diameter, loosely packed so that subcortical cells are visible in surface view (Fig. 11). The medulla is composed of densely aggregated stellate ganglia that are 450–880 μm in diameter (Fig. 10). The stipe is densely packed with medullary filaments (Fig. 9).

Reproductive morphology

Cystocarps (up to 2 mm in diameter) are scattered over the entire blade protruding at both sides. Carposporophytes are 320–450 μm in height and 600–800 μm in diameter when developing, and 500–550 μm in height and 850–1000 μm in diameter when mature (Figs 18, 21), and protrude from both sides of the blades. Carpogonial branches are initiated laterally from subcortical cells (Fig. 16). Spermatangial sori are scattered over one surface of lamina (Figs 22–24). Mature carposporangia are 12–20 μm wide by 25–40 μm long, obovate in shape (Figs 19, 20). Tetrasporophytes were not observed.

***Meredithia* [specific epithet under development] F.P.Cabrera, J.M.Huisman &**

A.R.Sherwood, sp. nov.

(Figs 25–32)

Description: Reniform to semi-peltate red blades associated in small clumps, 0.5–1.5 cm in diameter, typically wider than tall. Thalli stipitate or non-stipitate. Stipes 415–440 μm in diameter, 1–2 mm tall, densely packed with medullary filaments, bearing a single blade with smooth margins. Non-stipitate blades foliose with loosely undulate margins. Blades with 1–2 layers of subcortical cells, 8–17 μm in diameter. Blades multiaxial in structure, composed of a filamentous medulla with occasional lightly staining stellate medullary cells throughout the blade. Blades 55–108 μm thick through apical margins, 230–275 μm thick through median region, and 340–370 μm thick through basal portion. Cystocarps 100–200 μm diameter, immersed when developing and protuberant when mature, scattered throughout the blades. Male gametophytes and tetrasporophytes not observed.

Holotype: BISH xxxxxx (ARS 09958; Pearl and Hermes Atoll (Manawai), Hawai‘i (27.91062, –175.90483), 17 m, 31. VII.2019, leg. B. Hauk (NWHI-800)). GenBank accessions xxxxxxxx.

Etymology: specific epithet under development (will be available prior to manuscript submission to *European Journal of Phycology*).

Distribution: *Meredithia sp. nov.* has a geographical range extending from the PMNM at Pearl and Hermes Atoll (Manawai) (17 m), and Lisianski (Papa‘āpoho) (at 55 m) to the MHI (Maui) in the shallow intertidal (less than 1 m).

Habit and vegetative morphology

Thalli consist of simple, semi-peltate to lobed blades, 0.5–1.5 cm in diameter, typically wider than tall. Blades are erect and solitary. *In situ*, blades are rose pink to red magenta in color, turning to dark fuchsia when dried (Figs 25–26). Blades have smoother margins and are single-lobed when a stipe is present. Stipes are 415–440 μm in diameter and 1–2 mm tall (Fig. 28). Blade has more undulate margins and is irregularly lobed when lacking stipe. Blades are 230–275 μm thick (Fig. 29), progressively thickening at the basal part of the blade (340–370 μm) (Fig. 31), and becoming thinner at the margins (55–108 μm) (Fig. 27). Inner cortical cells are polygonal to sub-rounded in surface view, and are 5–15 μm in diameter (Fig. 11).

Reproductive morphology

Specimens included occasional blades with protruding cystocarps (0.2–0.4 mm in diameter), such as those observed in holotype herbarium voucher BISH xxxxxx (Figs 18, 21). However, observations of reproduction were limited by a paucity of material. Under this constraint, the carpogonial branches initiating laterally from subcortical cells was the sole observation that could be made regarding the carpogonial system (Fig. 32). Male gametophytes and tetrasporophytes were not observed.

Specimens Examined: BISH xxxxxx (ARS 02021) and BISH xxxxxx (ARS 09947).

Discussion

Morphological and Phylogenetic analysis

Small stipitate red blades of various morphologies are frequent finds on Hawaiian reefs. Floristic collections over the years in Hawai‘i have been biased towards large, upright macroalgal species,

and little attention has been paid to dwarf blades and their possible taxonomic significance, which explains the modest collection of specimens secured for the present study. Regardless, the samples analyzed represent a defined portion of the red algal diversity from a largely protected PMNM, and the more heavily impacted Main Hawaiian Islands (MHI). Sequence divergences of *P. laulamaula* and *Meredithia* sp. nov. within their respective genera exceed thresholds generally used to discriminate species within the order Gigartinales (Le Gall & Saunders, 2010). Only *Meredithia pulchella*, for which only LSU sequence data is available, was not included in our analyses; however, the species has been previously determined to be a sister species to *M. crenata* (Ballantine et al., 2015). The MAAT approach employed in this study demonstrated the two entities studied here as distinct and undescribed species: *Psaromenia laulamaula* sp. nov. and *Meredithia* sp. nov., which are presumed to be endemic to Hawai'i based on their phylogenetic affinities, geographic distributions and morphologies. These results have the effect of carving up several pan-oceanic taxa? into more regional species.

Psaromenia laulamaula represents the third species formally described in the genus, and the first to be described from the North Pacific, extending the known distribution of *Psaromenia* beyond Bermuda, New Zealand, Korea and Australia. Its closest relative in our analysis is “*Psaromenia* sp.1_Jeju”; an undescribed, novel Korean species (Schneider et al., 2014). Biogeographical links between Hawaiian and Korean material have been observed recently for other mesophotic red algal species in Hawai'i (i.e., *Martensia albida* Y.Lee, *Herposiphonia* Nägeli, *Gracilaria parvispora* I.A. Abbott; Sherwood et al., 2019; Koh et al., 2018; Kim et al., 2008).

Morphological characteristics were identified that are useful in differentiating *P. laulamaula* from other members of the genus (Table 2). In terms of gross morphology, *P.*

laulamaula is easily distinguished by its simple blade morphology, which contrasts with the foliose and erect blades of *P. berggrenii* and *P. septentrionalis*. Secondly, blades of *P. laulamaula* usually lie prostrate on the substratum. *Psaromenia laulamaula* grows solitarily with a single holdfast as a mode of attachment. Thirdly, the cystocarps of *P. laulamaula* are comparatively larger than all other described species (Table 2) and possess more densely packed carposporangia than other species. Similar to the generitype, *Psaromenia berggrenii*, *P. laulamaula* is a variable species, especially with respect to its blade morphology. The holotype material has clearly spatulate blades, whereas other collections of *P. laulamaula* display blades only slightly broadened at the apex. The morphology of *P. berggrenii* has been noted to be variable in relation to age and depth (D'Archino et al., 2010). Like all other members of *Psaromenia*, no tetrasporangial plants of *P. laulamaula* were observed (D'Archino et al., 2010; Schneider et al., 2019). Given the isolation of the PMNM and the uniqueness of its macrofloral community, we suspect that the new species is endemic to the reefs of Hawai'i.

Hawaiian *Meredithia* sp. nov. represents the twelfth species formally described in the genus, with species of *Meredithia* reported from the Atlantic, Indian and Pacific Oceans. The available material for *Meredithia* sp. nov. consists of fairly young blades, which are difficult to distinguish from other members of the genus. Gross and vegetative morphological observations demonstrated high polymorphism derived from substantial overlap among *Meredithia* species. Reproductive morphology of members of the genus provide little power in delineating species as reproductive structures in more than half of the species were either not observed, or observed but not described in sufficient detail. Species of *Meredithia* are often difficult to verify as morphological differences between the species are small and subtle, and thus weakly differentiated. Considerable morphological variability in *Meredithia* remains poorly reflected in

dichotomous identification keys and is not easily related to the phylogenetic results, which is why species within the genus are still largely distinguished from each other on a molecular basis (Schneider et al., 2014 Saunders et al., 2017). Nevertheless, we found that a small set of vegetative characters (blade shape, blade thickness and presence of stipe) (Table 2.3–4) that represent synapomorphies for *Meredithia* species.

Species overlap, Distribution and DRRH

Whether mesophotic reefs function as refugia largely depends on the extent of species overlap with shallow-water counterparts (Bongaerts & Smith, 2019). *Psaromenia laulamaula* is documented exclusively at greater mesophotic depths (> 60 m), similar to its Atlantic congener, *P. septentrionalis*. Similarly, at least for endemic Hawaiian flora, *Codium campanulatum* P.C.Silva & M.E.Chacana (95 m), *Martensia abbottiae* A.R.Sherwood & S.-M.Lin (65–93 m), *Martensia tsudae* A.R.Sherwood & S.-M.Lin, (64–126 m), *Martensia lauhiekoeloa* A.R.Sherwood & S.-M.Lin (61–67 m) have only been found at lower mesophotic depths while *Codium hawaiiense* P.C.Silva & M.E.Chacana (35 m) and *Codium intermedium* P.C.Silva & M.E.Chacana (45–55 m) have only been found at upper mesophotic depths (>30m).

In contrast, *Meredithia* sp. nov. was first collected in 2007 at intertidal depths on Maui, MHI and in 2018 on PMNM shallow reefs (17 m) of Pearl and Hermes Atoll (PHA) to mesophotic depths on reefs of Lisianski (55 m), exhibiting distributional overlap between shallow and mesophotic communities. Some of the endemic Hawaiian flora such as *Codium desultorium* P.C.Silva & M.E.Chacana (27-37m) exhibit a narrow range of distributional overlap, while *Martensia hawaiiensis* A.R. Sherwood & S.-M. Lin (1–65 m) and *Halimeda kanaloana* Vroom (1–85 m) have wider ranges of distributional overlap. The variability observed in terms of distributional overlap within shallow and mesophotic communities in this study corroborates

trends from other Hawaiian mesophotic studies mentioned above in that the evidence for connectivity among MCE and shallow macroalgal populations can differ among species. Clearly, connectivity between MCEs and shallow reefs needs to be evaluated not as an ecosystem-wide phenomenon but on a species-by-species basis, incorporating the relevant facets of life history and ecology where possible (Bongaerts et al., 2017).

