Phylogeography of *Amansia glomerata* C.Agardh (Ceramiales, Rhodomelaceae) in Hawai'i: A single species with high divergence

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ABSTRACT

Algal biogeography in Hawai'i has not been studied in detail for many species. The islands are home to swift-current deep-water channels separating suitable habitats. The ability of these channels to act as barriers to dispersal has been studied in several animal lineages and is assessed here using the widespread red alga *Amansia glomerata* C.Agardh. A variety of analytical techniques based on 129 mitochondrial COI barcoding sequences collected across *c.* 2500 km were used to assess the genetic diversity of *A. glomerata* in Hawai'i. Haplotype network analyses demonstrated that the species is split into four main lineages which overlap in large parts of their range, yet there is insufficient support to recognize the lineages as separate species. Measures of haplotype diversity, nucleotide diversity, and neutrality tests suggest that at least three of these lineages have undergone recent population expansion. Biogeographic barriers were found to largely match those of marine animal groups in the archipelago. No evidence was found for distinct haplotypes or lineages between shallow and mesophotic reefs. A number of potential collection locations are suggested for the 1822 lectotype of the species, which was included in the molecular analyses. Potential scenarios leading to observed diversity patterns in the archipelago are presented. *Amansia glomerata* exhibits a high degree of haplotypic variation in Hawai'i, suggesting it may exhibit vast molecular divergences across its broader range, which extends from Hawai'i to southeastern Africa.

KEY WORDS
Tropical Pacific,
haplotypes,
lineages,
lectotype,
mesophotic.

RÉSUMÉ
*Phylogéographie d'*Amansia glomerata *C.Agardh (Ceramiales, Rhodomelaceae) à Hawai'i: une seule espèce à forte divergence.*
La biogéographie des algues à Hawai'i n'a pas été étudiée en détail pour de nombreuses espèces. Les îles abritent des chenaux rapides d'eau profonde séparant les habitats appropriés. La capacité de ces chenaux à agir comme des barrières à la dispersion a été étudiée dans plusieurs lignées animales et est évaluée ici à l'aide de l'algue très répandue *Amansia glomerata* C.Agardh. Une variété de techniques analytiques basées sur 129 séquences de code-barres COI mitochondriales collectées sur environ 2500 km ont été utilisées pour évaluer la diversité génétique d'*A. glomerata* à Hawai'i. Les analyses du réseau d'haplotypes ont démontré que l'espèce est divisée en quatre lignées principales qui se chevauchent dans de grandes parties de leur aire de répartition, mais il n'y a pas suffisamment de soutien pour reconnaître les lignées comme plusieurs espèces. Les mesures de la diversité des haplotypes, de la diversité des nucléotides et des tests de neutralité suggèrent qu'au moins trois de ces lignées ont subi des expansions démographiques récentes. Les barrières biogéographiques correspondent largement à celles des groupes d'animaux marins de l'archipel. Aucune preuve n'a été trouvée pour des haplotypes ou des lignées distincts entre les récifs peu profonds et mésophotiques. Un certain nombre de lieux de collecte potentiels sont suggérés pour le lectotype de l'espèce de 1822, qui a été inclus dans les analyses moléculaires. Des scénarios potentiels conduisant à des modèles de diversité observés dans l'archipel sont présentés. *Amansia glomerata* présente un degré élevé de variation haplotypique à Hawai'i, ce qui suggère qu'il peut présenter de vastes divergences moléculaires sur son aire de répartition plus large, qui s'étend d'Hawai'i au sud-est de l'Afrique.

MOTS CLÉS
Pacifique tropical,
haplotypes,
lignées,
lectotype,
mésophotique.

INTRODUCTION

Biogeographic patterns inform our understanding of dispersal and natural history (Assis *et al.* 2022), explain the observed diversity of life (Bowen *et al.* 2016), and have implications for ecosystem management (Channell & Lomolino 2000). Assessment of the strength of barriers to dispersal that shape biogeographic patterns is essential for policy makers and stock managers who must define the appropriate spatial scales for management of marine ecosystems (Toonen *et al.* 2011). As the boundaries of marine ecosystems are typically not clear, management must consider the processes underlying dispersal and connectivity in order to adequately protect marine resources.

In the Hawaiian Archipelago, there is evidence that strong currents and winds in certain channels act as geographic barriers for various marine animal groups (Toonen *et al.* 2011). Namely, the 'Alenuihāhā channel between the islands of Hawai'i and the Maui Nui island complex, the Ka'ie'ie Waho Channel channel between the islands of O'ahu and Kaua'i, the separation between the Main Hawaiian Islands (MHI) and the Papahānaumokuākea Marine National Monument (PMNM), and the barrier between the far islands of the PMNM and the remainder of the group constitute significant barriers to dispersal (Toonen *et al.* 2011; Wren *et al.* 2016; Friedlander *et al.* 2020). Largely excluded thus far from studies on taxonomic breaks between inter-island intraspecies populations are the algae. Algae are essential components of benthic systems, contributing to primary production and sustaining reef food webs (Hilting *et al.* 2013). There is potential for geographic barriers to differ in red algae from those of the animal groups as they have different life histories and are non-flagellated in all

life stages (Norris 1988; Santelices 1990). Marine fauna and flora in the Hawaiian Archipelago must have either arrived from elsewhere or diversified locally (Bowen *et al.* 2013). One of the major introduction pathways of marine introductions to Hawai'i is suggested to be from island-hopping across the Pacific Ocean, eventually making their way north to the Line Islands and from there into Hawai'i (Craig *et al.* 2010). Johnston Atoll has also been suggested as a source of material into Hawai'i, focusing on the island of Lalo (French Frigate Shoals) in PMNM (Grigg 1981; Kobayashi 2006).

The red alga *Amansia glomerata* C.Agardh is an ideal model taxon for the study of Hawaiian marine phylogeography. It is widespread in tropical to warm-temperate waters, found from the coasts of Eastern Africa, Southeast Asia, Asia, Australia, New Zealand, Japan, and throughout the Pacific Islands (Guiry & Guiry 2022). In Hawai'i it is typically abundant (Doty 1971), perennial (Abbott 1999), provides food for herbivores including turtles (Miyal & Balazs 1993), forms expansive monospecific stands, and hosts diverse invertebrate populations (Russo 1989). Furthermore, the species was described from the "Sandwich Islands" (Hawai'i) and a mitochondrial cytochrome c oxidase subunit I (COI) sequence of the lectotype is available (Sherwood *et al.* 2011). Preliminary research into the molecular and morphological diversity of *A. glomerata* has raised a number of questions that remain unresolved. Sherwood *et al.* (2011) investigated the molecular signatures of the tribe Amansieae in the Hawaiian Islands and suggested the taxon may warrant splitting after more extensive molecular work. Of the markers used by Sherwood *et al.* (2011), COI was the most informative. Estimates of genetic divergence based on COI by Sherwood *et al.* (2011) demonstrated a divergence of up to 3.6% (22 out of 603 bp) between

lineages of *A. glomerata*, falling roughly between those values typical of intraspecific variation and interspecific variation. Typical intraspecific thresholds for COI in Rhodophyta are 0.0-0.7% while interspecific variation can be very broad from 2.6-10.5% (Saunders 2005; Robba *et al.* 2006; Clarkston & Saunders 2013; Savoie 2017).

Mesophotic coral ecosystems (MCEs) are located between 30 and 150 m depths in the tropics and subtropics, and represent areas where sampling has historically been minimal (Hinderstein *et al.* 2010). Given that *A. glomerata* is observed in the Hawaiian Archipelago from intertidal to mesophotic depths, patterns of distribution for haplotypes may inform connectivity dynamics across this potential threshold. Recent evidence suggested that MCEs may not act as a refugia for shallow water species under a warming climate as in order to act as a refugia the species must be distributed in both shallow and MCE habitats (Pyle *et al.* 2016; Smith *et al.* 2016) Further, several algal species unique to Hawaiian MCEs lacking shallow water representation have been described in recent years (Alvarado *et al.* 2022; Cabrera *et al.* 2022; Paiano *et al.* 2022; Sherwood *et al.* 2022). Conversely, a number of algal species do straddle this range, including *A. glomerata*, yet connectivity between these habitats remains unknown.

This study aims to: 1) investigate the phylogeography of *A. glomerata* in Hawai'i; 2) assess whether recognizing *A. glomerata* as multiple species in the Hawaiian Archipelago is warranted; and 3) investigate connectivity between shallow reefs and MCEs.

