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Uncovering diversity in the mesophotic zone of Hawaiʻi: species new to science in the genera *Halopeltis* and *Leptofauchea* (Rhodymeniales, Rhodophyta)

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Always, I am grateful for the land and water and to those fighting for their protection.

Abstract

Macroalgal surveys in the mesophotic zone shed light on undocumented diversity in the Hawaiian Islands. This study highlights more newfound Rhodophyta diversity through the uncovering of multiple species in the genus *Leptofauchea* and a species new to science in the genus *Halopeltis*. Both genera belong to the diverse order, Rhodymeniales. Neither *Halopeltis* nor *Leptofauchea* have been previously documented from the Hawaiian Islands prior to this study and were only observed in the mesophotic zone. Samples were gathered at depths of 72 – 104 m, with most samples collected off the west coast of Molokaʻi. One *Leptofauchea* sample included in this analysis was collected from the Papahānaumokuākea Marine National Monument, demonstrating the large range this genus has within the Hawaiian mesophotic zone. Species identification was performed using the COI-5P (mitochondrial), *rbcL* (plastid), and LSU (nuclear) molecular markers, in combination with morphological and anatomical characterization. Sequencing of multiple markers aided in phylogenetic reconstruction of these Hawaiian samples in relation to currently described species. Morphological comparisons complemented the molecular analyses and were used to describe two species total. All Hawaiian species explored are more closely related to species in different localities than they are to each other, suggesting multiple colonizations. Links between Hawaiian, Asian, and Australian species were found. This study contributes to the baseline of knowledge of the red algal flora found in the mesophotic zone.

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Chapter 1

Background and Introduction

Taxonomy

The Rhodymeniales is a monophyletic order known for having a complex taxonomic history with many unresolved relationships that have had recent clarification (Saunders et al. 1999; Le Gall et al. 2008; Filloramo & Saunders 2016). Currently there are unresolved relationships within higher red algal taxa and among related orders (Withall & Saunders 2007; Le Gall & Saunders 2007; Verbruggen et al. 2010). The Rhodymeniales encompasses morphologically diverse taxa with more than 400 current species (Guiry & Guiry 2021). This order is distinguished from other florideophyte taxa based on its distinct procarpy, with other defining characteristics including a medulla with large cells, small pigmented cells in the cortex, tetrasporangia divided either cruciately or tetrahedrally, and by having 3-4-celled carpogonial branches.

Described by Schmitz in 1889, this order has been refined multiple times, fluctuating in the numbers of families included since its description (Schmitz 1889; Oltmanns 1904; Sjöstedt 1926; Bliding 1928; Sparling 1957; Saunders et al. 1999; Le Gall et al 2008). The six families that currently make up this order are: Champiaceae, Faucheaceae, Fryeellaceae, Hymenocladiaceae, Lomentariaceae, and Rhodymeniaceae, which are all monophyletic (Le Gall and Saunders 2007). Each familial designation is based on tetrasporangial ontogeny (Le Gall et al. 2008; Filloramo & Saunders 2016), and differences in reproductive characters. Familial relationships within the Rhodymeniales were recently clarified by Filloramo and Saunders (2016). Fryeellaceae was found to be sister to Faucheaceae and the Lomentariaceae, while the Rhodymeniaceae is more closely related to Champiaceae and Hymenocladiaceae (Filloramo and Saunders 2016). The two genera explored in this thesis are classified within the families Faucheaceae and the Rhodymeniaceae.

Faucheaceae was described in 1999 by Saunders et al. with *Fauchea* as the type genus. The defining features of the family are three-celled carpogonial branches, terminal cruciately divided tetrasporangia, and the presence of well-developed *tela arachnoidea* (Saunders et al. 1999; Dalen & Saunders 2007). The family currently contains 58 species, and it is the third largest in the Rhodymeniales (Guiry & Guiry 2021). Three species new to science, one of which

is described herein, were collected from Hawai‘i and belong to the faucheacean genus *Leptofauchea*. This genus aligns closely to the small genus *Webervanbossea*, characterized by compound branching, an erect thallus, and the presence of constrictions (De Toni 1936). Morphological distinctions between *Webervanbossea* and *Leptofauchea* are most easily seen through the difference in branching. *Leptofauchea* has dichotomous branching, 2-3 cell layers in the medulla, large thin-walled medullary cells, small cortical cells, 3-celled carpogonial branches, and well-defined *tela arachnoidea* (Kylin 1931; Dalen & Saunders 2007).
Within the Rhodymeniales, the family Rhodymeniaceae is the largest with 193 species currently recognized species listed on AlgaeBase (Guiry & Guiry 2021). The Rhodymeniaceae is characterized by 3-4 celled carpogonial branches, erect or decumbent thallus construction, cruciate or tetrahedrally divided tetrasporangia, large medullary cells, and by having small pigmented cells in the cortex (Kamiya et al. 2017). One species new to science from the Rhodymeniaceae is described in Chapter 2 belonging to the genus *Halopeltis*, which was resurrected in 2010 (Saunders & McDonald 2010). *Halopeltis* has had an amended description with its resurrection and is described as having large medullary cells typically in two rows, small intercalating cells, sori produced only on one or both thallus surfaces, protuberant cystocarps, and tetrasporangia generated through swelling of intercalary and terminal cortical cells (Saunders & McDonald 2010).

Halopeltis and *Leptofauchea*, while belonging to different families, have comparable gross morphologies that appear similar to an untrained eye (Figure 1.1). Appearing very similar in color, branching, lack of apparent holdfast in some species, large medullary cells, and small cortical cells, these two genera share many similarities. The most easily distinguishable character that separates these two are the lack of intercalating cells in *Leptofauchea*, a character which is found in *Halopeltis*. In addition, the presence of a *tela arachnoidea* is listed as a character for Faucheaceae and *Leptofauchea*, but not for *Halopeltis*.

Studying these genera in the Hawaiian Islands will provide a more accurate representation of species richness and diversity contributing to previous macroalgal systematics work conducted by Isabella Aiona Abbott who documented many species of Rhodophyta in her book *Marine Red Algae of the Hawaiian Islands* (1999); since this book was published there have been more additions to the documented algal flora of Hawai‘i. In Abbott’s book she included 19 species from the Rhodymeniales (Abbott 1999), and the two species described herein contribute to the Rhodymeniales diversity known from the Hawaiian Islands.

Figure 1.1. Herbarium specimens analyzed in this study belonging to the genera *Leptofauchea* and *Halopeltis* within the Rhodymeniales displaying similar branching patterns and coloring. Scale bar = 5 cm.

Taxonomic Approaches

Historically, taxonomic studies were predominantly conducted by studying morphological characters. For classifying Rhodophyta, distinctions between groups were primarily based on differences in reproductive characters (Kylin, 1928; Figure 1.2). Morphology can be a useful tool for documenting differences between and within species. In addition, since

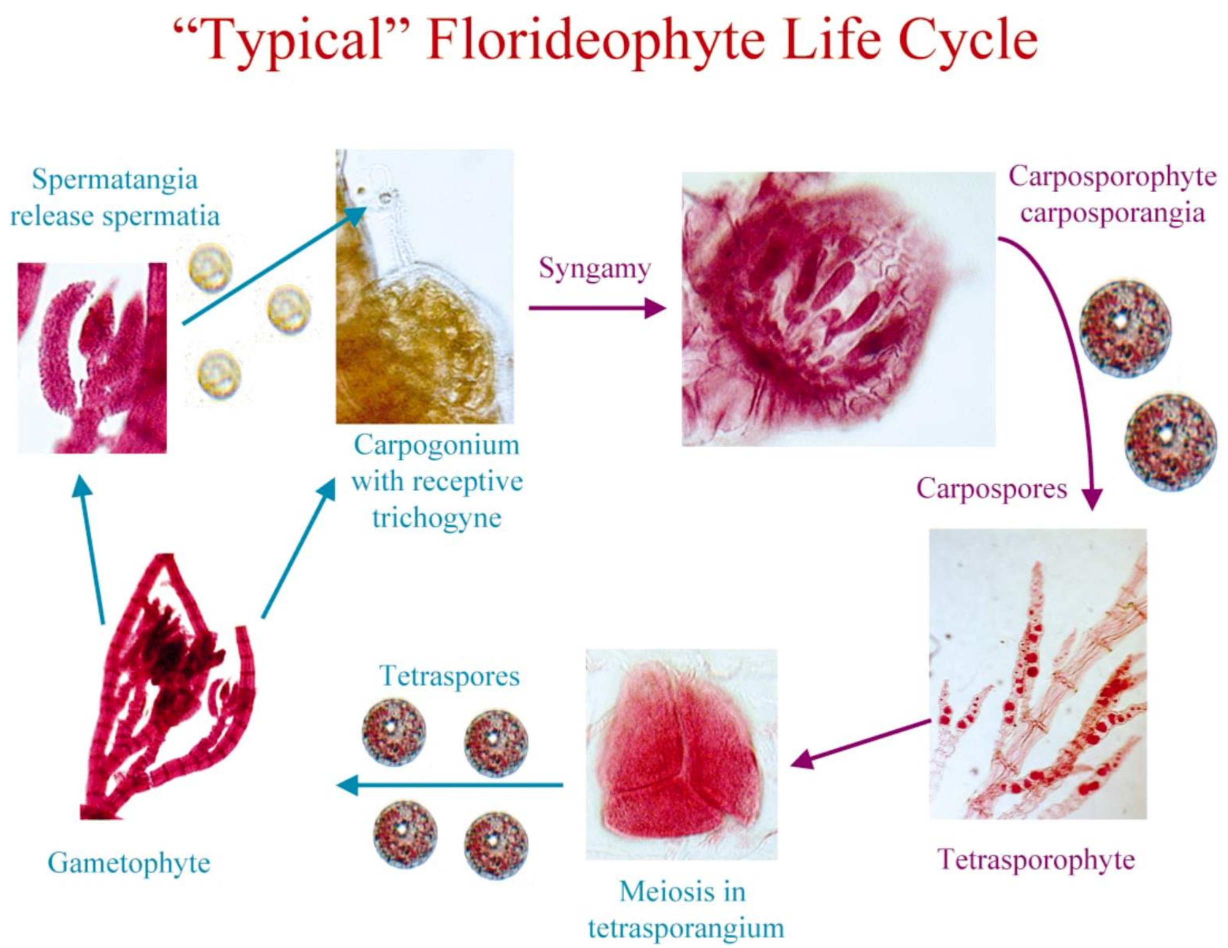

Figure 1.2. The life cycle of a typical Florideophyte distinguishes morphological characters that are typically used for species identifications. Blue labels depict haploid stages and purple labels identify diploid life stages; plants are stained for easy visualization. From Saunders and Hommersand (2004).

morphology was heavily relied upon before the advent of new technologies, there is a large repository of information that can be added to and compared against. However, relying on morphology alone can also pose problems. Morphology can vary based on life history traits, phenotypic plasticity, convergence, and the traits present in an individual are not always representative of the order or family to which it belongs (Saunders 2005). The main problem with this is that morphological classifications can be misleading, and molecular methods relieve

Table 1.1 Morphological characters analyzed for *Leptofauchea* species in this study. Includes general gross morphology (overall), vegetative anatomy, and characteristic components of the reproductive anatomy.

Overall	Vegative Anatomy	Reproductive Anatomy
Branching pattern	Shape of medullary cells	Tetrasporangia shape
Branch color	Size of medullary cells	Tetrasporangia size
Blade length	Number of medullar cell layers	Tetraspore size
Blade width	Size of cortex cells	Tetraspore division
Blade thickness	Number of cortex layers	Tetraspore locality
Presence of stipe	Thickness of blade	Cell number of paraphyses
Apex shape		Tetraspore development
Shape of budding branches		
Blade constriction width		

Table 1.2 Morphological characters analyzed for *Halopeltis* species in this study. Includes general gross morphology (overall), vegetative anatomy, and characteristic components of the reproductive anatomy.

Overall	Vegative Anatomy	Reproductive Anatomy
Branching pattern	Shape of medullary cells	Tetrasporangia shape
Branch color	Size of medullary cells	Tetrasporangia size
Blade length	Number of medullar cell layers	Tetraspore size
Blade width	Size of cortex cells	Tetraspore division
Blade thickness	Number of cortex layers	Tetraspore locality
Presence of stipe	Thickness of blade	Cell number of paraphyses
Apex shape	Intercalacating cell size	Tetraspore development
Shape of budding branches		
Blade constriction width		

problems associated with relying on morphology alone. Using both molecular and morphological observations is a widely used practice for characterizing species and phylogenetic relationships (e.g., Gavio and Fredericq 2005; Dalen & Saunders, 2007; Rodríguez-Prieto & De Clerck 2009; Saunders & McDonald 2010; Suzuki et al. 2010; Schneider et al. 2012; Filloramo & Saunders 2015; Paiano et al. 2020; Cabrera et al. 2021). Morphological traits that are assessed in this study are determined from characters reported in original species descriptions for *Leptofauchea* and *Halopeltis*. These characters are listed below in Table 1.1 and Table 1.2, respectively.

