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A new species of *Gibsmithia* (Dumontiaceae, Rhodophyta) from mesophotic depths of the Papahānaumokuākea Marine National Monument, Hawai'i, USA

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ABSTRACT
Molecular phylogenetic analyses of Hawaiian members of the red algal family Dumontiaceae were used to clarify the species diversity of *Dudresnaya* and *Gibsmithia* from Hawai'i. Although no new species of *Dudresnaya* were detected in the analyses, *D. babbittiana* is newly recorded by Lalo, Manawai, and O'ahu; however, this record remains tentative until the type material is sequenced. A new species of *Gibsmithia, G. punonomaewa* A.R. Sherwood, is described here and reported from the mesophotic depths (79–104 m) of the Papahānaumokuākea Marine National Monument, Hawai'i. This new species differs from all others in the genus based on the following combination of characters: moderate thallus size (up to 11 cm), smooth and terete gelatinous lobes, presence of a stipe (which is often branched), globose carposporangia, and a non-isodiametric shaped cell subtending the tetrasporangia. This new taxon increases the number of *Gibsmithia* species recorded from Hawai'i to three. Phylogenetically, *G. punonomaewa* is most closely related to *G. dotyi* (type locality, Lord Howe Island, Australia) and *G. larkumii* (type locality, One Tree Island, Queensland, Australia), which are both reportedly widespread in distribution. The relatively dark habitat of the mesophotic in Papahānaumokuākea Marine National Monument contrasts with the surface waters of tropical and subtropical habitats where most *Gibsmithia* species are found, further highlighting the uniqueness of the species.

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INTRODUCTION

The family Dumontiaceae contains 24 genera (19 of which contain currently recognized species) and is reportedly primarily cool- and cold-temperate in distribution (Kraft 1986; Guiry & Guiry 2021). Despite having characteristic post-fertilization events that unite its members, the family has an astounding degree of morphological and anatomical diversity (Robins & Kraft 1985), and has also been shown to harbour high levels of cryptic diversity (Saunders 2008). The two exceptions to the generally cooler-water distribution of the Dumontiaceae, *Gibsmithia* Doty and *Dudresnaya* P. Crouan & H. Crouan, are both known from the Hawaiian Islands (Abbott 1999). *Gibsmithia* was originally described based on specimens collected from O'ahu, Hawai'i (Doty 1963) and currently comprises seven species (Guiry & Guiry 2021). *Dudresnaya* is a relatively large genus, with 21 species currently recognized (Guiry & Guiry 2021). Although sharing a close phylogenetic relationship, the two genera differ in several aspects of their morphology and anatomy, including thallus construction (*Gibsmithia* is multiaxial, while *Dudresnaya* is uniaxial), and tetrasporangial division (tetrasporangia in *Gibsmithia* are cruciate, whereas those in *Dudresnaya* are zonate) (Abbott 1999).

Until now, Hawaiian representatives of the Dumontiaceae included two species of *Gibsmithia*: the generitype, *G. hawaiiensis* Doty, and *G. dotyi* Kraft & R.W. Ricker; and three species of *Dudresnaya*: *D. hawaiiensis* R.K.S. Lee, *D. littleri* I.A. Abbott and *D. babbittiana* I.A. Abbott & McDermid (Abbott 1999; Abbott & McDermid 2001). *Gibsmithia hawaiiensis sensu lato*, which differs morphologically from its congeners in having a 'hairy' appearance due to exserted cortical filaments (all other species of *Gibsmithia* are 'smooth', lacking the exserted cortical filaments), has been reported, based solely on morphology and anatomy, to have a particularly broad distribution, including South Africa, the Indian Ocean, Australasia, and throughout the Indo-Pacific (Guiry & Guiry 2021). Using multi-locus sequence comparisons, Gabriel *et al.* (2016, 2017) showed that specimens from a broad geographical distribution, and morphologically ascribed to *G. hawaiiensis*, formed a species complex harbouring high levels of cryptic and pseudocryptic diversity. Consequently, they described three species from the complex with distinctive geographical distributions, microscopic-level morphological features, and DNA signatures: *G. eilatensis* D. Gabriel & Fredericq (Gabriel *et al.* 2016), *G. malayensis* D. Gabriel, Draisma & Fredericq (Gabriel *et al.* 2017) and *G. indopacifica* D. Gabriel, Draisma & Fredericq (Gabriel *et al.*

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2017). *Gibsmithia hawaiiensis sensu stricto* was confirmed only from the Hawaiian Islands (as an endemic taxon), and other clades within the *G. hawaiiensis* complex were resolved as unique lineages that represent as-of-yet undescribed species (Gabriel *et al.* 2017). The three Hawaiian species of *Dudresnaya* are reasonably well represented in recent systematic and floristic works (Sherwood *et al.* 2010; Bárbara *et al.* 2013; Gabriel *et al.* 2016, 2017). Based on morpho-anatomical identifications, *Dudresnaya hawaiiensis* has been reported from Australia, Bangladesh and the Pacific Islands, while *D. littleri* and *D. babbittiana* have been reported thus far only from the Hawaiian Islands (McDermid & Abbott 2006; Guiry & Guiry 2021).

Recent surveys of Hawaiian mesophotic reefs have uncovered large numbers of undescribed algal species (e.g. studies of Kallymeniaceae by Cabrera *et al.* 2021, Peyssonneliales by Sherwood *et al.* 2020, 2021a, and Ulvales by Spalding *et al.* 2016, among others). Surveys in the Papahānaumokuākea Marine National Monument (PMNM) in 2016 by the National Oceanic and Atmospheric Administration (NOAA) divers yielded collections of an unknown species morphologically resembling members of the genus *Gibsmithia*, as well as collections resembling *Dudresnaya babbittiana*, a presumed endemic species known only from Midway Atoll, and for which confirmed molecular data have yet to be obtained. This study provides a phylogenetic analysis of *Gibsmithia* and *Dudresnaya* specimens from the Hawaiian Islands, and extends the known ranges for some of these species.

MATERIAL AND METHODS

Specimens morphologically identified as belonging to *Gibsmithia* and *Dudresnaya* were analysed in this study (Table S1); many of these were collected during a 2016 expedition to PMNM aboard the NOAA Ship, *Hi'ialakai* (HA-16-04). Other specimens were sampled from the Bernice P. Bishop Herbarium (BISH; herbarium acronyms follow the Index Herbariorum, Thiers 2022), or collected opportunistically from locations around the Hawaiian Islands during general surveys over the last several decades. Specimens were preserved in silica gel desiccant or pressed as herbarium vouchers and were assigned a Sherwood Lab accession number (a five-digit number prefixed by 'ARS'). Morpho-anatomical investigations were conducted on formalin-preserved material or by gently rehydrating small pieces lifted from herbarium sheets in Modified Pohl's Solution (https://www.eeob.iastate.edu/research/bamboo/pdf/anatomy_protocols.pdf) for 30 min. Squashes were prepared on glass slides, stained with 1% aniline blue and mounted in 30–50% Karo™. Photomicrographs were taken on a Zeiss AxioImager A1 compound light microscope (Pleasanton, California, USA) with an Infinity2-1RC digital camera (Lumenera Corporation, Ottawa, Ontario, Canada) using brightfield or differential interference contrast microscopy. Specimens were deposited in BISH under accession numbers BISH 783201–783225.

