Brilliantia kiribatiensis, a new genus and species of Cladophorales (Chlorophyta) from the remote coral reefs of the Southern Line Islands, Pacific Ocean[1]

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Running title: *Brilliantia kiribatiensis* gen. et sp. nov.

ABSTRACT

The marine green alga *Brilliantia kiribatiensis* gen. et sp. nov. is described from samples collected during two expeditions (2009, 2013) from the coral reefs of the Southern Line Islands, Republic of Kiribati, Pacific Ocean. Phylogenetic analysis of sequences of the large- and small-subunit rDNA and the rDNA internal transcribed spacer region revealed that *Brilliantia* is a member of the Boodleaceae (Cladophorales), containing the genera *Apjohnia, Boodlea, Cladophoropsis, Chamaedoris, Phyllodictyon* and *Struvea*. Within this clade it formed a distinct lineage, sister to *Struvea elegans*, but more distantly related to the bona-fide *Struvea* species (including the type *S. plumosa*). *Brilliantia* differs from the other genera by having a very simple architecture forming upright, unbranched, single-celled filaments attached to the substratum by a rhizoidal mat. Cell division occurs by segregative cell division only at the onset of reproduction. Based on current sample collection, *B. kiribatiensis* seems to be largely restricted to the Southern Line Islands, although it was also observed on neighboring islands, including Orona Atoll in the Phoenix Islands of Kiriati, and the Rangiroa and Takapoto Atolls in the Tuamotus of French Polynesia. This discovery highlights the likeliness that there is still much biodiversity yet to be discovered from these remote and pristine reefs of the central Pacific.

Key words: 18S nuclear ribosomal DNA; Chlorophyta; Cladophorales; molecular phylogeny; Siphonocladales; Ulvophyceae

Abbreviations: BISH, Bishop Museum; ML, maximum likelihood; SCR, Herbarium of Scripps Institution of Oceanography, University of California; SLIMPA, Southern Line Island Marine Protected Area.

INTRODUCTION

The Southern Line Islands in the central Pacific Ocean are some of the most remote islands on earth. They form the southern half of the Line Islands Archipelago in the Republic of Kiribati and consist of five islands and atolls: Flint, Vostok, Starbuck, Malden, and Millennium Atoll (known as Caroline Atoll prior to 2000). They are currently uninhabited and are seldom visited, with a history of sporadic human settlement. Having significant biodiversity value, the atolls and their coral reefs are considered some of the most pristine on the planet (Sandin and Charles 2009, Barott et al. 2010, Smith et al. 2016, Mangubhai et al. 2019). Information on marine biodiversity in these atolls, however, is fragmented (Kerr and Wragg 2008), and data on benthic macroalgal diversity is almost non-existent.

Limited surveys from the neighboring Northern Line Islands, including Palmyra Atoll, Jarvis Island, and Kingman Reef indicate a relatively low diversity of seaweeds, including six brown algae (Phaeophyceae), 28 green algae (Chlorophyta), and 83 red algae (Rhodophyta; Sandin et al. 2008, Braun et al. 2009, Tsuda et al. 2012). Many of these species were found to be widely recorded in the tropical Indo-Pacific, while a few others had narrower ranges within Polynesia.

Among the green algae reported from the Line Islands, species of Cladophorales are well represented with eleven species of *Cladophora*, *Cladophoropsis*, *Dictyosphaeria*, *Microdictyon*, *Phyllodictyon* and *Valonia* (Tsuda et al. 2012). Similar to the other algal groups, most of the memberof the Cladophorales have broad tropical ranges, with the exception of *Microdictyon setchellianum*, which is restricted to the Pacific Islands of Polynesia, Micronesia, French Polynesia, and Hawaii (Guiry and Guiry 2021). Tropical species of Cladophorales often have wide ranges as a result of high dispersal capacity (Leliaert et al. 2009b). Still, there are numerous species with more confined ranges. For example, *Struvea gardineri* and *Phyllodictyon orientale* are restricted to some Indian Ocean islands, *Struvea thoracica* occurs only in the Great Barrier Reef and New Caledonia, and *Struvea okamurae* is restricted to the Philippines, Japan, and a few other Pacific islands

(Leliaert and Coppejans 2007).