MATERIAL AND METHODS

In total 85 samples were collected and sequenced from 30 unique locations (Appendix 1). An additional 10 were subsampled from the Bishop Museum's Herbarium Pacificum (BISH). Fragments of vegetative plants were cleaned of epiphytes and then stored in silica gel. Genomic DNA was extracted following a modified cetyltrimethylammonium bromide (CTAB) protocol (Doyle & Doyle 1989) with a CTAB buffer and β-mercaptoethanol as a grinding solution. Samples were kept at 65°C overnight and purified using a 24:1 chloroform-isoamyl alchohol solution, then stored at -20°C overnight with 95% ethanol to precipitate DNA before extractions were completed using Qiagen (Valencia, California, United States) QIAquick spin columns, wash buffers AW1 and AW2, and elution buffer AE.

The mitochondrial COI region was amplified following Saunders (2005) with the GazF1 and GazR1 primers. Primers were designed for samples failing amplification: Amansia_GazF (5'-GGTTCAAATCCCGCCTCTCT-3') and Amansia_GazR (5'-TGCAAATACAGCACCCATTGA-3'), amplifying a larger segment surrounding the COI gene than GazF1, *c.* 790 bp. Amplification followed that of Saunders (2005) with the annealing temperature adjusted to 57°C. Successful PCR amplifications were confirmed using gel electrophoresis and were purified with ExoSAP-IT (Affymetrix, Santa Clara, California, United States) before submission to GENEWIZ (Azenta Corporation South Plainfield, New Jersey, United States) for Sanger sequencing. Forward and reverse sequencing reads were assembled in Geneious Prime 2021.1 (https://www.geneious.com) and consensus sequences were BLAST searched on the National Center for Biotechnology Information (NCBI) database to ensure there was no contamination. Consensus sequences were aligned using the MUSCLE 3.8.425 plugin (Edgar 2004) in Geneious Prime. An additional 34 samples, including the *A. glomerata* lectotype, were downloaded from GenBank to create a final alignment of 129 sequences of 597 bp. Sequences generated in this study were uploaded to GenBank and assigned accession numbers OQ079250-OQ079366 (Appendix 1).

Lineage designation was conducted in R (R Core Team 2021) using the rmaverick library (Verity 2018). Rmaverick is a generalized thermodynamic integration algorithm for generating population structure and numbers of sub-populations (K), and is considered more accurate than STRUCTURE (Pritchard *et al.* 2000) when dealing with relatively small data sets. FASTA files were converted to the necessary .str file format using xmfaconvertor and xmfa2struct. Markov Chain Monte Carlo (MCMC) runs were conducted for K = 1-6 with 10 000 samples and 1000 discarded as burn-in on 50 rungs with a convergence test every 100 generations. FASTA files were imported to R using the adegenet library (Jombart 2008; Jombart & Ahmed 2011) and haplotypes were assigned and plotted using the HaploNet function in the pegas library (Paradis 2010). Designations from the rmaverick analysis informed the boundaries between each lineage. Differences between lineages were calculated using an alignment file in Geneious. The best fit model for tree estimation was assessed using jModelTest2 on XSEDE via the CIPRES gateway (Miller *et al.* 2011). The best fit model for the alignment, GTRGAMMAI, was used in a Bayesian phylogenetic reconstruction on four threads on the University of Hawai'i High Performance Computing cluster (HPC) (https://datascience.hawaii.edu/hpc/). Bayesian analyses were conducted using MrBayes 3.2.7 (Ronquist *et al.* 2012) using 1 500 000 generations and four chains of Metropolis-coupled MCMC with sampling every 1000 generations. The alignment included three sequences of *Amansia fimbrifolia* (R.E.Norris) L.E.Phillips, two sequences of *A. multifida* J.V.Lamouroux, and one sequence of *A. pinnatifada* Harvey as outgroups, together with all COI sequences available for the genus.

A Generalized Yule Mixed Coalescent (GMYC) species delimitation model was run through the R library splits (Ezard *et al.* 2021) on an ultrametric tree containing only unique sequences and without outgroups. Bayesian Poission Tree Process (bPTP) species delimitation was carried out through the online server (Zhang *et al.* 2013; https://species.h-its.org/) on a newick tree containing no outgroups with 100 000 MCMC generations and 10 000 discarded as burn-in. Bayesian Phylogenetics and Phylogeography (BPP; Yang 2015) delimitation was carried out using all 129 sequences on both available species delimitation algorithms (Yang & Rannala 2010) following a tutorial from Flouri *et al.* (2020) without priors under 100 000 MCMC generations with 8000 discarded as burn-in.

Calculations of ΦST were conducted in R using the StrataG library (Archer *et al.* 2016) using pairwise tests with 10 000 iterations. Samples were split into their respective island groups to test the strength of inter-island channels as barriers to dispersal. Due to variable sampling, and to test only the strength of major barriers, several groupings were necessary. Samples from the islands of Maui, Kahoʻolawe, Lānaʻi, and Molokaʻi were grouped together as Maui Nui while Kauaʻi and Niʻihau samples were combined into a single group and those from PMNM were treated similarly. Genetic structure differences were calculated using the pegas and strataG libraries in R. Comparisons within and between lineages were reliant on Nei & Tajima's (1981) model. Tajima's D (Tajima 1989) and Fu's Fs (Fu 1997) were calculated to test for population expansion.

ABBREVIATIONS

BISH	Bishop Museum's Herbarium Pacificum;
BPP	Bayesian Phylogenetics and Phylogeography;
bPTP	Bayesian Poisson Tree Process;
COI	Cytochrome C Oxidase Subunit I;
CTAB	Cetyltrimethylammonium bromide;
GMYC	Generalized Yule Mixed Coalescent;
HPC	University of Hawaiʻi High Performance Computing cluster;
MCE	Mesophotic Coral Ecosystem;
MCMC	Markov Chain Monte Carlo;
MHI	Main Hawaiian Islands;
NCBI	National Center for Biotechnology Information;
PMNM	Papahānaumokuākea Marine National Monument.

RESULTS

In total, 129 sequences were assigned to 35 unique haplotypes (Fig. 1; Appendix 1). These were grouped into four subgroups by selecting the most probable value (K = 4) from the rmaverick analysis. Each subgroup forms a continuous line of descent in the bayesian phylogeny and thus each subgroup is termed a lineage (Fig. 2). Of the four lineages presented here, three were described by Sherwood *et al.* (2011) and we separate Lineage 4 from Lineage 1 based on its assignment by rmaverick as a distinct group. Lineages 2 and 3 were equidistant from the central lineage, 4, while Lineage 1 was closer to Lineage 4 (Fig. 1). The divergence between lineages was reduced from the 2.3-3.6% reported by Sherwood *et al.* (2011) to 1.3-2.3% through inclusion of several intermediate haplotypes.

The COI haplotype network revealed 35 distinct haplotypes corresponding to 16 singletons and four haplotypes with greater than 10 observations (Fig. 1). The genetic distance of haplotypes within a single lineage ranged from 1-9 bp (0.17-1.51%). Lineages 1, 2, and 4 exhibited high haplotypic diversity (h = 0.6732-0.8609) and low nucleotide diversity (π = 0.0022-0.0054) while Lineage 3 displayed low values for both parameters (h = 0.4837; π = 0.0043). Tajima's D and Fu's F_s were negative for Lineages 1, 2, and 4 while in Lineage 3 Tajima's D was negative and Fu's F_s positive (Table 1).

Mapping the four lineages of *A. glomerata* in Hawaiʻi revealed that some lineages were separated geographically as Lineage 3 was unique to PMNM and Lineage 4 was unique to the southeastern coastline of the island of Hawaiʻi (Fig. 3). The remaining Lineages, 1 and 2, were distributed throughout the MHI showing no clear pattern. Hawaiʻi Island and Oʻahu exhibited the highest genetic diversity (h = 0.8266-0.9012) followed by Maui Nui (h = 0.6157), PMNM (h = 0.4191), and Kauaʻi (h = 0.3333). All regions exhibited low nucleotide diversity (π < 0.0164) and the number of collected samples varied by location (Table 2). Three of these haplotypes occurred on multiple island groupings, H6 (L1), H10 (L1), and H29 (L2). Haplotype six (H6) in Lineage 1 was the best represented in the archipelago, with 23 sequences, and was found on the Kona and Waʻianae coasts on the western sides of the islands of Hawaiʻi and Oʻahu. It was not the only haplotype on these coasts, however. In Kona, several other haplotypes in Lineage 1 were present, while the Waiʻanae coast not only presented other haplotypes in Lineage 1 but several haplotypes in Lineage 2. Other coastlines with representation from both Lineages 1 and 2 include the north, south, and east sides of Oʻahu, and the western end of Molokaʻi. The haplotype with the highest representation in Lineage 2 was H29, found on the northern half of the MHI on the islands of Molokaʻi, Oʻahu, Kauaʻi, and Niʻihau. The most common haplotype in Lineage 3 was H16, found on Manawai (Pearl and Hermes Atoll), Kapou (Lisianski), and Kamole (Laysan).