Combining molecular sequence data with morphological data can illuminate species relationships, including both types of data compensates for the possibility of having weak morphological descriptions or a having a lack of molecular data (Will et al. 2005). Species identification through sequencing has been made simpler through the advent of molecular barcoding. DNA barcoding uses short sections of DNA that are relatively conserved to identify species; comparing multiple barcode sequences allows for the determination of how different or similar individuals are. Barcoding took off with the publication of two papers by Herbert et al. (2003a,b) focusing on the mitochondrial marker cytochrome oxidase subunit I (*cox1*), and this marker was refined for macroalgal applications shortly thereafter (Saunders 2005). Other non-barcode markers are also used for molecular assessments of macroalgae including the 18S ribosomal DNA (Bird et al. 1992a,b) and ribulose-1, 5-biphosphate carboxylase large subunit (*rbc*L) markers (Freshwater & Rueness 1994; Hommersand et al. 1994).

Using DNA barcoding as a starting point, multiple markers can then be sequenced to infer phylogenetic relationships among algal taxa (Saunders 2009; Walker et al. 2009; Le Gall & Saunders 2010). For both genera identified in this study four molecular markers are used: the mitochondrial marker COI-5P, plastid marker *rbc*L, nuclear ribosomal marker large subunit ribosomal DNA (LSU), and the Universal Plastid Amplicon (UPA). UPA is a good marker to use as a starting point for documenting diversity (Sherwood & Presting 2007). Using this marker is a viable way to narrow down taxonomic possibilities for the sample in question and can be used to fine tune following maker and primer choices (Sherwood et al. 2010; Saunders & Moore 2013). *Leptofauchea* and *Halopeltis* studies both use LSU, UPA, COI, and *rbc*L markers to document newly uncovered diversity, therefore there is already a database of sequences available for these markers. Utilizing this database of representative sequence for both genera and using previously created phylogenetic trees as a reference point (Schneider et al. 2012; Filloramo & Saunders 2015), it is possible to reconstruct phylogenies with the addition of the newly documented species from Hawaiian waters.

Mesophotic Coral Ecosystems (MCEs) and Endemism

Mesophotic Coral Ecosystems (MCEs) are found all over the world, but only recently have they been broadly accessible for research with the advent of new diving technology, remotely operated vehicles, and underwater submersibles that enable researchers to reach these depths (Lehnert & Fischer 1999). More than 70% of all MCE studies have been published in the past seven years, despite about 80% of coral reef habitat residing in this zone, highlighting a need to study these systems (Loya et al. 2019). The depth range for MCEs differs based on locality, but typically they are found at 30 – 150 m in tropical and subtropical areas (Lesser et al. 2009; Baker et al. 2016; Loya et al. 2019), with the lower depth range determined by the ~1% irradiance level (Lesser et al. 2009). Occurrence of these ecosystems is dependent on depth, light, temperature, substrate type, and substrate features (Lesser et al. 2009; Baker et al. 2016; Pyle et al. 2016). Organisms in MCEs are adapted to low light conditions and include creatures such as corals, macroalgae, fish, sponges, and invertebrates (Kahng & Maragos 2006; Kahng & Kelley 2007; Hinderstein et al. 2010; Baker et al. 2016; Loya et al. 2019). MCEs are home to a high level of organismal diversity (Rooney et al. 2010; Harris et al. 2013), and high rates of endemism have been found for mesophotic systems throughout the world (Kane et al. 2014; Baker et al. 2016; Pyle et al. 2016; Kosaki et al. 2017; Loya et al. 2019).

The Hawaiian archipelago is one of the most isolated places on earth, providing a unique location to study endemism (Grigg 1988; Fleischer et al. 1998; Friedlander & Brown 2019). While endemism is high in shallow waters, endemism in Hawaiʻi is especially high in MCEs (Kane et al. 2014; Pyle et al. 2016) and can even reach 100% for fish assemblages in the Papahānaumokuākea Marine National Monument (PMNM) located northwest of the Main Hawaiian Islands (Kosaki et al. 2017; Fig. 1.3). Recent studies have documented high numbers of endemic species in Hawaiian MCEs (DeMartini & Friedlander 2004; Kerswell 2006; Grigg et al. 2008; Rooney et al. 2010; Kane et al. 2014; Kosaki et al. 2017), including many recently described macroalgal species (Spalding et al. 2016; Sherwood et al. 2020a,b). Macroalgae are abundant in Hawaiian mesophotic systems and beds of algae have been found as deep as 160 m (Spalding 2012). In Hawaiian mesophotic zones Rhodophyta are found in both the shallow and deep mesophotic, with several species encompassing both habitats (Spalding 2012). Red algae are unique in that they can utilize blue light due to presence of a pigment called phycoerythrin, which is advantageous because blue light is dominant at deeper ocean depths. A macroalgal diversity study conducted by Spalding (2012), focused on discovering species composition in Hawaiian mesophotic zones, uncovered interesting red algal trends. Spalding (2012) noted that foliose red algae were the most diverse in both size and morphology. This study also mentioned the large size of individuals observed in the order Rhodymeniales. Because there is an abundance of uncharacterized diversity in Hawaiian MCEs, there is a need to document species found in these environments.

Influxes of cold water in the mesophotic zone in Hawaiʻi can provide suitable habitat for species typically found in temperate zones (Abbott & Huisman 2003). This could also apply to the mesophotic zone where water temperatures are lower than they are in areas shallower than 30 m. Documenting the algal species present in mesophotic systems will help provide an understanding of the biogeographic patterns and processes of the flora by comparing newfound algal taxa with closely related taxa and their distributions. In addition, species new to science in Hawaiian MCEs can provide insight to how currents and physical forces shape species distributions on a large scale (Abbott & Huisman 2003).

Biogeography

Distributions of algae worldwide are contingent upon a combination of water temperature (Van den Hoek 1984; Lobban & Harrison 1994) and other factors (Hommersand 2001). While temperature plays a role, physical movement of macroalgae also has an equally important role. Pathways for species distribution and barriers to distribution, known theoretically as vicariance biogeography, also plays a role in species patterns worldwide (Hommersand 2001). Macroalgal spore dispersal is limited due to short periods of viability combined with their tendency to sink (Kinlan & Gaines 2003). Conversely, algal dispersal can also occur over long distances through the transportation of buoyant individuals or through individuals hitchhiking on floating material or fauna (McDermid & Abbott 2006; Fraser et al. 2011; Saunders 2014; Macaya et al. 2016). Since the Hawaiian Islands are home to volcanic activity, there is a possibility of algae floating through hitchhiking upon buoyant pumice as has been noted in corals (Jokiel 1990). There is a consensus that macroalgal groups subject to speciation events typically spread to the Atlantic from the Pacific, and not the other way around (Bringloe & Saunders 2019). Hawaiʻi is a unique place to look at biogeography due to its isolation, high degree of endemism, and occurrence of species with distributions encompassing more than one country (Abbott & Huisman 2003; McDermid & Abbott 2006). Studies analyzing macroalgal biogeographic patterns are lacking in

Figure 1.3. Map of the Main Hawaiian Islands and the Papahānaumokuākea Marine National Monument. Map created by NOAA - https://www.papahanaumokuakea.gov/new-library/

the literature, this highlights a need for more studies that can uncover these patterns (Leliaert et al. 2018).

Hawai‘i is part of a large region known as the Tropical Indo-West Pacific, and while currently tropical over the span of geological time, the ocean waters have fluctuated in temperature. The current tropical climate of Hawai‘i includes oceanic temperatures higher than 25°C, (PacIOOS: Sea Surface Temperature (SST) Maximum Monthly Climatological Mean, 1985-2013 - Hawaii). About 40-15mya water temperatures were much lower and ranged from 16°C to 20°C mimicking a mild temperate climate (Grigg 1988). During this period of lower temperature many islands, including the northwestern most islands, were present or being created through geological processes in the region currently known as the PMNM (State of Hawaii Department of Land and Natural Resources 2005). As the ocean warmed, a potential refuge for shallow water species could have been deeper cooler waters that they are accustomed to. It has been noted that there are species present in the Hawaiian mesophotic zone that belong to temperate algal groups (Abbott & Huisman 2003). This idea provides clues to depth distribution and biogeographical patterns present in MCEs, which currently are severely understudied (Loya et al. 2019).

Many currents are present in the Indo-Pacific and likely come into play when discussing biogeography in isolated areas like Hawai‘i. In the present day a large gyre is present in the middle of the Pacific Ocean, created through the combination of currents found on all sides of the gyre (Schmitz 1996). The Kuroshio Current runs from west to east, flowing on the northern portion of the gyre (Qiu and Lukas, 1996). The California Current runs north to south and is found on the eastern side of the gyre (Kamikuri & Motoyama 2020). On the southern portion is the North Equatorial Current which runs east to west (Qu et al. 1998). Hawai‘i is found in the south-eastern portion of the gyre but many species found here are also found, or are closest relatives with, species present in eastern Asia and Australia (Abbott & Huisman 2003; McDermid & Abbott 2006; Paiano et al. 2020; Cabrera et al. 2021; Sherwood et al. 2020b; Spalding et al. 2016). McDermid & Abbott (2006) speculated that the reason there are so many species present in Hawai‘i with Japanese affinities is due to the Kuroshio Current. Locally in the Hawaiian Islands there are three main currents at play. One of these currents that could possibly aid in algal dispersal runs west to east and is known as the Hawaiian Lee Counter Current (Xie et al. 2001; McDermid & Abbott 2006; Yu et al. 2003). The North Hawaiian Ridge Current (NHRC) is another local current that flows northwest from the Main Hawaiian Islands (MHI).

The NHRC seems to be a likely mechanism to transport larvae from the MHI to PMNM (Firing & Brainard 2006). Though recruitment is likely to happen from the MHI to PMNM through the NHRC, there is possibility that it can occur the other way around as well (Firing et al. 2004). Lastly there is the Subtropical Counter Current (SCC) is impacted by seasonality but during March to July it runs eastward towards the middle of the Archipelago (Firing & Brainard 2006) (Figure 1.4).

While there is general information available for how algae disperse over long distances and for possible local currents that can facilitate this, there is still a glaring lack of literature on either of these topics for the Hawaiian Islands. While algal transport is important for species relationships through an evolutionary lens, it is also important to consider oceanic conditions. To adequately understand macroalgal biogeography in Hawaiʻi there needs to be more studies that focus on these topics. Learning about macroalgal diversity and relationships in Hawaiʻi is especially timely given the rising threats of a warming ocean, introductions of invasive species, and increasing human populations on the islands. For example, a recent species described from PMNM was shown to be overgrowing corals to an alarming extent (Sherwood et al. 2020c). It is hard to say if this species is non-native or from another area. More studies highlighting macroalgal biogeography could uncover clues of where this species originated and how best to manage this species. Algal biogeography can be a useful tool and there is a pressing need for more studies highlighting these relationships.

Rhodymeniales in Hawaiʻi: *Halopeltis* and *Leptofauchea*

In Hawaiʻi, seven genera from the family Rhodymeniaceae have been documented: *Botryocladia, Chrysymenia, Halichrysis, Rhodymenia, Coelarthrum, Erythrocolon,* and *Drouetia* (Abbott & Littler 1969; Abbott 1999). In the Faucheaceae, two genera have been documented in Hawaiʻi: *Gloiocladia* and *Gloioderma* (Abbott 1999). Both genera examined in this study from the mesophotic zone, *Leptofauchea* and *Halopeltis*, have not been before documented in the Hawaiian Islands. Known distributions of *Leptofauchea* and *Halopeltis* are illustrated in Figures 1.5 and 1.6, respectively. *Leptofauchea* (J. Agardh) Kylin is described based on *L. nitophylloides* (J. Agardh) as the type species. Originally a species poor genus, recent additions of newly described taxa have increased the known diversity (Gavio & Fredericq 2005; Dalen & Saunders 2007; Rodríguez- Prieto & De Clerck 2009; Suzuki et al. 2010, 2012; Filloramo & Saunders 2015). This is a small genus including nine currently accepted species (see below).

