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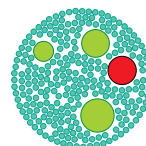
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Molecular confirmation and morphological reassessment of *Udotea geppiorum* (Bryopsidales, Chlorophyta) with ecological observations of mesophotic meadows in the Main Hawaiian Islands

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ABSTRACT
Udotea geppiorum is reported to form meadows in deep-water, soft sediment habitats in the Main Hawaiian Islands. The identification of the species, initially published as *U. argentea* in the archipelago, is revised and confirmed based on morphological assessment and comparative DNA sequencing with western Pacific specimens. *Udotea geppiorum* is fully corticated and newly shown to display discrete concentric segments arranged in a 'tongue and groove' manner, which are connected by medullary siphons running along the entire segment interface. *Udotea geppiorum* is phylogenetically nested among a cluster of *Udotea* spp. recently circumscribed within *Udotea sensu stricto*. This cluster of species is separate from the remainder of the family Udoteaceae, which includes several genera and *Udotea* spp. *sensu lato* that lack or exhibit incomplete cortication. Based on field observations conducted in O'ahu and Maui, *U. geppiorum* can be found from 20 m to > 90 m depth with a peak in abundance at mesophotic depths of 60–85 m, where it forms extensive meadows that support associated diversity.

Introduction

Although rarely explored, deep-water marine assemblages in the tropics have been shown to possess a remarkable biodiversity, including species new to science and new distributional records of macroalgae (e.g. Agegian & Abbott, 1985; Littler *et al.*, 1985; Hanisak & Blair, 1988; Ballantine, 1990; Norris & Olsen, 1991; Ballantine & Norris, 1994; Ballantine & Aponte, 2002; Graham *et al.*, 2007). However, because of their richer macroalgal biodiversity in comparison to soft bottom habitats, most studies of macroalgae in deep-water habitats have focused on assemblages attached to hard substratum (such as fore-reefs and pinnacles, e.g. Hillis-Colinvaux, 1986; Hanisak & Blair, 1988; Norris & Olsen, 1991; Aponte & Ballantine, 2001; Ballantine & Aponte, 2005; Graham *et al.*, 2007; Leichter *et al.*, 2008).

At these mesophotic depths (i.e. from 30–40 m to 150 m; Hinderstein *et al.*, 2010), large expanses of sand may provide habitat for the growth of extensive meadows formed by psammophytic siphonous green algae (Bryopsidales) that can anchor themselves in soft bottom substratum via rhizoids, such as *Halimeda incrassata* (Ellis) Lamouroux (Sangil *et al.*, 2018). These meadows may be defined as "relatively flat areas where sizeable and relatively dense upright living algal

populations (i.e. rhipsalian *Halimeda*) occur" (Freile *et al.*, 1995: 28), but the definition may also include other bryopsidalean genera, such as the Udoteacean taxon *Penicillus capitatus* Lamarck (e.g. at depths of 28–50 m in the Canary Islands, Sangil *et al.*, 2010). While mesophotic algal meadows are still poorly documented, they may be widespread, especially as they have been reported from a broad geographic range, spanning the Atlantic and the Pacific basin, in the Bahamas (Freile *et al.*, 1995), Canary Islands (Sangil *et al.* 2010), the Great Barrier Reef (Drew & Abel, 1988), the Marshall Islands (Hillis-Colinvaux, 1988) and Hawai'i (Smith *et al.*, 2005; Kahng & Kelley, 2007; Spalding, 2012).

In the Hawaiian Islands, the photic zone extends to at least 250 m (Agegian & Abbott, 1985) and consists of deep-water macroalgal assemblages and coral reefs (Doty *et al.*, 1974; Agegian & Abbott, 1985; Kahng & Kelley, 2007). During the course of baseline surveys conducted between 2000 and 2006 in the deep waters off O'ahu to explore the distribution of the invasive macroalga *Avrainvillea amadelpha* (Montagne) A. Gepp & E. Gepp (see Brostoff, 1989), a mesophotic *Udotea* species was discovered forming extensive meadows in sand at depths of 60–85 m. Since these surveys, the taxon was also encountered in 2007 by the City and

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County of Honolulu in recently dredged areas of Mamala Bay, and reported as a potentially introduced species by local scientists, who identified it as *U. argentea* Zanardini (Bailey-Brock & Magalhães, 2010). However, a pronounced morphological feature of these mesophotic *Udotea* specimens was the distinct concentric segmentation of the flabellum, a character that does not apply to *U. argentea* ("obsolete zonata" in Zanardini 1858, i.e. zonation scarcely apparent). A search for Pacific *Udotea* species exhibiting similar habits among the currently accepted *Udotea* species listed in AlgaeBase (Guiry & Guiry, 2019) and a review of the primary literature, both pointed at a rarely reported species known as *U. geppiorum* Yamada (1930). In congruence with this identification, was the discovery through the Macroalgal Herbarium Portal (macroalgae.org/portal/) of a Hawaiian specimen held at BISH (Thiers, 2019) collected in 2005 by Dr R. Okano off Kalaeloa, south-west of Oʻahu, that the late Dr I.A. Abbott had identified as *Udotea geppii* (subsequently annotated as *U. geppiorum* by Dr R. Tsuda in 2013, see Supplementary fig. S1).