Pairwise $Φ_{ST}$ values were significant across all boundaries tested with one exception (Table 3). From south to north these boundaries were: the ʻAlenuihāhā channel between the islands of Hawaiʻi and the Maui Nui island complex ($Φ_{ST}$ = 0.59; p < 0.01), the Kaʻiwi channel between the Maui Nui island complex and Oʻahu ($Φ_{ST}$ = 0.29; p < 0.01), the Kaʻieʻie Waho Channel between the islands of Oʻahu and Kauaʻi ($Φ_{ST}$ = 0.16; p = 0.07), and the separation between the MHI and PMNM ($Φ_{ST}$ = 0.87; p < 0.01).

Phylogenies including all available sequences of COI for the genus *Amansia* showed full support for each species and confirmed that the species *A. glomerata* as well as all of the lineages within the species form monophyletic groupings (Fig. 2). GMYC species delimitation analysis revealed three entities, grouping Lineages 1 and 4 together while designating the others as their own entities. Species delimitation with bPTP yielded 13 entities, delimiting all four lineages as distinct with several entities delimited within lineages while BPP revealed one entity for all sequences (Fig. 2).

Sequences were generated from 11 mesophotic specimens which were not unique to this zone. Mesophotic specimens in Lineage 1 were collected from south Oʻahu (H10, H11) and west Molokaʻi (H10) and these two haplotypes are also represented by shallow water representatives from south Oʻahu. Lineage 2 contained two mesophotic representatives both in the same haplotype (H29) that were collected from Honolulu and Niʻihau, *c.* 250 km apart. The haplotype is well represented in shallow water with 12 sequences from Kauaʻi, Oʻahu, and Maui Nui. Lineage 3 contained one mesophotic haplotype, H16, which was collected from the islands of Manawai (Pearl and Hermes Atoll), Kapou (Lisianski), and Kamole (Laysan) in PMNM and was also found in shallow water on Manawai.
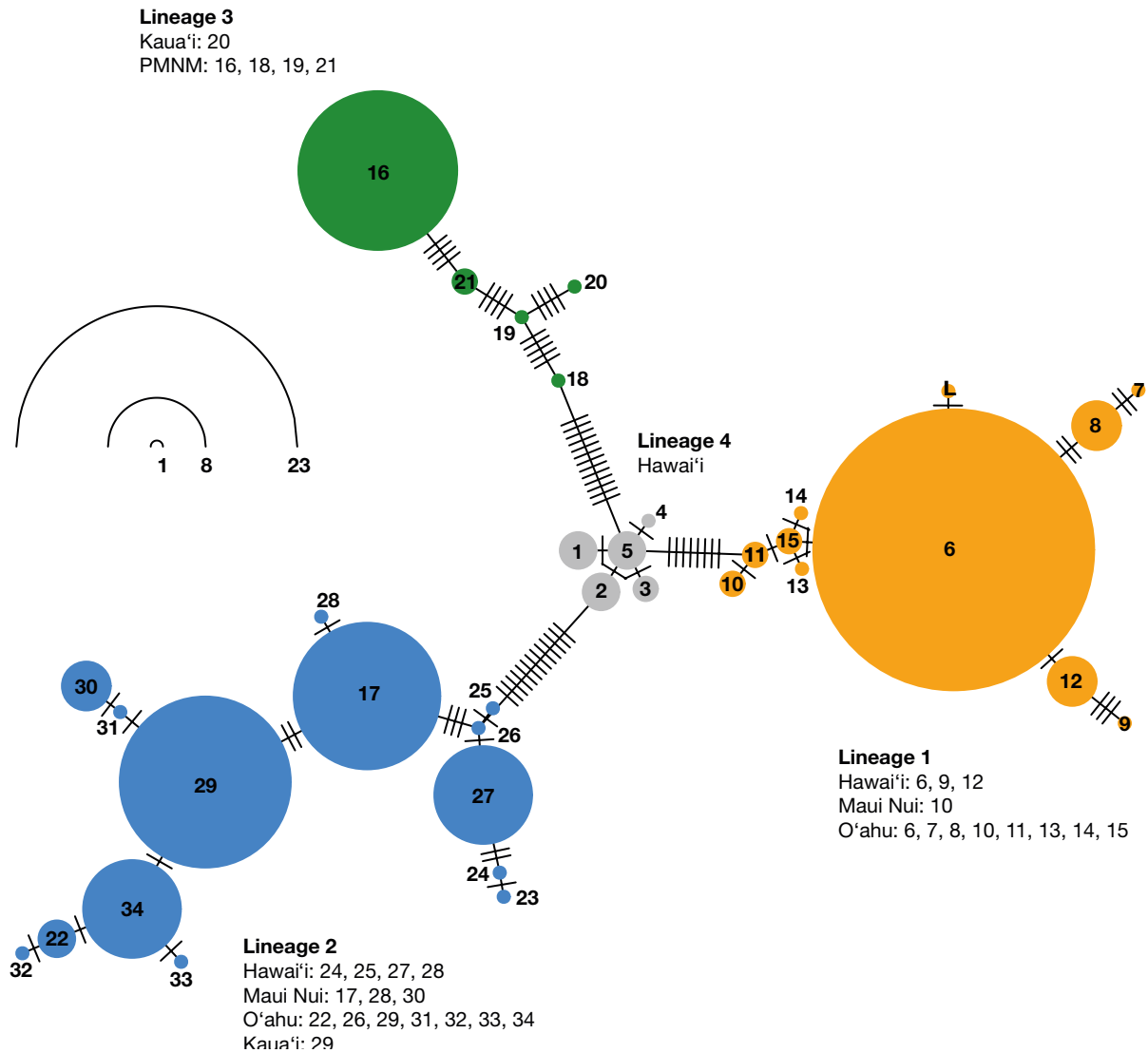
Lineage 3
Kaua'i: 20
PMNM: 16, 18, 19, 21

Lineage 4
Hawai'i

Lineage 1
Hawai'i: 6, 9, 12
Maui Nui: 10
O'ahu: 6, 7, 8, 10, 11, 13, 14, 15

Lineage 2
Hawai'i: 24, 25, 27, 28
Maui Nui: 17, 28, 30
O'ahu: 22, 26, 29, 31, 32, 33, 34
Kaua'i: 29

Fɪɢ. 1. — Haplotype network of *Amansia glomerata* C.Agardh from the Hawaiian Islands based on the mitochondrial COI region, including 129 samples. Each **circle** represents a unique haplotype and is scaled to size based on the number of sequences assigned to it. **Colors** are indicative of thermodynamically assigned subgroups and phylogenetically supported lineage designations: **orange**, Lineage 1; **blue**, Lineage 2; **green**, Lineage 3; and **gray**, Lineage 4. Haplotypes and lineages are arbitrarily numbered. Collection locations for each haplotype are listed alongside each lineage. The lectotype for *A. glomerata* is labeled **L**. Each **perpendicular line** bisecting the connecting lines between adjacent haplotypes represents one nucleotide difference. Haplotype numbers correspond to those in Appendix 1.

The lectotype for *A. glomerata* did not match any of the sequences from this analysis. It was, however, one bp divergent from the largest haplotype in Lineage 1 (H6) and this divergence was due to an ambiguous base code in the lectotype sequence. This haplotype includes collections from the west of Hawai'i island as well as the west and north shores of O'ahu.

DISCUSSION

Specimens of *A. glomerata* from Hawai'i exhibit a wide range of haplotypic variability, splitting into four lineages (Figs 1; 2). The divergence between each lineage was reduced from the 2.6-3.6% reported by Sherwood *et al.* (2011) to 1.3-2.3% through the inclusion of several intermediate haplotypes. As more samples are collected, missing haplotypes are filled

in, a process yielding results similar to those of other species (e.g. Dijoux *et al.* 2014). *Amansia glomerata* appears to follow a similar pattern, where increased numbers of specimens sequenced yields more lineages and closes the gaps between those lineages.

Measurements of genetic connectivity assessing the strength of barriers to dispersal are largely congruent with those observed in animal groups (Toonen *et al.* 2011). The 'Alenuihāhā channel between the islands of Hawai'i and the Maui Nui island complex, the Ka'iwi channel between the Maui Nui island complex and O'ahu, and the channel between the MHI and PMNM all are significant barriers to dispersal (Table 3). The Ka'ie'ie Waho Channel channel between the islands of O'ahu and Kaua'i is here not a significant barrier despite being the largest of the channels in the MHI and shown to be a barrier to dispersal for animal groups (Toonen *et al.* 2011). Mixing

TABLE 1. — Summary of molecular diversity and demographic analyses for the four lineages of *A. glomerata* C.Agardh from Hawai'i where **h** represents haplotypic diversity, **π** represents nucleotide diversity and **SD** refers to standard deviation.