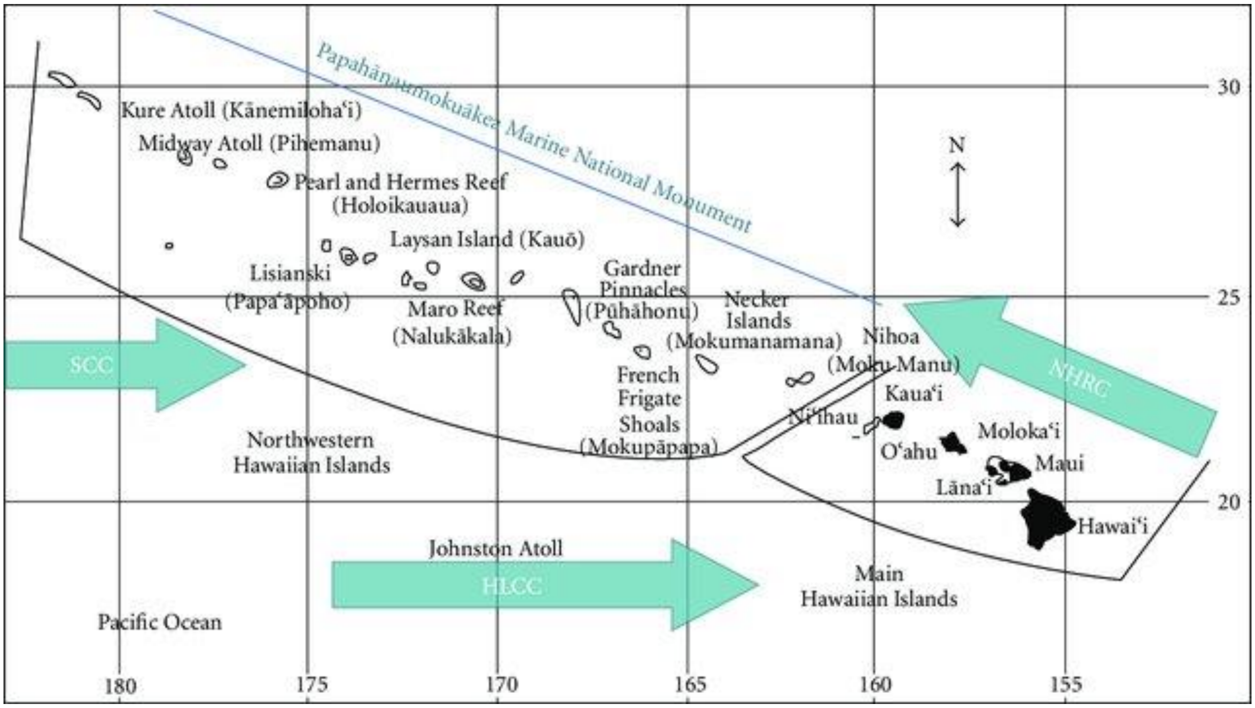

Figure 1.4. Major local currents found in the Hawaiian Islands that potentially aid in algal transport through hitchhiking. The flow directions of the Subtropical Countercurrent (SCC), the Hawaiian Lee Countercurrent (HLCC), and the North Hawaiian Ridge Current (NHRC) are depicted by the green colored arrows. The Papahānaumokuākea Marine National Monument (PMNM) encompasses the area shown by the blue labeled line. The Main Hawaiian Islands (MHI) are in black. (from Toonen et al. 2011).

Recently, *Leptofauchea rhodymenioides*, studied by Filloramo and Saunders (2015), was found to be very similar to *L. leptophylla*. Thus, it was suggested that this species be excluded from the genus until more molecular data could be generated for the material attributed to *L. rhodymenioides* (Filloramo & Saunders 2015); since 2015, there have not been any additions to the molecular database to test if this “species” is distinct or if it should be included in *L. leptophylla*. This genus has species described from both warm and temperate waters. Specimens from this study will be the first records of *Leptofauchea* in Hawai‘i. Adding these species to *Leptofauchea* will provide a clearer picture of the diversity within this genus.

Halopeltis belongs to the Rhodymeniaceae family and was recently resurrected as of 2010 (Saunders & McDonald 2010). This genus is based on the type species, *Halopeltis australis* (J. Agardh) G. W. Saunders and B. McDonald, was originally described as *Rhodymenia australis* (Sonder). The type species has many synonyms, both homotypic and heterotypic, which points to the complex history of the genus (Guiry & Guiry 2021). *Rhodymenia australis* was described by Sonder (1845), but the description was deemed superfluous (Saunders & McDonald 2010).

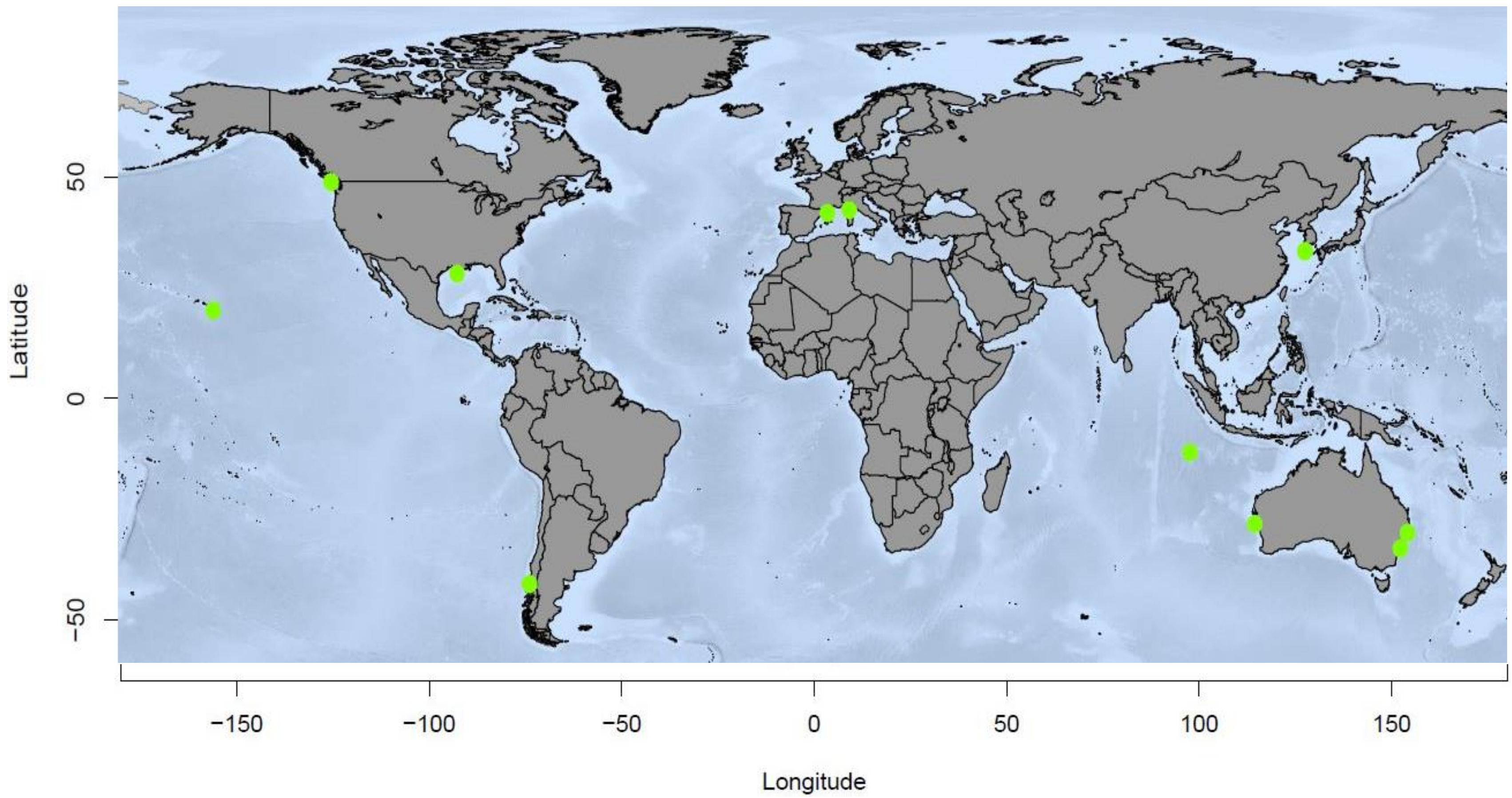

Figure 1.5. Species map for *Leptofauchea* spp. denoting locations of species reporting with a green circle.

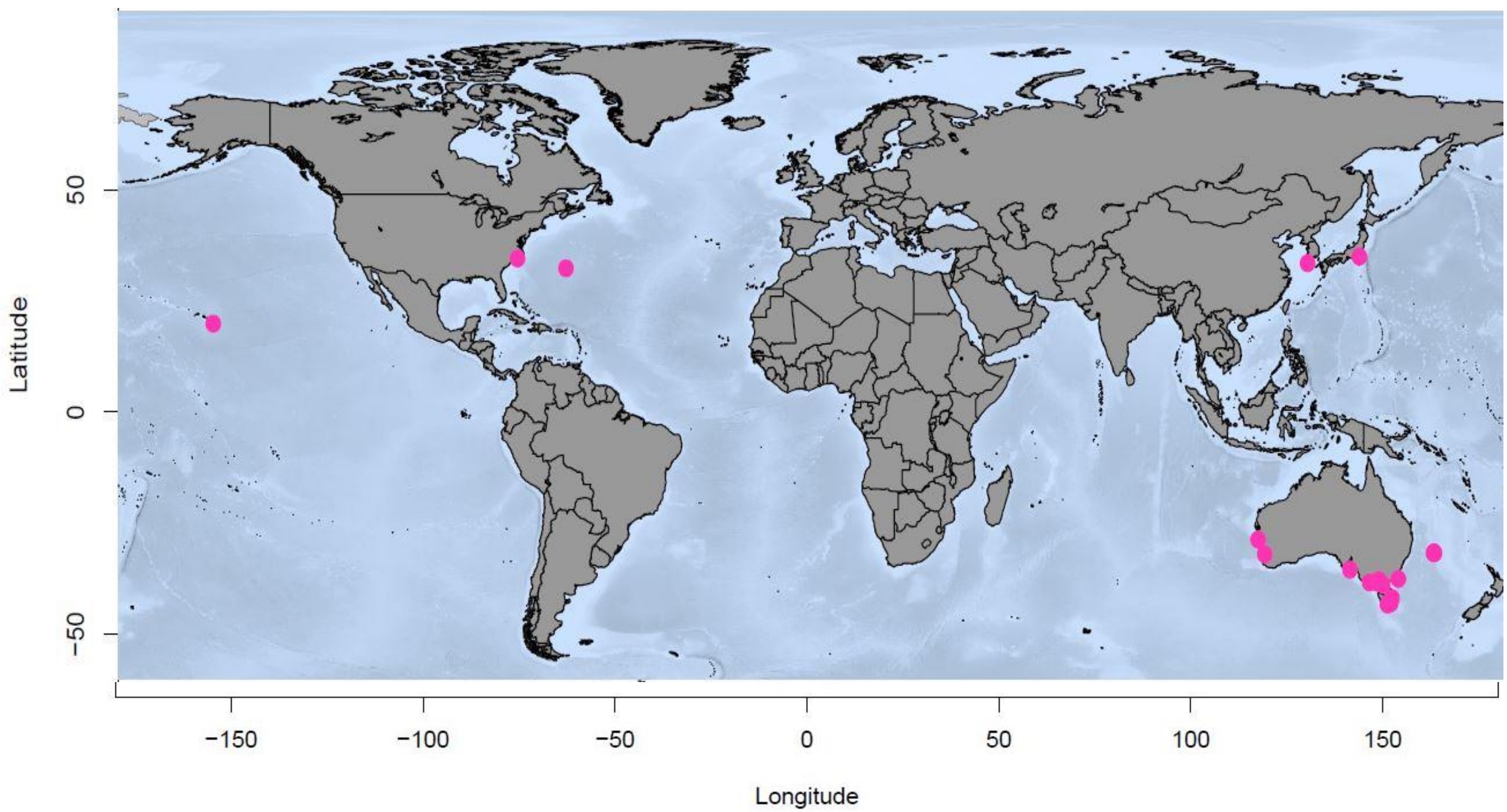

Figure 1.6. Species map for *Halopeltis* spp. denoting locations of species reporting with a dark pink circle.

This description was used by J. Agardh (1854) to establish *Halopeltis*, but because the name was superfluous there was action to correct this by changing the specific epithet (Silva et al. 1996; Womersley 1996; Saunders & McDonald 2010). Two different species names were suggested in published articles within three days of one another (Silva et al. 1996; Womersley 1996); despite these attempts an earlier name was available if the species was transferred to a different genus (Saunders & McDonald 2010). *Sphaerococcus australis* Kützing (1849) became the new name for this taxon at the time, which rid it of the superfluous description but, to make matters more complex, this new name was a homonym of an earlier described species of the same name (Harvey 1844; Saunders & McDonald 2010). To address this, *Sphaerococcus australis* Kützing was transferred to *Acropeltis* by J. Agardh (1852) and is now known as *Rhodymenia sonderi* P.C. Silva (Saunders & McDonald 2010). This became the new name upon which J. Agardh originally created the genus *Halopeltis* (Saunders & McDonald 2010). The genus was published by Agardh but the name *Halopeltis australis* was officially assigned to the genus by Saunders and McDonald (2010). This genus has few species, partially due to how recently it has been resurrected, with additions to this genus in occurring in recent years (Schneider et al. 2012; Saunders & McDonald 2010) and the genus is currently composed of nine taxonomically accepted species (see below for a summary of currently accepted species in both *Leptofauchea* and *Halopeltis*).