The Cladophorales is a diverse order of about 500 species in 32 genera, which have been traditionally distinguished by their thallus architecture and more recently through molecular phylogenetic analyses (Leliaert et al. 2007a, Boedeker et al. 2016). The order is predominantly marine with a number of species occurring in freshwater habitats (Škaloud et al. 2018). Most species are macroscopic, although recently a number of microscopic species have been assigned to the order based on DNA sequence data (Leliaert et al. 2009a, Johnston et al. 2018). Species in the Cladophorales are characterized by a siphonocladous thallus architecture, which means that the multicellular thalli are composed of multinucleate cells with regularly-spaced nuclei in a stationary cytoplasm, and multiple chloroplasts are interconnected by delicate strands forming a parietal network or a more or less continuous layer (McNaughton and Goff 1990). The basic thallus architectures are branched or unbranched filaments, but tropical marine species exhibit a remarkable diversity of morphologies, including blade- and net-like forms, as well as giant-celled thalli with unique cytomorphological traits and modes of cell division (Mine et al. 2008). The Boodleaceae (sesu Huisman and Leliaert 2015; *Chamaedoris* clade sensu Leliaert et al. 2007) is one of the most diverse clades morphologically, including cushion-like forms (*Boodlea*, *Cladophoropsis*), net-like blades with stipes (*Struvea*, *Phyllodictyon*), and stipitate capitula (*Apjohnia*, *Chamaedoris*).

Expeditions in 2009 and 2013 to the remote Southern Line Islands (Fig. 1, a and b) were undertaken with the primary goal of categorizing reef community structure and productivity (Smith et al. 2016, Fox et al. 2018, Johnson et al. 2020). During these surveys we observed a conspicuous and common green alga composed of densely clustered, stiff, erect unbranched single-celled filaments attached to coral or rocky substrate with a matted rhizoidal mass. The erect filaments could have crustose coralline algae and other epiphytes toward the base. The algae had a siphonocladous appearance but could not readily be assigned to any of the known species or genera of Cladophorales. Here we describe the morphology, ecology, geographical distribution, and

phylogenetic relationships of this undescribed species, which also represents a new genus.

MATERIALS AND METHODS

Sampling, morphological observations, and species distribution

Sample collections and surveys were done under two Scientific Research Permits issued by the Republic of Kiribati for March 24 – May 5, 2009 and October 9 – November 15, 2013. All collections were obtained using SCUBA, and samples were photographed fresh on board the research vessel, pressed as herbarium specimens, and preserved in 4% formalin/seawater. Clean portions of the thalli were desiccated and stored in silica gel for subsequent molecular analysis. Voucher specimens, including holo- and paratypes, are deposited at the Bernice Pauahi Bishop Museum's Herbarium Pacificum (BISH), and additional specimens are housed in the herbarium of the Scripps Institution of Oceanography, University of California (SCR). Specimens were examined with an Olympus 40 bright field light microscope and an Olympus 16ZXstereo microscope, and photographs were taken with an integratd Olympus 10 MP digital camera mounted on the light or stereo microscope. Three measurements of the thallus were taken on preserved and dried specimens collected from each island: range in diameter of the stipe, range in diameter of the rhizoids, and total height of the alga.

Distribution and abundance of the new species and the composition of the algal community of Flint, Vostok, Starbuck, Malden, and Millennium (Fig. 1) were determined through a series of photographic benthic surveys conducted around each island. Varying based on island circumference, 6-14 survey sites were established at each island. Photoquadrats (1 m$_2$) were taken every 2 m along a 25 m belt transect at 10 m depth at each site. Photographs were analyzed using the image analysis program PhotoGrid 1.0 in which 100 stratified random points per photo were identified to genus level for fleshy macroalgae or functional group for turf algae, crustose coralline

algae, and cyanobacteria. The percent cover of the new cladophoralean species and other algal groups were recorded for each site at all five islands (Table S1 in the Supporting Information).

Additional observations were made and photographs were taken from Orona Atoll, Phoenix Islands (Kiribati) in May 2018, and from the Rangiroa and Takapoto Atolls, Tuamotus (French Polynesia) in September 2021.