Moreover, we also cannot discard the hypothesis that finding algal species such as *Psaromenia laulamaula* and *P. septentrionalis* only at mesophotic depths could just be a consequence of either the insufficient taxonomic effort directed towards existing research collections (i.e. herbarium vouchers that are not as easily accessible as they should be) of these blades that have gone undetected or misidentified; or that floral collections in general are focused on more sizeable charismatic groups. Or that sampling is strongly seasonal and further limited by being based on available ship-time.

Biogeographic links

The patterns of distribution of the *Psaromenia-Meredithia* clade show different aspects of the natural history of both genera. Biogeographic patterns are complex as species are not clustered by biogeographic region, suggesting alternative biogeographic patterns or dispersal routes (McDermid & Abbott, 2006). Bringloe and Saunders (2019) proposed that amphitropical florideophytes established from refugial populations during the late Pleistocene may have crossed the tropics along deep-water refugia coincident with global cooling. With the Hawaiian Islands located in the path of the North Equatorial Current, which would have provided greater opportunities for colonization (Gosline, 1955), Hawai'i was in a favorable position to have been colonized by these refugial populations. Hawaiian Kallymeniaceae, however, appear to be more closely allied with related taxa in Australasia. McDermid and Abbott (2006) discuss how

Hawaiian algal populations with disjunct global distributions such as the *Psaromenia-Meredithia* clade can be explained not only by palaeoclimatic vicariance but also with present climatic vicariance, because distributions of putative algal species are frequently only exclusively observed at MCEs that could have acted as climatic refuges having more or less the same conditions as those of the shallow to mesophotic temperate regions.

Species threats

Some populations of *P. laulamaula* and *Meredithia* sp. were collected at PHA, PMNM. Recently, a mat-forming *Chondria* species with strong invasive characteristics was detected overgrowing shallow reefs of PHA and smothering coral and other native organisms (Sherwood et al., in revision). Ongoing research to determine the scale and impact of relative risks associated with invasive behavior of this alga, assessing risks of dispersal pathways to neighboring reefs, and additional taxonomic research to underpin accurate recognition of invasive and native species, is required. This report stresses urgency for further research aimed at continued collections and work to understand taxonomic placement of dwarf red blades such as *Psaromenia* and *Meredithia* that as demonstrated by the present study. There is an enormous potential for hidden biodiversity.

Conclusion

The discovery of the two novel species in Hawai'i, *Psaromenia laulamaula* and *Meredithia* sp., represents a case of species being overlooked because of their relatively small size that often goes undetected in the field and in existing research collections. While species differences at the extremes of morphological variation within the clade can be very clear, e.g. *Psaromenia laulamaula*. vs. all other *Psaromenia* species, some species are very difficult to separate, e.g.

Hawaiian *Meredithia* vs. all other *Meredithia* species, associated with polymorphic characters within the genus requiring molecular data to confirm their taxonomic placement. Most species of *Psaromenia* are restricted geographically to either lower mesophotic depths of the Western Pacific (*P. laulamaula*) or Atlantic Ocean (*P. septentrionalis*), suggesting that paleoclimatic vicariance has played a role in speciation (McDermid & Abbott, 2006; Bringloe & Saunders, 2019). However, worldwide floristic collection intensity is too low to judge whether this is accidental or a true pattern of distribution. We suggest that even with the further clarification of the generic taxonomy, the general trends in the distribution patterns of Kallymeniaceae will largely remain the same, with the occurrence of a majority of genera in broad geographical ranges with prevalence in temperate regions. Our work on Hawaiian stipitate red blades is inconclusive with respect to the DRRH: while *Meredithia* in Hawai'i is known from both shallow and mesophotic habitats (M. [species epithet]), *Psaromenia laulamaula* was collected only from mesophotic depths. Nevertheless, this study represents a step towards increasing our understanding of mesophotic diversity and taxonomy, tripling the number of known genera in the Family Kallymeniaceae in Hawai'i (Abbott, 1999). Additionally, the present study underscores how much undescribed biodiversity remains in the archipelago that even dwarf stipitate red blades deserve the systematic attention for detection of biodiversity. Further phycological studies, particularly material that still remains unidentified and material associated with mesophotic environments, may further increase known algal biodiversity of Hawai'i. In conclusion, this study joins a growing body of work in characterizing the algal diversity in Hawaiian MCEs (Sauvage et al., 2019; Sherwood et al., 2019; Silva & Chacana, 2014; Spalding, 2012; Wade et al., 2018).

References

- Abbott, I.A. (1999). *Marine Red Algae of the Hawaiian Islands*. Bishop Museum Press, Honolulu.
- Agardh, J. (1892). *Analecta algologica*. *Acta Universitatis Lundensis* **28**:1–182.
- Agardh, J. (1899). *Analecta algologica, Continuatio V*. *Acta Universitatis Lundensis*, **35**:1–160.
- Baker, E.K., Puglise, K.A. & Harris, P.T. (2016). *Mesophotic coral ecosystems — A lifeboat for coral reefs?* The United Nations Environment Programme and GRID-Arendal, Nairobi and Arendal.
- Ballantine, D. L., Ruíz, H., & Norris, J. N. (2015). Notes on the benthic marine algae of Puerto Rico, XI: new records including new *Meredithia* (Kallymeniaceae, Rhodophyta) species. *Botanica Marina*, **58**:355–365.
- Beckwith, M. (1951). *The Kumulipo, a Hawaiian creation chant*. University of Chicago Press, Chicago.
- Bongaerts, P., Riginos, C., Brunner, R., Englebert, N., Smith, S. R., & Hoegh-Guldberg, O. (2017). Deep reefs are not universal refuges: reseeding potential varies among coral species. *Science Advances*, **3**:e1602373.
- Bongaerts, P., & Smith, T. B. (2019). Beyond the “Deep Reef Refuge” hypothesis: a conceptual framework to characterize persistence at depth. In *Mesophotic Coral Ecosystems* (881–895). Springer International Publishing, Cham.
- Bringloe, T. T., & Saunders, G. W. (2019). Trans-Arctic speciation of Florideophyceae (Rhodophyta) since the opening of the Bering Strait, with consideration of the “species pump” hypothesis. *Journal of Biogeography*, **46**:694–705.
- D'Archino, R., Nelson, W. A., & Zuccarello, G. C. (2010). *Psaromenia* (Kallymeniaceae, Rhodophyta): a new genus for *Kallymenia berggrenii*. *Phycologia*, **49**:73–85.
- Edgar, R. C. (2004). MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research*, **32**:1792–1797.
- Evans, J. R., & Saunders, G. W. (2017). PCR fishing for red endophytes in British Columbia Kallymeniaceae (Gigartinales, Florideophyceae) uncovers the species-rich *Kallymenicola* gen. nov. (Palmariales, Nemaliophycidae). *Journal of Phycology*, **53**: 577–588.
- Freshwater, D. W., & Rueness, J. (1994). Phylogenetic relationships of some European *Gelidium* (Gelidiales, Rhodophyta) species, based on *rbcL* nucleotide sequence analysis. *Phycologia*, **33**: 187–194.

- Gavio, B., & Fredericq, S. (2002). *Grateloupia turuturu* (Halymeniaceae, Rhodophyta) is the correct name of the non-native species in the Atlantic known as *Grateloupia doryphora*. *European Journal of Phycology*, **37**:349–359.
- Gosline W. A. (1955). The inshore fish of Johnston Island, a central Pacific atoll. *Pacific Science*, **9**:442–480.
- Guiry, M. D. & Maggs, C. A. (1982). The life history of *Meredithia microphylla* (J. Agardh) J. Agardh (Rhodophyta) in culture. *British Phycological Journal*. **17**:232–233.
- Guiry, M. D., & Maggs, C. A. (1984). Reproduction and life history of *Meredithia microphylla* (J. Agardh) J. Agardh (Kallymeniaceae, Rhodophyta) from Ireland. *Plant Biosystems*, **118**:105–125.
- Hansen, G. I., & Lindstrom, S. C. (1984). A Morphological Study of *Hommersandia maximicarpa* gen. et sp. nov. (Kallymeniaceae, Rhodophyta) From the North Pacific. *Journal of Phycology*, **20**:476–488.
- Kim, M. S., Kim, M., Terada, R., Yang, E. C., & Boo, S. M. (2008). *Gracilaria parvispora* is the correct name of the species known as *G. bursa-pastoris* in Korea and Japan. *Taxon*, **57**:231–237.
- Koh, Y. H., Kim, M. S., Koh, Y. H., & Kim, M. S. (2018). Taxonomic revision of the genus *Herposiphonia* (Rhodomelaceae, Rhodophyta) from Korea, with the description of three new species. *Algae*, **33**:69–84.
- Lanfear, R., Calcott, B., Ho, S. Y., & Guindon, S. (2012). PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution*, **29**:1695–1701.
- Le Gall, L., & Saunders, G. W. (2010). DNA barcoding is a powerful tool to uncover algal diversity: A case study of the Phylloporaceae (Gigartinales, Rhodophyta) in the Canadian flora. *Journal of Phycology*, **42**: 374–389.
- Lesser, M. P., Slattery, M., Laverick, J. H., Macartney, K. J., & Bridge, T. C. (2019). Global community breaks at 60 m on mesophotic coral reefs. *Global Ecology and Biogeography*, **28**: 1403–1416.
- Letunic, I., & Bork, P. (2019). Interactive Tree of Life (iTOL) v4: Recent updates and new developments. *Nucleic Acids Research*, **47**:W256–W259.
- Ma, B., Zimmermann, T., Rohde, M., Winkelbach, S., He, F., Lindenmaier, W., & Dittmar, K. E. (2007). Use of autostitch for automatic stitching of microscope images. *Micron*, **38**:492–499.
- McDermid, K. J., & Abbott, I. A. (2006). Deep subtidal marine plants from the Northwestern Hawaiian Islands: new perspectives on biogeography. *Atoll Research Bulletin*, **543**:525–532.
- Nylander, J. A., Wilgenbusch, J. C., Warren, D. L., & Swofford, D. L. (2008). AWTY (are we

there yet?): a system for graphical exploration of MCMC convergence in Bayesian phylogenetics. *Bioinformatics*, **24**:581–583.