Genus *Leptofauchea*

Leptofauchea chiloensis J. L. Dalen & G. W. Saunders, 2007
Leptofauchea cocosana G. V. Filloramo & G. W. Saunders, 2015
Leptofauchea coralligena C. Rodríguez-Prieto & O De Clerck, 2009
Leptofauchea earleae B. Gavio & S. Fredericq, 2005
Leptofauchea leptophylla M. Suzuki & al., 2012
Leptofauchea lucida J. Huisman & G. W. Saunders, 2020
Leptofauchea munseomica G. V. Filloramo & G. W. Saunders, 2015
Leptofauchea nitophylloides (J. Agardh) Kylin, 1931
Leptofauchea pacifica E. Y. Dawson, 1945

Genus *Halopeltis*

Halopeltis adnata (Okamura) C. W. Schneider, D. W. Freshwater & G. W. Saunders, 2012
Halopeltis australis (J. Agardh) G. W. Saunders & B. McDonald, 2010
Halopeltis austrina (Wormersley) G. W. Saunders & B. McDonald, 2010
Halopeltis cuneata (Harvey) G. W. Saunders, 2010
Halopeltis gracilis G. W. Saunders, 2010
Halopeltis pellucida C. W. Schneider, D. W. Freshwater & G. W. Saunders, 2012
Halopeltis prostrata G. W. Saunders & B. McDonald, 2010
Halopeltis verrucosa (Womersley) G. W. Saunders & B. McDonald, 2010
Halopeltis willisii C. W. Schneider, D. W. Freshwater & G. W. Saunders, 2012

Study Overview

A combination of morphological data and molecular analysis will be used to examine relationships amongst currently described and collected samples from the Hawaiian mesophotic zone. The general study approach follows the flow charts above (Figure 1.7).

Questions

The aim of this project was to contribute to the existing baseline knowledge for the order Rhodymeniales in the Hawaiian mesophotic zone by exploring all collected specimens of Rhodymeniales with constrictions in their branches. Documentation of these new species provides a larger context for the overall Rhodophyta diversity in one of the most well sampled mesophotic zones in the world for macroalgae. The questions addressed by this study are shown below.

1) Which species of Rhodymeniales are present in shallow and mesophotic zones, and how closely related are they to each other?
2) Are endemic species of Rhodymeniales present in MCEs in Hawai'i?
3) Can Rhodymeniales taxa provide clues to biogeographical links?

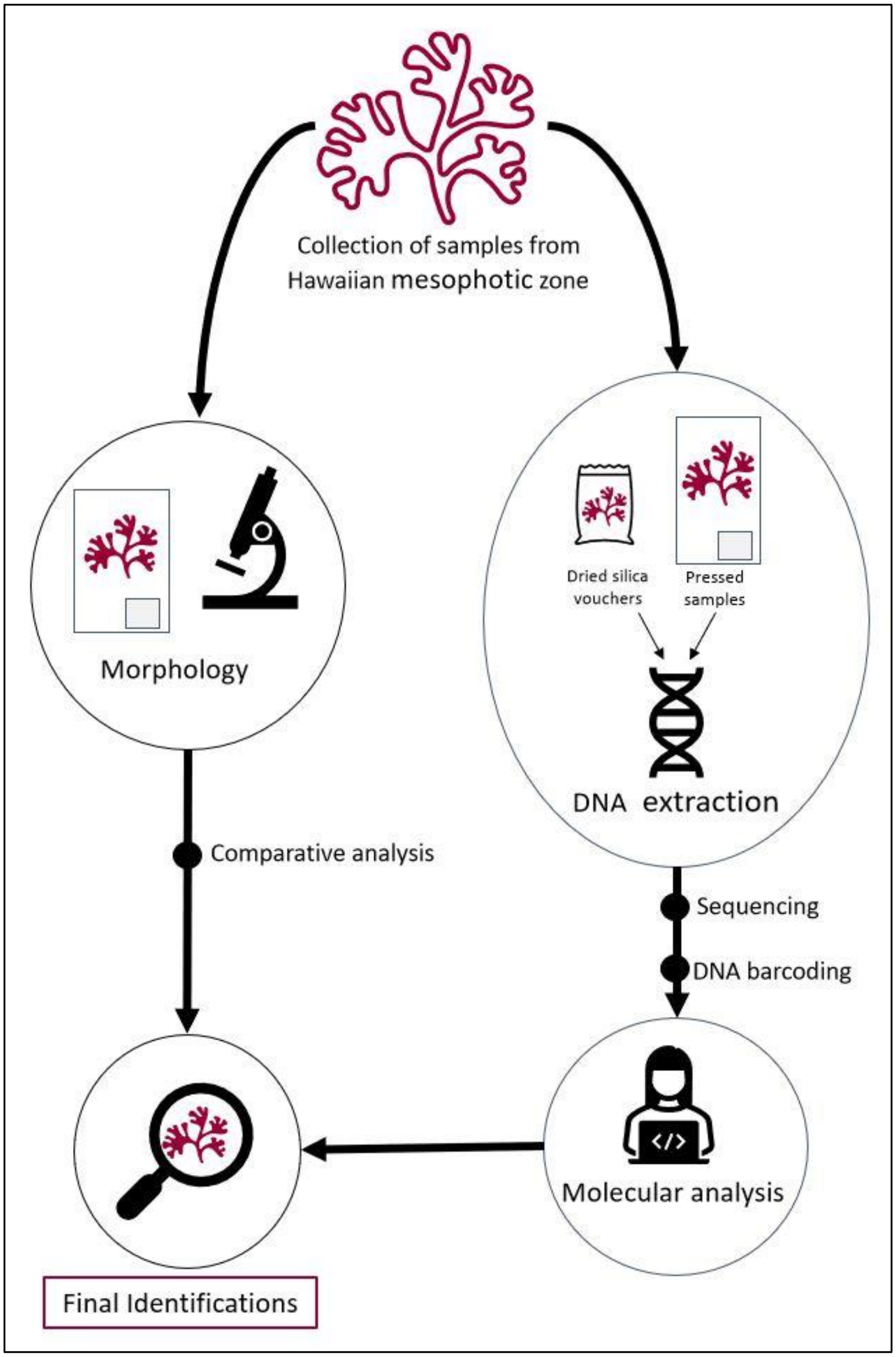

Figure 1.7. Work-flow chart depicting general procedure used in this study.

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Chapter 2

Uncovering diversity in the mesophotic zone of Hawai‘i: species new to science in the genera *Halopeltis* and *Leptofauchea* (Rhodymeniales, Rhodophyta)

Introduction

The Rhodymeniales is a diverse order, both in species richness and morphological diversity encompassing six monophyletic families: Champiaceae, Faucheaceae, Fryeellaceae, Hymenocladiaceae, Lomentariaceae, and the Rhodymeniaceae (Filloramo & Saunders 2016). This order has had a complicated taxonomic history which includes the addition and redaction of families over the years, beginning shortly after its inception by Schmitz in 1889 (Schmitz 1889; Le Gall et al. 2008). Recent studies have clarified the taxonomic relationships within the order and have resulted in new additions at multiple levels including (but not limited to) the introduction of the family Fryeellacae and Hymenocladiacae, the introduction of the genera *Neogastroclonium* L. Le Gall, Dalen & G. W. Saunders and *Pseudohalopeltis* G. W. Saunders, resurrection of the genus *Halopeltis* (J Agardh) G. W. Saunders 2010, and the introduction of multiple new species (Gavio & Fredericq 2005; Afonso-Carrillo et al. 2006; Dalen & Saunders 2007; Le Gall et al. 2008; Schneider & Lane 2008; Rodríguez-Prieto & de Clerck 2009; Saunders & McDonald 2010; Suzuki et al. 2010; Schneider et al. 2012; Filloramo & Saunders 2015; Santiago et al. 2016).

The Rhodymeniales is known for containing substantial cryptic diversity (Saunders et al. 2006; Lozada-Troche & Ballantine 2010), even between non-sister families such as the Rhodymeniaceae and Faucheaceae (Le Gall et al. 2008). The Faucheaceae is the third largest family within the order and is characterized by three-celled carpogonial branches, terminal cruciate tetrasporangia, and by having small cells within the cortex (Saunders et al. 1999). The difference between the Faucheaceae and the Rhodymeniaceae lies with their reproductive structures. The Rhodymeniaceae is the largest family in the Rhodymeniales and is characterized by typically having four-celled carpogonial branches (Dalen & Saunders 2007; Le Gall et al. 2008). It differs from the Faucheaceae by having terminal or intercalary cruciately or tetrahedrally divided tetrasporangia (Dalen & Saunders 2007; Le Gall et al. 2008). Observing individuals at the level of gross morphology makes it nearly impossible to tell the two families apart since they can have very similar coloring, branching patterns, and habit. The introduction of molecular-assisted alpha taxonomy (MAAT) has clarified relationships which would have been otherwise difficult to discern using morphology alone. Combining taxonomy and morphology has proven to be a tangible way to describe many algal species (Dalen & Saunders, 2007; Suzuki et al. 2010; Filloramo & Saunders 2015; Paiano et al. 2020; Cabrera et al. 2021).

Mesophotic coral ecosystems (MCEs) host substantial biodiversity, much of it unique, and research in these ecosystems has resulted in newfound species descriptions that help clarify diversity within algal groups (e.g., Sherwood et al. 2010; Paiano et al. 2020; Cabrera et al. 2021). The depth range for MCEs differs based on locality, but typically they are found at 30 – 150 m in tropical and subtropical areas (Lesser et al. 2009; Baker et al. 2016; Loya et al. 2019), with the lower depth range determined by the ~1% irradiance level (Lesser et al. 2009). MCEs are home to a high level of organismal diversity (Rooney et al. 2010; Harris et al. 2013), and high levels of endemism have been found for mesophotic systems throughout the world (Kane et al. 2014; Baker et al. 2016; Pyle et al. 2016; Kosaki et al. 2017; Loya et al. 2019). This is also true in Hawaiʻi where endemism levels are typically high due to the isolation of the archipelago (Grigg 1988), and a number of new-to-science endemic algal species have recently been described from Hawaiian mesophotic zones (e.g., Spalding et al. 2016; Sherwood et al. 2020a,b). Macroalgae are abundant in Hawaiian mesophotic systems and beds of algae have been found as deep as 160 m (Spalding 2012). Influxes of cold water in the mesophotic in Hawaiʻi can provide suitable habitat for species typically found in temperate zones (Abbott & Huisman 2003), resulting in certain groups that are only found in the mesophotic and absent from shallow waters (Cabrera et al. 2021).

Both genera examined in this study, *Leptofauchea* and *Halopeltis*, are reported from the mesophotic, and have not been previously documented in the Hawaiian Islands. In Hawaiʻi, seven genera from the family Rhodymeniaceae have been reported: *Botryocladia, Chrysymenia, Halichrysis, Rhodymenia, Coelarthrum, Erythrocolon,* and *Drouetia* (Abbott & Littler 1969; Abbott 1999). In the Faucheaceae, two genera have been documented: *Gloiocladia* and *Gloioderma* (Abbott 1999). The genus *Leptofauchea* is part of the family Faucheaceae and is composed of 9 taxonomically accepted species (Guiry & Guiry 2021). *Halopeltis* belongs to the Rhodymeniaceae and is a resurrected genus as of 2010 (Saunders & McDonald 2010). Species new to science in Hawaiian MCEs (DeMartini & Friedlander 2004; Kosaki et al. 2017) provide insight into how currents and physical forces shape species distributions on a large scale (Abbott & Huisman 2003). This study describes the phylogenetic relationships for three species new to science within the genera *Leptofauchea* and *Halopeltis* of the Rhodymeniales and examines biogeographic patterns for these new additions.

Materials & Methods

All mesophotic samples were gathered via technical diving or submersible excursions within the Main Hawaiian Islands or the Papahānaumokuākea Marine National Monument (PMNM). Most specimens were collected in 2006 off the west coast of Molokaʻi in a locality known as Penguin Bank. Two samples were collected from the island of Hawaiʻi in 2015, and one sample was collected from the PMNM in 2019. After collection, samples were dried as herbarium specimens and preserved in silica gel for molecular analysis. Sample collection information is listed in Table 3.