DNA sequencing and phylogenetic analyses

In order to determine the phylogenetic affiliation of the cladophoralean species from the Southern Line Islands, we inferred molecular phylogenetic analyses based on partial small subunit (SSU) and large subunit (LSU) rDNA, and rDNA internal transcribed spacer (ITS1-5.8S-ITS2) sequences derived from a sample collected off the coast of Millennium Island, on 11 September 2013. Total genomic DNA was extracted by using Power Soil DNA Extraction Kit following the manufacturer instructions, with a 10 min vortexing step. SSU rDNA was amplified (annealing for 30 s at 52°C and extension for 2 min at 72°C for 35 cycles) by the universal eukaryotic primers Euk-A 5'-AACCTGGTTGATCCTGCCAGT-3' (Medlin et al. 1988) and 18SRU 5'-CWGGTTCACCWACGGAAACCTTGTTACG-3' (Tikhonenkov et al. 2016), gel-purified, and cloned bysing the pGEM-T PCR cloning vector (Promega) in One Shot TOP10 E. coli (Invitrogen). Ten bacterial clones were sequenced by Sanger dideoxy sequencing. Based on nucleotide BLAST searches (megablast, https://blast.ncbi.nlm.nih.gov/Blast.cgi), five belonged to Cladophorales and the remaining five to diverse red algae observed at the sampling site (e.g., *Peyssonnelia* spp.). Four genetic variants of the cladophoralean species SSU rDNA were found with 4 to 10 nucleotide differences. rDNA ITS and partial LSU rDNA were amplified (PCR conditions as above) by using a specific forward primer for the cladophoralean species, 5'-TGCGAAAGTGCATCGTGATG-3', and the ulvophyte reverse primer D2FL 5'-GGTCCGTGTTTCAAGACGG-3' (Leliaert et al. 2007a), purified, and Sanger-sequenced from the primary PCR product (two polymorphic sites were

identified). Sequences were deposited in NCBI GenBank under the accessions KU359232-KU359236.

Sequences were added to updated phylogenetic datasets used previously (Leliaert et al. 2007a, 2009b; Table S2 in the Supporting Information), aligned in MAFFT v7.215 (Katoh and Standley 2013), and stripped of hypervariable sites in BMGE v1.1 (Criscuolo and Gribaldo 2010) by using the -h 0.4 -g 0.35 parameters. Alignments were visually checked and concatenated in Seaview v4.4.2, and are available from the Zenodo open access repository https://doi.org/10.5281/zenodo.5584487. Maximum likelihood phylogenies were computed in IQ-Tree v1.3.8 (Nguyen et al. 2015) under the GTR+G4+I model with 1000 ultrafast and 300 nonparametric bootstrap replicates. Bayesian phylogenies were computed in MrBayes v3.2.2 (Ronquist and Huelsenbeck 2003) by using the nst = 6, rates=invgamma, and ngammacat = 4 priors, 2 chains, and 25% burnin after convergence to stopval = 0.01.

RESULTS

Phylogenetic analysis

Maximum likelihood (ML) phylogenies from single gene datasets corresponding to the SSU rDNA and the LSU rDNA moiety of the ITS/LSU fragment congruently placed the Southern Line Island cladophoralean species (described hereunder as a new genus and species, *Brilliantia kiribatiensis*) inside the Boodleaceae (*Chamaedoris* clade) of the Cladophorales as a sister taxon to *Struvea elegans* (Fig. S1 in the Supporting Information). Concatenated ML and Bayesian phylogenies based on SSU, ITS, and LSU in selected representatives (Table S2) were in line with these results: *B. kiribatiensis* was unambiguously placed within the Boodleaceae, and specifically affiliated with *Struvea elegans,* though with weak support (Fig. 2). *Struvea elegans* and the Southern Line Island species formed a separate lineage from the *Struvea plumosa* clade (containing the type of *Struvea,*

S. plumosa), as reported previously (Leliaert et al. 2007, 2009). Other genera in the clade, *Phyllodictyon, Apjohnia, Chamaedoris,* and *Boodlea* were monophyletic and well separated from one another. No environmental sequences closely similar to SSU and ITS/LSU rDNAs of *B. kiribatiensis* were found in environmental clone libraries in GenBank.