Genomic DNA was extracted using an OMEGA E.Z.N.A.® Plant DNA DS Kit (OMEGA Biotek, Norcross, Georgia, USA) or the NucleoSpin Plant II Kit (Macherey-Nagel, Düren, Germany) following the manufacturer's protocol. A portion of the DNA barcode region near the 5′ end of the mitochondrial COI gene was generated using the GazF1 and GazR1 primer pair (Saunders 2005). The *rbc*L (ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit) marker was amplified into two overlapping fragments using the primer pairs rbcLF7 and R898 (Gavio & Fredericq 2002; Kang & Kim 2013), and rbcLF762 and rbcLR1381 (Kim *et al.* 2010). Sequences of the UPA (Universal Plastid Amplicon) marker were generated with the primer pair p23SrV_f1 and p23SrV_r1, and cycling conditions as described in Sherwood & Presting (2007). Successful PCR products were submitted for sequencing by GENEWIZ (South Plainfield, New Jersey, USA). Raw sequence reads for each gene were assembled, edited and aligned using the MUSCLE v3.8.425 plug-in (Edgar 2004) in Geneious Prime 2019.1.3 (http://www.geneious.com) with other available sequences for *Gibsmithia, Dudresnaya* and related genera of the Dumontiaceae from GenBank (Table S2). For the *rbc*L and COI phylogenetic analyses, sequences were analysed with PartitionFinder 2 (Lanfear *et al.* 2017). Maximum Likelihood (ML) analyses were performed on all alignments using RAxML-HPC2 on XSEDE v8.2.10 (Stamatakis 2014) via the CIPRES gateway (Miller *et al.* 2010) with 1,000 bootstrap replicates and using the GTRCAT model. Similar methods were followed for the UPA phylogenetic analyses, except that the HKY85 model was employed. Bayesian inference was performed using MrBayes v3.2.7a (Huelsenbeck *et al.* 2003) through the CIPRES Science Gateway (Miller *et al.* 2010) using four chains of Metropolis-coupled Markov Chain Monte Carlo for 5,000,000 generations (for COI and *rbc*L) or 1,000,000 generations (UPA), sampling every 500 generations, and other parameters set as default. Twenty-five per cent of sampled trees were discarded as burn-in to determine posterior probabilities. Tracer v1.7.1 was used to estimate the burn-in cut-off and to check if further runs were required to reach convergence, with the average standard deviation of split frequencies = 0.010032 (COI), 0.009332 (*rbc*L), or 0.0076 (UPA) (Rambaut *et al.* 2018). For the *rbc*L and COI analyses, and as per Gabriel *et al.* (2017), a member of the Kallymeniaceae was used as the outgroup taxon and numerous additional representatives of the Dumontiaeae were included to provide phylogenetic context for the two genera under consideration. The short length and high level of conservation of the UPA marker made it less useful for interpreting phylogenetic relationships (Sherwood *et al.* 2010), and thus this analysis was presented without outgroups, and with a focus on visualizing lineages of *Gibsmithia*.

RESULTS

Phylogenetic analyses

Phylogenetic analyses of the COI marker revealed distinct clades corresponding to *Gibsmithia* and *Dudresnaya* (Fig. 1). This was not surprising since topotype sequences of the generitype of *Gibsmithia* (*G. hawaiiensis*) were included in the analyses, providing confirmation for that genus and anchoring that clade. Confirmed sequences are

Fig. 1. Maximum likelihood phylogenetic tree of COI sequences for *Gibsmithia* and *Dudresnaya*, along with representatives of the Dumontiaceae. Numbers along branches indicate nodal support (first value = bootstrap support, second value = Bayesian posterior probabilities). Nodes with full support are indicated with an asterisk. Scale bar indicates number of substitutions per site. For sequences included from GenBank, accession codes are listed before taxonomic names, with

still not available for the generitype of *Dudresnaya* (*D. verticillata*) given that there is a lack of clarity about the type locality of the lectotype; all available sequences ascribed to *Dudresnaya* specimens remain provisionally identified as members of that genus until definitive *D. verticillata* material is sequenced. Needless to say, all sequences ascribed to *Dudresnaya* specimens included in our analyses are resolved in the same clade. Of the newly generated Hawaiian *Dudresnaya* sequences, one corresponded to specimens ascribed to *D. hawaiiensis* and eight to *D. babbittiana* (including the previously published HQ423130 '*D. hawaiiensis*'). No sequence data for *D. littleri* were obtained in this study, and no potentially undescribed species of Hawaiian *Dudresnaya* were suggested by these analyses. In terms of diversity within *Gibsmithia*, the COI analyses revealed three clades containing Hawaiian specimens: two sequences corresponding to *G. dotyi*, nine matching *G. hawaiiensis* and three forming a well-supported clade sister to *G. dotyi* and not matching available sequences from Hawai'i or other parts of the world (Fig. 1). Species of *Gibsmithia* are labelled using the system of Gabriel *et al.* (2016, 2017); with the remaining undescribed lineages labelled as *Gibsmithia* sp. 1 through *Gibsmithia* sp. 5), and groups are consistent with the analyses presented in those two publications.

Similar to the COI analyses, the *rbc*L analyses supported two groups of Hawaiian *Dudresnaya* specimens (with no representation for the third Hawaiian species, *D. littleri):* *D. hawaiiensis* and *D. babbittiana* (Fig. 2). One published sequence labelled *D. verticillata* (MW275859, from Taiwan), grouped with the Hawaiian *D. babbittiana* sequences. Nine *Gibsmithia* clades were recovered, which was topologically consistent with Gabriel *et al.* (2016, 2017). One of these clades represented novel diversity from the Hawaiian Islands, one corresponded to *G. dotyi*, and the remainder of the Hawaiian specimens were members of the large *G. hawaiiensis* clade. As shown by the COI analyses, this new species clade was sister to *G. dotyi*.

Analyses of the UPA marker revealed a less well-resolved phylogeny than the previous two markers, especially with respect to the distinction of species closely related to *G. hawaiiensis* (Fig. 3). This marker was included in the analysis because it is the only marker for which sequence data are available for *G. larkumii* Kraft. For this reason, and because no novel Hawaiian *Dudresnaya* diversity was detected with COI or *rbc*L, only *Gibsmithia* was included in the UPA analysis. As for the two previous markers, a clade bearing three specimens of undescribed *Gibsmithia* was recovered in this analysis, which was sister to *G. larkumii* (Fig. 3). The undescribed *Gibsmithia* and *G. larkumii* formed a clade that was sister to *G. dotyi*. Below, we describe this clade as a new species within the genus *Gibsmithia*, based on the molecular phylogenetic comparisons of COI, *rbc*L and UPA, and the morpho-anatomical comparisons presented in the next section.

Gibsmithia punonomaewa* A.R. Sherwood *sp. nov.