Robuchon, M., Le Gall, L., Gey, D., Valero, M., & Vergés, A. (2014). *Kallymenia crouaniorum* (Kallymeniaceae, Rhodophyta), a new red algal species from the *Laminaria hyperborea* understory community. *European Journal of Phycology*, **49**:493–507.

Ronquist, F., Teslenko, M., Van Der Mark, P., Ayres, D. L., Darling, A., Höhna, S., ... & Huelsenbeck, J. P. (2012). MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology*, **61**:539–542.

Saunders, G.W. (2005). Applying DNA barcoding to red macroalgae: a preliminary appraisal holds promise for future applications. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **360**: 1879–1888.

Saunders, G.W. (2008). A DNA barcode examination of the red algal family Dumontiaceae in Canadian waters reveals substantial cryptic species diversity. 1. The foliose *Dilsea*–*Neodilsea* complex and *Weeksia*. *Botany (Botanique)*, **86**: 773–789.

Saunders, G. W., Huisman, J. M., Vergés, A., Kraft, G. T., & Le Gall, L. (2017). Phylogenetic analyses support recognition of ten new genera, ten new species and 16 new combinations in the family Kallymeniaceae (Gigartinales, Rhodophyta). *Cryptogamie, Algologie*, **38**:79–132.

Sauvage, T., Ballantine, D. L., Peyton, K. A., Wade, R. M., Sherwood, A. R., Keeley, S., & Smith, C. (2019). Molecular confirmation and morphological reassessment of *Udotea geppiorum* (Bryopsidales, Chlorophyta) with ecological observations of mesophotic meadows in the Main Hawaiian Islands. *European Journal of Phycology*, DOI: 10.1080/09670262.2019.1668061.

Schneider, C. W., Saunders, G. W., & Lane, C. E. (2014). The monospecific genus *Meredithia* (Kallymeniaceae, Gigartinales) is species rich and geographically widespread with species from temperate Atlantic, Pacific, and Indian Oceans. *Journal of Phycology*, **50**: 167–186.

Schneider, C. W., Popolizio, T. R., & Saunders, G. W. (2019). Collections from the mesophotic zone off Bermuda reveal three species of Kallymeniaceae (Gigartinales, Rhodophyta) in genera with transoceanic distributions. *Journal of Phycology*, **55**: 415–424.

Sherwood, A. R., Lin, S. M., Wade, R. M., Spalding, H. L., Smith, C. M., & Kosaki, R. K. (2019). Characterization of *Martensia* (Delesseriaceae; Rhodophyta) from shallow and mesophotic habitats in the Hawaiian Islands: description of four new species. *European Journal of Phycology*, DOI: 10.1080/09670262.2019.1668062.

Silva, P. C., & Chacana, M. E. (2014). Validation of names of four Hawaiian species of *Codium* (Chlorophyta). *Nova Hedwigia*, **98**:253–256.

Spalding, H.L. (2012). Ecology of mesophotic macroalgae and *Halimeda kanaloana* meadows in the main Hawaiian Islands. Ph.D. Dissertation, University of Hawai‘i, Honolulu.

Spalding, H. L., Amado-Filho, G. M., Bahia, R. G., Ballantine, D. L., Fredericq, S., Leichter, J. J., et al. (2019). Macroalgae. In *Mesophotic Coral Ecosystems*. Springer International Publishing, New York.

Spalding, H.L., Conklin, K.Y., Smith, C.M., O’Kelly, C.J. & Sherwood, A.R. (2016). New Ulvaceae (Ulvophyceae, Chlorophyta) from mesophotic ecosystems across the Hawaiian Archipelago. *Journal of Phycology*, **52**:40–53.

Stamatakis, A. (2014). RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*, **30**:1312–1313.

Tokida, J., & Masaki. (1956). Studies on the reproductive organs of red algae II. On *Erythrophyllum gmelini* (Grun.) Yendo. *Bulletin of the Faculty of Fisheries Hokkaido University* **7**:63–71.

Wade, R., Spalding, H., Peyton, K., Foster, K., Sauvage, T., Ross, M., & Sherwood, A. (2018). A new record of *Avrainvillea* cf. *erecta* (Berkeley) A. Gepp & E. S. Gepp (Bryopsidales, Chlorophyta) from urbanized estuaries in the Hawaiian Islands. *Biodiversity Data Journal*, **6**:e21617.

Womersley, H.B.S. (1994). *The Marine Benthic Flora of Southern Australia, Part IIIA, Bangiophyceae and Florideophyceae (Acrochaetiales, Nemaliales, Gelidiales, Hildenbrandiales and Gigartinales sensu lato)*. Australian Biological Resources Study, Canberra.

Womersley, H. B. S. (1973). Further studies on Australian Kallymeniaceae (Rhodophyta). *Transactions of the Royal Society of South Australia*, **97**: 253–256.

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Figures and Captions

Figure 1. Combined COI and *rbcL* maximum likelihood tree of Hawaiian *Meredithia* and *Psaromenia* specimens in the context of published GenBank sequences. Outgroup taxa (*Dudresnaya hawaiiensis*) pruned to facilitate presentation. Taxa Scale bar = substitutions per site. Numbers at nodes greater than 70% (bootstrap, first value) and 0.9 (Bayesian posterior probabilities, second value) are shown. Full support is indicated by *. Support values are shown if one or both of the support methods reach the cutoff value.

Figures 2 – 5. *Psaromenia laulamaula* sp. nov. *in situ* and habit images.

Figure 2. Holotype specimen (BISH 776061) *in situ*, collected at Lisianski at 84 m. Scale bar = 5 cm.

Figure 3. Live holotype specimen (BISH 776061) cleaned of epiphytes. Scale bar = 5 cm.

Figure 4. Part of the herbarium voucher of the holotype specimen (BISH 776061), female blades. Scale bar = 5 cm.

Figure 5. Isotype (BISH 776062), male and female blades. Scale bar = 5 cm.

Figures 6 – 11. Vegetative anatomy of mature blades of *Psaromenia laulamaula* sp. nov.

Figure 6. Cross-section through thickened margin in the median part of the blade. Scale bar = 100 μ m (BISH 776061).

Figure 7. Cross-section through apex of the blade. Scale bar = 100 μ m (BISH 776061).

Figure 8. Cross section through basal part of the blade. Scale bar = 100 μ m (BISH 776061).

Figure 9. Cross section through basal part of stipe. Scale bar = 300 μ m (BISH 776061).

Figure 10. Fully developed stellate ganglia. Scale bar = 50 μ m (BISH 776061).

Figure 11. Surface view of cortical cells. Scale bar = 50 μ m (BISH 776061).

Figures 12 – 21. Female reproductive anatomy of *Psaromenia laulamaula* sp. nov. Scale bar = 50 μ m.

Figure 12. Early development of carpogonial branch (detached) in squash preparation. Scale bar = 50 μ m (BISH 776062).

Figure 13. Early-intermediate development of carpogonial branch (detached) in squash preparation. Scale bar = 50 μ m (BISH 776062).

Figure 14. Late-intermediate development of carpogonial branch (detached) in squash preparation. Scale bar = 50 μ m (BISH 776062).

Figure 15. Late development phase of carpogonial branch (detached) in squash preparation. Scale bar = 50 μ m (BISH 776062).

Figure 16. View of a developing carpogonial branch attached near inner cortical cells. Scale bar = 50 μ m (BISH 776062).

Figure 17. Subsurface view of a carpogonial branch. Scale bar = 50 μ m (BISH 776062).

Figure 18. Cross section through an immature carposporophyte. Scale bar = 200 μ m (BISH 776062).

Figure 19. Close up of an immature carposporophyte showing carposporangia in loose clusters. Scale bar = 50 μ m (BISH 776062).

Figure 20. Close up of a mature carposporophyte showing carposporangia in compact clusters. Scale bar = 50 μ m (BISH 776062).

Figure 21. Cross section through a mature carposporophyte. Scale bar = 200 μ m (BISH 776062).

Figures 22 – 24. Male reproductive structures of *Psaromenia laulamaula* sp. nov.

Figure 22. Cross section through a spermatangial sorus. Scale bar = 100 μm (BISH 776063).

Figure 23. Spermatangial sorus showing the formation of spermatangia. Scale bar = 50 μm (BISH 776063).

Figure 24. Surface view of spermatangial sorus. Scale bar = 50 μm (BISH 776063).

Figures 25 – 32. *Meredithia* [species name] sp. nov. habit photos.

Figure 25. Live holotype specimen (BISH xxxxxx), collected at Lisianski at 55 m. Scale bar = 2.5 cm.

Figure 26. Herbarium voucher of the holotype specimen (BISH 776061) cleaned of epiphytes. Scale bar = 2.5 cm.

Figure 27. Cross-section through margins of the blade. Scale bar = 100 μm (BISH xxxxxx).

Figure 28. Cross-section through stipe. Scale bar = 100 μm (BISH xxxxxx).

Figure 29. Cross-section through apex of the blade. Scale bar = 100 μm (BISH xxxxxx).

Figure 30. Cross-section through basal part of the blade. Scale bar = 100 μm (BISH xxxxxx).

Figure 31. Cross-section through basal part of the blade showing a carpogonial branch. Scale bar = 100 μm (BISH xxxxxx).