Morphological observations

Brilliantia kiribatiensis formed bright green mats (Figs. 3, a-d, 4, a-d), up to 5 cm across, composed of intertwined, irregularly branched rhizoids that were firmly attached to the substratum, from which densely clustered, erect, unbranched, single-celled, wider diameter filaments developed. Rhizoidal cells were 100-150 µm in diameter (Fig. 5, e-g). The upright filaments were 2.5–6 cm high and 750-1100 µm in diameter (Figs. 3, e-f, 5, a-d). In some erect cells, segregative cell division was observed at the distal end of the cell, in which the protoplast divided into several, rounded or elongated daughter protoplasts, which subsequently formed new cell walls, resulting in 5 to 6 cells of more or less equal length (Fig. 4c arrows, Fig. 6, a-e). In some of these cells, cytoplasmic aggregation was observed (Fig. 6, c-f), as well as developing crater-like pores in the cell wall (Fig. 6f, arrowhead). These cells can thus be interpreted as zoosporangia or gametangia, which develop from vegetative cells following segregative cell division, with pores through which the zoospores or gametes are released. Although we did not observe release of zoids directly, some filaments were found with empty apical cells indicative of zoid release (Fig. 4, d and e, arrowheads). Older empty cells were found to be degraded (Fig. 4f, double arrowheads). Some other cells contracted their protoplasts into cytoplasmic spheres that produced new cell walls, possibly in response to mechanical damage by collecting (Fig. 3f, asterisks). Chloroplasts in the rhizoidal and upright cells contained a single pyrenoid, and formed an open parietal reticulum (Fig. 6, g-h). No crystalline cell inclusions were observed.

Habitat and geographical distribution.

Specimens of *Brilliantia kiribatiensis* were found firmly attached to hard substratum primarily on carbonate reef structures or dead coral skeletons. Other seaweeds found in these communities include species of *Halimeda*, *Lobophora,* Peyssonneliaceae, abundant populations of several genera of crustose coralline algae, and highly grazed and cropped mixed filamentous turf algal communities. Abundance and distribution of *B. kiribatiensis* in the Southern Line Islands varied (Fig. 1c), but the species was among the most common macroalgal taxa observed in subtidal habitats from 5-25 m (Fig. S2 in the Supporting Information) on four of the five islands, Flint, Vostok, Malden, and Millennium, with lower relative abundance in the algal community on Starbuck probably as a result of the predominance of *Halimeda* spp. (Smith et al. 2016). *Brilliantia kiribatiensis* may be more widely distributed throughout the Pacific as other sightings were confirmed from neighboring islands, including Orona Atoll in the Phoenix Islands of Kiribati in May 2018, and Rangiroa and Takapoto Atolls in the Tuamotus of French Polynesia in September 2021 (Figs. 1b, 4).

Taxonomic proposals.

Based on the distinct phylogenetic position of the cladophoralean species from the Southern Line Islands in the Boodleaceae (*Chamaedoris* clade sensu Leliaert et al. 2007) and the morphological features that clearly distinguish it from other genera in that clade (*Apjohnia*, *Boodlea*, *Chamaedoris*, *Cladophoropsis*, *Phyllodictyon*, *Struvea*; Table 1), we propose a new species and genus in the Boodleaceae.

Brilliantia Leliaert, E.Kelly & Jen.E.Smith **gen. nov.**

Diagnosis: Genus of Boodleaceae distinguished from other genera in the family by rhizoidal mats producing upright, unbranched, elongate, single-celled filaments, with zoidangia formed by segregative cell division.

Etymology: Referring to the bright color of the thallus.

Type species. B. kiribatiensis Leliaert, E.Kelly & Jen.E.Smith

Brilliantia kiribatiensis Leliaert, E.Kelly & Jen.E.Smith **sp. nov.**

Diagnosis: Thallus forming bright green mats, up to 5 cm across, composed of irregularly branched rhizoids and upright, unbranched, elongate, single-celled filaments, 2.5–6 cm high and 750-1100 µm in diameter. Segregative cell division in the upper part of the upright filaments resulting in uniseriate rows of cells that transform into zoidangia.

Etymology: Referring to the Republic of Kiribati to which the Southern Line Islands (type locality) belong.

Holotype: Republic of Kiribati, Southern Line Islands, Millennium, coral reef, 4 m deep, leeward side of island, 09°57' S 150°13' W, 10 October 2013, leg. Emily Kelly SLI 035 (BISH 777505).

Representative DNA sequences: KU359234 (SSU ribosomal RNA gene), KU359236 (partial SSU ribosomal RNA gene, internal transcribed spacer region, and partial LSU ribosomal RNA gene). Sequences from a silica gel preserved specimen (labelled 'SP1' and housed in SCR) collected from the type locality on 11 September 2013.

Additional specimens examined (paratypes): Republic of Kiribati, Southern Line Islands, Millennium, forereef on coral, 10 m deep, 09°57' S 150°13' W, 17 April 2009, leg. Jennifer E. Smith 0148A (BISH 782311); Vostok, forereef on coral, 12 m deep, 10°03' S 152°18' W, 3 April

2009, leg. Jennifer E. Smith 0161B (BISH 782308); Vostok, coral reef, 10 m deep, leeward side of island, 10°04' S 152°18' W, 23 October 2013, leg. Emily Kelly SLI 045 (SCR); Flint, coral reef, 4 m deep, leeward side of island, 11°26' S 151°48' W, 19 October 2013, leg. Emily Kelly SLI 042 A-E (BISH 782306); Malden, coral reef, 10 m deep, leeward side of island, 04°01' S 154°59' W, 02 November 2013, leg. Emily Kelly SLI 049 (SCR).