Figs 4–18

DESCRIPTION: Thallus up to 11 cm high, bright orange-red when living, sometimes green-brown or tan-coloured in parts, drying to a pinkish-green colour near the stalk. Stalk firm, dark and cartilaginous, 8–25 mm long and 1–6 mm wide (typically 1–2 mm), slender, either simple or branched, and producing a single blade with up to six terminal mucilaginous branches. Mucilaginous branches smooth, mostly long and thin, and branched irregularly, cortical filaments not exserted. Cortical filaments dichotomously branched every 1–3 cells, with ultimate branches 1–3 cells long. Cells of cortical filaments subclavate to cylindrical, 11.0–21.1 µm long × 3.8–6.9 µm wide. Medullary filament cells mostly cylindrical, 18.4–36.6 µm long × 3.5–5.0 µm wide. Slender rhizoidal filaments present in medulla. Plants dioecious. Auxiliary cell filaments 7–14 cells long and terminating in a series of unmodified filaments. Mature cystocarps 150–360 µm diameter, carposporangia globose, 16–29 µm long and up to 20 µm in diameter. Spherical spermatangia produced in clusters at tips of cortical fascicles, 2–4 µm in diameter.

HOLOTYPE: BISH 783203 (ARS 09867/NWHI-570; Lalo – French Frigate Shoals); Papahānaumokuākea Marine National Monument, Hawai'i, USA, 23°39.951′N, 166°18.045′W, collected 11 June 2016 by R. Kosaki & D. Wagner; tetrasporic specimen.

ISOTYPES: BISH 783204, BISH 783205, HAW-A-02881 (ARS 09867/NWHI-570; collection information as for holotype).

TYPE GENBANK ACCESSIONS: COI, OK448452; *rbc*L, OK448474; UPA, OK448430.

OTHER EXAMINED MATERIAL: BISH 783201, BISH 783202 (ARS 09865/NWHI-557, ARS 09866/NWHI-594).

ETYMOLOGY: Named by the PMNM Cultural Working Group (Nomenclature Subcommittee). The epithet *punonomaewa* is a noun used in apposition formed by a combination of several Hawaiian words. 'Pūnono', meaning 'gorgeously red, ever-beautiful, flushed red', is representative of this alga's most striking feature – its colour. From its anchored base, the alga stands upright making it one of the taller algae in this habitat. 'Māewa', which means 'swaying, as something with an anchored base, as seaweed', reflects this alga's graceful and elegant hula-like moves in the ocean's current. 'Ewa' is intended to represent the term 'lewa', which aludes to two different aspects: 'lewa', meaning 'limber-jointed, of admired hula dancers', suggests the likeness of this alga to a beautiful hula dancer. In a second allusion to its growth as one of the deepest algae, 'lewa' defines the deep ocean in which this alga thrives. This final part of the name defines the epithet as a noun and thus is not declinable in Latinized form.

DISTRIBUTION: Lalo (French Frigate Shoals) and Kapou (Lisianski), Papahānaumokuākea Marine National Monument, Hawai'i, USA, 79–104 m depth.

DNA SEQUENCE DATA (GENBANK ACCESSIONS): COI, OK448850, OK448851, OK448852; *rbc*L, OK448472, OK448473, OK448474; UPA, OK448428, OK448429, OK448430.

specimen voucher codes listed afterwards. Sequences generated in the current study are indicated in bold, and are listed by specimen voucher code, type status, location, depth, and then GenBank accession. Species names applied to lineages (to the right of the tree) are followed by a '?' in instances where the taxonomic name is equivocal (i.e. not confirmed through type or topotype comparisons).

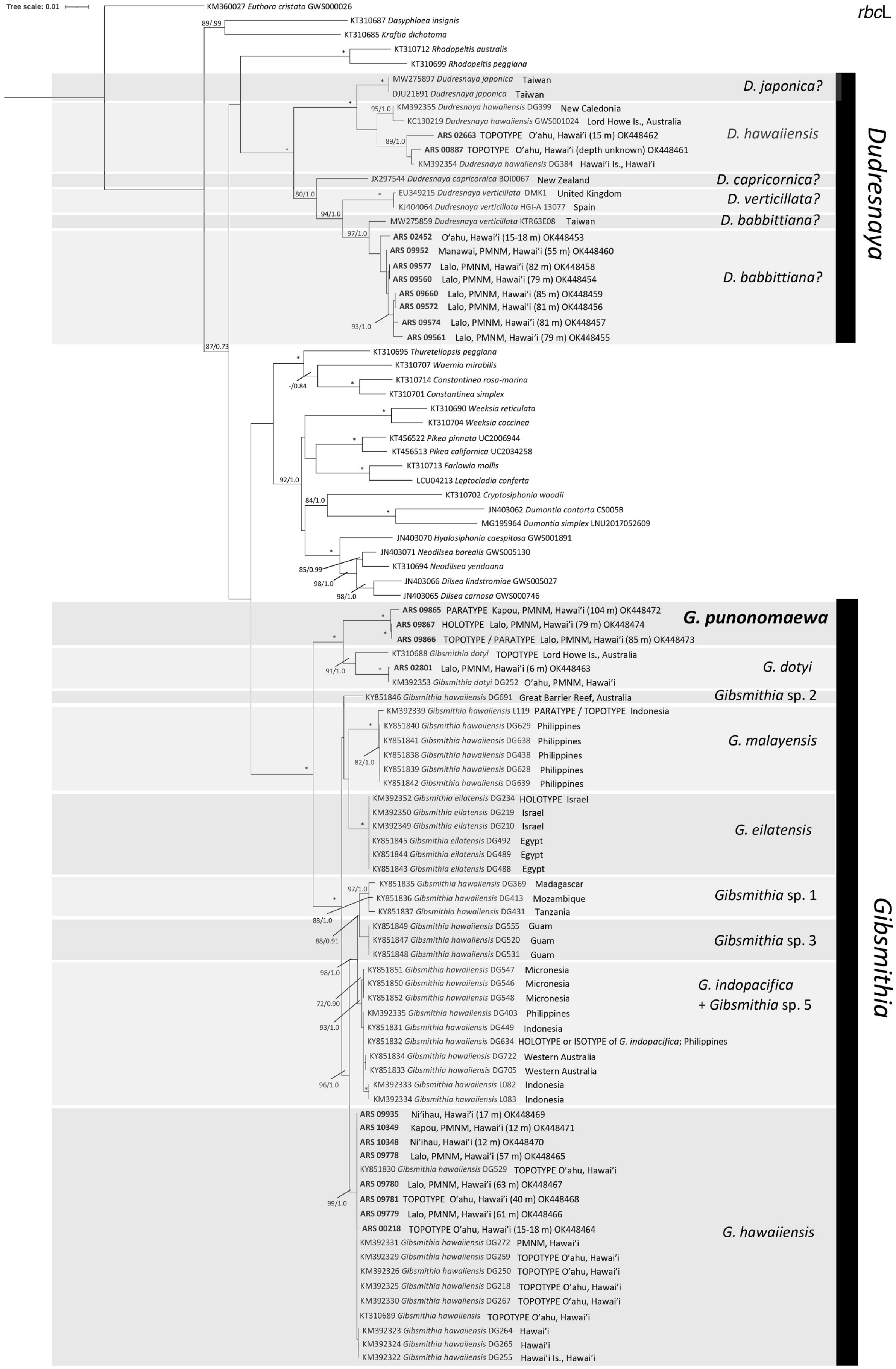

Fig. 2. Maximum likelihood phylogenetic tree of *rbc*L sequences for *Gibsmithia* and *Dudresnaya*, along with representatives of the Dumontiaceae. Numbers along branches indicate nodal support (first value = bootstrap support, second value = Bayesian posterior probabilities). Nodes with full support are indicated with an asterisk. Scale bar indicates number of substitutions per site. For sequences included from GenBank, accession codes are listed before taxonomic names, with