Lineage	N	Nh	h ± SD	π ± SD	Tajima's D	Fu's Fs
1	41	10	0.6732 ± 0.0780	0.0027 ± 0.0018	-1.4728	-3.3959
2	57	14	0.8609 ± 0.0222	0.0054 ± 0.0031	-0.0378	-2.4312
3	18	5	0.4837 ± 0.1392	0.0043 ± 0.0027	-0.9878	1.2002
4	12	5	0.8485 ± 0.0470	0.0022 ± 0.0016	-0.0974	-1.2702

TABLE 2. — Genetic diversity of populations of *A. glomerata* C.Agardh from Hawai'i. **N** represents the number of individuals assigned to that group, **Nh** is the number of haplotypes observed on that island. **Lineages** lists the lineage numbers observed on that island. Haplotype diversity and standard deviation are given under **h ± SD**; and nucleotide diversity is displayed under **π ± SD**.

Location	N	Nh	Lineages	h ± SD	π ± SD
Hawai'i	44	12	1, 2, 4	0.8266 ± 0.0426	0.0153 ± 0.0080
Maui Nui	20	5	1, 2	0.6157 ± 0.1060	0.00436 ± 0.0027
O'ahu	41	15	1, 2	0.9012 ± 0.0224	0.0164 ± 0.0085
Kaua'i	6	2	2, 3	0.3333 ± 0.2187	0.0112 ± 0.0071
PMNM	17	4	3	0.4191 ± 0.1425	0.0036 ± 0.0024

TABLE 3. — Fst values for *Amansia glomerata* C.Agardh across channels of the Hawaiian Archipelago. Numbers **below** the diagonal are Fst values and those **above**, in *italics*, are p-values. Maui Nui includes samples collected from the islands of Maui, Kaho'olawe, Lāna'i, and Moloka'i. Kaua'i includes samples from Ni'ihau. All samples from Papahānaumokuākea Marine National Monument (PMNM) are included as one group.

Location	Hawai'i	Maui Nui	O'ahu	Kaua'i	PMNM
Hawai'i	–	*<0.001*	*<0.001*	*<0.001*	*<0.001*
Maui Nui	0.59	–	*<0.001*	*1.00*	*<0.001*
O'ahu	0.18	0.29	–	*0.079*	*<0.001*
Kaua'i	0.46	0	0.16	–	*<0.001*
PMNM	0.68	0.94	0.69	0.88	–

across this channel may be facilitated by the North Hawai'i Ridge Current which runs from Southeast to Northwest through most of the year (Qiu *et al.* 1997; Firing *et al.* 1999). The dispersal of organisms across this channel based on oceanographic currents has been demonstrated for fish species with longer pelagic larval durations (Vaz 2012) but may be more complex for the non-motile and negatively buoyant (Norris 1988; Santelices 1990). Toonen *et al.* (2011) suggested the Ka'iwi channel as only a potential barrier as it is a significant barrier to spread for approximately half of the animal species assessed in that study. We found the Ka'iwi channel to be a significant barrier to dispersal for *A. glomerata* yet testing additional species of Rhodophyta across these boundaries is essential for assessing the channels of the Hawaiian Islands as barriers to dispersal for this phylum. The phylogeography of *A. glomerata* across its entire range will additionally inform whether certain lineages are introduced to the archipelago and allow evaluation of potential routes of introduction to Hawai'i.

BPP, GMYC, and bPTP suggest one, three, and 13 entities exist in Hawai'i, respectively (Fig. 2). Consensus among these methods would provide sufficient support for species delimitation as each method must contain simplifications or assumptions of evolutionary parameters and their results must be reconciled (Carstens *et al.* 2013; Dellicour & Flot 2018). Markers from the nucleus and chloroplast are notably less divergent for *A. glomerata* than the mitochondrial COI marker (Sherwood *et al.* 2011). A comparison of these delimitation tools suggests that GMYC and bPTP are prone to false positive entity designation when only one entity exists (Luo *et al.* 2018). Further, species splits should be made conservatively when dealing with cryptic lineages as failing to split species is preferable to false delimitation in most contexts (Carstens *et al.* 2013). As *A. glomerata* is widespread across the Pacific and Indian Oceans it is possible that future sequencing across the range of this species will support splitting yet with currently available sequences there is insufficient evidence to support splitting the species into multiple entities.

Amansia glomerata exhibits shared haplotypes across the mesophotic-shallow threshold and exceptional diversity in the archipelago, following several of the hypotheses suggested by Copus *et al.* (2022) as the habitat persistence hypothesis. The presence of *A. glomerata* in its current diversity of habitats suggests the possibility of being a persistent species with a long diversification period in Hawai'i, using certain parts of its range during periods of climactic variability. Specifically, the species exhibits strong population structure and further mesophotic sampling should be undertaken to assess whether population expansion has also occurred in this more stable habitat (Copus *et al.* 2022). Haplotypes containing samples collected from mesophotic depths in Hawai'i are almost always shared with identical shallow water samples, an indication the mesophotic may act as a refugia for the species (Bongaerts *et al.* 2010; Hinderstein *et al.* 2010; Bongaerts & Smith 2019; Pyle & Copus 2019). Some haplotypes occur at mesophotic
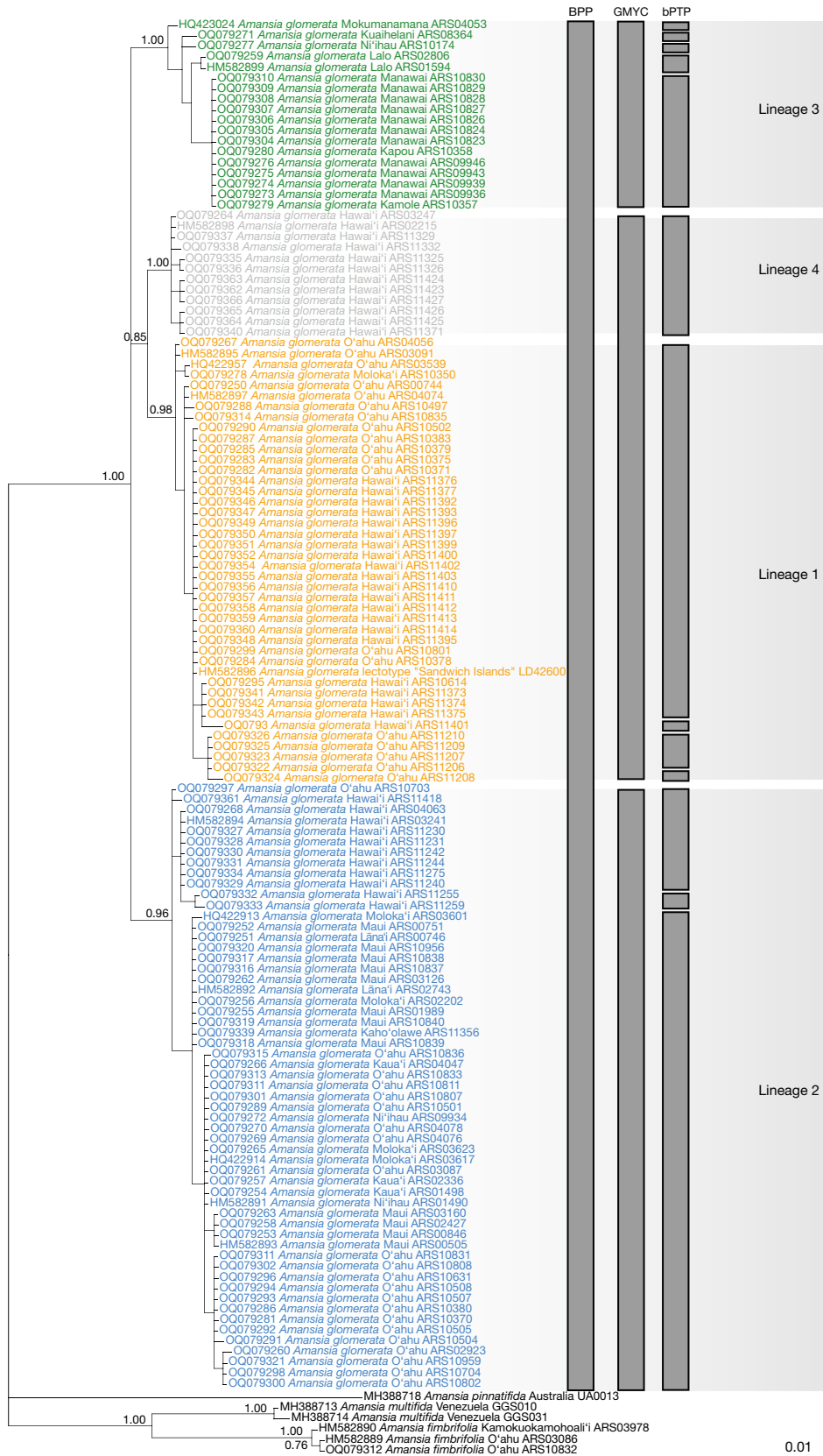
FIG. 2. — Bayesian phylogeny of the genus *Amansia* J.V.Lamouroux based on the mitochondrial COI marker. *Amansia glomerata* C.Agardh makes up a majority of the sequences available for the genus. Lineages of *A. glomerata* are colored **orange**, **blue**, **green**, and **gray** to represent Lineages 1, 2, 3, and 4, respectively (as per Fig. 1). **Bars** represent the results from species delimitation models derived from Bayesian Phylogenetics and Phylogeography (BPP), Generalized Mixed Yule Coalescent (GMYC), and bayesian Poisson Tree Process (bPTP).