Habitat and geographical distribution: *Brilliantia kiribatiensis* is found subtidally at 5-25 m depth, on hard substratum primarily on carbonate reef structures or dead coral skeletons, and is currently known from the five Southern Line Islands Flint, Vostok, Starbuck, Malden, and Millennium (Kiribati), Orona in the Phoenix Islands (Kiribati), and the Rangiroa and Takapoto Atolls in the Tuamotus (French Polynesia).

Discussion

The new genus *Brilliantia* is represented by the single species *B. kiribatiensis*, which formed a distinct clade within the Boodleaceae. Previous molecular phylogenetic studies have indicated that most boodleacean genera as originally circumscribed, including *Boodlea*, *Chamaedoris*, *Cladophoropsis*, *Phyllodictyon*, and *Struvea*, were non-monophyletic. A series of systematic studies has aimed to revise the taxonomy of the group so that it better reflects evolutionary relationships (Kooistra et al. 1993, Kraft and Wynne 1996, Leliaert and Coppejans 2007, Leliaert et al. 2007b,c). A number of taxonomically problematic groups remain however, including the *Boodlea* complex (including species of *Boodlea*, *Phyllodictyon*, *Cladophoropsis* and *Struveopsis*), where traditional and phylogenetic species definitions are in complete disagreement with each other due to a combination of cryptic diversity and intraspecific morphological variation (Leliaert et al. 2009b).

Our phylogenetic analyses indicated that *Brilliantia kiribatiensis* is sister to *Struvea elegans*, a species from the tropical Western Atlantic, typically growing in deeper waters, down to 40 m

(Taylor 1960, Børgesen 1912, Littler and Littler 2000). The species has also been reported from the Indian and Pacific Ocean (Guiry and Guiry 2021), although these records need verification by DNA sequence data. *Struvea elegans* is characterized by delicate, erect, stipitate, net-like blades, thus clearly differing from *B. kiribatiensis* which lacks such blades.

Brilliantia may be confused with juvenile, unbranched stages of *Struvea* species (Fig. 7). However, during our surveys across all islands and different years and seasons no blade-like structures were observed in any of the *B. kiribatiensis* populations, providing confidence that branching is absent in this species, at least in situ. Moreover, the observation that reproductive cells were formed in the apical part of the upright filaments ('stipes') indicates that these morphological forms represent the full-grown stage of the species. Nevertheless, we cannot exclude the possibility that *B. kiribatiensis* may take a different form in unexplored environments such as mesophotic habitats, as has for example been shown in a species of *Caulerpa* (Sauvage et al. 2021). *Brilliantia* is also morphologically clearly distinct from the other genera within the family Boodleaceae (Table 1). It superficially resembles some coarse *Cladophoropsis* species, such as *C. magna* or *C. philippinensis*, but can be clearly distinguished by the unbranched filaments (Leliaert and Coppejans 2006). Thus the morphological distinctness and separate phylogenetic position warrants the designation of a new species and genus.

Our phylogenetic analysis agrees with previous studies and indicates that *Struvea* is currently paraphyletic, with the type of the genus (*S. plumosa*) grouping separately from *S. elegans* and *Brilliantia kiribatiensis*. *Struvea elegans* may thus need to be transferred to another genus, possibly *Brilliantia*. However, statistical support for the sister relationship between *S. elegans* and *B. kiribatiensis* was low, so we prefer to await phylogenetic analyses with additional taxon sampling (e.g., adding *S. elegans* samples from the Indian and Pacific Oceans) before proposing formal taxonomic changes.