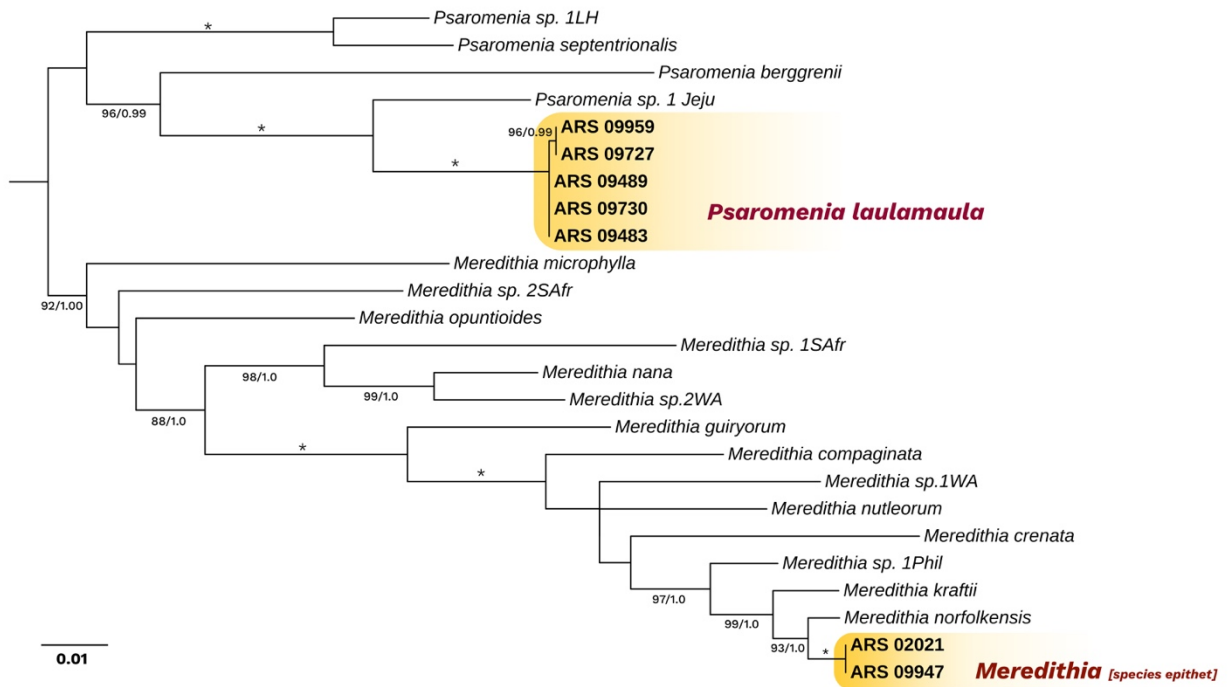


Figure 2. 1

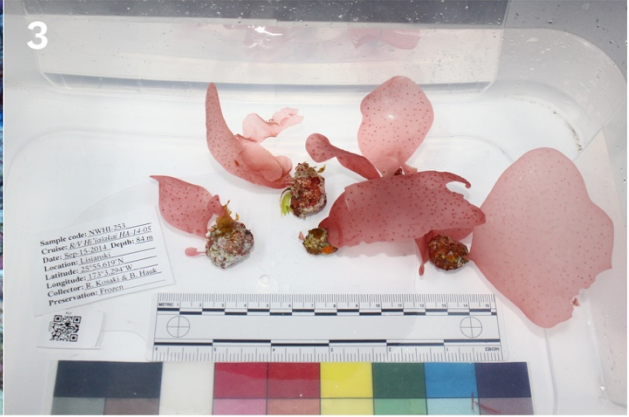
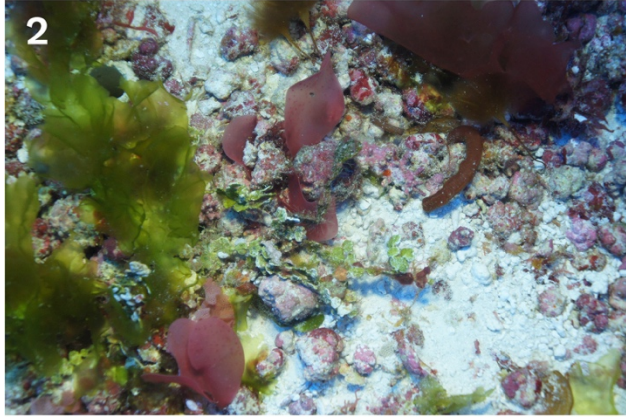