FIG. 3. — *Amansia glomerata* C.Agardh specimen sampling locations across the Hawaiian Archipelago. Each **pie graph** is scaled in size to the number of samples from each island or island group. An inset of the Main Hawaiian Islands is included and at a separate scale from the main map. The islands of Maui, Moloka'i, Lāna'i, and Kaho'olawe are grouped together, as are the islands of Kaua'i and Ni'ihau. Channels and Islands of interest are labeled. **Colors** are representative of lineage designations (as per Figs 1; 2). Pie chart scaling does not change in inset map.

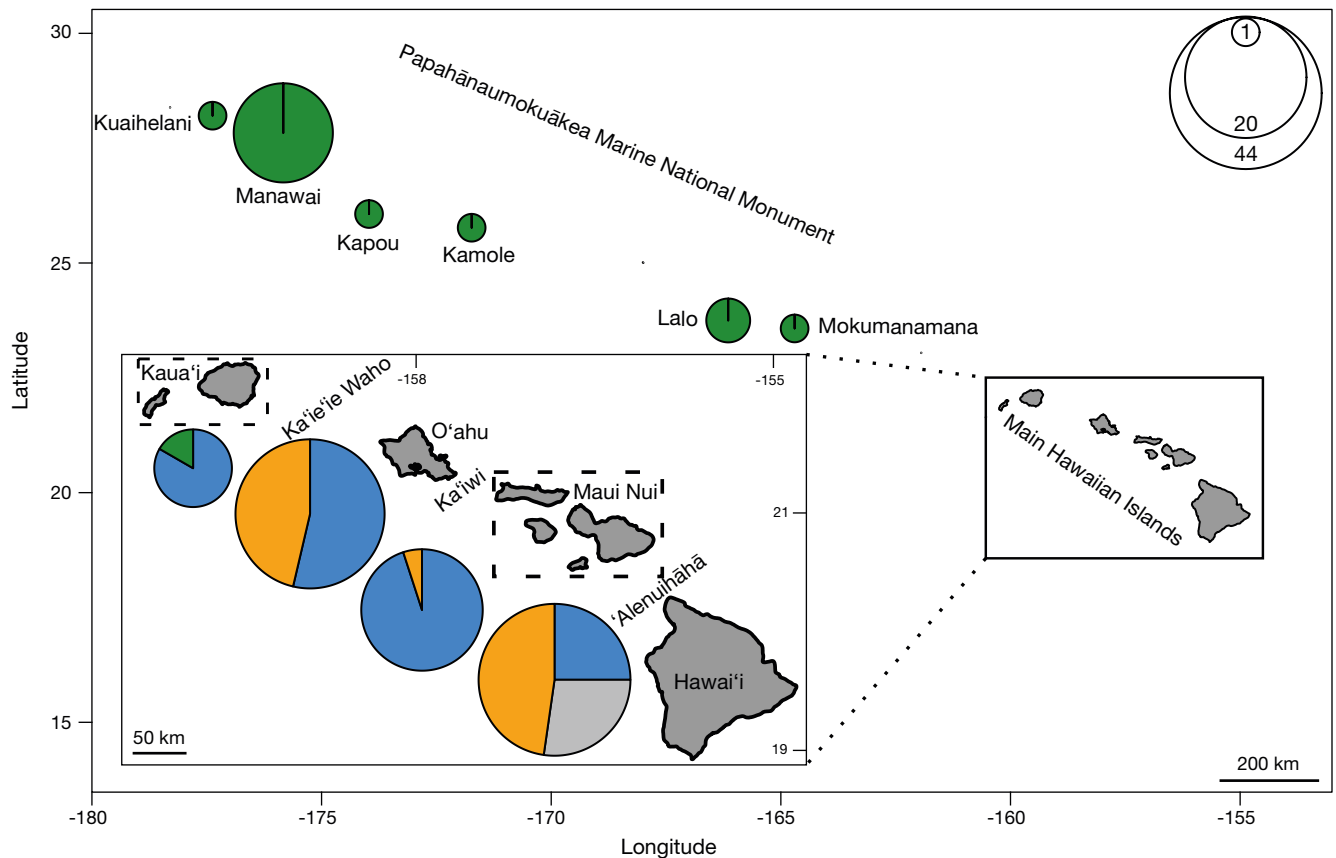depths on several islands separated by great distances. However, the number of mesophotic sequences included in this study (n = 11) implies that mesophotic-shallow comparisons can only be made in a preliminary manner.

The lectotype of *A. glomerata* sampled from the "Sandwich Islands" in 1822 is a member of Lineage 1 (Fig. 1). The sequence falls 1 bp away from the most common haplotype of the lineage, haplotype six, which is broadly distributed on the west and north sides of O'ahu as well as the west side of the island of Hawai'i. As the sequence was generated recently (Sherwood *et al.* 2011) but the lectotype was collected in the early 19th century, it is possible that DNA degradation occurred in this sample (Staats *et al.* 2011; Hughey & Gabrielson 2012; Saunders & McDevit 2012) and led to the ambiguous nucleotide. Samples from H6 exhibited the largest geographic spread of any single haplotype in the MHI. It is therefore possible that the lectotype is a member of haplotype six, and was collected from the islands of Hawai'i or O'ahu.

Amansia glomerata lineages yielded low nucleotide diversity, high haplotypic diversity, and negative values for both Tajima's D and Fu's F_s with the exception of Lineage 3's low haplotypic diversity and positive Fu's F_s (Table 1). For Lineages 1, 2, and 4, the lineages that occur in the MHI, this suggests recent population expansion in Hawai'i, where land

use change and increased nutrient runoff have been recorded over the last several hundred years (Bishop *et al.* 2017). It is unclear whether the abundance of *A. glomerata* in nutrient rich areas of the MHI is dependent on increased nutrient exposure as there is a lack of information on fine scale algal distributions in Hawai'i. Several waves of natural introduction from the Line Islands (Craig *et al.* 2010) or Johnston Atoll (Grigg 1981; Kobayashi 2006) may explain this diversity as well. Non-linear patterns of colonization may be underway, however, as the genetic differentiation from the MHI towards the northwest is not linear with distance. Introduced lineages may be a source of diversity as this scenario was observed in other algal taxa in Hawai'i (e.g. *Asparagopsis taxiformis*, Sherwood 2008). Additionally, the MHI are composed of many more microhabitats than PMNM (Parrish & Boland 2004). Cryptic diversity may be created through lineage-specific selection and subsequent adaptation to microhabitats as has been observed in marine fishes (Herler 2007; Tornabene *et al.* 2015). Samples anchored to the top and sides of a single rock contained individuals belonging to Lineages 1 and 2, respectively. Microhabitat selection could be indicative of cryptic diversity in *A. glomerata* similar to *Bostrychia intricata* (Bory) Montagne in southeastern Australia (Muangmai *et al.* 2022) and further work should focus on reproductive crosses

(Zuccarello & West 2003). Regardless of the mechanism of dispersal it is apparent that interisland mixing, though rare, does occur in the Hawaiian Islands. This is evidenced not only by Φ_{ST} values suggesting occasional mixing, but also by shared haplotypes across channels. Indeed, studies suggesting low divergences in species of Rhodophyta tend to be in regions of recent glaciation and may be artifacts of recent range expansion while considerable genetic variability exists in closely separated tropical regions where habitat persistence is higher (Assis *et al.* 2014; Francisco *et al.* 2014; Guillemin *et al.* 2018; Ferrer *et al.* 2019, 2020; Zhong *et al.* 2020; Nielsen *et al.* 2021).