Brilliantia shares a specialized mode of cell division – segregative cell division – with some

other species of Cladophorales. In segregative cell division the protoplasm cleaves into spherical portions, which later expand and develop into new cells. Segregative cell division is fundamentally different from other cell division types in the green algae, both at the macroscopic and ultrastructural levels (Okuda et al. 1997, Mine et al. 2008). In some genera, such as *Siphonocladus* and *Struvea*, these cells remain inside the mother cell, expand and form new vegetative branches (Kraft and Wynne 1996, Okuda et al. 2016), while in other taxa, such as *Boergesenia* and *Valonia ventricosa*, the segregated cells are released from the degenerated mother cell, settle and form new thalli (Olsen and West 1988). In *Brilliantia*, the new cells remain in the parent cell, but do not form new lateral branches as in *Struvea*. Instead, some of these cells transform into zoidangia. Reproduction by zoids is rarely observed in the Boodleaceae, but in some species, including *Cladophoropsis membranacea*, *Phyllodictyon pulcherrimum*, and *Struvea elegans*, cells in the terminal branch systems have been observed to transform into zoidangia with lateral conical projections through which the zoids are released (Leliaert 2004), similar to the structures observed in *B. kiribatiensis*.

The intensive grazing pressure by fish and sea urchins, which is typical in tropical shores, has led to the evolution of a special mode of wounding reaction in some cladophoralean species, which was also observed in *Brilliantia*. This reaction resembles segregative cell division where after mechanical damage, cells rapidly contract and separate their cytoplasm into numerous spherical protoplasts, which later secrete new cell walls and grow into new cells (La Claire 1982, Mine et al. 2008). The whole process takes place in a couple of seconds. The cells either remain within the mother cell or are released and develop into new thalli.

Brilliantia kiribatiensis is currently only known from the Southern Line Islands although observations (without voucher specimens) suggest that it may also occur in the neighboring Phoenix Islands (also in the Republic of Kiribati), as well as the Tuamotus of French Polynesia. Given the vast number of islands and atolls that exist across the Pacific that have never had thorough biodiversity assessments, *Brilliantia* could have a broader distribution than described here. It is also

possible that *Brilliantia* has been overlooked in other regions of the Indo-Pacific or has been misidentified as young forms of other cladophoralean species; however, its striking morphological appearance and conspicuous habit makes this unlikely. It is therefore reasonable to assume that *B. kiribatiensis* has a relatively narrow geographical range, possibly restricted to the remote central Pacific. Some other species of Boodleaceae have similarly restricted ranges, including the mesophotic *Struvea gardineri* and *Phyllodictyon orientale*, only known from a few Indian Ocean islands, including the Maldives, Seychelles, Cargados Carajos, and Socotra Island (Leliaert and Coppejans 2007), *Struvea thoracica*, which has only been found in Queensland and New Caledonia (Kraft and Millar 2005), and *Apjohnia laetevirens*, which is restricted to Southern Australia and Tasmania (Womersley 1984). Conversely, other species in the famil, mainly in the *Boodlea* complex, have wider ranges, sometimes spanning different ocean basins, which has been explained by the ability of these species to form free-floating thalli that facilitate long distance dispersal (van den Hoek 1987, van der Strate et al. 2002, Leliaert et al. 2009b).

Based on significant overlap of marine species diversity, the Line Islands are considered part of the larger Indo-Polynesian, or Eastern Indo-Pacific marine provinces (van den Hoek 1984, Briggs and Bowen 2012, Kulbicki et al. 2014, Cowman et al. 2017). Within these larger regions, the Line Islands have been variously allied biogeographically depending on the criteria or taxonomic groups used for drawing biogeographic divisions (Stoddart 1992). In the scheme of Spalding (2007), the Northern Line Islands, Malden, Starbuck are grouped with the Phoenix, Tokelau, Northern Cook, and Samoa Islands in the Central Polynesia marine ecoregion, while the three most southern Islands, Vostok, Millennium and Flint, are grouped in the Southeast Polynesia marine ecoregion along with the Tuamotus, Pitcairn Islands, Southern Cook and Austral Islands, and Society Islands (Fig. 1a). The geographical isolation of these oceanic island groups is reflected in the relatively low marine biodiversity, especially compared with islands in the Central Indo-Pacific (Cowman et al. 2017, Etti and Schils 2016).