Figure 2. 2-5

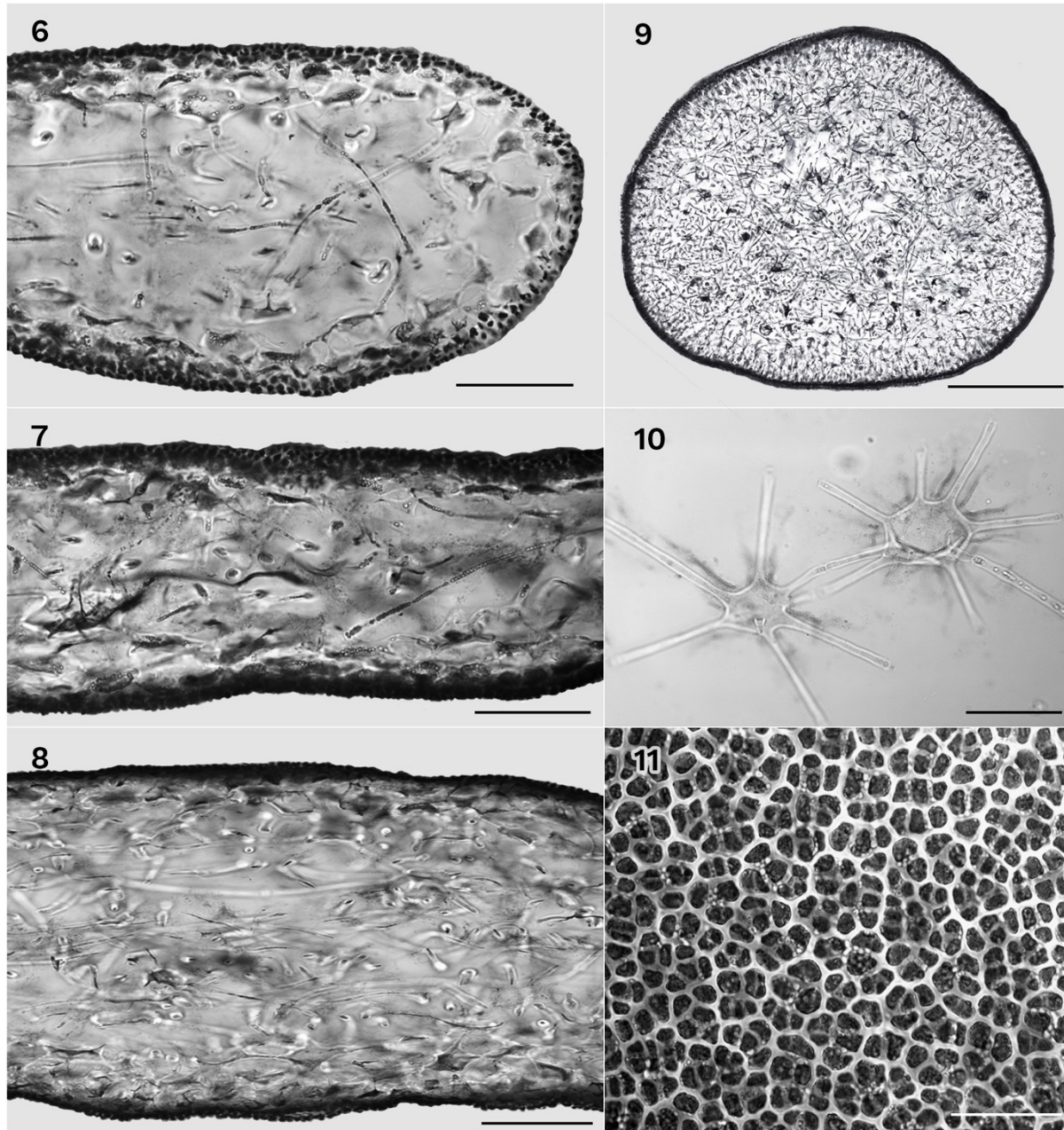


Figure 2. 6-11

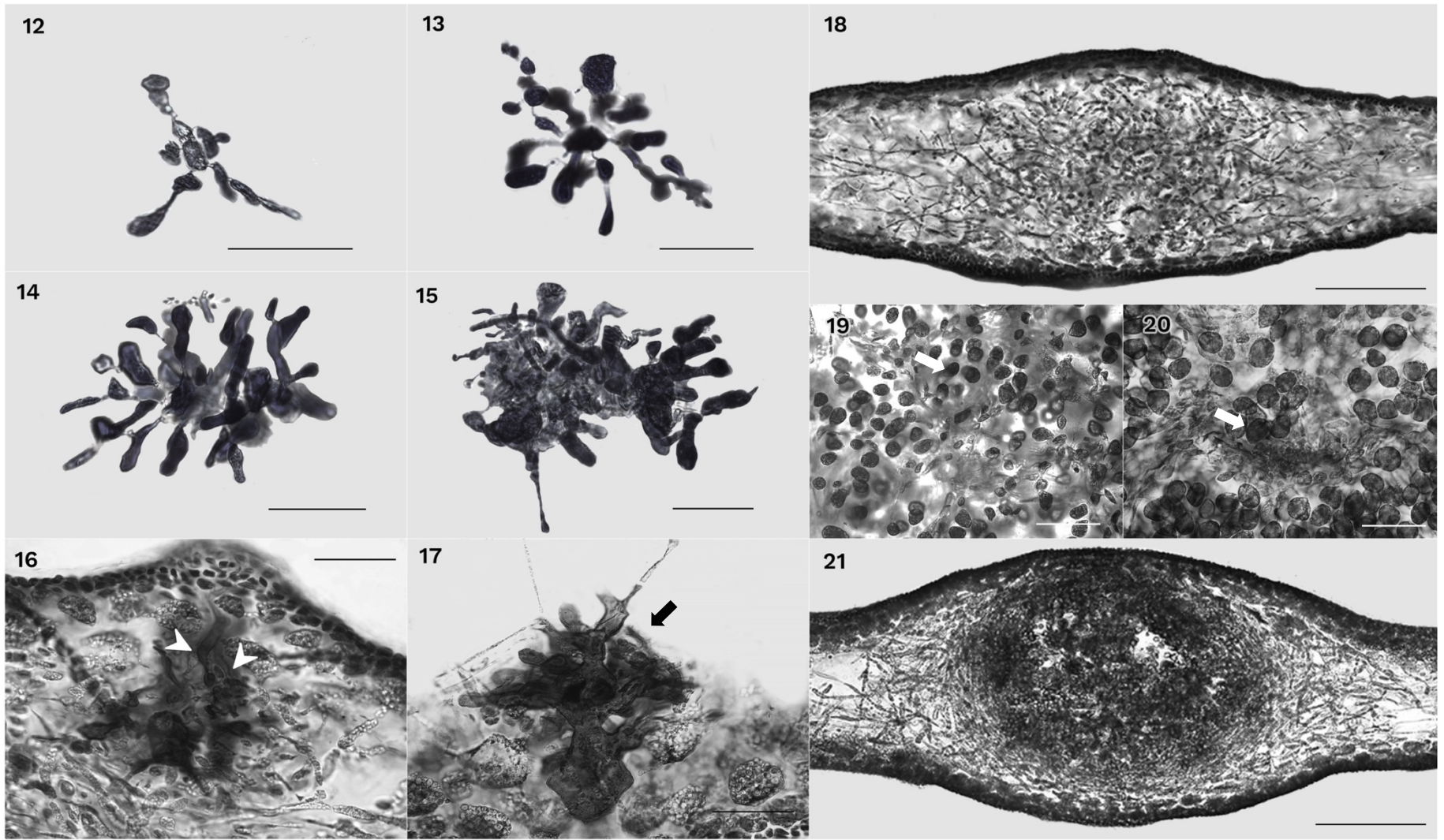


Figure 2. 12–21

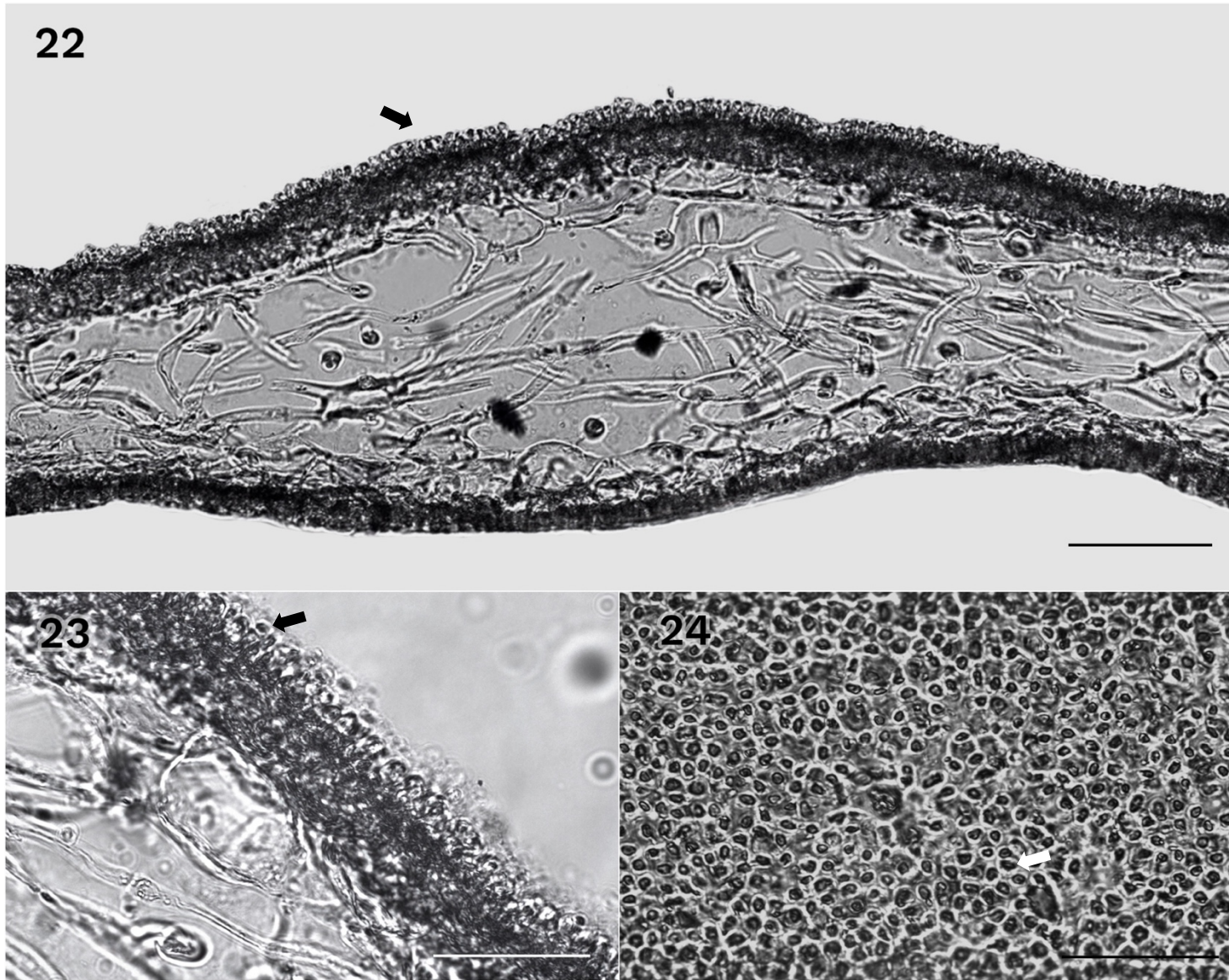


Figure 2. 22–24

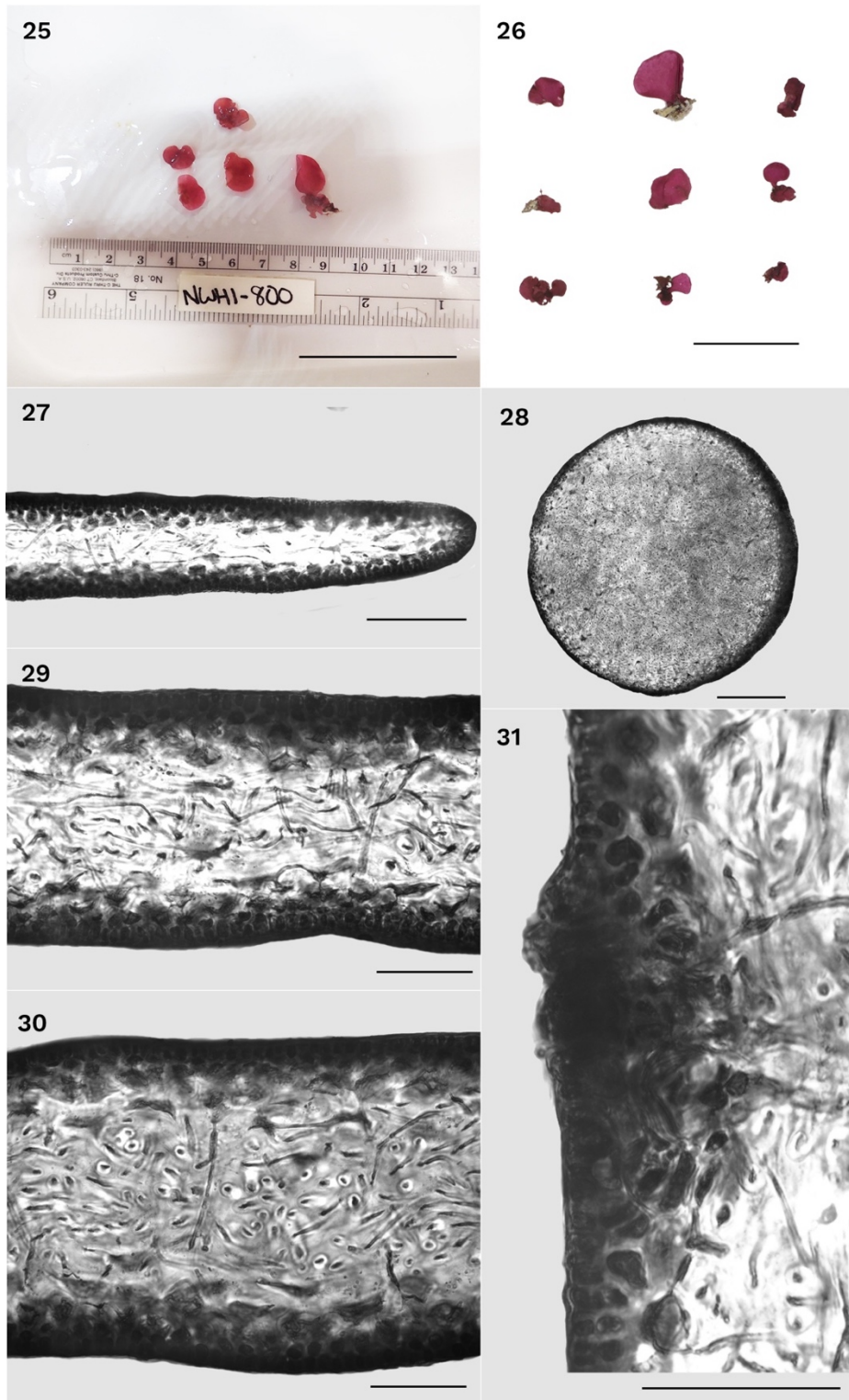


Figure 2. 25–31

Table 2. 1 List of species used in combined COI and rbcL analysis and accession numbers in GenBank (ND = no data).