DNA was extracted from individuals pressed onto herbarium sheets or from tissue preserved in silica gel. The extraction method followed instructions provided with the OMEGA E.Z.N.A® Plant DNA Kit (OMEGA Biotek, Norcross, GA, USA). Amplification for the COI-5P barcoding marker was accomplished using two sets of primers: GazF1 and GazR1 using cycling conditions from Saunders (2005), or GWSFn and GWSRx using cycling conditions from Saunders & Moore (2013). The plastidial *rbc*L gene was amplified in three fragments. The first fragment was amplified using F57 and R562 with the cycling conditions listed in Freshwater & Rueness (1994); alternately, the primer pair F8 and R646 were used with the cycling conditions from Wang et al. (2000). The second fragment was amplified with F481 and R1150, and F765 R1381 was used to amplify the third fragment, with cycling conditions for both fragments listed in Wang et al. (2000). For the ribosomal LSU marker, four pairs of primers and cycling conditions were used: T16 and T24, T25 and T20, T05 and T15 (Saunders and Moore 2013), and nu28SF and nu28SR (Sherwood et al. 2010). Sequencing of successfully amplified PCR products was performed at the Advanced Studies in Genomics, Proteomics and Bioinformatics (ASGPB) sequencing facility of the University of Hawaiʻi at Mānoa or through Genewiz® (https://www.genewiz.com/).

Editing and aligning of sequence data was conducted using Geneious Prime with downloaded sequence data from GenBank and BOLD (Tables 1, 2). Sequences of the COI-5P, *rbcL*, and LSU markers were aligned using MUSCLE (Edgar 2004) using the default settings; sequences were aligned separately for each marker to check for any inconsistencies in the data before being combined into a concatenated alignment. *Halichrysis* was used as an outgroup taxon for the *Halopeltis* phylogeny (Schneider et al. 2012) and *Webervanbossea* was used as the outgroup for *Leptofauchea* (Filloramo & Saunders 2015). The concatenated alignments were then transferred to CIPRES (Miller et al. 2010), where JModelTest2 was used to determine the best fit model of evolution for each alignment. RAxML phylogenies were run on Geneious Prime using the suggested models from the JModelTest2 while keeping all other setting on default. Bayesian analyses were also run using Geneious Prime and the suggested model. Figures 2.1 and 2.2 display the statistical output from both analyses. Sequences from this study can be accessed on Genbank (XXXXXX-XXXXXX; to be obtained).

Gross morphological observations of pressed herbarium specimens were made using a ruler. Smaller features were observed by mounting specimens onto microscope slides and viewing through a Zeiss AxioImager A1 compound light microscope (Pleasanton, CA) with an Infinity2-1RC digital camera (Lumenera Corporation, Ottawa, Ontario, Canada) used for taking images. Slides were made by sectioning material by hand using a single-edged razor blade and hydrating in water for 5 min followed by a 5-min staining period with 0.5% aniline blue. Final slide mounting was accomplished by applying 30% Karo™ syrup around the edges of the coverslip. Digitization of pressed sheets was performed in the Joseph F. Rock Herbarium (HAW) with a Canon EOS 5D Mark II Digital Camera mounted on a MK Direct Photo-eBox PLUS 1419 setup.

Results

Phylogenetic analyses

Phylogenetic analyses of the genus *Leptofauchea* revealed that the mesophotic samples analyzed in this study are molecularly distinct from all but one described species *Leptofauchea lucida* represented by the sample *Leptofauchea* sp. 1WA G0400. Mesophotic samples in this study formed four clades labeled by color for ease of reference (Figure 2.1). Two of the clades consist of only a single herbarium sheet with small individuals, for this reason they are not formally described here and await analysis of additional specimens before being described in future work. The closest relatives for each of these clades are species that are currently described, indicating that while samples of all four clades were collected from the Hawaiian Islands, they are more genetically similar to species found in other localities than to each other. The sample from the PMNM (ARS 10013) is most closely related to *L. leptophylla*, which is described from Japan (Segawa 1941) and was recently transferred to the genus (Suzuki et al.

2012). All mesophotic samples analyzed here do not originate from branches deep within the phylogeny, except for the red clade, suggesting that this red clade species may be older in comparison to recently branched clades. The blue clade, containing ARS 10228, ARS 09905, ARS 09895, ARS 09909, ARS 09900, and ARS 09907, is most closely related to *L. cocosana*, a recently described species from the Cocos (Keeling) Islands in the Indian Ocean (Filloramo & Saunders 2015). The other large clade, the green clade, includes a recently described species, (*L. lucida*) collected from Australia (Huisman & Saunders 2020).

All currently described species of *Halopeltis* are represented in the concatenated phylogeny Figure 2.2. The samples sequenced in this study from the mesophotic for *Halopeltis* appear in a small clade separate from currently described species in the genus, and represent a species new to science. There are four samples within this clade, and they are most closely related to *H. adnata* described by Schneider et al. (2012). *Halopeltis adnata* was reported from Korea and Japan representing (at the time) the first species in the genus to be reported in the Northern Hemisphere in a non-tropical climate (Schneider et al. 2012).

Three of the four new-to-science species represented in these phylogenies are most closely related to species in the Western Pacific, with only a single species aligning closely with a species described from the Indian Ocean. The species from the Hawaiian mesophotic zone outlined here are the first reports of the genera *Halopeltis* and *Leptofauchea* in the Hawaiian Islands.

<u>Morphological analysis and Taxonomic Treatment</u>

Leptofauchea sp.1 **E.A. Alvarado & A.R. Sherwood (Blue Clade) [name under development in collaboration with the NOAA-PMNM Cultural Working Group]**

Description: Individual plants up to 21 cm tall × 18 cm wide, lacking a stipe, deep red-pink to light pink in color. Spreading habit with irregular to loosely dichotomous branching. Blades 2–4 mm broad with some constrictions present below or above branching points, constrictions range in breadth <1–1.75 mm. Lengths of branches between points of branching up to 6.4 cm in length. Thallus flat and thin ranging from 120–150 μm thick, no clear main axis. Young fruiting blades ovular and typically lighter in color than the surrounding thallus. Few attenuate apices, most apices rounded and constricted just below the apex. Medulla is 2–3 layers, irregularly arranged, cells visible from surface view. Medullary cells axially elongated, large, 27–81 μm in length × 18–47 μm in width, lightly staining, and thin-walled. Medullary layers grade into an incomplete layer of inner cortical cells, this is especially apparent in marginal cross sections; inner cortex composed of 1–2 layers total. Cortical cells loosely arranged in surface view, with a single layer in the outer cortex, spherical to slightly axially elongated, 7.4–17 μm in diameter. Cortical cells darkly pigmented when stained with aniline blue. Tetrasporanigal nemathecia, when observed, slightly darker than the surrounding thallus, positioned near blade apices, round to elliptical in shape and elongated towards the branch apex, the only patch observed was 1370 × 740 μm. Tetrasporangia tetrahedrally divided and spherical, ranging from 7.6–29.8 μm in diameter where observed. Gametangial reproduction not observed.

Holotype: ARS 09895; West Molokaʻi, Hawaiʻi (21.022172, -157.216848), 72m, 17 .XI.2006, leg.Terry Kerby (P4-184 #185).

Isotype: ARS 09900 West Molokaʻi, Hawaiʻi (21.022172, -157.216848), 72m, 17 .XI.2006, leg. Terry Kerby (P4-184 #171).

Specimens examined: ARS 09895, ARS 09900, ARS 09905, ARS 09907, ARS 09909, ARS 10228

GenBank accession numbers: XXXXXX-XXXXXX; to be obtained

Individuals are loosely dichotomously loosely dichotomously branched, with several exceptions to a strict dichotomous branching pattern (Figures 2.3, 2.4). Specimens have a deep pink-red color that lightens to a very light pink nearing most branch apices. There are long branch lengths found between points of branching, giving a lanky appearance. Branches are thin and range from 120–150 μm thick (Figure 2.7) with large medullary cells and small pigmented cortical cells (Figures 2.7, 2.5, 2.6). In surface view cortical cells are loosely arranged and medullary cells can be seen faintly under the cortical cells (Figure 2.5); they become more apparent when adjusting the fine-tuning knob of the light microscope (Figure 6). Medulla is 2–3 layers, with thin-walled cells, 27–81 μm long by 18–47 μm wide (Figure 2.7). Cortical cells are very small, mostly round, and 7.4–17 μm in diameter (Figures 2.5, 2.7). Only tetrasporangial reproduction observed. Tetrasporania formed in nemathecia (Figure 2.10) elongated towards a branch apex, 1370 × 740 μm in size. Paraphyses composed of 4-7 cells (Figures 2.8, 2.11). Young tetrasporangia divided in half, otherwise mature sporangia tetrahedrally divided, which is characteristic for the genus.

Halopeltis sp. **E.A. Alvarado & A.R. Sherwood (Purple Clade) [name under development in collaboration with the NOAA-PMNM Cultural Working Group]**

Description: Whole plants up to 10.5 cm tall and 7.3 cm wide, including anastomosed portions. Plants layered on top of each other creating a tangled appearance *in situ*, spreading, no stipe observed. Thalli deep pink-purple to medium-pink in color. Thallus flat and thin, up to 191 μm thick. Branches range from thin to broad, 1–4 mm. Subdichotomously branched, constrictions in the branches present typically under apices and sometimes under points of branching. Apices attenuate, spatulate, rounded, or ovular to elliptical depending on degree of development. Medullary cells round to slightly elongated axially, 40–117 μm long by 40–79 μm wide, cells present in 2–3 layers, thin, lightly staining. Cortical cells small and darkly staining, positioned irregularly. Outer cortex composed of 1–2 layers, and inner cortical layer incomplete, cells 11–17 μm by 8.5–13 μm. Young tetrasporophytic with underdeveloped paraphyses present only, no sori or tetrasporangia observed.

Holotype: ARS 09898; Hawai‘i, Hawai‘i (20.016600, -155.843550), 90m, 11.XI.2015, leg. Randall Kosaki & Jason Leonard (MHI-20).

Paratype: ARS 09912; ‘Au‘Au Channel Maui, Hawai‘i (20.458500, -156.396000), 104 m, 29.XI.2006, leg. Terry Kerby (P4-190 #443).

Representative samples from this study: ARS 09898, ARS 09912, ARS 10478, ARS 10479

GenBank accession numbers: XXXXXX-XXXXXX; to be obtained

Specimens have different a range of morphologies, from broadly branched, to less so (Figure 2.18, 2.19). Specimens range in color from dark (ARS 09898; Figure 2.18) to lighter medium to light pink (ARS 09112; Figure 2.19). Apices are varied and present a variety of shapes: rounded, attenuate, and ovular. From a surface view cortical cells are arranged loosely and irregularly, darkly staining (Figure 2.20). The outer cortex is composed of 1–2 layers with an incomplete inner layer, cells ranging in size from 11–17 μm by 8.5–13 μm (Figures 2.21, 2.22, 2.23). Medullary cells round to axially elongated, large and thin-walled, 40–117 μm long by 40–79 μm in width (Figures 2.21, 2.22, 2.23). Medulla composed of 2–3 layers of these lightly staining cells. Young tetrasporophytic specimens were observed, and the beginnings of paraphysis development were captured (Figures 2.22, 2.23).

Discussion

Leptofauchea and *Halopeltis* are new genus records for the Hawaiian Islands, contributing to the recent addition of such records for Hawai‘i (e.g., Huisman & Abbott 2003; Kraft et al. 2004; Sherwood et al. 2010; Sherwood & Carlile 2012; Conklin et al. 2014).

Interestingly, these specimens have very similar gross morphology and were collected from similar environments. Hawaiian *Leptofauchea* specimens were found growing in clumps among other macroalgal species, dispersed loosely in a sandy habitat, which resembles the reported habitat for other *Leptofauchea* species (Dalen & Saunders 2007). While *Leptofauchea* has been reported from depths greater than 30 m (Gavio & Fredericq 2005; Dalen & Saunders 2007; Rodríguez-Prieto & De Clerck 2009) and *Halopeltis* has been reported at 30 m depths (Saunders & McDonald 2010, Schneider et al. 2012), our samples from the Hawaiian Islands are the deepest reported collections for both genera. *Leptofauchea* and *Halopeltis* are (thus far) only known from the mesophotic zone of Hawaiʻi, and have not been documented from depths shallower than 30 m in the Islands. These genera add to our knowledge of algae present in the mesophotic of Hawaiʻi which has a high level of endemism for many groups of organisms found there (Kane et al. 2014; Baker et al. 2016; Pyle et al. 2016; Kosaki et al. 2017).

<u>Morphological and Phylogenetic Analysis</u>

Molecular analysis provided good support for the recognition of *Leptofauchea sp.1*_Blue described herein as new species. A threshold of 70% support was to determine clade significance as in Filloramo & Saunders (2015). The red and yellow clades representing two species of *Leptofauchea* collected from Hawaiʻi (Figure 2.1) have only low support in the molecular phylogenetic analyses (the addition of more DNA sequence data in the future may help resolve this issue) and thus are not formally recognized here as new species. While three of the clades are distinct from all other sequences reported in GenBank, the green clade encompasses Hawaiian samples together with a recently described species: *Leptofauchea lucida*. This species was described in Huisman & Saunders (2020) and reported in Dalen & Saunders (2007) from western Australia. Since this species is shared from such a far away locality, is it is possible that it was transported here and there has not been sufficient time for it to speciate, or that this species may have a wider distribution than suspected. *Halopeltis* collected from the Hawaiian Islands resolved as a single clade, and collected samples have different degrees of maturation and morphology, which provide a lens into the plasticity of these features within the species.