VEGETATIVE MORPHOLOGY AND ANATOMY

Plants comparatively large, up to 11 cm in height, most commonly 5–9 cm high (Figs 4–9), attached to coral rubble (Figs 4, 5). Colour of live plants distinctively orange-red, with a dark red stalk (Figs 5, 6). Colour of dried plants substantially different from living plants; including areas of light rose-pink (usually in the mid- to upper regions of the branches), and lime- to olive-green close to the stalk and sometimes also at the branch tips (Figs 7–9). Stalk dries to a brick red colour and can be branched or unbranched (Figs 7–9). Up to six main branching points of the mucilaginous branches from the single blade, each branching further in an irregular manner, giving the plants a look of many long, cylindrical branched branches (Figs 7–9). Thalli appear smooth, lacking exserted cortical filaments. Cortical fascicles composed of filaments that branch every 1–3 cells to form a cluster-like appearance at their tips; this appearance also emphasized by short ultimate branches that are 1–3 cells in length (Figs 11, 12). Cells of the cortical fascicles range from cylindrical to subclavate in shape, longer (11.0–21.1 μm) than broad (3.8–6.9 μm; Fig. 12). Medulla composed of mostly cylindrical cells (18.4–36.6 μm long × 3.5–5.0 μm wide) and with numerous slender rhizoidal filaments (Fig. 12).

REPRODUCTIVE MORPHOLOGY AND ANATOMY

Subspherical or ovoid tetrasporangia arising in a terminal position on lateral filaments (Figs 13, 14), subtended by a cylindrical to subconical cell (i.e. not isodiametric; Fig. 14). Tetrasporangial divisions either decussate or cruciate (Figs 13, 14). Clusters of small (2–4 μm), spherical spermatangia produced at the tips of cortical fascicles (Fig. 15). Pre-fertilization (female) and early stages of post-fertilization not observed. Numerous auxiliary cell filaments present in specimens, 7–14 cells long (Figs 16, 17), terminating in sterile extension of unmodified vegetative cells. Auxiliary cell variable in shape. Mature cystocarps 150–360 μm in diameter, producing numerous rounded, globose carposporangia (Fig. 18).

DISCUSSION

In this study, analyses of the COI, *rbc*L and UPA markers were used to clarify the phylogenetic relationships of Hawaiian representatives of the Dumontiaceae (*Dudresnaya* and *Gibsmithia*), including recognition of a new species (*G. punonomaewa*) from the Hawaiian mesophotic. This represents the eighth species in the genus and the fourth outside of the *G. hawaiiensis* species complex (Guiry & Guiry 2021). Three *Gibsmithia* species are now known from the Hawaiian Islands (*G. dotyi, G. hawaiiensis* and *G. punonomaewa*) and two are believed to be endemic (*G. hawaiiensis sensu stricto* and *G. punonomaewa*) (Abbott 1999; Abbott & McDermid 2001; Gabriel *et al.* 2017; this study). Additionally, two of the three Hawaiian species ascribed to *Dudresnaya* are confirmed in our analyses (*D. babbittiana* and *D. hawaiiensis*). The status of the third, *D. littleri*, is discussed in more detail below. It is important to reiterate that generitype sequences for *Dudresnaya* remain unavailable (given the lack of clarity on the type locality of the lectotype); all available sequences ascribed to *Dudresnaya* specimens therefore remain provisionally identified as members of that genus.

A combination of molecular and morpho-anatomical data was used to examine the distinctiveness of *G. punonomaewa* from other members of the genus. Our COI and *rbc*L phylogenies were consistent in topology with those published by Gabriel *et al.* (2016, 2017), which allowed phylogenetic positioning of the new *Gibsmithia* species. These analyses demonstrated that *G. punonomaewa* is sister to *G. dotyi* (based on both COI and *rbc*L), and sister to *G. larkumii* in the UPA barcoding analysis (with *G. larkumii* and *G. punonomaewa* sister to *G. dotyi* based on UPA). *Gibsmithia punonomaewa* and *G. dotyi* share 90.2%–92.4% identity based on COI and 95.8%–96.0% based on *rbc*L. The two species differ in gross morphology; *G. punonomaewa* thalli consist of terete, cylindrical gelatinous lobes, whereas *G. dotyi* typically has flattened lobes (Kraft 1986; this study). The only sequence data available for *G. larkumii* are a set of three UPA sequences from Yemen, Tanzania, and a topotype sequence from Australia, which we used in a phylogenetic analysis of the genus to demonstrate the distinction between *G. punonomaewa* and *G. larkumii* (they exhibited 98.7% sequence similarity based on the highly conserved UPA marker). *Gibsmithia punonomaewa* and *G. larkumii* also differ in a key morphological character; *G. punonomaewa* possesses a stipe, which is absent in *G. larkumii* (Kraft 1986; Schils & Coppejans 2002; this study). In addition to a clear phylogenetic distinction, *G. punonomaewa* differs morphologically from *G. hawaiiensis sensu lato* by its smooth gelatinous lobes that lack exserted cortical filaments (Gabriel *et al.* 2016, 2017). Of the described *Gibsmithia* species, only *G. womersleyi* Kraft & R.W. Ricker (in Kraft 1986) lacks DNA sequence data for comparison. Although *G. punonomaewa* and *G. womersleyi* share superficial morphological similarity, they have a number of characters that differ, or that have overlapping but different ranges in value: *G. womersleyi* can be much larger (up to 48 cm in height vs 11 cm in *G. punonomaewa*); has an unbranched stalk (the stalk of *G. punonomaewa* can be branched or unbranched); auxiliary cell branches 9–19 cells in length (vs 7–14 cells in *G. punonomaewa*); a trapezoidal profile shape of auxiliary cells (vs variably shaped auxiliary cells in *G. punonomaewa*); smaller cystocarps (120–250 μm diameter vs 150–360 μm in *G. punonomaewa*); and a different shape of cells subtending tetrasporangia (stout and nearly isodiametric in *G. womersleyi* vs cylindrical or subconical in *G. punonomaewa*) (Kraft 1986; this study). Nonetheless, the generation of DNA sequence data for *G. larkumii* and *G. womersleyi* in the future will greatly aid these taxonomic comparisons.

Phylogenetic analyses of Hawaiian *Dudresnaya* samples probably confirm two of the three currently recognized species in this genus in Hawai‘i, *D. hawaiiensis* and

specimen voucher codes listed afterwards. Sequences generated in the current study are indicated in bold, and are listed by specimen voucher code, type status, location, depth, and then GenBank accession. Species names applied to lineages (to the right of the tree) are followed by a '?' in instances where the taxonomic name is equivocal (i.e. not confirmed through type or topotype comparisons).