Udotea geppiorum was originally described as *U. geppii* Yamada (1930) in honour of Gepps; however according to Guiry & Guiry (2019), the currently accepted epithet with proper genitive plural is *Udotea geppiorum*. Yamada's (1930: 141) description was based on type material collected from the Caroline Islands, Palau in SAP (Supplementary fig. S2) and material from the Friendly Islands (Tonga) distributed by Harvey as "Friendly Isl. Alg. No. 94" in BM, and labelled *Udotea flabellata*, which Yamada considered to be conspecific with his Caroline Islands material. Both of Harvey's *U. flabellata* specimens in NY and MICH display similar external features to *U. geppiorum* (Supplementary figs S3-S4). The name *Udotea flabellata* was introduced by Lamouroux (1816: 311) who included *Corallina flabellum* J.Ellis & Solander (1786: 124, pl. 24) in synonymy, rendering Lamouroux's name superfluous and illegitimate. The correct combination *Udotea flabellum* (J.Ellis & Solander) M.Howe was later made by Howe (1904: 94, pl. 6). Yamada (1930) did not designate a holotype in the protologue of *U. geppiorum* and the type specimens from Palau and Tonga thus represent syntypes (see M.J. Wynne annotation, Supplementary fig. S4).

Recently, Lagourgue et al. (2018) explored Udoteaceae diversity in the Caribbean region based on morphometrics and DNA sequencing of chloroplast barcodes (*tuf*A and *rbc*L) and nuclear markers (18S rRNA). While these authors noted the polyphyly of the genus *Udotea* (as previously reported by Kooistra, 2002), their assessment showed that species exhibiting complete cortication by lateral appendages throughout the thalli (stipe and blade) were monophyletic and included the generitype *U. flabellum* (see fig. 4, p. 766 in Lagourgue et al., 2018). Based on these findings, these authors delimited

Udotea sensu stricto in which they included *U. dixonii* Littler & Littler, *U. dotyi* Littler & Littler, *U. flabellum* and *U. occidentalis* Gepp & Gepp, while proposing that *U. argentea*, *U. geppiorum* and *U. norrisii* Littler & Littler should also be included pending further DNA sequencing for verification. In contrast, the remainder of *Udotea* spp. sequenced to date (i.e. those phylogenetically remote from *Udotea sensu stricto*) are incompletely corticated by lateral appendages (i.e. not forming a continuous cortex, e.g. *U. spinulosa* Howe), or lack cortication on the blade (although cortication may be present in the stipe, e.g. *U. conglutinata* (J.Ellis & Solander) J.V.Lamouroux and *U. cyathiformis* Decaisne).

Since Yamada's (1930) description, *U. geppiorum* was recorded from a few south-east Asian locations such as Indonesia (e.g. Atmadja & Prud'homme van Reine, 2014; Philippines: Silva et al., 1987) and Pacific Islands (e.g. Micronesia: Lobban & Tsuda, 2003; Fiji: Littler & Littler, 2003; Mariana Islands: Tsuda, 2003) and may thus be broadly distributed in the western Pacific. In Palau, Ohba et al. (2007) reported that the species was common, growing solitary or in clusters in shallow and deeper lagoons, and outer reefs from 0.5 to 35 m depth. By contrast, in Southern Pacific localities, Littler & Littler (2003) reported that it is not found below 10 m depth. In spite of these records and field observations, the taxon still eludes broader recognition and *U. geppiorum* may often be misidentified in Pacific localities, i.e. as *U. argentea* or *U. flabellum*. This may stem from the fact that the protologue of *U. geppiorum* is very brief and lacking in morphological details (Yamada, 1930), and that the taxon has not previously been sequenced to determine its molecular identity. Reports of *U. flabellum* in the Pacific probably originate from the use of *U. flabellata* J.V.Lamouroux *nom. illeg.* by Harvey (see taxonomic history above), an error that has probably been propagated in the literature. Such records appear biogeographically incongruent because *U. flabellum* represents a common species in the western Tropical Atlantic (type locality West Indies, Ellis & Solander, 1786), and appears to be restricted to this region based on published molecular data (e.g. Kooistra, 2002; Lagourgue et al., 2018). Similarly, *U. argentea*, whose type locality is the Red Sea (Zanardini, 1858), may represent a misidentification of *U. geppiorum* in the region, considering that it represents a more commonly known epithet due to its widespread recognition in the Indo-Pacific (Guiry & Guiry, 2019).

In the present study, we aim to clarify the molecular identity of *U. geppiorum* in order to revise the record of *Udotea* in the Hawaiian archipelago with proper identification (i.e. previously published as *U. argentea* in Bailey-Brock & Magalhães, 2010). We also reassess morphological features of this still poorly documented taxon for overlooked characters. Finally, we report ecological

observations on mesophotic meadows discovered in the Main Hawaiian Islands.

Materials and methods

Field observations

Observations were made during several submersible (~50–210 m) and/or remotely operated vehicle (~40–230 m) dives conducted between 2000 and 2006 along the western and southern shores of Oʻahu, Hawaiʻi (12. ix.2000, 03.ix.2004, 13.xii.2004, 27.xi.2006), and the vicinity of Maui Island (15.xi.2006 to 19.xi.2006). Submersible dives utilized the Hawaiʻi Underwater Research Laboratory's *Pisces IV* and *Pisces V* three-person submersibles and the Remote Operated Vehicle 150 with surface support from the Research Vessel *Kaimikai-o-Kanaloa*. Submersibles were equipped with two colour video cameras mounted externally that recorded entire dives, allowing image capture and later review of ecological observations. Additional field observations were made via scuba where possible (i.e. patches or meadows at accessible depths).