The Central Polynesian islands have been characterized by high marine faunal endemism

(Cowman et al. 2017), but for marine macroalgae, endemism in the region has been less well studied. Checklists from Samoa (Skelton and South 2004), the Phoenix islands (South et al. 2001), Northern Line Islands (Tsuda et al. 2012), and other islands of Central Polynesia (Tsuda and Walsh 2013) indicate that most seaweed species are widely distributed. Notable examples include *Halimeda fragilis, Dictyopteris repens,* and *Antithamnionella breviramosa,* which are widely recorded in the tropical Indo-Pacific, and *Bryopsis pennata, Antithamnion antillanum,* and *Heterosiphonia crispella* with pantropical ranges. Other species,ncluding *Ceramium krameri, Chondria simpliciuscula, Corallophila kleiwegii, Cryptonemia yendoi, Dotyella hawaiiensis, Herposiphonia pacifica, Polysiphonia homoia,* and *P. upolensis,* have narrower ranges within Polynesia, consistent with the notion that macroalgal assemblages on Pacific Islands display higher within-archipelago similarities than between-archipelago similarities (Schils et al. 2013, Tsuda 2014). This biogeographical clustering indicates the importance of evolutionary processes such as dispersal and speciation in seaweed biodiversity patterns, as has been illustrated by molecular data in some red and brown seaweeds (Payo et al. 2013, Vieira et al. 2017, Leliaert et al. 2018, Yip et al. 2020). These studies have also shown that a lot of diversity on remote oceanic islands likely remains to be discovered, which has implications for our knowledge of provincialism of seaweeds in the Pacific Ocean. Recently a number of new species of marine macroalgae, restricted to Pacific islands, have been identified or described based on morphological and DNA-sequence data (Vieira et al. 2014, Spalding et al. 2016, Gabriel et al. 2020, Sherwood et al. 2020, Sherwood et al. 2021), including the red alga *Dissimularia withallii* from Jarvis Island, Northern Line Islands (Kraft and Saunders 2014). The new genus and species *Brilliantia kiribatiensis* adds to our knowledge of endemic species in the region.

The geographical origin of *Brilliantia kiribatiensis* is unclear (its candidate sister species, *Struvea elegans,* has a tropical western Atlantic distribution). It is possible the species emerged by founder speciation in a relative recent timeframe, corresponding to the age (ca. 8 Ma) of the Line Islands (Neallnd Trewick 2008) or alternatively *B. kiribatiensis* may have persisted in the broader

Pacific over a longer time frame as a metapopulation (Heads 2018), after which it became geographically isolated. Additional sampling (possibly uncovering more closely related species to *B. kiribatiensis*) will be needed to further elucidate the biogeographical history of the group.

Finally, the finding of a new, possibly endemic species and genus of green macroalgae in the Southern Line Islands has consequences for marine conservation in these remote and pristine reef ecosystems. Our findings confirm previous reports highlighting the uniqueness of these ecosystems and their ability to support previously undescribed macroalgal diversity. Given the paucity of scientific data from many remote, uninhabited islands across the central Pacific there is likely much diversity yet to be discovered in this region. However, the potential impacts of global change threaten these unique habitats and the species they support, underlining the need for more research and for the protection of these as of yet pristine habitats. Several recent studies have shown the dramatic impact of coral bleaching and the demise of *Acropora* colonies on reefs in the central Pacific, including Kiribati, indicating that these reefs may be on the forefront of climate change (Cannon et al. 2021). In light of this, the Kiribati government recognizes the threats and benefits from this wealth of rich natural resources and has developed the Southern Line Island Marine Protected Area (SLIMPA) Management Plan 2020-2024 and Regulations 2020, which implies that the islands of Flint, Vostok, Starbuck, Malden, and Millennium will be a no-take marine protected area, similar to the Phoenix Islands Protected Area (PIPA). These efforts demonstrate Kiribati's commitment to protect these pristine ecosystems, and fulfill its obligations towards the Convention on Biological Diversity (CBD, https://www.cbd.int/).

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Data availability statement

DNA sequences were deposited in NCBI GenBank under accession numbers KU359232-KU359236. Sequence alignments, and data from the photographic benthic surveys, and morphological measurements are available as supplementary material, and on the Zenodo open access repository https://doi.org/10.5281/zenodo.5584487.

Author contributions

F. Leliaert: Writing- original draft (lead); Investigation (equal). **E.L.A. Kelly**: Conceptualization (equal); Investigation (equal); Writing- original draft (supporting). **J. Janouškovec**: Investigation (equal); Writing- review & editing (supporting). **M.D. Fox**: Investigation (supporting); Writing- review & editing (supporting). **M.D. Johnson**: Investigation (supporting); Writing- review & editing (supporting). **F.M. Redfern**: Writing- review & editing (supporting). **T. Eria**: Writing- review & editing (supporting). **A.F. Haas**: Conceptualization (supporting); Funding acquisition (supporting); Writing- review & editing (supporting). **E. Sala**: Conceptualization (supporting);Funding acquisition (supporting); Writing- review & editing (supporting). **S.A. Sandin**: Conceptualization (supporting); Funding acquisition (supporting); Writing- review &