Species	Voucher	rbcL	COI	Location	Depth (m)	Collector and Date	Coordinates
<i>Meredithia</i> sp. nov.	ARS 02021	ND	XXXXXXXXXX	Maui, Honolua Bay	0	Thomas Sauvage, 2/28/07	-
<i>Meredithia</i> sp. nov.	ARS 09947	XXXXXXXXXX	XXXXXXXXXX	Lisianski	55	Brian Hauk, 7/30/19	N 26° 1' 30.9972" W 174° 9' 24.984"
<i>Psaromenia laulamaula</i> sp. nov.	ARS 09958	XXXXXXXXXX	XXXXXXXXXX	Pearl and Hermes Atoll	17	H. Spalding and T. Williams	N 27° 54' 38.232" W 175° 54' 17.388"
<i>Psaromenia laulamaula</i> sp. nov.	ARS 09483	XXXXXXXXXX	XXXXXXXXXX	Lisianski	84	R. Kosaki & B. Hauk, 9/15/14	N 25° 56.619' W 173° 3.294'
<i>Psaromenia laulamaula</i> sp. nov.	ARS 09489	XXXXXXXXXX	ND	French Frigate Shoals	83	B. Hauk, 5/26/16	N 23° 38' 17.196" W 166° 13' 55.308"
<i>Psaromenia laulamaula</i> sp. nov.	ARS 09485	XXXXXXXXXX	XXXXXXXXXX	Lisianski	84	R. Pyle & D. Wagner, 9/15/14	N 25° 52.942' W 173° 57.727'
<i>Psaromenia laulamaula</i> sp. nov.	ARS 09727	XXXXXXXXXX	XXXXXXXXXX	Pearl and Hermes Atoll	85	R. Kosaki & B. Hauk, 9/18/14	N 27° 44.386' W 175° 57.692'
<i>Psaromenia laulamaula</i> sp. nov.	ARS 09730	XXXXXXXXXX	XXXXXXXXXX	Midway	84	D. Wagner, 9/19/15	N 28° 13' 7.788" W 177° 26' 56.148"
<i>Psaromenia laulamaula</i> sp. nov.	ARS 09732	XXXXXXXXXX	XXXXXXXXXX	Kure	88	R. Kosaki & B. Hauk, 9/21/15	N 28° 29' 24.252" W 178° 17' 28.068"
<i>Psaromenia laulamaula</i> sp. nov.	ARS 09959	ND	XXXXXXXXXX	Pearl and Hermes Atoll	94	R. Kosaki & S. Matadobra, 8/6/19	N 27° 44' 18.996" W 175° 57' 48.096"

Table 2. 2 Comparison of morphological characters of members of the genus
Psaromenia (J. Agardh) D'Archino, W.A.Nelson & Zuccarello (-o.n.d = observed but not described)

	<i>P. laulamaula</i> F. Cabrera, A.R. Sherwood & J.M. Huisman	<i>P. bergrenii</i> D'Archino, W.A.Nelson & Zuccarello ¹ - genotype	<i>P. septentrionalis</i> C.W. Schneider, Popolizio & G.W. Saunders ²
<i>Gross morphology</i>			
Blade shape	narrowly to broadly spatulate	lobed, lacinate to foliose	ligulate
Branching	non-branching	subdichotomously branched	subdichotomously branched
Margins	smooth to undulate	smooth to eroded margins	marginal proliferations
Blade dimensions	0.3–8 x 1–13 cm	up to 38 x 26 cm	up to 13 cm tall
Blade color	rose pink to magenta red	dark red to dark brown	rosy red
Blade thickness	220–300 µm	220–650 µm	300–500 µm thick
Stipe	always present, 0.2-1 x 1–5 mm	if present, 0.5 cm	absent
<i>Vegetative structures</i>			
Outer cortical cells	narrowly to broadly spatulate	polyhedral; 5–9 µm	polyhedral; 3.5–7.5 µm
Inner cortical cell layers	3–7 x 8–14 µm	2–4 cell layers	1–2 cell layers
Inner cortical cells	smooth to undulate	isodiametric; 28–32 µm	subglobose; 33.5–67.5 µm
medulla	filamentous	filamentous	filamentous
Stellate cells	rose pink to magenta red	200–300 µm	–o.n.d
<i>Reproductive structures</i>			
Carpogonial branch system	polycarpogonial	variable, mono-polycarpogonial branches	monocarpogonial
Cystocarp	~ 2.0 mm	1–1.5 mm	~ 1.3 mm
Carpospores	12–20 x 25–40 µm	18–21.5 µm	obpyriform to spherical; 9.5–17.0 µm
Spermatangia	3–7 x 5–10 µm	ovoid spermatangia; 2.6–4.3 x 4.4–7 µm	–o.n.d
Tetrasporangia	not observed	not observed	not observed
<i>Occurrence</i>			
Geographic distribution	NWHI	New Zealand	Bermuda
Depth distribution	84–94 m	3–25 m	90 m

References:
D'Archino et al., 2010¹
Schneider et al., 2019²

Table 2. 3 Comparison of morphological characters of members of the genus *Meredithia* J.Agardh (-o.n.d = observed but not described)

	<i>Meredithia</i> Cabrera, A.R. Sherwood & J.M. Huisman	<i>Meredithia microphylla</i> (J.Agardh) J.Agardh ⁵ - generitype	<i>Meredithia krafftii</i> G.W.Saunders & C.W.Schneider ²	<i>Meredithia opuntioides</i> G.W.Saunders & C.W.Schneider ²	<i>Meredithia pseudopeltata</i> G.W.Saunders & C.W.Schneider ²	<i>Meredithia pulchella</i> Ballantine, Ruiz & Norris ³
<i>Gross morphology</i>						
<i>Blade shape</i>	reniform, semi-peltate to foliose	semi-peltate, auriculate	prostrate	opuntoid	Spiraling oval to elongate	peltate, irregularly circular
<i>Branching pattern</i>	non branching	alternate to marginal branching	anastomosing	marginal branching with secondary stipes	marginal branching	marginal branching with secondary stipes
<i>Margins</i>	smooth	smooth to crenulated	undulate to crispate	smooth to irregular	broadly undulate	Irregular to crenulated
<i>Blade dimensions</i>	0.5–1.5 cm	–o.n.d	1–3 cm	2.0–3.5 cm	2.5–4.0 cm x 2.5 cm.	
<i>Blade color</i>	red magenta to dark fuchsia	rose-red, brick red to purplish	–o.n.d	–o.n.d	–o.n.d	3 cm tall
<i>Blade thickness</i>	55–370 µm	–o.n.d	200–275 µm	200–350 µm	250– 400 µm	110 µm
<i>Stipe</i>	0.02–0.4 mm x 1–2 mm	10–30 mm	1.5–2.0 mm x 1.5–2.0 mm	~1 mm x 1–2 mm	1.5 mm wide	–o.n.d
<i>Vegetative structures</i>						
<i>Outer cortical cells</i>	2.5–5.0 µm x 5.0–7.5 µm	3.5–6 µm	dimorphic; 3–6 µm x 5–8 µm	–o.n.d	obclavate; 2.5–3.5 µm x 5.0–10 µm	–o.n.d
<i>Inner cortical cell layers</i>	1–2 cell layers	2–4 cell layers	2–3 cell layers	2–3 cell layers	2–3 cell layers	2–3 cell layers
<i>Inner cortical cells</i>	polygonal to sub-rounded; 5–15 µm	3.5–6 µm	isodiametric	dimorphic; 3–5 µm x 5–9 µm	–n.d	–n.d
<i>medulla</i>	filamentous; 3– 6 µm	filamentous	filamentous	moderately filamentous; 8 µm wide	densely filamentous	filamentous; 2 µm
<i>stellate cells</i>	150–200 µm	200–300 µm	–o.n.d	–o.n.d	–o.n.d	–o.n.d
<i>Reproductive structures</i>						
<i>Carpogonial branch system</i>	not observed	monocarpogonial	not observed	monocarpogonial ?	not observed	not observed
<i>Cystocarp</i>	1 mm	2–3 mm	not observed	not observed	not observed	not described
<i>Carpospores</i>	not observed	7.5–15 µm	not observed	not observed	not observed	not observed
<i>Spermatangia</i>	not observed	oval to spherical; 1.5–3.5 µm	not observed	not observed	not observed	not observed
<i>Tetrasporangia</i>	not observed	–n.d.	not observed	not observed	not observed	not observed
<i>Occurrence</i>						
<i>Geographic distribution (*Type locality)</i>	NWHI* and MHI	British Isles*, Canary Isles, Bardsley Island, and Western Mediterranean	Lord Howe Island*, Australia	Southeastern Tasmania*, Australia	Rottneest Island*, Pt. Peron in Western Australia	Bermuda, Florida, Caribbean, Puerto Rico*
<i>Depth distribution</i>	~ 55 m	1.5–30 m	15 m	6 m	2.5 m	17– 70 m

References:

Womersley, 1994¹
 Schneider et al., 2014²
 Ballantine et al., 2015³
 Saunders et al., 2017⁴
 Womersley, 1973⁵

Table 2. 4 Cont. of Comparison of morphological characters of members of the genus *Meredithia* J.J. Agardh (-o.n.d = observed but not described)

	<i>Meredithia nana</i> J.Agardh ¹	<i>Meredithia norfolkensis</i> G.W.Saunders & C.W.Schneider ²	<i>Meredithia nutleorum</i> G.W.Saunders & C.W.Schneider ²	<i>Meredithia compaginata</i> G.W.Saunders ⁴	<i>Meredithia crenata</i> C.W.Schneider, G.W.Saunders & C.E.Lane ²	<i>Meredithia guiryorum</i> G.W.Saunders & C.W.Schneider ²
<i>Gross morphology</i>						
Blade shape	flattened	opuntoid	foliose	peltate	reniform to flattened	non-peltate
Branching	regularly alternately to subdichotomously branched	marginal branching	Rarely, marginal branching	marginal branching	Marginal branching with secondary stipes crenulated with finger-like projections	non-branching
Margins	smooth to slightly irregular	-o.n.d	loosely undulate to prostrate	smooth to irregularly crenulate		irregular
Blade length	5–15 cm	1–2 cm	1.0–2.5 cm	0.25–1.20 cm	1.5–6 cm	1.0–2.5 cm
Blade color	dark red	-o.n.d	-o.n.d	-o.n.d	-o.n.d	-o.n.d
Blade thickness	200–450 µm	200–300 µm	200–300 µm	140–210 µm	300 µm	200–270 µm
Stipe	0–o.n.d	<1 mm x 2–4 mm	~1 mm x 1–2 mm	0.5–0.8 mm x 0.5–0.8 mm	-o.n.d	~0.5–1.0 mm x 1–2 mm
<i>Vegetative structures</i>						
Outer cortical cells	ovoid, 1.5–2 µm	2.5–5.0 µm x 5.0–7.5 µm	3–6 µm x 3–8 µm	4–8 µm	-n.d.	2.5–5.0 µm x 5.0–7.5 µm
Inner cortical cell layers	1–2 cell layers	2–3 cell layers	1–2 cell layers	2–3 cell layers	4–5 cell layers	2–3 cell layers
Inner cortical cells medulla	-o.n.d	-o.n.d	-o.n.d	7–10 µm x 5–7 µm	-o.n.d	-o.n.d
	moderately dense filamentous; 2–6 µm	moderately filamentous	Moderately filamentous	Rectilinear filaments; 3–6 µm	finely filamentous; 1.5 µm	-o.n.d
Stellate cells	150–200 µm	-o.n.d	-o.n.d	-o.n.d	-o.n.d	-o.n.d
<i>Reproductive structures</i>						
Carpogonial branch system	not identified	not observed	not observed	polycarpogonial	not observed	not observed
Cystocarp	1–2 mm	not observed	not observed	not observed	400 µm	not observed
Carpospores	10–15 µm	not observed	not observed	not observed	3 µm	not observed
Spermatangia	-o.n.d	not observed	not observed	2.5 µm	2 µm	not observed
Tetrasporangia	25–38 µm	not observed	not observed	not observed	not observed	not observed
<i>Occurrence</i>						
Type locality	Port Phillip Head*, Australia	Norfolk Island*, Australia	Fish Bowl, Nepean Island, Norfolk* Island, Australia	Cocos (Keeling) Islands*, Australia	Bermuda*, Western Atlantic	Lord Howe Island, Australia
Depth distribution		12 m	10 m	5 m	2–6 m	
References						

Supplementary Data

Table S1. List of species used in combined COI and rbcL analysis and accession numbers in GenBank.