For the yellow and red clade of *Leptofauchea*, the lack of vegetative material makes it difficult to distinguish differences in morphology from other currently recognized species in the genus. Strikingly, very noticeable differences occur for the two species of *Leptofauchea* described herein – the blue clade and the *L. lucida* clade. *Leptofauchea* sp. (blue) is distinct from other species in the genus due to the presence of long branch lengths between points of branching (Figures 2.3, 2.4). These long branch lengths are not seen in other *Leptofauchea* species (Gavio & Fredericq 2005; Dalen & Saunders 2007; Rodríguez-Prieto & de Clerck 2009; Suzuki et al. 2010; Filloramo & Saunders 2015). Similarly, the Hawaiian *L. lucida* individuals have a morphological trait that makes it distinct from other described *Leptofauchea* species: the presence of many constrictions, which are present in other species but not to the same extent (Gavio & Fredericq 2005; Dalen & Saunders 2007; Rodríguez-Prieto & de Clerck 2009; Suzuki et al. 2010; Filloramo & Saunders 2015). Hawaiian specimens belonging to *L. lucida* typically have two constrictions between branching points, making the thalli appear tangled (Figures 2.12, 2.13). These constrictions may help the plants to have more flexibility and to attach to other branches through anatomizing connections, which would be helpful in a sandy environment where clumps of other algae are the only places to attach. These traits are seen in the Hawaiian collections of *L. lucida* but are not seen in Australian collections of *L. lucida* as described by Huisman & Saunders (2020). These differences may arise due to the larger size of the Hawaiian samples when compared to the samples collected in Australia, or because of abiotic factors which play a role in macroalgal phenotypic plasticity (Saunders 2005).

Similarities and differences arise between the Hawaiian collections of *Halopeltis* and *Leptofauchea* when compared to the gross morphology of species in each genus. For *Halopeltis* there are a range of morphologies presented by the mesophotic Hawaiian collections. Differences in these morphologies could arise due to life history stage (Schneider et al. 2012), but in other *Halopeltis* species there tends to be varying morphologies regardless of the life history stage (Saunders & McDonald 2010). The most distinctive difference between the clade of *Halopeltis* described here and currently described species are the constrictions present along the branches of the Hawaiian specimens. While constrictions are present in *H. pellucida* (Schneider et al. 2012), *H. verrucosa,* and *H. prostrata* (Saunders & McDonald 2010), they occur more often in the Hawaiian collections from the mesophotic zone and are especially evident in ARS 09898 (Figure 2.18).

<u>Biogeography</u>

Hawaiian *Leptofauchea* specimens were collected from a broad geographical range, from the Hawaiʻi island to PMNM. *Halopeltis* specimens were collected from a smaller range, from Maui to Hawaiʻi island. Interestingly, a *Leptofauchea lucida* collected from the Hawaiian mesophotic was collected mesophotic depths, while other documented individuals in the genus are from shallower waters (Dalen & Saunders 2007). This species was described from collected samples from Bynoe Island which is part of small group of islands known as the Easter Group in western Australia (Huisman & Saunders 2020). At this locality water temperatures average around 23°C (Australian Government (Bureau of Meteorology): Australian Climate Variability & Change – Average Maps). *Leptofauchea* sp. (blue) also has affinities with Australia and is most closely related to a species only reported from Australia thus far (Filloramo & Saunders 2015). Many species in Hawaiʻi have species from Australia or the Northwestern Pacific as their closest relatives (Abbott & Huisman 2003; McDermid & Abbott 2006; Paiano et al. 2020; Cabrera et al. 2021; Sherwood et al. 2020b; Spalding et al. 2016), so these findings are not surprising. *Halopeltis* sp. is allied most closely with *Halopeltis adnata*, described from Korea (Schneider et al. 2012), showing yet again a link between Hawaiian species and species from eastern Asia. Possible mechanisms for the natural transport of *Leptofauchea* and *Halopeltis* to Hawaiʻi include macroalgal "hitchhiking" on fauna or floating materials to move this great distance (McDermid & Abbott 2006; Fraser et al. 2011; Macaya et al. 2016; Saunders 2014) or unintentional human-mediated introductions like what happens with invasive species (Smith et al. 2004). All Hawaiian species of *Leptofauchea* have their closest relationships to species reported from other areas, rather than to each other. This type of relationship is seen to a lesser extent in other genera in Hawaiʻi (Spalding et al. 2016). This suggests that there may have been multiple colonizations of *Leptofauchea* to the Hawaiian Islands. Many of the Hawaiian *Leptofauchea* species are reported from one locality – Penguin Bank, while Hawaiian *Halopeltis* was collected from a few different locations. A study conducted by Toonen et al. (2011) describes genetic boundaries within the Hawaiian Islands that suggest a lack of gene flow for multiple taxa. Comparing these boundaries with *Leptofauchea* distributions supports the genetic boundary the encapsulates Penguin Bank except for Hawaiian *Leptofauchea lucida* which was reported from Penguin Bank and the far end of PMNM. The support for most of the *Leptofauchea* species following these boundaries is likely partially due to the lack of *Leptofauchea* collections from other locations within Hawaiʻi. *Halopeltis* mostly fits into the genetic boundaries described by Toonen et al. with the exception of one sample collected off the west coast of Hawaiʻi Island. This may suggest that algal species may not be easily grouped with other marine taxa when defining boundaries for connectivity.

Significance

While about 80% of all coral reef habitat is included in MCEs (Loya et al. 2019), most studies focus on shallow reefs, providing a very biased understanding of ocean diversity, function, and ecology. Uncovering hidden diversity in these ecosystems can help to understand evolutionary and biogeographic patterns by adding to the current body of knowledge. Similarly, having adequate representation of mesophotic species to use for analyses can provide links between shallow and deep-water species. There is still much work to be done in terms of categorizing macroalgal diversity of the deep. Recently there here have been multiple newly described species from the mesophotic of Hawaiʻi (Spalding et al. 2016, Sherwood et al. 2020a,b, Paiano et al. 2020, Cabrera et al. 2021) and these species represent just a sliver of the diversity that has yet to be categorized (Sherwood et al. 2010). *Leptofauchea* and *Halopeltis* have never been documented in the Hawaiian Islands prior to this study, demonstrating a need for a better understanding of the diversity in these deep reefs.

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Figures and Captions

Figure 2.1. Phylogenetic tree for the genus *Leptofauchea* using a concatenated alignment of COI-5P, LSU, and *rbcL* sequences. Bayesian and Maximum Likelihood support values shown at the nodes. The first value represents the Maximum Likelihood bootstrap support value and the second is the Bayesian posterior probability support value. *Webervanbossea* was used as the outgroup taxon.

Figure 2.2. Phylogenetic tree for the genus *Halopeltis* using a concatenated alignment of COI-5P, LSU, and *rbcL* sequences. Bayesian and Maximum Likelihood support values shown at the nodes. The first value represents the Maximum Likelihood bootstrap support value and the second is the Bayesian posterior probability support value. *Halichrysis* was used as the outgroup taxon.

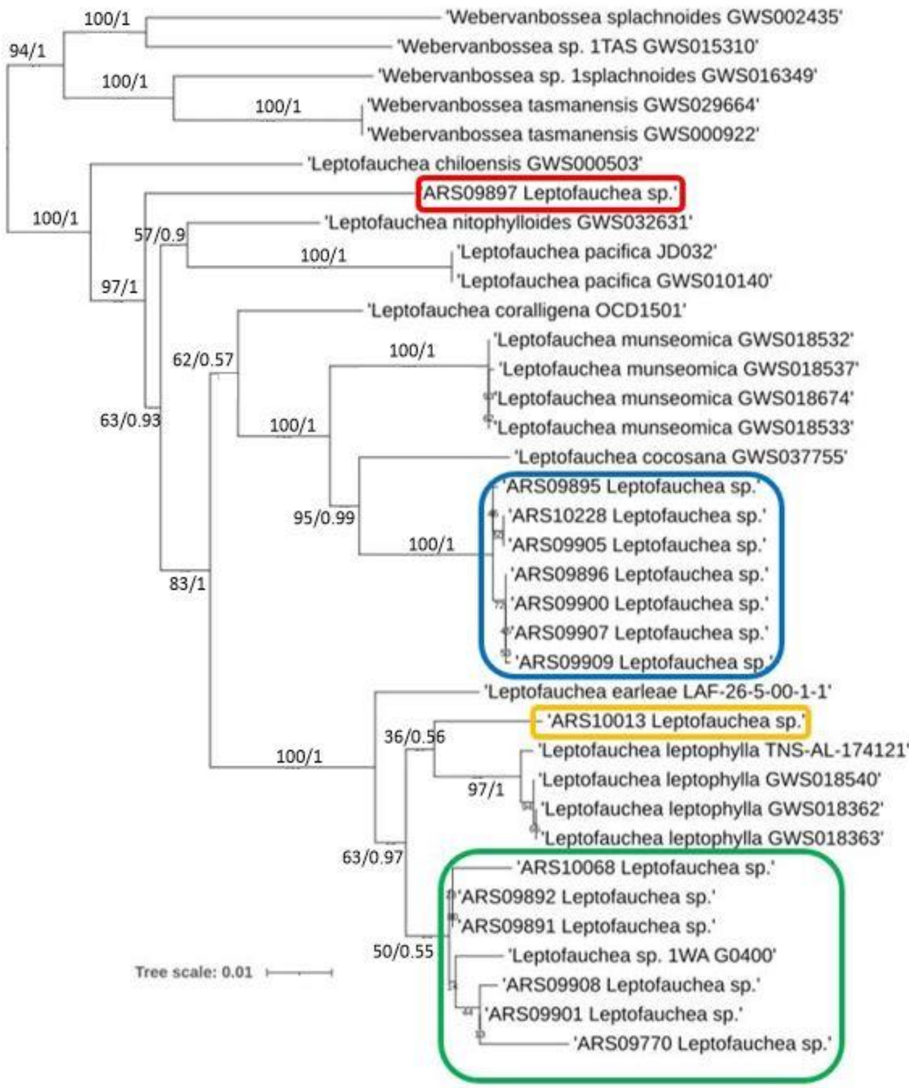
Figures 3–7. Morphology of *Leptofauchea* sp. (blue).
Figures 3–4. Tetrasporophyte representatives for *Leptofauchea* sp. (blue) displaying spreading habit, scale bars =10cm (ARS 09895, ARS 09905; respectively).
Figures 5–6. Surface view of the thallus, deeply stained cortical cells, and underlying medullary cells (ARS 09895). Scale bar = 50 µm.
Figure 7. Cross section showing medullary and cortical cell arrangement at the blade margin (ARS 09895). Scale bar = 100 µm.

Figures 8–11. Morphology of *Leptofauchea* sp. (blue).
Figure 8. Cross section exposing paraphyses and tetrasporangia (ARS 09907). Scale bar = 50 µm.
Figure 9. Thallus surface depicting tetrasporangial sori (ARS 09907). Scale bar = 50µm.
Figure 10. Nemathecia housing tetrasporangia (ARS 09907). Scale bar = 100 µm.
Figure 11. Cross section demonstrating paraphyses and tetrasporangia (ARS 09907). Scale bar = 100 µm.

Figures 12–17. Morphology for *Leptofauchea lucida*.
Figure 12–13. Habit of multiple branches connected by anastomization, both images also have *Halimeda* present which is commonly found growing among this species *in situ* (ARS 09892, ARS 09891; respectively). Scale bars = 10cm.
Figure 14. Surface view of the thallus, deeply stained cortical cells, and underlying medullary cells (ARS 09892). Scale bars = 50 µm.
Figures 15–16. Cross sections depicting shape of medullary cells at different thallus thickness (ARS 09892). Scale bar = 100 µm.
Figure 17. Tetrasporangial paraphyses growing from the thallus surface (ARS 09891). Scale bar = 50 µm.