This study is one of the few focused on patterns of algal phylogeography in the Hawaiian Archipelago and confirms a complex pattern of introduction and diversification amongst the lineages. With 129 sequences of *A. glomerata* we confirmed a wide molecular diversity across the archipelago but insufficient evidence to recognize multiple species. Sequencing many specimens from neighboring islands allowed us to make direct comparisons with animal groups and highlights the need for further algal phylogeographic studies in Hawai'i.

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APPENDIX

APPENDIX 1. — Collection information for specimens of *Amansia* J.V.Lamouroux used in this study sorted by accession number.

Species name	Sherwood Lab accession number	GenBank accession	Collection Details	Lineage (Haplotype)	Source
Amansia glomerata C.Agardh	ARS00505	HM582893	D.T. Fleming Beach Park, Maui, 21.V.2005, coll. A. Sherwood & G. Presting, Intertidal	2 (30)	Sherwood *et al.* 2011
A. glomerata	ARS00744	OQ079250	Kahe Point, Oʻahu, 18.V.2001, coll. R. Okano, Intertidal	1 (15)	Sherwood *et al.* 2011
A. glomerata	ARS00746	OQ079251	Kaiolohia Bay, Lānaʻi, 06.II.2003, coll. J. Fisher, B. Kennedy & S. James, Intertidal	2 (17)	Sherwood *et al.* 2011
A. glomerata	ARS00751	OQ079252	Hana Boat Harbor, Maui, 07.II.2001, coll. C. Puttock, Intertidal	2 (17)	Sherwood *et al.* 2011
A. glomerata	ARS00846	OQ079253	Honokeana Bay, Maui, 04.IV.2006, coll. A. Carlile, Intertidal	2 (30)	Sherwood *et al.* 2011
A. glomerata	ARS01490	HM582891	Nanina Beach, Niʻihau, 25.X.2006, coll. J. Matthews, Intertidal	2 (29)	Sherwood *et al.* 2011
A. glomerata	ARS01498	OQ079254	Salt Pond Park, Kauaʻi, 24.X.2006, coll. J. Matthews, Intertidal	2 (29)	Sherwood *et al.* 2011
A. glomerata	ARS01594	HM582899	Census of Marine Life CReefs cruise to Lalo (French Frigate Shoals) sample ID 159, Papahānaumokuākea Marine National Monument, 17.X.2006, coll. K. Coontz, from bait trap	3 (21)	Sherwood *et al.* 2011
A. glomerata	ARS01989	OQ079255	Lipoa St. (beach wash), Maui, 27.I.2007, coll. T. Chandrasekharan, Intertidal	2 (17)	Sherwood *et al.* 2011
A. glomerata	ARS02202	OQ079256	Honolui Maloʻo Bay Beach, Molokaʻi, 11.II.2007, coll. T. Chandrasekharan, Intertidal	2 (17)	Sherwood *et al.* 2011
A. glomerata	ARS02215	HM582898	South Point (Green Sand Beach), Hawaiʻi, 25.II.2007, coll. K. Conklin, Intertidal	4 (5)	Sherwood *et al.* 2011
A. glomerata	ARS02336	OQ079257	Keʻe Beach, Kauaʻi, 17.III.2007, coll. K. Conklin, Intertidal	2 (29)	Sherwood *et al.* 2011
A. glomerata	ARS02427	OQ079258	Outside of Honolua, Maui, 28.I.2007, coll. T. Chandrasekharan, Intertidal	2 (30)	Sherwood *et al.* 2011
A. glomerata	ARS02743	HM582892	North end of Keomoku Rd., Lānaʻi, 03.III.2007, coll. T. Sauvage & A. Kurihara, Intertidal	2 (17)	Sherwood *et al.* 2011
A. glomerata	ARS02806	OQ079259	Census of Marine Life CReefs cruise to Lalo (French Frigate Shoals) sample ID 314, Papahānaumokuākea Marine National Monument, 23.X.2006, coll. K. Coontz, from bait trap	3 (21)	Sherwood *et al.* 2011
A. glomerata	ARS02923	OQ079260	Hauʻula Beach Park, Oʻahu, 18.IX.2007, A. Kurihara, Intertidal	2 (32)	Sherwood *et al.* 2011
A. fimbrifolia	ARS03086	HM582889	Kewalo Basin, Oʻahu, 09.X.2007, coll. D. Pence	NA	Sherwood *et al.* 2011
A. glomerata	ARS03087	OQ079261	Kewalo Basin, Oʻahu, 09.X.2007, coll. D. Pence, 60 m	2 (29)	Sherwood *et al.* 2011
A. glomerata	ARS03091	HM582895	Kewalo Basin, Oʻahu, 09.X.2007, coll. D. Pence, 60 m	1 (11)	Sherwood *et al.* 2011
A. glomerata	ARS03126	OQ079262	Hamoa Beach, Maui, 11.XII.2007, coll. K. Conklin, Intertidal	2 (17)	Sherwood *et al.* 2011
A. glomerata	ARS03160	OQ079263	Honokowai, Maui, 17.XI.2007, coll. S. Hau, Intertidal	2 (30)	Sherwood *et al.* 2011
A. glomerata	ARS03241	HM582894	Laupahoehoe, Hawaiʻi, 23.I.2008, coll. K. Conklin, Intertidal	2 (27)	Sherwood *et al.* 2011
A. glomerata	ARS03247	OQ079264	Richardson Beach Park, Hawaiʻi, 23.I.2008, coll. K. Conklin, Intertidal	4 (5)	Sherwood *et al.* 2011
A. glomerata	ARS03539	HQ422957	South Oʻahu, 27.XI.2006, coll. H. Spalding, 70 m	1 (10)	Sherwood *et al.* 2011

Appendix 1. — Continuation.