Collection information and specimens examined

Specimens included a collection by M. Ross via scuba from Barber's Point, south-west Oʻahu (ARS08534, 36 m depth, 30.ix.2013), another from submersible dives in mesophotic habitats found in the vicinity of Maui Island (ARS01416, > 40 m depth, 16.xi.2006, coll. Terry Kerby), and a third originating from a strain maintained for several months in a marine aquarium in the C. Smith laboratory (TS0398, location of collection in Hawaiʻi and collector not available). Two frozen specimens (BBM1 and BBM2) previously collected from Mamala Bay, Oʻahu, and published as 'U. argentea' by Bailey-Brock & Magalhães (2010) were also made available for molecular confirmation by Dr Bailey Brock, Biology Department, University of Hawaiʻi at Manoa. For comparison with Hawaiian material (see Fig. 1 for a typical habit), two western Pacific specimens were also examined and sequenced; these included a silica preserved specimen identified as *U. geppii* from Fiji collected by D.S and M.M. Littler (DML40191, Great Astrolabe Reef; ~18°51′59.8″S, 178°30′60.0″E; 37 m depth, 27.ii.1996), and an unidentified herbarium specimen fitting the habit of *U. geppiorum* collected from Guam by C. Squair (CS405A, Asan unit of the National Park; 13°28′54.8″N, 144°42′13.3″E, 18 m depth, 08.vi.2006, Fig. 2) (National Park-Assigned Permit Number WAPA-2004-SCI-0001). Anatomical observations (Figs 3–9) were made from a live specimen collected from south-west Oʻahu by H. Spalding (HS174, 35 m, 30.iv.2006). Specimen ARS08534 from southern Oʻahu was deposited at BISH under accession BISH775782 (Supplementary fig. S5).
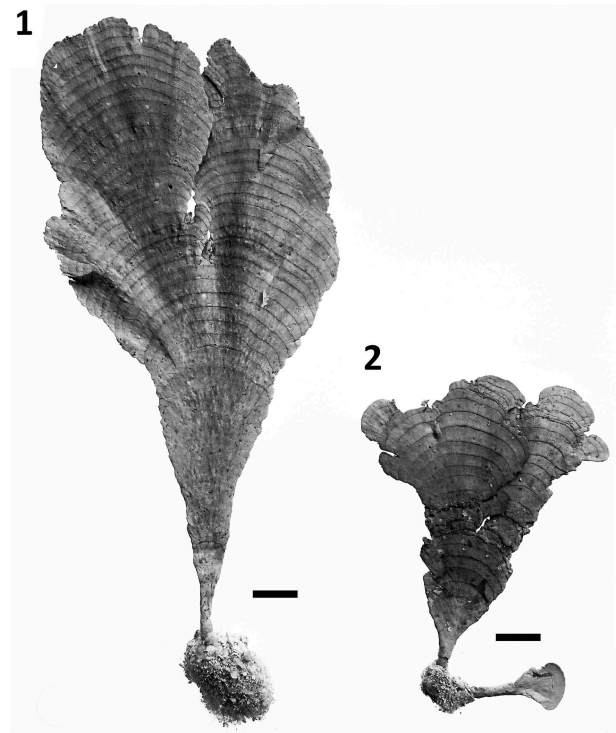

Figs 1–2. Habit of *Udotea geppiorum*. **Fig. 1**. Typical habit of a mesophotic specimen collected from south-west Oʻahu Hawaiʻi. **Fig. 2**. Specimen CS405A collected from 18 m depth in Guam. Note the conspicuous concentric segments on both specimens and the more relaxed/elongated holdfast and blade of the mesophotic specimen from Hawaiʻi. Specimens display proportional size difference. Scale bars = 1 cm.