Figures 18–23. Morphology for *Halopeltis* sp.
Figure 18–19. Habit showing different morphologies from two presses collected from different localities at different times (ARS 09898, ARS 09912; respectively). Scale bar = 10cm.
Figure 20. Surface view of the thallus with darkly stained cortical cells (ARS 09898). Scale bar = 100 µm.
Figure 21. Cross section showing large medullary cells and cortical cell organization at the

margin (ARS 09912). Scale bar = 100 µm.
Figures 22–23. Early stages of paraphysis development (ARS 09898). Scale bar = 100 µm.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'Webervanbossea tasmanensis GWS029664'
'Webervanbossea tasmanensis GWS000922'
'Leptofauchea chiloensis GWS000503'
ARS09897 Leptofauchea sp.'
'Leptofauchea nitophylloides GWS032631'
'Leptofauchea pacifica JD032'
'Leptofauchea pacifica GWS010140'
'Leptofauchea coralligena OCD1501'
'Leptofauchea munseomica GWS018532'
'Leptofauchea munseomica GWS018537'
'Leptofauchea munseomica GWS018674'
'Leptofauchea munseomica GWS018533'
'Leptofauchea cocosana GWS037755'
'ARS09895 Leptofauchea sp.'
'ARS10228 Leptofauchea sp.'
'ARS09905 Leptofauchea sp.'
'ARS09896 Leptofauchea sp.'
'ARS09900 Leptofauchea sp.'
'ARS09907 Leptofauchea sp.'
'ARS09909 Leptofauchea sp.'
'Leptofauchea earleae LAF-26-5-00-1-1'
'ARS10013 Leptofauchea sp.'
'Leptofauchea leptophylla TNS-AL-174121'
'Leptofauchea leptophylla GWS018540'
'Leptofauchea leptophylla GWS018362'
'Leptofauchea leptophylla GWS018363'
'ARS10068 Leptofauchea sp.'
'ARS09892 Leptofauchea sp.'
'ARS09891 Leptofauchea sp.'
'Leptofauchea sp. 1WA G0400'
'ARS09908 Leptofauchea sp.'
'ARS09901 Leptofauchea sp.'
'ARS09770 Leptofauchea sp.'
100/1
94/1
100/1
100/1
100/1
57/0.9
100/1
97/1
62/0.57
100/1
63/0.93
100/1
95/0.99
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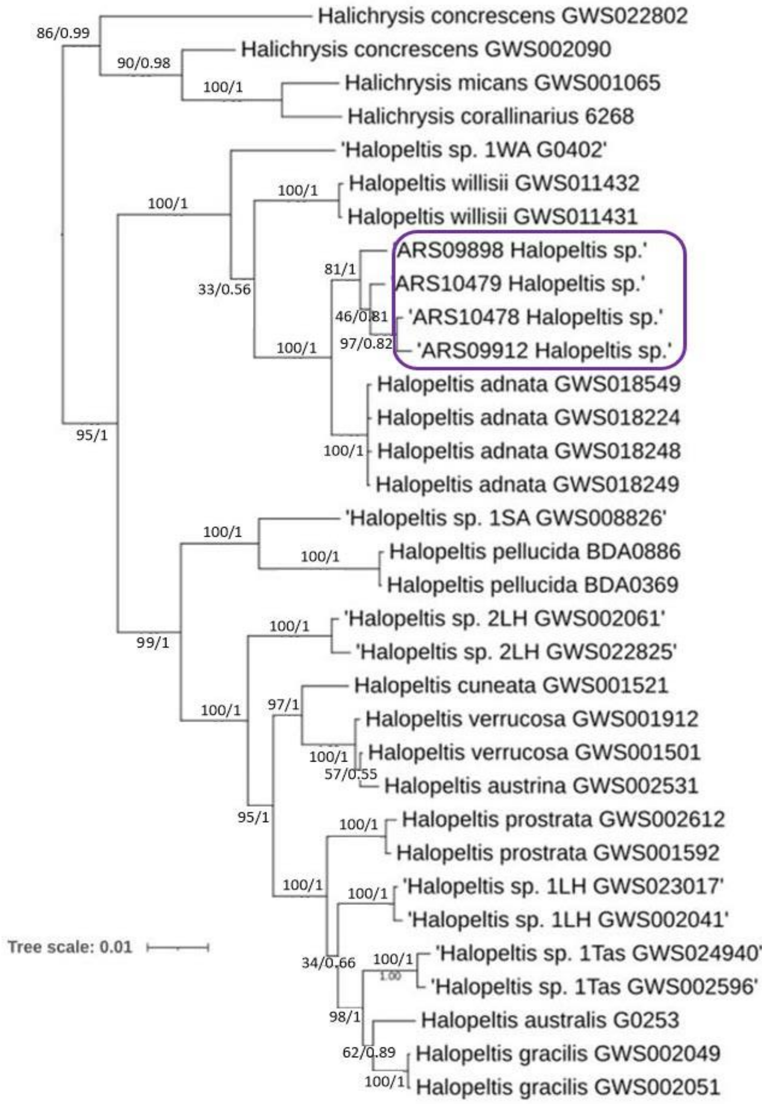
83/1
36/0.56
100/1
97/1
63/0.97
50/0.55
Tree scale: 0.01

Figure 2.1


Halichrysis concrescens GWS022802
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Halichrysis micans GWS001065
Halichrysis corallinarius 6268
'Halopeltis sp. 1WA G0402'
Halopeltis willisii GWS011432
Halopeltis willisii GWS011431
ARS09898 Halopeltis sp.'
ARS10479 Halopeltis sp.'
'ARS10478 Halopeltis sp.'
'ARS09912 Halopeltis sp.'
Halopeltis adnata GWS018549
Halopeltis adnata GWS018224
Halopeltis adnata GWS018248
Halopeltis adnata GWS018249
'Halopeltis sp. 1SA GWS008826'
Halopeltis pellucida BDA0886
Halopeltis pellucida BDA0369
'Halopeltis sp. 2LH GWS002061'
'Halopeltis sp. 2LH GWS022825'
Halopeltis cuneata GWS001521
Halopeltis verrucosa GWS001912
Halopeltis verrucosa GWS001501
Halopeltis austrina GWS002531
Halopeltis prostrata GWS002612
Halopeltis prostrata GWS001592
'Halopeltis sp. 1LH GWS023017'
'Halopeltis sp. 1LH GWS002041'
'Halopeltis sp. 1Tas GWS024940'
'Halopeltis sp. 1Tas GWS002596'
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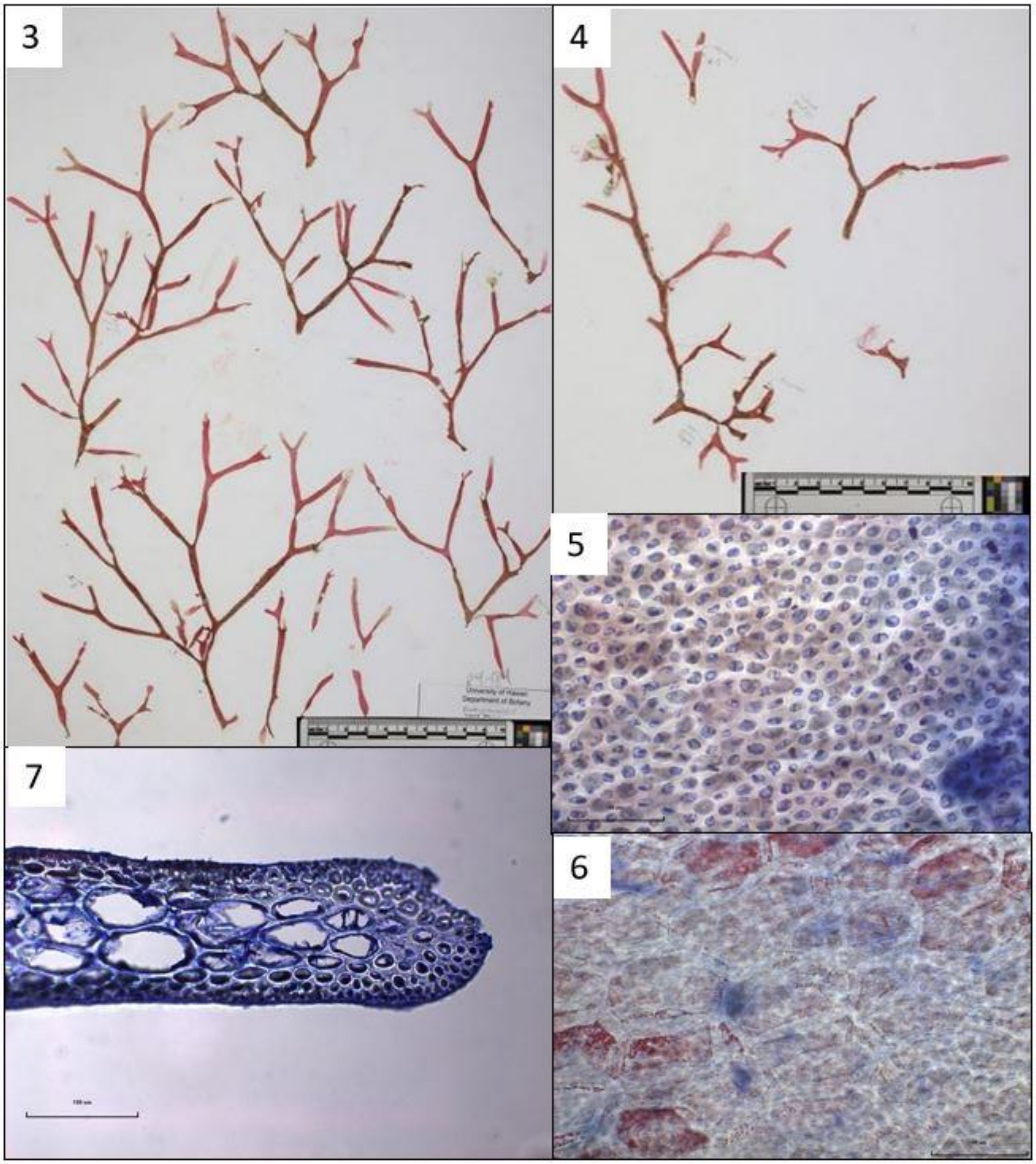
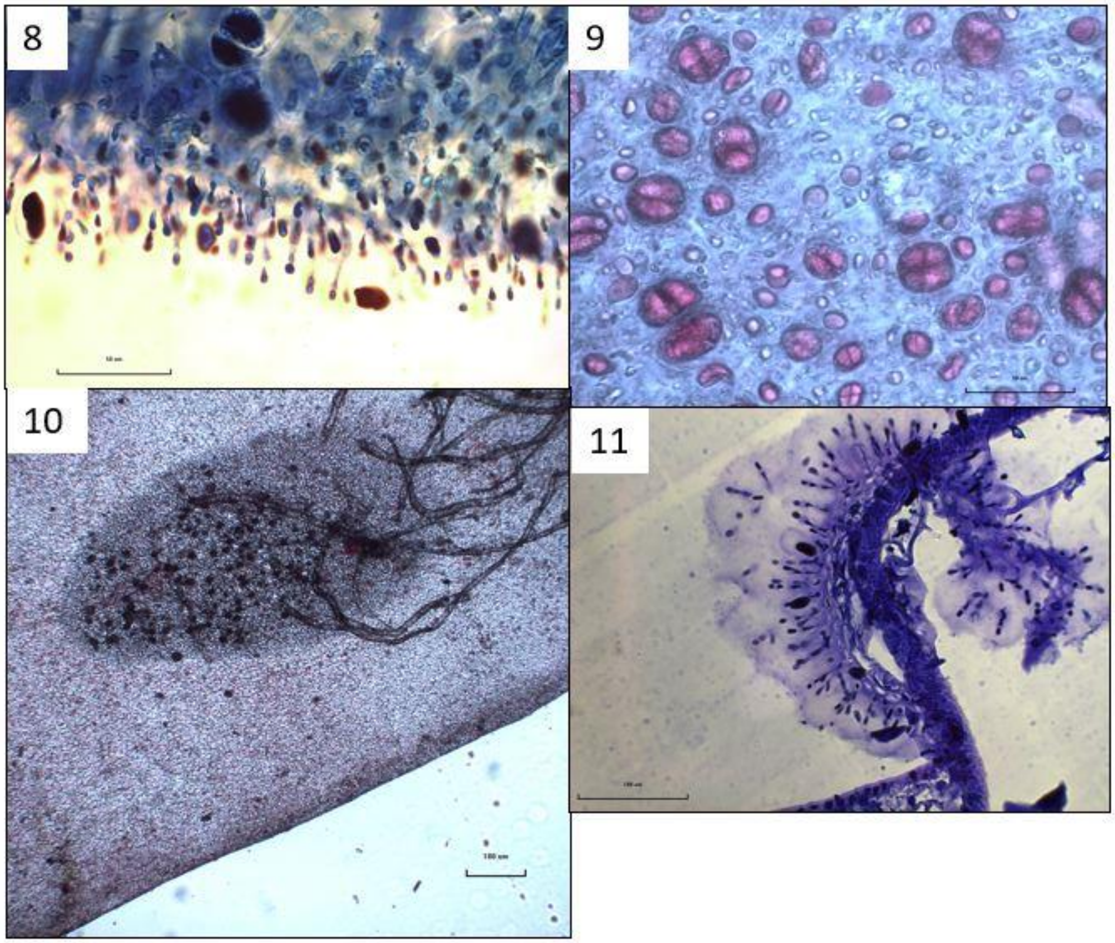
Halopeltis gracilis GWS002049
Halopeltis gracilis GWS002051
Tree scale: 0.01