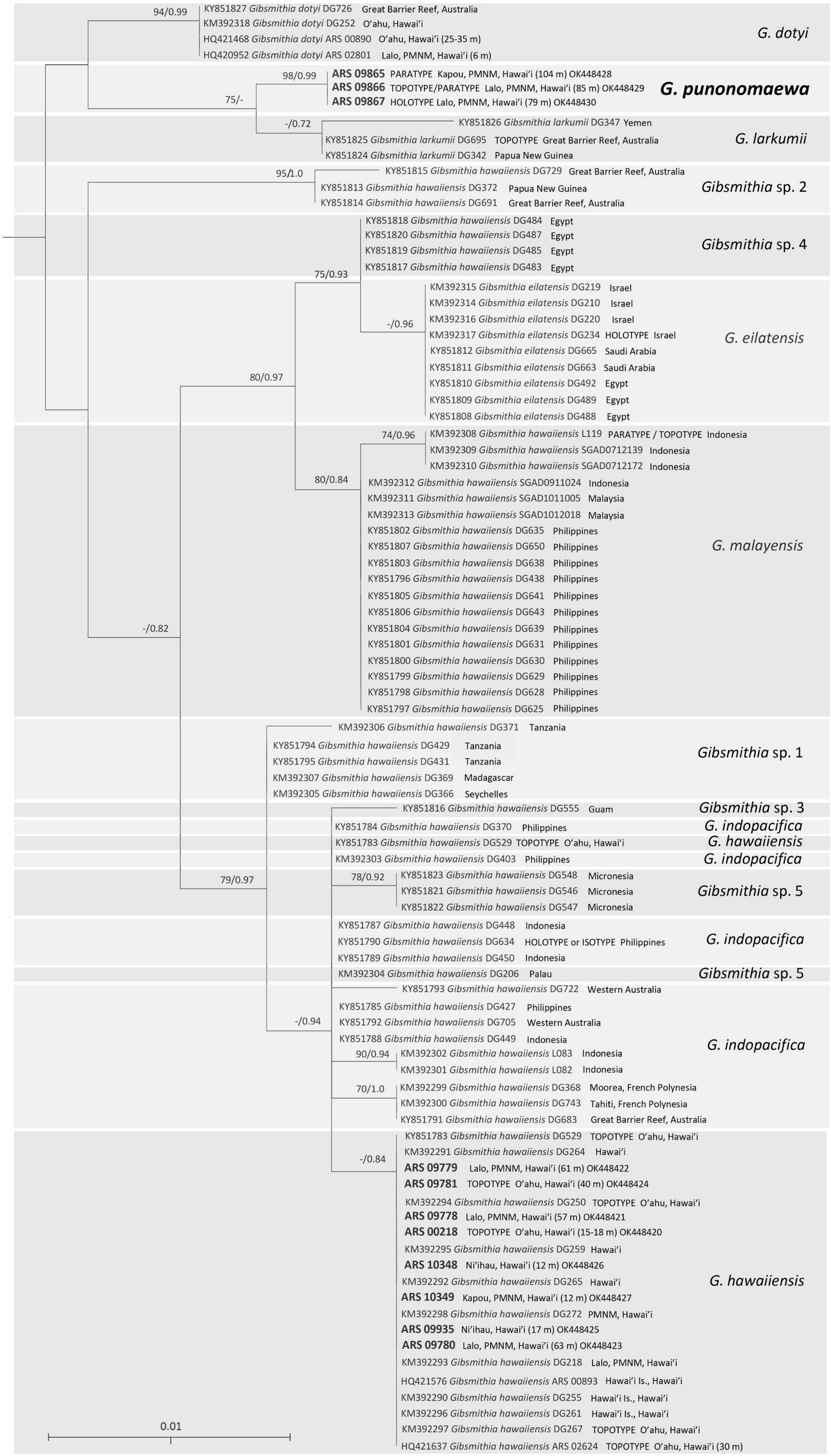

Fig. 3. Maximum likelihood phylogenetic tree of *Gibsmithia* UPA sequences. Numbers along branches indicate nodal support (first value = bootstrap support, second value = Bayesian posterior probabilities). Nodes with full support are indicated with an asterisk. Scale bar indicates number of substitutions per site. For sequences

D. babbittiana, pending sequencing of the type material of the latter species and the generitype specimen; no new species were revealed. Topotype material for *D. hawaiiensis* was included to confirm the phylogenetic position of that taxon; however, no type or topotype material has yet been sequenced for *D. babbittiana*, and so our conclusions for this taxon remain tentative. A number of mesophotic specimens were identified as *D. babbittiana* based on the key morpho-anatomical characters for that species (plant size 10–18 cm high at maturity, habit of irregular branching and slender branches, axial cell diameter of primary axes 30–80 µm, few slender medullary rhizoids, zonate tetrasporangia in outer parts of cortical filaments, cylindrical outer cortical cells, and the ability to distinguish order of branching from pressed specimens) (Abbott & McDermid 2001). Our specimens, morpho-anatomically corresponding to *D. babbittiana*, are from Lalo (French Frigate Shoals), Manawai (Pearl & Hermes Atoll) and Oʻahu, and are from as deep as 85 m. *Dudresnaya verticillata* (Withering) Le Jolis, which is not a member of the Hawaiian flora, was phylogenetically placed in two distinct lineages on the COI tree; one sister to all other *Dudresnaya* sequences (KT307584, from Ireland), and one sister to *D. babbittiana* (KM406848, from the United Kingdom). In the *rbc*L analyses, *D. verticillata* was also represented by two lineages: one with a single specimen from Taiwan (MW275859), which was sister to *D. babbittiana*, and one containing two specimens (EU349215 and KJ404064) from European localities, which was sister to the above two lineages. The Taiwanese specimen may represent a range extension of *D. babbittiana*, but more data are needed to confirm this. Given that the exact location of the lectotype specimen of *D. verticillata* is unknown, but believed to be on the south coast of England (Robins & Kraft 1985; Guiry & Guiry 2021), additional molecular and morphological data for *D. verticillata* are needed to confirm which of the two COI lineages corresponds to this species (if either). Regardless, the Hawaiian specimens morpho-anatomically corresponding to *D. babbittiana* appear to be a well-defined lineage, and separate from *D. verticillata*. No specimens of *D. littleri*, the third species of *Dudresnaya* reported from Hawaiʻi, were available for inclusion in this study. However, two LSU sequences were included in Sherwood *et al.* (2010) that illustrated that these specimens are quite distinct from other members of the genus, and research is underway to propose *D. littleri* as a member of a new genus (Masakazu Hoshino, personal communication).

Most *Gibsmithia* species have been reported from shallow waters, sometimes as deep as the upper mesophotic zone (35 m), while *G. hawaiiensis* occurs in the mid-mesophotic (e.g. ARS 09780 is from 63 m depth) as well as shallow depths. *Gibsmithia punonomaewa* represents the first report of an exclusively mesophotic species for the genus and joins a growing list of Hawaiian mesophotic algae that appear to be depth specialists restricted to deep waters (some species of *Codium*, Silva & Chacana 2013; some Ulvales, Spalding *et al.* 2016; some *Martensia*, Sherwood *et al.* 2019; *Sonderophycus*, Sherwood *et al.* 2020; *Haraldiophyllum*, Paiano *et al.* 2020; *Psaromenia*, Cabrera *et al.* 2021; *Halopeltis* and *Leptofauchea*, Alvarado 2021; *Ethelia*, Sherwood *et al.* 2021b; *Croisettea*, Cabrera *et al.* 2022). Most new algal species descriptions from the Hawaiian mesophotic zone have been for those found only at these depths; thus, little support is emerging for the concept of shallow-to-deep connectivity and mesophotic reefs acting as refuges for shallow reefs (the Deep Reef Refuge Hypothesis; Bongaerts & Smith 2019).