Molecular sequencing

DNA was extracted from the six *U. geppiorum* specimens listed in the previous section using a DNeasy Plant Minikit (Qiagen, Valencia, California, USA) and amplified in 25 µl-reactions with MangoTaq™ DNA polymerase (Bioline USA Inc., Taunton, Massachusetts, USA) with previously published primers and cycling conditions for the chloroplast *tuf*A marker (~800 bp, Händeler *et al.*, 2009). PCR products were purified using ExoSAP-IT® (Affymetrix, Inc., Cleveland, Ohio, USA) and Sanger sequenced in both directions at LAB (Smithsonian Institution, Washington, DC) or the ASGPB core facility (University of Hawaiʻi at Mānoa). Individual chromatograms were assembled into contigs and edited using Sequencher™ v.4.8 (Gene Codes, Ann Arbor, Michigan, USA). For tree building, the newly generated *tuf*A sequences were merged with a comprehensive selection of Udoteacean (ingroup) and Bryopsidalean (outgroup, not shown) taxa downloaded from GenBank (alignment length of 1230 bp); most from Lagourgue *et al.* (2018) and a few others from Cremen *et al.* (2018), Sauvage *et al.* (2016) and Wade & Sherwood (2017) (Supplementary table S1). *Udotea geppiorum tuf*A data was deposited on GenBank under accessions MK460430–MK460432 and MN202614–MN202616. Additional DNA barcodes were generated and deposited for reference on GenBank but
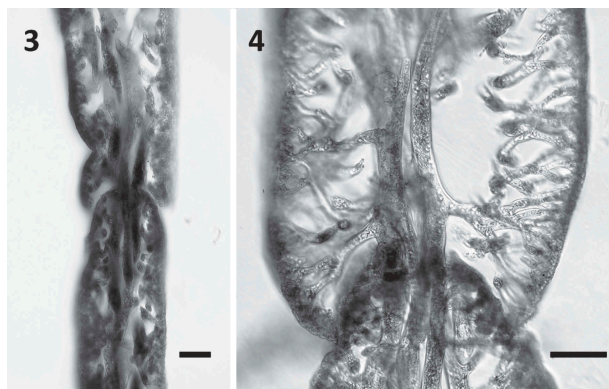
Figs 3–4. Blade anatomy of *Udotea geppiorum*. **Fig. 3**. Longitudinal section emphasizing the 'tongue and groove' arrangement of concentric segments and bridging medullary siphons between an inferior and a superior segment. **Fig. 4**. Longitudinal section emphasizing lateral branching of medullary siphons and the dense cortex. Scale bars = 50 μm.
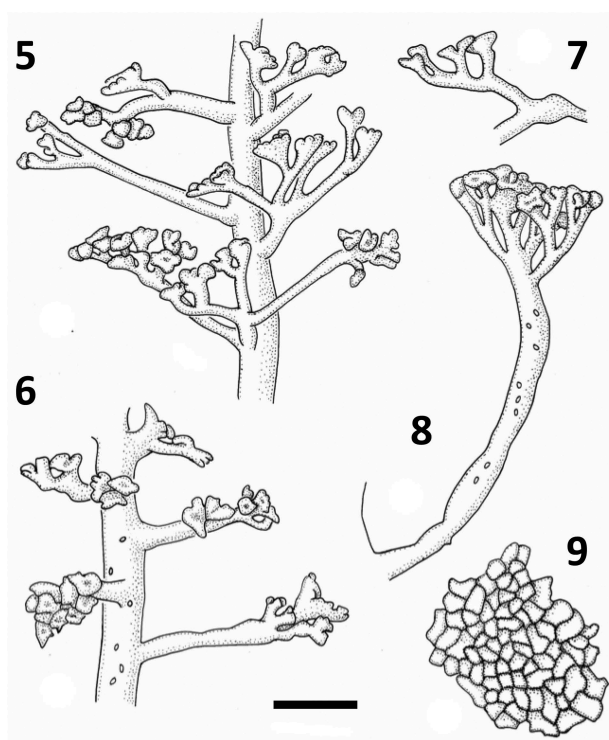
Figs 5–9. Line drawings of *Udotea geppiorum* medullary siphons. **Fig. 5**. Blade siphon with lateral branchlets. **Fig. 6**. Distal blade siphon with lateral branchlets and chloroplasts. **Fig. 7**. Lateral branchlets from apical region of the blade. **Fig. 8**. Stipe lateral branchlet with chloroplasts. **Fig. 9**. Surface view of decalcified blade showing siphon apices. Scale bar = 30 μm.

not analysed herein, *rbc*L-5P (MK460427–MK460429) and nuclear 18S rRNA (MK426759).

Phylogenetic analyses

Bayesian and Maximum likelihood analyses were conducted in MrBayes v.3.1.2 (Ronquist &

Huelsenbeck, 2003) and RAxML-HPC2 on CIPRES computer cluster (http://www.phylo.org, Stamatakis, 2014) using a GTR+I+G model of evolution partitioned per codon position as determined by PartitionFinder (Lanfear *et al.*, 2012). The Bayesian analysis consisted of two independent runs of four increasingly heated chains run for a maximum of 10 million generations sampled every 1000 generations (resulting in 2×20 000 trees). The runs converged rapidly as demonstrated by the average standard deviation of split frequencies reaching < 0.01 following ~450 000 generations, and large ESS values (≫ 200) indicating more than sufficient sampling. The first 25% of sampled trees (default) were then discarded as burn-in (n = 10 000 trees) and a majority-rule consensus tree was computed with the remaining trees to obtain node support (posterior probabilities from n = 30 000 trees). The Maximum likelihood analysis consisted of 1000 topological searches from random restarts and 1000 bootstrap replicates for node support estimation (bootstrap).

Results

Molecular analyses

For the *U. geppiorum* specimens sequenced from Hawai'i, Fiji and Guam, no genetic variation was observed based on *tuf*A (Fig. 10). Bailey-Brock & Magalhães (2010) specimens from O'ahu, previously published as '*U. argentea*' (BBM1 and BBM2), were also identical to those of *U. geppiorum* and were genetically distinct from a Pacific specimen of *U. argentea* sequenced from Papua New Guinea by Cremen *et al.* (2018). *Udotea geppiorum* was also distinct from *U. flabellum* from the western Tropical Atlantic. The *tuf*A phylogeny shows that *U. geppiorum* branches among a poorly resolved cluster (polytomy) of completely corticated *Udotea* spp. including the generi-type *U. flabellum*, and *U. dixonii, U. dotyi* and *U. occidentalis* recently proposed as *Udotea sensu stricto* by Lagourgue *et al.* (2018). However, the addition of *U. argentea* from Cremen *et al.* (2018) in the phylogeny shows that it branches sister to a large group of partially corticated species (or lacking cortication), which includes *Udotea* spp. *sensu lato* (e.g. *U. cyathiformis, U. conglutinata*) and other genera (*Rhipidosiphon* Montagne, *Rhipocephalus* Kützing and *Penicillus* Lamarck), rendering the topology of *Udotea sensu stricto* paraphyletic (albeit with low to very low support, BI, 83%, ML, 53%) (Fig. 10).