Taxon	Voucher	rbcL	COI	Location	Collector and Date	Coordinates
<i>Meredithia compaginata</i> G.W.Saunders	GWS037856	KX783123	KX808100	Australia, Cocos (Keeling Islands)	G.W. Saunders & K. Dixon, 12/09/13	S 12°09'14.4" E 96°52'48.0"
<i>Meredithia crenata</i> G.W.Saunders, C.W.Schneider & C.E.Lane	GWS001247	KC157632	KC157617	Bermuda: Walsingham Pond	G. Saunders, 11/12/01	N 32°20'52.8" W 64°42'36.0"
<i>Meredithia guiryorum</i> G.W.Saunders & C.W.Schneider	GWS023411	KC157628	KC157607	Australia: New South Wales, Algae Hole North, Lord Howe	G.W. Saunders, K. Dixon & R. Withall; 11/21/10	S 31°31'30.0" E 159°03'36.0"
<i>Meredithia kraftii</i> G.W.Saunders & C.W.Schneider	GWS023522	KC157630	KC157613	Australia: New South Wales, Algae Hole North, Lord Howe I.	G.W. Saunders, K. Dixon & R. Withall; 11/24/10	S 31°31'30.0" E 159°03'36.0"
<i>Meredithia microphylla</i> J. Agardh (J. Agardh)	LLG1538	KC157626	KC157605	France: Brittany, Ille-et-Vilaine, Les Buharats, Saint-Malo	Line Le Gall, Jose Maria Utge, Florence Rousseau, 7/14/08	N 48°40'01.9" W 2°07'05.0"
<i>Meredithia nana</i> J. Agardh	GWS028599	KF280977	KF280927	Australia: South Australia, Shell Bay, Yorke Peninsula	K. Dixon, 11/23/11	S 35°11'31.2" E 136°52'19.2"
<i>Meredithia norfolkensis</i> G.W.Saunders & C.W.Schneider	GWS032281	KF280971	KF280922	Australia: Norfolk Island, Simon's Water Flats	G.W. Saunders & K. Dixon; 11/30/12	S 29°01'19.2" E 167°59'09.6"
<i>Meredithia nutleorum</i> G.W.Saunders & C.W.Schneider	GWS032241	KF280969	KF280921	Australia: Norfolk Island, Fish Bowl, Nepean Island	G.W. Saunders & K. Dixon; 11/29/12	S 29°04'19.2" E 167°58'01.2"
<i>Meredithia opuntioides</i> G.W.Saunders & C.W.Schneider	GWS015287	KC157635	HM917677	Australia: Tasmania, Burying Ground Point	G.W. Saunders & K. Dixon; 1/21/10	S 43°26'24.0" E 146°59'20.4"
<i>Meredithia pseudopeltata</i> Ballantine, Ruiz & Norris	GWS025546	KF280984	KF280937	Australia: Western Australia, Ricey Beach, Rottneest I.	G.W. Saunders & K. Dixon; 11/17/10	S 32°00'00.0" E 115°29'24.0"
<i>Meredithia</i> sp.1SAfr	GWS036510	KX783095	KX808068	South Africa: KwaZulu-Natal, Aliwal Shoal, inner reef	K. Dixon & J. Ferreira Costa, 12/14/14	S 30°15'00.0" E 30°49'01.2"
<i>Meredithia</i> sp.1WA	GWS025460	KC157634	KC157619	Australia: Western Australia, Pt. Peron	G.W. Saunders & K. Dixon, 11/13/10	S 32°16'15.6" E 115°40'55.2"
<i>Meredithia</i> sp.2SAfr	GWS036327	KX783068	KX808035	South Africa: Western Cape, Oudekraal	K. Dixon & J. Ferreira Costa, 11/22/14	S 33°59'13.2" E 18°20'52.8"
<i>Meredithia</i> sp.2WA	GWS025159	KC157633	KC157618	Australia: Western Australia, Canal Rocks	G.W. Saunders & K. Dixon, 11/11/10	S 33°40'08.4" E 114°59'42.0"
<i>Psaromenia berggrenii</i> D'Archino, W.A.Nelson & Zuccarello D'Archino, W.A.Nelson & Zuccarello	GGCZ-2009a	GQ376532	ND	New Zealand: Bay of Islands, Moturoa, Rangiatea	-	-
<i>Psaromenia septentrionalis</i>	BDA1993	MH777607	MH777603	Bermuda: North northeast ledge	C.W. Schneider, C.E. Lane, 7/1/16	N 32°28'58.8" W 64°35'06.0"

<i>Psaromenia sp. IJeju</i>	GWS018239	KC157636	KC157623	South Korea: Cheju-do, Rocky Reef at Lighthouse 'Point' Piyangdo Island	G.W. Saunders & H-G. Choi, 5/20/10	N 33°25'12.0" E 126°13'26.4"
<i>Psaromenia sp. 1LH</i>	GWS002058	KC157627	HM915984	Australia: New South Wales, Leeward side of Balls Pyramid, Lord Howe I.	G.W. Saunders, 2/1/04	S 31°43'37.2" E 159°15'28.8"

To: Alison Sherwood, Interim Associate Dean, College of Natural Sciences, UH Mānoa
Feresa Cabrera, MS Graduate Student, Botany Department, UH Mānoa
CC: Randy Kosaki, Research Coordinator, NOAA PMNM
From: Kalani Quioco, Native Hawaiian Program Specialist, PMNM
Re: Naming *Psaromenia* species
Date: 29 October, 2019 / Hoaka: Welehu

laulama‘ula (lau-lama-‘ula)

My naming methodology derives partly from the Hawaiian creation chant, Kumulipo. During much of the Kumulipo, creation is articulated through a series of births which, for part of the chant, occurs in pairs with one living thing birthed in the sea and the other on land.

Common names for plants, fish, birds, and other creatures have various naming conventions, but some naming conventions are unknown. Some naming conventions are based on physiology, function, the sound it makes, similarity to something animate or inanimate, or some other cultural convention.

One interpretation of lau-lama-‘ula is the “red ebony tree leaf”. The lama (ebony, *Diospyros sandwicensis*) tree is a Hawai‘i endemic found in dry and mesic forests from 15 to 4,000 feet on all islands, except Ni‘ihau and Kaho‘olawe. The young leaves can be red, magenta, pink, and orange.

The following definitions provide additional insight into the meaning of the name.

lau

1. nvi. Leaf, frond, leaflet, greens; to leaf out. ho‘o.lau To grow leaves; to leaf out. (PPN lau.)
2. n. Sheet; surface; blade, as of grass.
3. num. To be much, many; very many, numerous; four hundred.
4. n. Pattern, as for quilts; design; print of a cloth.
5. n. Tip, as of the tongue; top (probably related to wēlau and ‘ēlau, tip).

lama n.

1. All endemic kinds of ebony (*Diospyros*, synonym Maba), hardwood trees with small flowers and fruits. (Neal 674.) Also ēlama. (PNP lama.)
2. Torch, light, lamp. Lama wood was used in medicine and placed in hula altars because its name suggested enlightenment.

lau-lama n. Design on a Ni‘ihau mat. *Lit.*, many torches or lama tree leaves.