Species name	Sherwood Lab accession number	GenBank accession	Collection Details	Lineage (Haplotype)	Source
A. glomerata	ARS03601	HQ422913	Honouli, Moloka'i, 22.III.2008, coll. E. Conklin, Intertidal	2 (28)	Sherwood *et al.* 2011
A. glomerata	ARS03617	HQ422914	Kanalukaha, Moloka'i, 23.III.2008, coll. E. Conklin, Intertidal	2 (29)	Sherwood *et al.* 2011
A. glomerata	ARS03623	OQ079265	Hale O Lono Harbor, Moloka'i, 23.III.2008, coll. K. Conklin, Intertidal	2 (29)	Sherwood *et al.* 2011
A. fimbrifolia	ARS03978	HM582890	Kamokuokamohoali'i (Maro Reef), Papahānaumokuākea Marine National Monument, 16.IX.2002, coll. K. McDermid	NA	Sherwood *et al.* 2011
A. glomerata	ARS04047	OQ079266	Kipukai, Kaua'i, 09.IX.1979, coll. W. Magruder, Intertidal	2 (29)	Sherwood *et al.* 2011
A. glomerata	ARS04053	HQ423024	Mokumanamana (Necker) lobster trap sample, Papahānaumokuākea Marine National Monumen1t, 02.VII.2004, coll. R. Moffitt	3 (18)	Sherwood *et al.* 2011
A. glomerata	ARS04056	OQ079267	Honolulu, O'ahu, 04.XI.1981, coll. Hunt, 15-27 m	1 (11)	Sherwood *et al.* 2011
A. glomerata	ARS04063	OQ079268	Leleiwi, Hawai'i, 14 & 16.VI.2000, coll. R. Okano, Intertidal	2 (27)	Sherwood *et al.* 2011
A. glomerata	ARS04074	HM582897	Ala Moana Beach Park, O'ahu, 10.II.2009, coll. A. Kurihara, Intertidal	1 (15)	Sherwood *et al.* 2011
A. glomerata	ARS04076	OQ079269	Kewalo Basin Beach Park, O'ahu, 10.II.2009, coll. A. Kurihara, Intertidal	2 (29)	Sherwood *et al.* 2011
A. glomerata	ARS04078	OQ079270	Kewalo Basin Beach Park, O'ahu, 10.II.2009, coll. A. Kurihara, Intertidal	2 (29)	Sherwood *et al.* 2011
A. glomerata	ARS08364	OQ079271	Kuaihelani (Midway Atoll), Papahānaumokuākea Marine National Monument, 16.IX.2012, coll. C. Clark, 55 m	3 (19)	This study
A. glomerata	ARS09934	OQ079272	Keyhole, Lehua islet, Ni'ihau, 24.VII.2019, coll. H. Spalding, 17 m	2 (29)	This study
A. glomerata	ARS09936	OQ079273	Manawai (Pearl & Hermes Atoll), Papahānaumokuākea Marine National Monument, 03.VIII.2019, coll. J. Leonard, 92 m	3 (16)	This study
A. glomerata	ARS09939	OQ079274	Manawai (Pearl & Hermes Atoll), Papahānaumokuākea Marine National Monument, 05.VIII.2019, coll. R. Kosaki, 85 m	3 (16)	This study
A. glomerata	ARS09943	OQ079275	Manawai (Pearl & Hermes Atoll), Papahānaumokuākea Marine National Monument, 06.VIII.2019, coll. R. Kosaki, 94 m	3 (16)	This study
A. glomerata	ARS09946	OQ079276	Manawai (Pearl & Hermes Atoll), Papahānaumokuākea Marine National Monument, 06.VIII.2019, coll. B. Hauk, 75 m	3 (16)	This study
A. glomerata	ARS10174	OQ079277	Ni'ihau, 24.VII.2019, coll. R. Kosaki, 62 m	3 (20)	This study
A. glomerata	ARS10350	OQ079278	West Moloka'i, 02.XI.2015, coll. R. Kosaki, 61 m	1 (10)	This study
A. glomerata	ARS10357	OQ079279	Kamole (Laysan), Papahānaumokuākea Marine National Monument, 25.V.2013, coll. K. Gleason, 62 m	3 (16)	This study
A. glomerata	ARS10358	OQ079280	Kapou (Lisianski), Papahānaumokuākea Marine National Monument, 14.IX.2014, coll. J. Leonard, 59 m	3 (16)	This study
A. glomerata	ARS10370	OQ079281	Makua Beach, O'ahu, 11.XI.2020, coll. C. Walsh, Intertidal	2 (34)	This study
A. glomerata	ARS10371	OQ079282	Makua Beach, O'ahu, 11.XI.2020, coll. C. Walsh, Intertidal	1 (6)	This study
A. glomerata	ARS10375	OQ079283	Makua Beach, O'ahu, 01.XI.2021, coll. J. Fumo, Intertidal	1 (6)	This study
A. glomerata	ARS10378	OQ079284	Makua Beach, O'ahu, 01.XI.2021, coll. J. Fumo, Intertidal	1 (6)	This study
A. glomerata	ARS10379	OQ079285	Makua Beach, O'ahu, 01.XI.2021, coll. J. Fumo, Intertidal	1 (6)	This study
A. glomerata	ARS10380	OQ079286	Makua Beach, O'ahu, 01.XI.2021, coll. J. Fumo, Intertidal	2 (34)	This study
A. glomerata	ARS10383	OQ079287	Makua Beach, O'ahu, 01.XI.2021, coll. J. Fumo, Intertidal	1 (6)	This study
A. glomerata	ARS10497	OQ079288	Magic Island, Honolulu, O'ahu, 15.II.2021, coll. B. Weeks, Intertidal	1 (13)	This study
A. glomerata	ARS10501	OQ079289	Kahe Beach Park, O'ahu, 03.V.2021, coll. A. Innes-Gold, Intertidal	2 (29)	This study
A. glomerata	ARS10502	OQ079290	Kahe Beach Park, O'ahu, 03.V.2021, coll. C. Stephenson, Intertidal	1 (6)	This study
A. glomerata	ARS10504	OQ079291	Laniakea, O'ahu, 08.V.2021, coll. J. Fumo, Intertidal	2 (33)	This study

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Species name	Sherwood Lab accession number	GenBank accession	Collection Details	Lineage (Haplotype)	Source
A. glomerata	ARS10505	OQ079292	Laniakea, Oʻahu, 08.V.2021, coll. J. Fumo, Intertidal	2 (34)	This study
A. glomerata	ARS10507	OQ079293	Laniakea, Oʻahu, 08.V.2021, coll. J. Fumo, 19 m	2 (34)	This study
A. glomerata	ARS10508	OQ079294	Waialua, Oʻahu, 13.V.2021, coll. J. Fumo, Intertidal	2 (34)	This study
A. glomerata	ARS10614	OQ079295	Holualoa, Hawaiʻi, 25.IV.2021, coll. J. Smith, Intertidal	1 (12)	This study
A. glomerata	ARS10631	OQ079296	Waialua, Oʻahu, 13.V.2021, coll. J. Fumo, Intertidal	2 (34)	This study
A. glomerata	ARS10703	OQ079297	Lāʻie, Oʻahu, 22.VII.2021, coll. F. Cabrera, Intertidal	2 (26)	This study
A. glomerata	ARS10704	OQ079298	Kahana Bay, Oʻahu, 22.VII.2021, coll. A. Edwards, Intertidal	2 (22)	This study
A. glomerata	ARS10801	OQ079299	Kawailoa, Oʻahu, 23.V.2021, coll. M. Schelvis, Intertidal	1 (6)	This study
A. glomerata	ARS10802	OQ079300	Kahana Bay, Oʻahu, 22.V.2021, coll. J. Fumo, Intertidal	2 (22)	This study
A. glomerata	ARS10807	OQ079301	Wawamalu, Oʻahu, 10.VI.2021, coll. O. Presting, Intertidal	2 (29)	This study
A. glomerata	ARS10808	OQ079302	Kawela Bay, Oʻahu, 25.VI.2021, coll. B. Spry, Intertidal	2 (34)	This study
A. glomerata	ARS10811	OQ079303	Kaʻalawai (Cromwell's Beach), Oʻahu, 02.IX.2021, coll. S. Whitner, Intertidal	2 (29)	This study
A. glomerata	ARS10823	OQ079304	Manawai (Pearl & Hermes Atoll), Papahānaumokuākea Marine National Monument, 15.VII.2021, coll. J. Fumo, 12 m	3 (16)	This study
A. glomerata	ARS10824	OQ079305	Manawai (Pearl & Hermes Atoll), Papahānaumokuākea Marine National Monument, 15.VII.2021, coll. J. Fumo, 12 m	3 (16)	This study
A. glomerata	ARS10826	OQ079306	Manawai (Pearl & Hermes Atoll), Papahānaumokuākea Marine National Monument, 15.VII.2021, coll. J. Fumo, 12 m	3 (16)	This study
A. glomerata	ARS10827	OQ079307	Manawai (Pearl & Hermes Atoll), Papahānaumokuākea Marine National Monument, 15.VII.2021, coll. J. Fumo, 12 m	3 (16)	This study
A. glomerata	ARS10828	OQ079308	Manawai (Pearl & Hermes Atoll), Papahānaumokuākea Marine National Monument, 15.VII.2021, coll. J. Fumo, 12 m	3 (16)	This study
A. glomerata	ARS10829	OQ079309	Manawai (Pearl & Hermes Atoll), Papahānaumokuākea Marine National Monument, 15.VII.2021, coll. J. Fumo, 12 m	3 (16)	This study
A. glomerata	ARS10830	OQ079310	Manawai (Pearl & Hermes Atoll), Papahānaumokuākea Marine National Monument, 15.VII.2021, coll. J. Fumo, 12 m	3 (16)	This study
A. glomerata	ARS10831	OQ079311	ʻEhukai, Oʻahu, 30.VII.2021, coll. A. Meier, Intertidal	2 (34)	This study
A. fimbfrifolia	ARS10832	OQ079312	Maunalua Bay (Turtle Canyons), Oʻahu, 07.VIII.2021, coll. J. Fumo, 10 m	NA	This Study
A. glomerata	ARS10833	OQ079313	Maunalua Bay (Turtle Canyons), Oʻahu, 07.VIII.2021, coll. L. Maki, 6 m	2 (29)	This study
A. glomerata	ARS10835	OQ079314	Maunalua Bay (China Walls), Oʻahu, 07.VIII.2021, coll. J. Fumo, Intertidal	1 (14)	This study
A. glomerata	ARS10836	OQ079315	Maunalua Bay (China Walls), Oʻahu, 07.VIII.2021, coll. J. Fumo, Intertidal	2 (31)	This study
A. glomerata	ARS10837	OQ079316	Hamoa, Maui, 15.VIII.2021, coll. B. Spry, Intertidal	2 (17)	This study
A. glomerata	ARS10838	OQ079317	Hamoa, Maui, 15.VIII.2021, coll. B. Spry, Intertidal	2 (17)	This study
A. glomerata	ARS10839	OQ079318	Hamoa, Maui, 15.VIII.2021, coll. B. Spry, Intertidal	2 (17)	This study
A. glomerata	ARS10840	OQ079319	Hamoa, Maui, 15.VIII.2021, coll. B. Spry, Intertidal	2 (17)	This study
A. glomerata	ARS10956	OQ079320	Mala Wharf, Maui, 31.X.2021, coll. J. Fumo, 7 m	2 (17)	This study
A. glomerata	ARS10959	OQ079321	Mokoliʻi, Oʻahu, 09.I.2022, coll. L. McPherson, Intertidal	2 (22)	This study
A. glomerata	ARS11206	OQ079322	Popoia, Oʻahu, 18.V.2022, coll. J. Fumo, Intertidal	1 (8)	This study
A. glomerata	ARS11207	OQ079323	Popoia, Oʻahu, 18.V.2022, coll. J. Fumo, Intertidal	1 (8)	This study
A. glomerata	ARS11208	OQ079324	Popoia, Oʻahu, 18.V.2022, coll. J. Fumo, Intertidal	1 (7)	This study
A. glomerata	ARS11209	OQ079325	Popoia, Oʻahu, 18.V.2022, coll. J. Fumo, Intertidal	1 (8)	This study
A. glomerata	ARS11210	OQ079326	Popoia, Oʻahu, 18.V.2022, coll. J. Fumo, Intertidal	1 (8)	This study
A. glomerata	ARS11230	OQ079327	Laupahoehoe Point, Hawaiʻi, 30.V.2022, coll. J. Fumo, Intertidal	2 (27)	This study
A. glomerata	ARS11231	OQ079328	Laupahoehoe Point, Hawaiʻi, 30.V.2022, coll. J. Fumo, Intertidal	2 (27)	This study
A. glomerata	ARS11240	OQ079329	Laupahoehoe Point, Hawaiʻi, 30.V.2022, coll. J. Fumo, Intertidal	2 (27)	This study
A. glomerata	ARS11242	OQ079330	Laupahoehoe Point, Hawaiʻi, 30.V.2022, coll. J. Fumo, Intertidal	2 (27)	This study
A. glomerata	ARS11244	OQ079331	Laupahoehoe Point, Hawaiʻi, 30.V.2022, coll. J. Fumo, Intertidal	2 (27)	This study