Morphological observations

The thalli of examined Hawaiian specimens of *U. geppiorum* included a calcified stipe and calcified segmented blade reaching up to 24 cm in height.
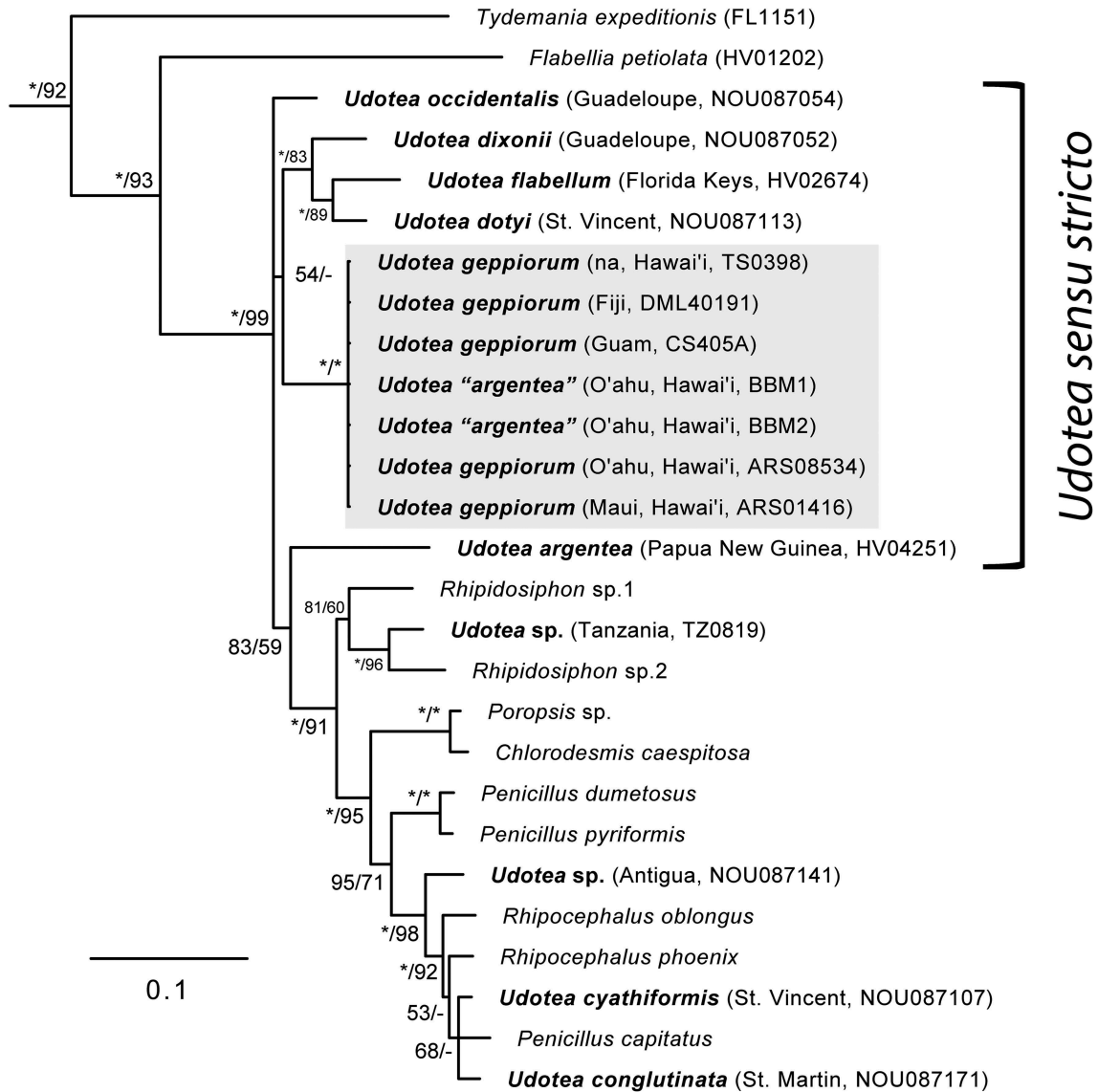
Fig. 10. Bayesian majority-rule consensus tree depicting the phylogenetic position of *Udotea geppiorum* in the family Udoteaceae inferred by the chloroplast genes *tuf*A. Values shown at nodes represent posterior probabilities scaled on 100% (before forward slash) and bootstrap support determined via Maximum likelihood analysis (after forward slash). Full support (> 99%) is noted by an asterisk (*) while values below 50% are indicated by an hyphen (-). *Udotea* spp. are displayed in bold with their sampling locality. Newly sequenced specimens of *U. geppiorum* are shaded in grey. Note the paraphyly of *Udotea sensu stricto* caused by the position of *U. argentea*, and the polyphyly of *Udotea* spp. *sensu lato*.

The holdfast is bulbous to elongate with attached sediment among the rhizoids, and measures up to 6 cm long and 1–2 cm in width. One to several stipes may arise from a single holdfast. *Udotea geppiorum* is generally anchored in sediment and occasionally on sediment-covered rock. When alive, blades are greyish-olive-green, becoming olive-green with senescence or upon drying. Stipes are variable in length, 0.5–4.0 cm long and occasionally highly reduced (i.e. lacking elongation and thus not forming a clear separation from the blade). The stipe, when present, is subterete, 1.0–4.0 mm in diameter near holdfasts, widening as it joins with the lowermost segment of the blade. The basal subterete segment is generally elongate, up to 40 mm long. Above the basal segment

are a series of short concentric (arc-shaped) segments, 1.0–4.0 mm long, each spanning the width of the blade as seen on the type specimens (Supplementary figs S2–S4). Blades are flat and narrowly fan-shaped, to 21 cm long by 1.5–8 cm wide often becoming dissected with overlapping lobes distally, measuring 0.4–1.6 mm thick. All segments possess a proximal groove into which the distally constricted lower segment is inserted, appearing as a 'tongue and groove' arrangement (Figs 3–4). The segmentation gives the entire blade a strongly zonate appearance (Fig. 1) with new segments being added apically. Despite segmentation, the flabellum is mostly rigid. Medullary siphons pass through the segments' interface (Figs 3–4) along the entire length

of the segment and are emergent (to 50 μm) beyond the apical-most (growing) margin of the distal segment prior to the deposition of calcium carbonate and formation of a new segment.