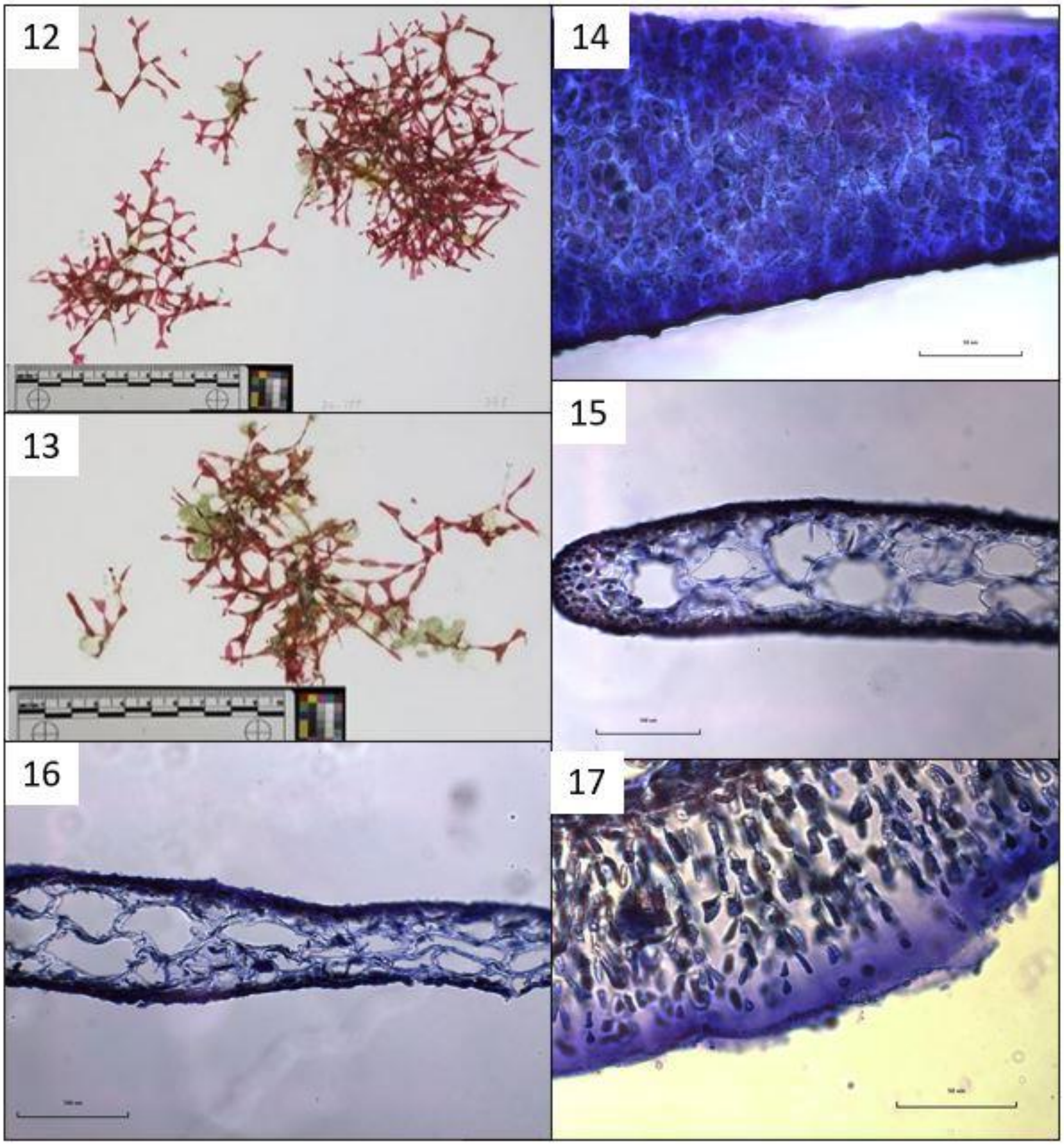
Figure 2.2



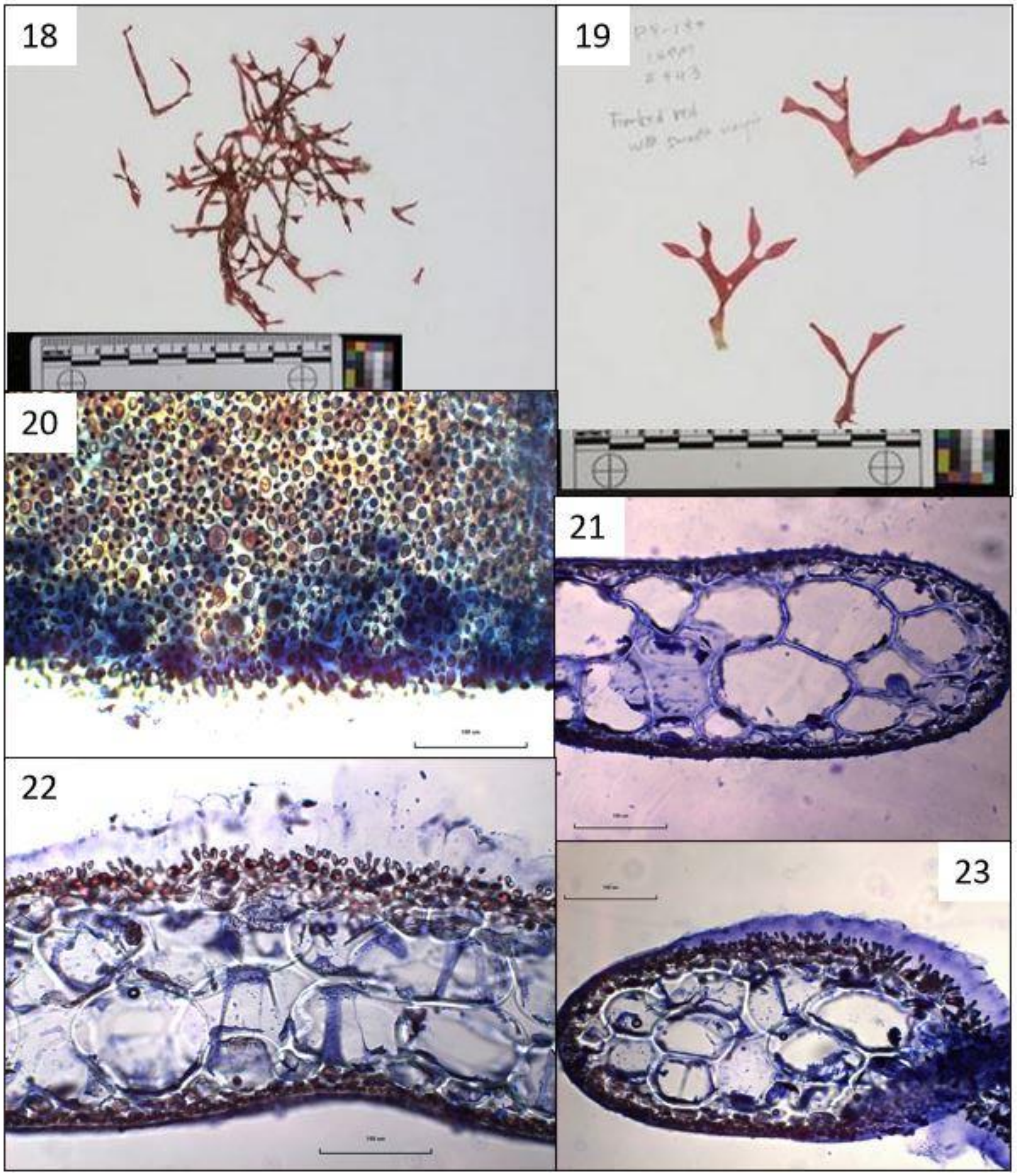
Figures 3–7



Figures 8–11



Figures 12–17



Figures 18–23

Table 2.1. List of species used for phylogenetic analysis of *Leptofauchea* accessed from Genbank or sequenced for this study

Species	Voucher	COI-5P	*rbcL*	LSU
Leptofauchea chiloensis Dalen & G. W. Saunders	GWS000503	ND	DQ873285	KR140338
Leptofauchea cocosana Filloramo & G. W. Saunders	GWS037755	KR140330	KR140334	KR140340
Leptofauchea coralligena Rodríguez-Prieto & De Clerck	OCD1501	ND	EU418774	ND
Leptofauchea earleae Gavio & Fredericq	LAF-26-5-00-1-1	ND	ND	HQ400570
Leptofauchea leptophylla (Segawa) Mas. Suzuki, Nozaki, R. Terada, Kitayama, Tetsu. Hashimoto & Yoshizaki	GWS018362	KR140329	KR140333	ND
	GWS018363	KR140328	ND	ND
	GWS018540	KR140327	ND	KR140336
	TNS-AL-174121	ND	AB677823	AB693120
Leptofauchea munseomica Filloramo & G. W. Saunders	GWS018532	HQ544094	KR140331	KR140337
	GWS018533	HQ544095	ND	ND
	GWS18537	HQ544099	ND	ND
	GWS018674	HQ544137	ND	ND
Leptofauchea nitophylloides (J. Agardh) Kylin	GWS032631	KR085173	KR085179	KR085190
Leptofauchea pacifica E. Y. Dawson	JD032	AY970566	DQ873286	ND
	GWS010140	HM916176	ND	KR085195
Leptofauchea lucida	G0400	HM915831	DQ873287	KR085194
Leptofauchea sp. (blue)	ARS09895		X	X
	ARS09896	X	X	X
	ARS09900	X	X	X
	ARS09905	X	X	
	ARS09907	X	X	X
	ARS10228	X	X	X
Leptofauchea lucida Huisman & Saunders	ARS09891		X	X
	ARS09892		X	X
	ARS09770		X	
	ARS09901		X	X
	ARS09908		X	X
	ARS10068		X	
Leptofauchea sp. (yellow)	ARS10013	X	X	X
Leptofauchea sp. (red)	ARS09897	X	X	X
Webervonbossea sp._1splachnoides	GWS016349	HM918169	KR085175	KR085185
Webervanbossea sp._1TAS	GWS015310	HM917690	KR085182	KR085193
Webervanbossea splachnoides (Harvey) De Toni	GWS002435	HM916011	DQ873288	KR085186
Webervanbossea tasmanensis Womersley	GWS000922	HM915887	ND	KR085196
	GWS029664	KR085171	KR085178	ND

Table 2.2. List of species used for phylogenetic analysis of *Halopeltis* accessed from Genbank or sequenced for this study

Species	Voucher	COI-5P	*rbcL*	LSU
Halichrysis concrescens (J. Agardh) DeToni	GWS002090	ND	DQ343672	ND
	GWS022802	JQ907536	ND	JQ907551
Halichrysis corallinaria D. L. Ballant., G. W. Saunders & H. Ruiz	6268	AY970628	DQ343674	JQ858279
Halichrysis micans (Hauptfl.) P. Huvé	GWS001065	EF101937	DQ343673	JQ858278
Halopeltis adnata (Okamura) G. W. Saunders et C. W. Schneid.	GWS018224	JQ907537	ND	ND
	GWS018248	JQ907539	ND	ND
	GWS018249	JQ907538	JQ907563	JQ907552
	GWS018549	HQ544106	ND	ND
Halopeltis australis (J. Agardh) G. W. Saunders	G0253	HM033026	EU624152	JQ858281
Halopeltis cuneata (Harv.) G. W. Saunders	GWS001521	HM033032	HM033164	JQ858283
Halopeltis gracilis G. W. Saunders	GWS002049	HM033033	HM033165	ND
	GWS002051	HM915982	ND	JQ907554
Halopeltis pellucida C. W. Schneid. et G. W. Saunders	BDA0369	HQ933375	JQ907565	JQ907555
	BDA0886	JQ907541	ND	ND
Halopeltis prostrata G. W. Saunders	GWS001592	HM033044	HM033166	JQ858280
Halopeltis sp._1LH	GWS002041	EF101938	DQ343678	ND
	GWS023017	JQ907542	ND	JQ907556
Halopeltis sp._1SA	GWS008826	HM918824	JQ907562	JQ907549
Halopeltis sp._1Tas	GWS002596	HM033050	HM033167	ND
	GWS024940	JQ907543	ND	JQ907557
Halopeltis sp._1WA	G0402	EF101939	DQ343679	ND
Halopeltis sp._2LH	GWS002061	HM033051	HM033168	ND
	GWS022825	JQ907544	ND	JQ907558
Halopeltis verrucosa (Womersley) G. W. Saunders	GWS001501	HM033062	HM033169	ND
	GWS001912	HM033060	ND	JQ858282
Halopeltis willisii Freshwater et G. W. Saunders	GWS011431	HM915686	HQ907564	JQ907553
	GWS011432	JQ907540	ND	ND
Halopeltis sp. (purple)	ARS09898	X	X	
	ARS09912	X	X	
	ARS10478		X	
	ARS10479	X	X	X