The biogeographical affinities of *G. punonomaewa* are difficult to discern; its closest relatives (*G. dotyi* and *G. larkumii*) are widespread in reported distribution (Australia, the western Pacific, Africa, the Middle East, and Hawaiʻi; Guiry & Guiry 2021), albeit a large majority of these distributional records are based solely on morphological and anatomical records, and have not been confirmed by DNA sequencing. Most *Gibsmithia* species (except *G. womersleyi*) are known only from tropical and/or subtropical waters (Kraft 1986; Gabriel *et al.* 2017), and thus the mesophotic habitats of the Papahānaumokuākea Marine National Monument represent somewhat of an anomaly for members of this genus. Although water temperatures at the locations where the *G. punonomaewa* specimens were collected ranged from 21.8°C to 23.3°C (at 79–104 m depth), only a small fraction of surface irradiance is available to these plants at these depths (Kahng *et al.* 2019), and these dark environments contrast strongly with the more typical shallow water habitats of *Gibsmithia*. Further explorations of understudied regions and habitats are likely to yield additional species of *Gibsmithia*. Their inclusion in comparative analyses will be critical for understanding the biogeography and evolutionary history of this charismatic red algal genus.

Key to the ‘smooth’ species of *Gibsmithia* (i.e. those lacking exserted cortical filaments)

1a. Thallus lacking a cartilaginous stalk and attached by a filamentous pad .... *G. larkumii*
1b. Thallus with an obvious cartilaginous stalk 2
2a. Stalk (or stalk branches) giving rise to a single blade, which divides into multiple terete, branched lobes.. *G. punonomaewa*
2b. Stalk (or stalk branches) giving rise directly to several to many terete to compressed, branched lobes 3
3a. Plants up to 5 cm in height, with broadly lobed blades or blades with short branches .... *G. dotyi*
3b. Plants up to 48 cm in height, with linear to subdichotomous branches .... *G. womersleyi*

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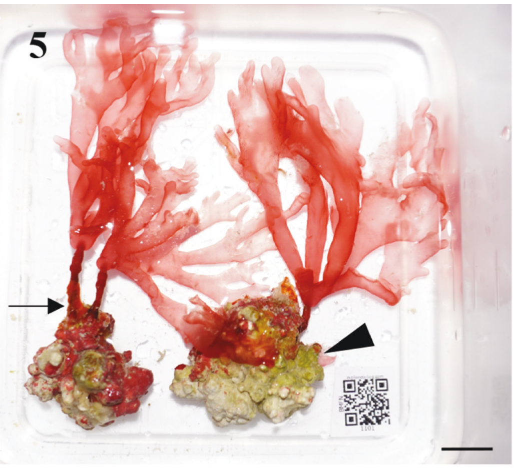
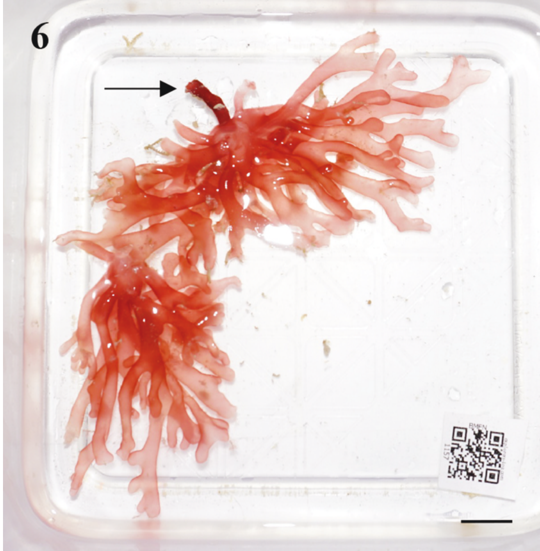
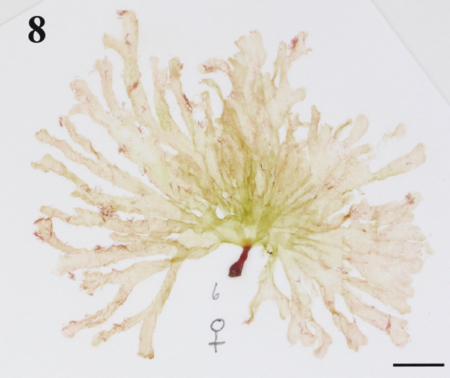
included from GenBank, accession codes are listed before taxonomic names, with specimen voucher codes listed afterwards. Sequences generated in the current study are indicated in bold, and are listed by specimen voucher code, type status, location, depth, and then GenBank accession. Species names applied to lineages (to the right of the tree) are followed by a ‘?’ in instances where the taxonomic name is equivocal (i.e. not confirmed through type or topotype comparisons).