Medullary siphons in the blade are 20–42.5 μm diameter, and are oriented parallel to one another length-wise in the flabellum. They possess infrequent dichotomies lacking constrictions. The siphons produce abundant lateral branchlets (Figs 5–8). These are irregularly spaced and project mostly alternately from the siphon, being situated perpendicularly to the plane of the blade surface (Fig. 6). Lateral branchlets are 10–17.5 μm in diameter, and range from 50–165 μm long distally to 160–600 μm proximally. Lateral branchlets are simple or dichotomously divided, terminating in stubby, truncated ends (Figs 7–8), as reported for closely related species such as *U. dixonii* D.S. Littler & Littler, *U. dotyi* D.S. Littler & Littler, *U. flabellum* (J. Ellis & Solander) M. Howe and *U. occidentalis* A. Gepp & E.S. Gepp (Littler & Littler, 1990). The distal expanded portions of the lateral siphon branchlets (i.e. their outer surface) are adherent to one another forming a coherent cortex (Fig. 4) and in surface view are laterally coherent in a jigsaw fashion (Fig. 9). Reproduction is unknown.

Habitat

Submersible and ROV observations revealed that *U. geppiorum* formed extensive meadows on sandy bottoms in deep water between 60–85 m (Figs 11–12). These extensive monospecific meadows spread to an estimated 500 m or more in extent. In these dense meadows, the blades of *U. geppiorum* were tightly spaced, forming a closed canopy with mean ± SE canopy heights of 13.3 ± 0.6 cm (range: 4.9–23.5 cm). At the shallower (from 20–60 m) and deepest edges of its vertical distribution (85 to at least 92 m), *U. geppiorum* formed sparse patches that range from < 1.0 m to ~15 m in extent. *Udotea geppiorum* was observed predominately anchored in sand. However, at two locations (at 87 and 50 m depth), the alga was growing on sediment-covered carbonate in assemblages with erect, boring sponges, including *Spirastrella* spp. (Spirastrellidae). No ecological observations are available for shallow specimens (6 m depth, BISH1053691, Supplementary fig. S1).

Narrower blades were commonly collected within the denser portions of *U. geppiorum* meadows, while wider-bladed plants were encountered at the meadow margins. At the deeper and shallower limits of its distribution, *U. geppiorum* formed round to oval-shaped discrete patches. In some locations it appeared that the patches were coalescing, which may represent the mechanism by which continuous meadows form. On the edge and within occasional gaps in the meadow, other indigenous

Figs 11–12. Submersible views of mesophotic meadows formed by *Udotea geppiorum* in south-west O'ahu. **Fig. 11**. Overlooking an extensive meadow (Dive P4-188). **Fig. 12**. Edge of a meadow (Dive P5-606). Note that the meadows are so densely vegetated that the sand substrate is not visible among the blades. Picture taken by submersible pilot Terry Kerby in Kaiwi, O'ahu.

macroalgae were observed, including *Caulerpa mexicana*, *Halimeda distorta*, *Distromium flabellatum*, *Cladophora* sp. and *Microdictyon* sp. At many of the shallower sites visited, the invasive alga *Avrainvillea amadelpha* was also present. *Udotea geppiorum* also occurred as discrete patches within deep-water *Halophila decipiens* seagrass meadows. Assemblages of small fish (Pomacentridae and Labridae) were commonly observed hovering above the *Udotea* canopy. On submersible approach, these fish sought refuge among the closely spaced blades. On several occasions, the predatory barred jack *Carangoides ferdau* was observed to aggressively chase and presumably feed on the smaller fish hovering above the algal meadow.

Discussion

Revised record

In the present study, we confirm the revised record of the genus *Udotea* in Hawai'i as *U. geppiorum* and its phylogenetic affinity with *Udotea sensu stricto*

(Fig. 10, Lagourgue *et al.*, 2018). The phylogeny shows that *U. geppiorum* from Hawai'i is conspecific with specimens sampled from Fiji and Guam that clearly fit the habit of Yamada's type from Palau (see Figs 1–2 vs. the syntype in Supplementary fig. S2), and that the species is separate genetically from other *Udotea* spp., especially *U. argentea* and *U. flabellum*, two names that have potentially been applied to it in the Pacific. Morphologically, *U. geppiorum* appears to be unique among *Udotea* spp. in having well-defined concentric segments (Yamada, 1930) newly shown to be articulated in a 'tongue and groove' manner with medullary siphons running between segments and developing a contiguous cortex (Figs 3–4, 9). This conspicuous feature, giving the blade a strong zonation, represents an important morphological character separating *U. geppiorum* from *U. argentea* (see Zanardini, 1858).

Our phylogeny including all previously sequenced *Udotea* species that exhibit complete cortication, renders *Udotea sensu stricto* paraphyletic rather than monophyletic because of the positioning of *U. argentea*. Although the paraphyletic topology receives overall low to very low support with *tuf*A, a recent plastome-scale phylogenomy including *U. argentea* and *U. flabellum* shows a similar topology and has high support (Cremen *et al.*, 2018, fig. 1, p. 3). Nonetheless, until similar phylogenomies are re-analysed with increased taxon sampling to further assess the monophyly (or paraphyly) of fully corticated *Udotea* spp., the genus *sensu stricto* as currently defined remains valid (Lagourgue *et al.*, 2018). In contrast, *Udotea* spp. lacking or exhibiting incomplete cortication, and clustering remotely in the tree among other Udoteacean genera such as *Penicillus*, *Rhipidosiphon* and *Rhipocephalus*, are in need of taxonomic revision.