Appendix 1. — Continuation.

Species name	Sherwood Lab accession number	GenBank accession	Collection Details	Lineage (Haplotype)	Source
A. glomerata	ARS11255	OQ079332	Onomea Bay, Hawai'i, 31.V.2022, coll. J. Fumo, Intertidal	2 (24)	This study
A. glomerata	ARS11259	OQ079333	Onomea Bay, Hawai'i, 31.V.2022, coll. J. Fumo, Intertidal	2 (23)	This study
A. glomerata	ARS11275	OQ079334	Onomea Bay, Hawai'i, 31.V.2022, coll. J. Fumo, Intertidal	2 (27)	This study
A. glomerata	ARS11325	OQ079335	Richardson Ocean Park, Hawai'i, 01.VI.2022, coll. S. Champion, Intertidal	4 (3)	This study
A. glomerata	ARS11326	OQ079336	Richardson Ocean Park, Hawai'i, 01.VI.2022, coll. T. Marsh, Intertidal	4 (3)	This study
A. glomerata	ARS11329	OQ079337	Richardson Ocean Park, Hawai'i, 01.VI.2022, coll. A. Kelly, Intertidal	4 (5)	This study
A. glomerata	ARS11332	OQ079338	Richardson Ocean Park, Hawai'i, 01.VI.2022, coll. J. Fumo, Intertidal	4 (4)	This study
A. glomerata	ARS11356	OQ079339	Hakioawa Iki, Kaho'olawe, 02.VI.2022, coll. N. Goo, Intertidal	2 (17)	This study
A. glomerata	ARS11371	OQ079340	South Point (Green Sand Beach), Hawai'i, 02.VI.2022, coll. J. Fumo, Intertidal	4 (2)	This study
A. glomerata	ARS11373	OQ079341	Manukā Bay, Hawai'i, 05.VI.2022, coll. J. Fumo, Intertidal	1 (12)	This study
A. glomerata	ARS11374	OQ079342	Manukā Bay, Hawai'i, 05.VI.2022, coll. J. Fumo, Intertidal	1 (12)	This study
A. glomerata	ARS11375	OQ079343	Manukā Bay, Hawai'i, 05.VI.2022, coll. J. Fumo, Intertidal	1 (12)	This study
A. glomerata	ARS11376	OQ079344	Manukā Bay, Hawai'i, 05.VI.2022, coll. J. Fumo, Intertidal	1 (6)	This study
A. glomerata	ARS11377	OQ079345	Manukā Bay, Hawai'i, 05.VI.2022, coll. J. Fumo, Intertidal	1 (6)	This study
A. glomerata	ARS11392	OQ079346	Manukā Bay, Hawai'i, 05.VI.2022, coll. J. Fumo, Intertidal	1 (6)	This study
A. glomerata	ARS11393	OQ079347	Miloli'i, Hawai'i, 06.VI.2022, coll. J. Fumo, Intertidal	1 (6)	This study
A. glomerata	ARS11395	OQ079348	Manini Beach, Hawai'i, 06.VI.2022, coll. J. Fumo, Intertidal	1 (6)	This study
A. glomerata	ARS11396	OQ079349	Manini Beach, Hawai'i, 06.VI.2022, coll. J. Fumo, Intertidal	1 (6)	This study
A. glomerata	ARS11397	OQ079350	Manini Beach, Hawai'i, 06.VI.2022, coll. J. Fumo, Intertidal	1 (6)	This study
A. glomerata	ARS11399	OQ079351	Lyman's Point, Hawai'i, 04.VI.2022, coll. J. Fumo, Intertidal	1 (6)	This study
A. glomerata	ARS11400	OQ079352	Lyman's Point, Hawai'i, 04.VI.2022, coll. J. Fumo, Intertidal	1 (6)	This study
A. glomerata	ARS11401	OQ079353	Lyman's Point, Hawai'i, 04.VI.2022, coll. J. Fumo, Intertidal	1 (9)	This study
A. glomerata	ARS11402	OQ079354	Lyman's Point, Hawai'i, 04.VI.2022, coll. J. Fumo, Intertidal	1 (6)	This study
A. glomerata	ARS11403	OQ079355	Lyman's Point, Hawai'i, 04.VI.2022, coll. J. Fumo, Intertidal	1 (6)	This study
A. glomerata	ARS11410	OQ079356	Wawaionū Bay, Hawai'i, 11.VI.2022, coll. J. Fumo, Intertidal	1 (6)	This study
A. glomerata	ARS11411	OQ079357	Wawaionū Bay, Hawai'i, 11.VI.2022, coll. J. Fumo, Intertidal	1 (6)	This study
A. glomerata	ARS11412	OQ079358	Wawaionū Bay, Hawai'i, 11.VI.2022, coll. J. Fumo, Intertidal	1 (6)	This study
A. glomerata	ARS11413	OQ079359	Wawaionū Bay, Hawai'i, 11.VI.2022, coll. J. Fumo, Intertidal	1 (6)	This study
A. glomerata	ARS11414	OQ079360	Wawaionū Bay, Hawai'i, 11.VI.2022, coll. J. Fumo, Intertidal	1 (6)	This study
A. glomerata	ARS11418	OQ079361	Keokea Beach Park, Hawai'i, 10.VI.2022, coll. J. Glazner, Intertidal	2 (25)	This study
A. glomerata	ARS11423	OQ079362	South Point (Green Sand Beach), Hawai'i, 02.VI.2022, coll. J. Fumo, Intertidal	4 (1)	This study
A. glomerata	ARS11424	OQ079363	South Point (Green Sand Beach), Hawai'i, 02.VI.2022, coll. J. Fumo, Intertidal	4 (1)	This study
A. glomerata	ARS11425	OQ079364	South Point (Green Sand Beach), Hawai'i, 02.VI.2022, coll. J. Fumo, Intertidal	4 (2)	This study
A. glomerata	ARS11426	OQ079365	South Point (Green Sand Beach), Hawai'i, 02.VI.2022, coll. J. Fumo, Intertidal	4 (2)	This study
A. glomerata	ARS11427	OQ079366	South Point (Green Sand Beach), Hawai'i, 02.VI.2022, coll. J. Fumo, Intertidal	4 (1)	This study
A. multifida J.V.Lamouroux	GGS010	MH388713	Cabo San Ramon, Venezuela, 06.X.2012, coll. G. Garcia-Soto	NA	Garcia-Soto & Lopez-Bautista 2018

Appendix 1. — Continuation.

Species name	Sherwood Lab accession number	GenBank accession	Collection Details	Lineage (Haplotype)	Source
A. multifida	GGS031	MH388714	Cabo San Ramon, Venezuela, 06.X.2012, coll. G. Garcia-Soto	NA	Garcia-Soto & Lopez-Bautista 2018
A. glomerata	LD42600	HM582896	Sandwich Islands, 1822, Lectotype	1 (L)	This study
A. pinnatifida Harvey	UA0013	MH388718	Kangaroo Island, South Australia, Australia, 07.XI.1995, coll. M.H. & F.C. Hommersand	NA	Garcia-Soto & Lopez-Bautista 2018