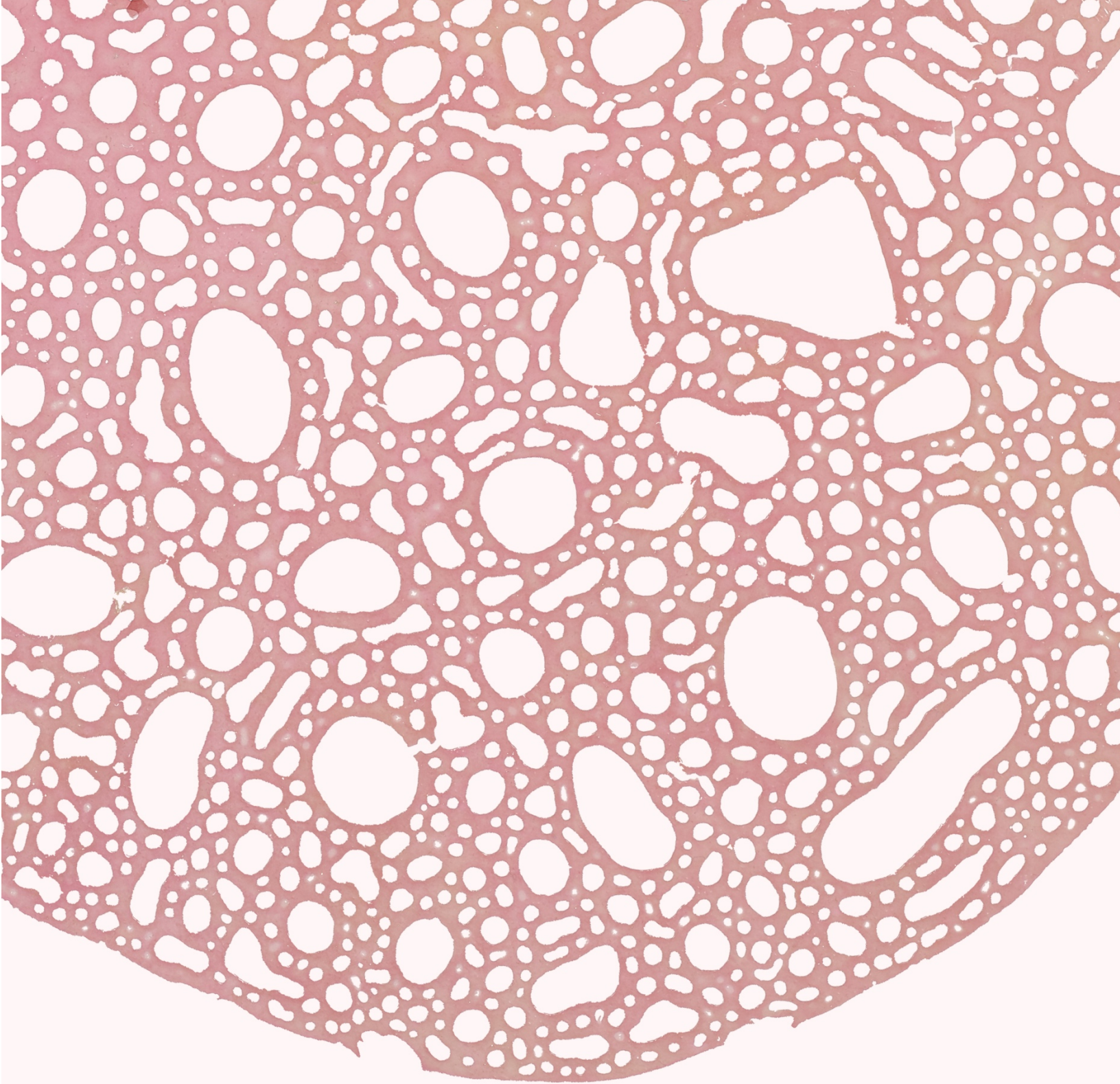
‘ula

1. nvi. Red, scarlet; brown, as skin of Hawaiians; to appear red.
2. nvs. Sacred; sacredness; regal, royal (probably so called because red was a sacred color).



Figure . Left. Young leaves of endemic lama tree. Right. Hawaiian *Psaromenia* species
Reflection

The color of the limu is most striking to me. The Hawaiian word for magenta, *'ulamāku'e*, made me think of a Hawai'i endemic fern, *māku'e*, that has a similar shape as some of the limu specimens. The name *'ulamāku'e* did not feel quite right because of various reasons. The color red is not associated to the *māku'e* fern and even though it followed the Kumulipo pairing concept, the play on Hawaiian words wasn't quite fitting. The single blade-like body also seemed to be an important feature. So I took the word *lā'ulamāku'e* which means "magenta leaf" and I removed all the diacritical marks to see if other names would arise. This is an appropriate method because related sounds in Hawaiian language have related meanings. English language is similar in these regards. For instance, in Hawaiian language *'ai* means "to eat" or "to conquer" and *ai* means "to have sex" and in Hawaiian thought these are all related concepts. Therefore, *lā'ulamāku'e* without the diacriticals became *laulamakue* which provided other references that eventually led to *laulama'ula*. I confirmed the name through various traditional and customary practices.



CHAPTER 3

*Living things don't all require light in the same degree.
Some of us make our own light.*

Louise Glück, *Lamium*

3.1 Conclusions

Numerous studies on the Kallymeniaceae based on comprehensive molecular and morphological datasets conducted by Saunders and colleagues have greatly furthered our understanding of the phylogeny and evolution of the family, particularly at the family and genus level (2017). We are now in a position to integrate this knowledge with an extensive study of comparative morphology of Hawaiian material and address the shortcomings in the representation of Hawaiian material in most of the earlier taxonomic studies.

This research initially pursued the following questions to provide insight into the expansive Hawaiian mesophotic red blades as members of the marine flora, and are answered in the context of the results of Chapter 2: Diversity of Kallymeniaceae (Gigartinales, Rhodophyta) associated to the mesophotic reefs of Hawai'i with description of *Psaromenia laulamaula* sp. nov. and *Meredithia* [*species epithet*] sp. nov.

— Do these species contain cryptic diversity?

Yes. The discovery of the two novel species in Hawaii: *Psaromenia laulamaula* and *Meredithia* sp. represents a typical case of species being overlooked because of their relatively small size and highly polymorphic characters that are often misidentified in field and existing research collections. While species differences at the extremes of morphological variation within the clade can be very clear e.g. *Psaromenia laulamaula* vs. all other *Psaromenia* species, some species are very difficult to separate, e.g. Hawaiian *Meredithia* vs. all other *Meredithia* species, due to subtle and uninformative morphological variation within the genus needing genetic data to confirm their taxonomic placement.

- Are there species shared among shallow and deep communities?

Species overlap is found to be variable. While *Meredithia* in Hawai‘i are known from both shallow and mesophotic habitats (*M.* [species epithet]), *Psaromenia laulamaula* are collected only from mesophotic depths.

- Are red blade species shared among biogeographic realms in the Pacific and elsewhere?

Yes. *Psaromenia laulamaula* sp. and *Meredithia* sp. have congeners in Atlantic and Indo-Pacific regions, rendering both genera pan-oceanic.

Nevertheless, further sampling in deep waters of Hawai‘i and scouring of unidentified existing research collection of stipitate red blades would be necessary to have a complete picture of the geographical distribution of this clade.

Although our work of the Hawaiian red blades remains inconclusive with respect to the DRRH, 64% of the Hawaiian putative endemic flora associated with MCEs is restricted to mesophotic depths with the remaining 36% shared in both shallow and mesophotic communities. On the other hand, emerging biogeographic patterns are complex as *Psaromenia* and *Meredithia* species are not clustered by biogeographic region, suggesting speciation within these genera may be due to alternative biogeographic patterns or dispersal routes (McDermid & Abbott, 2006) or associated with paleoclimatic vicariance, a pattern proposed for many other members of the Family Kallymeniaceae, e.g. *Callophyllis* Kützing (Bringloe and Saunders, 2019).

Hawaiian mesophotic reefs are rich platforms of marine biodiversity in the tropics with extraordinarily high rates of endemism (Spalding, 2012). Yet these MCEs are also at the forefront of ecosystem transitions, with the effects of climate change manifesting themselves in a

particularly pronounced manner through repetitive, global coral bleaching and ocean acidification events (Smith et al., 2019). This research will facilitate future studies on red blades by allowing more robust identification and contributing to more accurate geographic distributions, and enabling connectivity assessments and guide conservation efforts to protect these poorly understood MCEs.

Chronicling the world's biodiversity has perhaps never been more timely. Added to this is the enormous, and ever-increasing, volume of data and information being produced in ecology and evolution that requires a solid taxonomic system within which to reside if the data are to effectively contribute to the cumulative knowledge of the planet's marine biodiversity. Overall, this thesis presents a detailed, region-specific revision of the taxonomy, tripling the known generic diversity of the family Kallymeniaceae in Hawai'i, and should be seen as a small contribution to the task of ensuring that species remain anchored within a framework that represents our best current understanding of the world's biodiversity.

3.2 Directions for future research

Monographic treatments such as this one are a critical step in clarification of the taxonomy of the Family Kallymeniceae in Hawai'i, as they provide a comprehensive synopsis of current knowledge as well as a launching pad for the investigation of uncertainties that are highlighted in the treatments. We are far from understanding the true genetic diversity and biogeography of seaweeds in the Hawaiian archipelago, especially in the foliose reds. Continued exploratory work on both shallow reef and MCEs of Hawai'i is poised to redefine our understanding of algal diversity in the Archipelago, i.e., its community structure and vulnerability to changes in future, and has far-reaching implications for our understanding of biogeographic patterns in the Pacific. Additional efforts on the following are needed to achieve this:

- Generating a broader framework for understanding phylogenetic relationships of Hawaiian Kallymeniaceae in a wider context and describing unrecognized taxa when detected.
- Extensiveness of diversity among members of the Family Kallymeniaceae invites reconsideration of their center of origin which can be tested through rigorous dating with denser taxon sampling and reliable calibration points in a time-calibrated phylogeny. This will also provide insights to elucidate evolution and species divergences that encompass all recognized kallymeniacean taxa.
- Examining factors that favor the diversity and speciation of these simple blades with algal hologenomics and structural variation through microsatellites or other variable loci could be explored using high throughput sequencing.

References

- Bringloe, T. T., & Saunders, G. W. (2019). Trans-Arctic speciation of Florideophyceae (Rhodophyta) since the opening of the Bering Strait, with consideration of the “species pump” hypothesis. *Journal of Biogeography*, **46**:694–705.
- McDermid, K. J., & Abbott, I. A. (2006). Deep subtidal marine plants from the Northwestern Hawaiian Islands: new perspectives on biogeography. *Atoll Research Bulletin*, **543**:525–532.
- Saunders, G. W., Huisman, J. M., Vergés, A., Kraft, G. T., & Gall, L. Le. (2017). Phylogenetic analyses support recognition of ten new genera, ten new species and 16 new combinations in the Family Kallymeniaceae (Gigartinales, Rhodophyta). *Cryptogamie, Algologie*, **38**:79–132.
- Spalding, H.L. (2012). *Ecology of mesophotic macroalgae and Halimeda kanaloana meadows in the main Hawaiian Islands*. Ph.D. Dissertation, University of Hawai'i, Honolulu.
- Smith, T. B., Holstein, D. M., & Ennis, R. S. (2019). Disturbance in mesophotic coral ecosystems and linkages to conservation and management. In *Mesophotic Coral Ecosystems* (911–929). Springer International Publishing, Cham.