In future investigations, continued sequencing of fan-shaped taxa currently included in *Udotea* spp. will also be important to further resolve/delimit *Udotea sensu stricto*. For instance, *U. norrisii*, a completely corticated *Udotea* species, has not yet been sequenced for *tuf*A or *rbc*L, although an 18S sequence available on GenBank from Kooistra (2002) suggests that it might be circumscribed within *U. flabellum* (TS, personal observations). Likewise, the western Atlantic taxon recognized as *U. wilsonii* Gepp, Gepp & Howe and *U. goreaui* Littler & Littler, which are heavily calcified could be closely related to *Udotea* spp. *sensu stricto*. While in these two species, cortication is not as conspicuous (complete), they display blunt interlocking knobby lateral appendages/papillae throughout the thalli (terminology *sensu* Littler & Littler, 1990 but also referred to as lateral protuberances by Lagourgue *et al.*, 2018) that are somewhat reminiscent of those observed in *Udotea sensu stricto* spp., albeit shorter. Recent morphological

illustrations of such appendages/protuberances in *U. wilsonii* from Yucatan (Acosta-Calderón *et al.*, 2018, figs 81–84, p. 209) would also suggest a potential affinity of this taxon with *Udotea sensu stricto*, as do the morpho-anatomical analyses displayed in Lagourgue *et al.* (2018, fig. 2, p. 763).

Morphologically, *Udotea sensu stricto* species have continuous cortication from the stipe to the distal portions of the blade. Notably, after decalcification of *U. geppiorum*, and others such as *U. dixonii* and *U. flabellum*, it is difficult to tease apart the blade's lateral appendages without breaking many of them in the process. However, in the case of *U. flabellum* and *U. dixonii*, this is due to the interwoven or crowded nature of such lateral appendages (Gepp & Gepp, 1911; Littler & Littler, 1990). In contrast, the blade's lateral appendages of *U. geppiorum* terminate in utricle-like flattened tips that firmly adhere to one another at their edges and form a smooth surface, giving this species its distinctive contiguous cortex (Figs 4, 9), reminiscent of the cortex of *Halimeda* spp. However, as opposed to the mostly polygonal pattern observed in surface view of *Halimeda* spp., *U. geppiorum*'s surface shows an irregular, jigsaw-like pattern (Fig. 9). Another unique feature of *U. geppiorum* is the development of concentric articulated segments (Figs 1–4) joined together in a 'tongue and groove' arrangement that spans the entire width of the blade. Comparatively, the somewhat zonate appearance of *U. dotyi*, *U. dixonii* and *U. flabellum* is only superficial rather than due to genuine segmentation. Overall, the morphological similarity of the thalli of western Pacific specimens (including the syntypes in Supplementary figs S2–S4) with those collected from O'ahu, added to our molecular results (100% identity of Hawaiian and western Pacific specimens) and the paucity of *Udotea* species exhibiting complete cortication in the Pacific (e.g. *U. geppiorum* and *U. argentea*), provide strong evidence for the correct identification of the present revised new record in Hawai'i as *U. geppiorum*. Our account of medullary siphon characteristics and measurements closely agreed with those reported in the original description of *Udotea geppiorum* (Yamada 1930, fig. 3, p. 141). Based on the present study and review of the taxonomic history of *U. geppiorum*, reports of *U. flabellum* in the Pacific should be interpreted carefully. Likewise, in Pacific herbarium collections, some records of *U. argentea* may correspond to *U. geppiorum*.

A meadow-forming Udotea

Although Udoteaceae species have been previously reported as members of the deep-water flora (Gepp & Gepp, 1908, 1911; Hillis-Colinvaux, 1986; Hanisak & Blair, 1988; Littler & Littler, 1990; Norris & Olsen, 1991; Freile *et al.*, 1995; Leichter *et al.*, 2008) and in

some cases have been documented to form meadows (such as *Penicillus capitatus* in the Canary islands, Sangil *et al.*, 2010), to our knowledge, the present study represents the first documented mesophotic meadows formed by a *Udotea* species, here *U. geppiorum* (Figs 11–12).

Carbonate and biomass production have been well characterized for other bryopsidalean genera, particularly *Halimeda* (Hillis-Colinvaux, 1980, 1991 and references cited therein; Drew, 1983; Payri, 1988) and *Penicillus* (Stockman *et al.*, 1967), and given the extensive standing crop of *U. geppiorum* in Hawai'i, this calcified species is also likely to contribute to aragonitic lime mud production (as seen from the broken calcified remnants of old fronds found in the algal meadow, sediment also agglomerated within the rhizomatous holdfast of the collected specimens; field observations, K. Peyton). The overall contribution of the meadow to lime mud and sediments through thalli growth and death may also play an important role in nutrient cycling and carbonate budget in this mesophotic habitat (i.e. $CaCO_3$ production/erosion/dissolution) and represent potential avenues of research (e.g. see Granier, 2012 for a review on the contribution of green algae to limestone production).