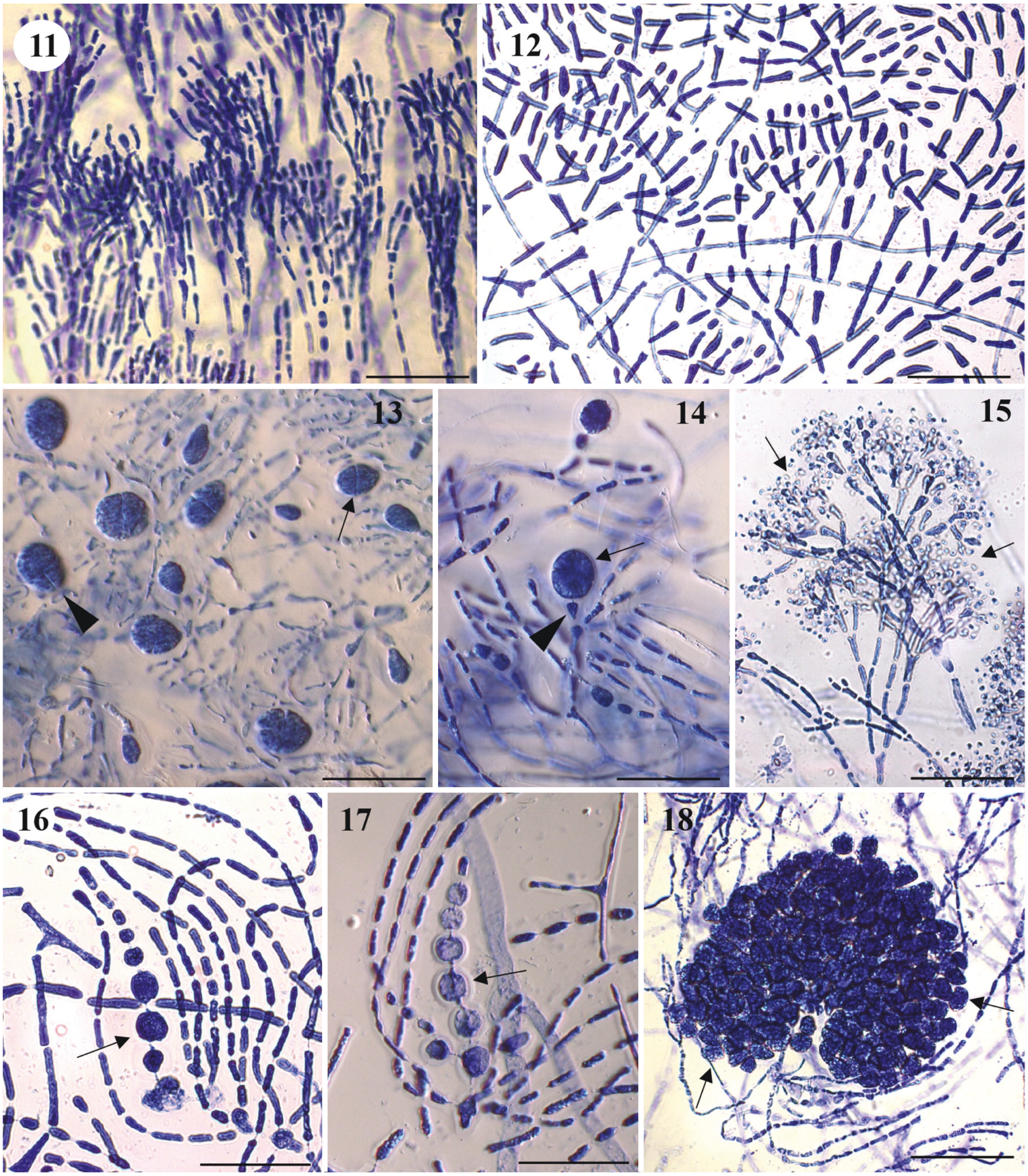




Figs 4–10. General morphology of *Gibsmithia punonomaewa* from *in situ* photographs, and images of live and pressed specimens. Scale bars = 1 cm.

Fig. 4. *In situ* photograph of *G. punonomaewa* growing attached to coral rubble at 79 m depth at Lalo (French Frigate Shoals), Hawaíi (BISH 783205; ARS 09867).

Fig. 5. Post-collection photograph of specimens while still living, illustrating distinctive orangey-red colour of thallus, branched stipe (arrow), and attachment to coral rubble (arrowhead) (BISH 783201; ARS 09865).



Figs 11–18. Morphology and anatomy of *G. punonomaewa*, LM.

Fig. 11. Cortical fascicles composed of filaments that branch every 1–3 cells (BISH 783205; ARS 09867). Scale bar = 50 µm.

Fig. 12. Slender rhizoidal filaments of the medulla (BISH 783205; ARS 09867). Scale bar = 50 µm.

Fig. 13. Subspherical or ovoid-shaped tetrasporangia that exhibit either cruciate (arrow) or decussate (arrowhead) divisions (BISH 783205; ARS 09867). Scale bar = 50 µm.

Fig. 14. Tetrasporangia (arrow) arise from a terminal position on lateral filaments (BISH 783201; ARS 09865). Tetrasporangium is subtended by a subconical-shaped cell (arrowhead). Scale bar = 50 µm.

Fig. 15. Clusters of small spherical spermatangia (arrows) produced at the tips of cortical fascicles (BISH 783201; ARS 09865). Scale bar = 50 µm.

Fig. 16. Auxiliary cell filament (arrow) of a female gametophyte (BISH 783205; ARS 09867). Scale bar = 50 µm.

Fig. 17. Auxiliary cell filament (arrow) of a female gametophyte (BISH 783205; ARS 09867). Scale bar = 50 µm.

Fig. 18. A mature cystocarp with numerous, globose carposporangia (arrows) (BISH 783201; ARS 09865). Scale bar = 100 µm.

Fig. 6. Post-collection photograph of specimens while still living, illustrating stipe (arrow) (BISH 783202; ARS 09866).

Fig. 7. Image of holotype specimen (tetrasporangial, BISH 783203; ARS 09867).

Fig. 8. Image of isotype specimen (carposporangial, BISH 783204; ARS 09867).

Fig. 9. Image of isotype specimens (BISH 783205; ARS 09867).

Fig. 10. Image of isotype specimen (tetrasporangial, HAW-A-02881; ARS 09867). Cartilaginous stalk with multiple branches (arrow).

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REFERENCES

Abbott I.A. 1999. *The marine red algae of the Hawaiian Islands*. Bishop Museum Press, Honolulu, Hawaii, USA. 477 pp.

Abbott I.A. & McDermid K.J. 2001. *Dudresnaya babbittiana* (Dumontiaceae, Gigartinales), a new red algal species from Midway Atoll, North Central Pacific. *Cryptogamie, Algologie* 22: 249–261. DOI:10.1016/S0181-1568(01)01061-3.

Alvarado E.A. 2021. *Uncovering diversity in the mesophotic zone of Hawaiʻi: species new to science in the genera* Halopeltis *and* Leptofauchea (*Rhodymeniales, Rhodophyta*). MSc thesis. University of Hawaiʻi at Mānoa, Honolulu, Hawaii, USA. 59 pp.

Bárbara I., Gallardo T., Cremades J., Barreiro R., Maneiro I. & Saunders G.W. 2013. *Pseudopolyides furcellarioides* gen. et sp. nov. (Gigartinales, Rhodophyta) an erect member of the Cruoriaceae based on morphological and molecular evidence. *Phycologia* 52: 191–203. DOI:10.2216/12-040.1.

Bongaerts P. & Smith T.B. 2019. Beyond the "Deep Reef Refuge" hypothesis: a conceptual framework to characterize persistence at depth. In: *Mesophotic coral ecosystems* (Ed. by Y. Loya, K.A. Puglise & T.C. L. Bridge), pp 881–895. Springer, New York, New York, USA.

Cabrera F.P., Huisman J.M., Spalding H.L., Kosaki R.K. & Sherwood A.R. 2021. Diversity of Kallymeniaceae (Gigartinales, Rhodophyta) associated with Hawaiian mesophotic reefs. *European Journal of Phycology* 57: 1–11.

Cabrera F.P., Huisman J.M., Spalding H.L., Kosaki R.K., Smith C.M. & Sherwood A.R. 2022. Cryptic diversity in the genus *Croisettea* (Kallymeniaceae, Rhodophyta) from Hawaiian mesophotic reefs. *Phycologia* 61: in press.

Doty M.S. 1963. *Gibsmithia hawaiiensis* gen. n. et sp. n. *Pacific Science* 17: 458–465.

Edgar R.C. 2004. MUSCLE: a multiple sequence alignment method with reduced time and space complexity. *BMC Bioinformatics* 5: Article 113. Article 113. DOI:10.1186/1471-2105-5-113.

Gabriel D., Draisma S.G.A., Sauvage T., Schmidt W.E., Schils T., Lim P.-E., Harris D.J. & Fredericq S. 2016. Multilocus phylogeny reveals *Gibsmithia hawaiiensis* (Dumontiaceae, Rhodophyta) to be a species complex from the Indo-Pacific, with the proposal of *G. eilatensis sp. nov*. *Phytotaxa* 277: 1–20. DOI:10.11646/phytotaxa.277.1.1.

Gabriel D., Draisma S.G.A., Schmidt W.E., Schils T., Sauvage T., Maridakis C., Gurgel C.F.D., Harris D.J. & Fredericq S. 2017. Beneath the hairy look: the hidden reproductive diversity of the *Gibsmithia hawaiiensis* complex (Dumontiaceae, Rhodophyta). *Journal of Phycology* 53: 1171–1192. DOI:10.1111/jpy.12593.