Vegetative reproduction by below-ground rhizomes (or stolons) is well recognized as an important attribute for growth and persistence of both macroalgae and seagrasses on a range of scales from ramets to meadows (Stockman *et al.*, 1967; Tomlinson, 1974; Friedmann & Roth, 1977; Hillis-Colinvaux, 1988; Littler & Littler, 1990, 1992; Williams, 1990; Fourqurean & Rutten, 2004; Littler *et al.*, 2005). Littler & Littler (1990) reported on the existence of a translucent and delicate but macroscopic subterranean rhizomatous system used for lateral vegetative reproduction in *Udotea* spp.; however, their observations seem to be restricted to 'false' *Udotea* spp. (e.g. *U. cyathiformis, U. fibrosa* Littler & Littler, and *U. unistratea* Littler & Littler). Here, *U. geppiorum* specimens maintained in an aquarium for > 36 months (C. Smith Laboratory) started to produce shoots remotely, but we could not identify macroscopic rhizoids linking thalli, a behaviour we also previously witnessed for *Halimeda* species (T. Sauvage and K. Peyton, unpublished data). Recent metabarcoding studies of endolithic communities have shown that vegetative reproduction of members of the Bryopsidales may also occur by microscopic rhizoids as deduced by the molecular detection of otherwise macroscopic taxa such *Halimeda* species in this microhabitat (Sauvage *et al.*, 2016). Such microscopic siphons could thus mediate the lateral spread of *U. geppiorum*, although the existence of a macroscopic rhizomatous system (or lack thereof) also needs to be further investigated in well-developed mesophotic meadows. Overall, we hypothesize that in the field, the ramets of

U. geppiorum can radiate into patches by rhizoids spreading laterally in the substratum (via macroscopic or microscopic rhizoids; to be determined), leading to the establishment of patches, which then further coalesce into meadows, similar to the vegetative propagation described for seagrass meadows via rhizomes/stolons (Williams, 1990; Rasheed, 2004).

Udotea geppiorum meadows are more reminiscent of seagrass canopies than those of *Halimeda* spp. Indeed, like seagrass species, the blades of *U. geppiorum* form a nearly closed canopy. In contrast, *H. kanaloana* Vroom, which also forms extensive meadows in the Hawaiian archipelago, off Maui, Moloka'i, Kaho'olawe and Lāna'i (Abbott & Huisman, 2004; Kahng & Kelley, 2007) exhibit a somewhat uniform dispersion pattern in which individuals are spaced > 7.0 cm apart (K. Peyton, unpublished data) and may thus not provide as much habitat or potential shelter for fauna as the dense blades of *U. geppiorum*. The ecological services of the meadows formed by *U. geppiorum* in Hawai'i, including the support of associated biodiversity and trophic interactions, from detritivores to apex predators (e.g. *Carangoides ferdau*), are poorly known and thus offer opportunities for further research in mesophotic habitats of the archipelago.

Status in Hawai'i

Bailey-Brock & Magalhães (2010) reported '*U. argentea*' from O'ahu (revised here as *U. geppiorum*) as a possible introduced species because of the proximity of their collections to the Honolulu's south shore harbours which are subjected to intense shipping, and the absence of an existing *Udotea* record in Hawai'i prior to 2007. Our collections of *U. geppiorum*, dating from as early as 2000, span two regions of the Main Hawaiian Islands (O'ahu and Maui), including pristine mesophotic habitats distant from harbours, anchoring or recently dredged areas, which would warrant recognition of the species as an indigenous member of the Hawaiian marine flora. In contrast, the recent record of the Bryopsidalean taxa *Avrainvillea* cf. *erecta* (Wade *et al.*, 2018) is indeed of ambiguous indigeneity because it has so far only been documented in the vicinity of harbours in disturbed dredged habitats. Future genomics efforts focused on deciphering population-level differences across Indo-Pacific localities will represent key approaches in determining the natural history of such taxa and their biogeographic origin.

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Disclosure statement

No potential conflict of interest was reported by the authors.

Supplementary information

The following supplementary material is accessible via the Supplementary Content tab on the article's online page at http://dx.doi.org/10.1080/09670262.2019.1668061

Supplementary fig. S1. Photographic illustration of a shallow *U. geppiorum* specimen collected off Kalaeloa southwest O'ahu from ~6 m depth by Dr R. Okano (BISH720045). Image downloaded from the Macroalgal Herbarium Portal.

Supplementary fig. S2. Photographic illustration of Yamada's syntype from Palau (SAP8601). The specimen is identical to fig. 1 in Yamada (1930).

Supplementary fig. S3. Photographic illustration of *Udotea flabellata* J.V.Lamouroux nom. illeg. syntype held at NY (Harvey's 'Friendly Isl. Alg. no. 94', NY02112272). Image downloaded from the Macroalgal Herbarium Portal.

Supplementary fig. S4. Photographic Illustration of *U. flabellata* J.V.Lamouroux nom. illeg. at MICH (Harvey's 'Friendly Isl. Alg no.94', MICH1306130). Note M.J. Wynne's annotation 'Udotea geppiorum Yamada 1930 syntypes!'. Image downloaded from the Macroalgal Herbarium Portal.

Supplementary fig. S5. Photographic illustration of Hawaiian specimen of *U. geppiorum* (ARS08534) sequenced for the present study and deposited in BISH under accession BISH775782.

Supplementary table S1. Newly and previously published *tuf*A accessions included in the phylogenetic analyses.

Author contributions

T. Sauvage, K.A. Peyton and D.L. Ballantine wrote the manuscript. K.A. Peyton conducted submersible dives and field observations. D.L. Ballantine conducted morphological observations. T. Sauvage and R.M. Wade conducted molecular work and phylogenetic analyses. A.R. Sherwood, S. Keeley and C. Smith contributed to data interpretation and revision of the manuscript. All authors read and approved the final manuscript.

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