Gavio B. & Fredericq S. 2002. *Grateloupia turuturu* (Halymeniaceae, Rhodophyta) is the correct name of the non-native species in the Atlantic known as *Grateloupia doryphora*. *European Journal of Phycology* 37: 349–359. DOI:10.1017/S0967026202003839.

Guiry M.D. & Guiry G.M. 2021. *AlgaeBase*. World-wide electronic publication, National University of Ireland, Galway. http://www.AlgaeBase.org; searched on 01 October 2021.

Huelsenbeck J.P., Ronquist F. & Hall B. 2003. MrBayes: a program for the Bayesian inference of phylogeny. *Version* 3: b4.

Kahng S.E., Akkaynak D., Schlesinger T., Hochberg E.J., Wiedenmann J., Tamir R. & Tchernov D. 2019. Light, temperature, photosynthesis, heterotrophy, and the lower depth limits of mesophotic coral ecosystems. In: *Mesophotic coral ecosystems* (Ed. by Y. Loya, K.A. Puglise & T.C.L. Bridge), pp 801–828. Springer, New York, New York, USA.

Kang J.C. & Kim M.S. 2013. A novel species *Symphyocladia glabra sp. nov*. (Rhodomelaceae, Rhodophyta) from Korea based on morphological and molecular analyses. *Algae* 28: 149–160. DOI:10.4490/algae.2013.28.2.149.

Kim M.S., Kim S.Y. & Nelson W.A. 2010. *Symphyocladia lithophila sp. nov*. (Rhodomelaceae, Ceramiales), a new Korean red algal species based on morphology and *rbc*L sequences. *Botanica Marina* 53: 233–241. DOI:10.1515/BOT.2010.031.

Kraft G.T. 1986. The genus *Gibsmithia* (Dumontiaceae, Rhodophyta) in Australia. *Phycologia* 25: 423–447. DOI:10.2216/i0031-8884-25-4-423.1.

Lanfear R., Frandsen P.B., Wright A.M., Senfeld T. & Calcott B. 2017. PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. *Molecular Biology and Evolution* 34: 772–773. DOI:10.1093/molbev/msw260.

McDermid K.J. & Abbott I.A. 2006. Deep subtidal marine plants from the Northwestern Hawaiian Islands: new perspectives on biogeography. *Atoll Research Bulletin* 543: 525–532.

Miller M.A., Pfeiffer W. & Schwartz T. 2010. *Creating the CIPRES Science Gateway for inference of large phylogenetic trees*. 2010 Gateway Computing Environments Workshop (GCE). IEEE, New Orleans, USA. 8 pp.

Paiano M.O., Huisman J.M., Cabrera F.P., Spalding H.L., Kosaki R.K. & Sherwood A.R. 2020. *Haraldiophyllum hawaiiense sp. nov*. (Delesseriaceae, Rhodophyta): a new mesophotic genus record for the Hawaiian Islands. *Algae* 35: 337–347. DOI:10.4490/algae.2020.35.11.5.

Rambaut A., Drummond A.J., Xie D., Baele G. & Suchard M.A. 2018. Posterior summarisation in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology* 67: 901–904.

Robins P.A. & Kraft G.T. 1985. Morphology of the type and Australian species of *Dudresnaya* (Dumontiaceae, Rhodophyta). *Phycologia* 24: 1–34. DOI:10.2216/i0031-8884-24-1-1.1.

Saunders G.W. 2005. Applying DNA barcoding to red macroalgae: a preliminary appraisal holds promise for future applications.

Philosophical Transactions of the Royal Society, Series B 360: 1879–1888. DOI:10.1098/rstb.2005.1719.

Saunders G.W. 2008. A DNA barcode examination of the red algal family Dumontiaceae in Canadian waters reveals substantial cryptic species diversity. 1. The foliose *Dilsea-Neodilsea* complex and *Weeksia*. *Botany* 86: 773–789. DOI:10.1139/B08-001.

Schils T. & Coppejans E. 2002. Gelatinous red algae of the Arabian Sea, including *Platoma hetermorphum sp. nov*. (Gigartinales, Rhodophyta). *Phycologia* 41: 254–267. DOI:10.2216/i0031-8884-41-3-254.1.

Sherwood A.R., Kurihara A., Conklin K.Y., Sauvage T. & Presting G.G. 2010. The Hawaiian Rhodophyta biodiversity survey (2016-2010): a summary of principal findings. *BMC Plant Biology* 10: Article 258. DOI: 10.1186/1471-2229-10-258.

Sherwood A.R., Lin S.M., Wade R.M., Spalding H.L., Smith C.M. & Kosaki R.K. 2019. Characterization of *Martensia* (Delesseriaceae; Rhodophyta) from shallow and mesophotic habitats in the Hawaiian Islands: description of four new species. *European Journal of Phycology* 55: 172–185. DOI:10.1080/09670262.2019.1668062.

Sherwood A.R., Paiano M.O., Spalding H.L. & Kosaki R.K. 2020. Biodiversity of Hawaiian Peyssonneliales (Rhodophyta): *Sonderophycus copusii sp. nov*., a new species from the Northwestern Hawaiian Islands. *Algae* 35: 145–155. DOI:10.4490/algae.2020.35.5.20.

Sherwood A.R., Paiano M.O., Wade R.M., Cabrera F.C., Spalding H.L. & Kosaki R.K. 2021a. Biodiversity of Hawaiian Peyssonneliales (Rhodophyta). 1. Two new species in the genus *Ramicrusta* from Lehua Island. *Pacific Science* 75: 185–195. DOI:10.2984/75.2.2.

Sherwood A.R., Paiano M.O., Cabrera F.P., Spalding H.L., Hauk B.B. & Kosaki R.K. 2021b. *Ethelia hawaiiensis* (Etheliaceae, Rhodophyta), a new mesophotic marine alga from Manawai (Pearl and Hermes Atoll), Papahānaumokuākea Marine National Monument, Hawai'i. *Pacific Science* 75: 237–246. DOI:10.2984/75.2.6.

Sherwood A.R. & Presting G.G. 2007. Universal primers amplify a 23S rDNA plastid marker in eukaryotic algae and cyanobacteria. *Journal of Phycology* 43: 605–608. DOI:10.1111/j.1529-8817.2007.00341.x.

Silva P.C. & Chacana M.E. 2013. Validation of names of four Hawaiian species of *Codium* (Chlorophyta). *Nova Hedwigia* 98: 253–256. DOI:10.1127/0029-5035/2013/0139.

Spalding H.L., Conklin K.Y., Smith C.M., O'Kelly C.J. & Sherwood A.R. 2016. New Ulvaceae (Ulvophyceae, Chlorophyta) from mesophotic ecosystems across the Hawaiian Archipelago. *Journal of Phycology* 52: 40–53. DOI:10.1111/jpy.12375.

Stamatakis A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30: 1312–1313. DOI:10.1093/bioinformatics/btu033.

Thiers B. 2022. *Index Herbariorum: a global directory of public herbaria and associated staff.* New York Botanical Garden's Virtual Herbarium [continuously updated]. http://sweetgum.nybg.org/ih/.