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1

Table 1. Morphological comparison of *Brilliantia* with the other genera of Boodleaceae

	Apjohnia	*Boodlea*	*Chamaedoris*	*Cladophoropsis*	*Phyllodictyon*	*Struvea*	*Brilliant...*
Habit	Erect, robust, stipitate capitula composed of a whorl or cluster of branches	Netlike cushions or blades, composed of densely branched filaments	Erect, robust, stipitate capitula, composed of whorls of branched filaments	Mats or cushions composed of long, irregularly branched cells; attached by rhizoids or tenacular cells	Erect, delicate, stipitate blades, composed of regularly branched filaments	Erect, delicate, stipitate blades or capitula, composed of regularly branched filaments	Filamen... mat and erect unbranch... filament...
Stipe	Prominent, single-celled, unbranched, with annular constrictions near base	Absent or inconspicuous, with or without annular constrictions	Prominent, single-celled, unbranched, with annular constrictions over the entire length	Absent or inconspicuous	Prominent, unbranched or branched, with or without annular constrictions.	Prominent, generally single-celled and unbranched, with or without annular constrictions.	Erect filament... regarded... homolog... to the sti... in the ot... genera
Thallus reinforcement	Older cells of the capitulum producing hapteroid cells at their base	Cells connecting by tenacular cells	Entangling of capitulum filaments and cells connecting by tenacular cells	Entangling of filaments or cells connecting by tenacular cells	Cells connecting by tenacular cells at the apical or basal poles of cells.	Cells connecting by tenacular cells at the apical poles of cells.	Tenacula... cells abs...
Mode of cell division	Centripetal wall ingrowths	Centripetal wall ingrowths	Centripetal wall ingrowths (capitulum filaments) and segregative cell division (distal end of stipe cell)	Centripetal wall ingrowths. Protoplasts formation (resembling segregative cell division) may occur as a wounding response.	Centripetal wall ingrowths	Segregative cell division or centripetal wall ingrowths	Segregat... cell divis...
Crystalline cell inclusions (Leliaert and Coppejans 2004)	Elongate rod-shaped calcium oxalate (CaOx) crystals	Elongate prismatic CaOx crystals	Elongate prismatic or diamond shaped CaOx crystals (some species), Tetrahedral protein crystals (some species)	Elongate prismatic CaOx crystals	Diamond-shaped or hexagonal CaOx crystals	Diamond-shaped or hexagonal CaOx crystals (absent in *S. elegans*)	Absent

	Deep intertidal pools or subtidal	Intertidal or shallow subtidal	Intertidal or subtidal	Intertidal or subtidal	Generally subtidal	Intertidal or subtidal	General subtidal
Habitat	Deep intertidal pools or subtidal	Intertidal or shallow subtidal	Intertidal or subtidal	Intertidal or subtidal	Generally subtidal	Intertidal or subtidal	General subtidal
Geographical distribution	Australia, New Caledonia, Norfolk Island	Global (sub)tropical	Global (sub)tropical	Global (sub)tropical	Global (sub)tropical	Global (sub)tropical	Souther Line Isla
Number of species	2	c. 10	3	c. 8	c. 7	5	1
Taxonomic notes		Included in the *Boodlea* complex		Non-monophyletic genus; *C. membranacea* (lectotype) included in the *Boodlea* complex. Other species (e.g. *C. herpestica*) are now placed in *Lychaete*.	Non-monophyletic genus; some species, e.g. *P. anastomosans*, included in the *Boodlea* complex	Non-monophyletic genus; *S. elegans* with separate phylogenetic position	
References	(Womersley 1984)	(Leliaert et al. 2009b)	(Leliaert et al. 2007c)	(Leliaert and Coppejans 2006, Leliaert et al. 2009b, Boedeker et al. 2016)	(Leliaert and Coppejans 2007, Leliaert et al. 2008)	(Kraft and Wynne 1996, Leliaert et al. 2007c, Okuda et al. 2016)	This stud

1

Fig. 1. Geographical location of the Southern Line Islands with indication of marine provinces (different colors) and ecoregions as defined in Spalding et al. (2007) (a); islands were *Brilliantia kiribatiensis* was recorded (b); and sampling and monitoring sites of the five Southern Line Islands with average percent cover of *B. kiribatiensis* (green circles) (c) (data available in Table S1).

Fig. 2. Maximum likelihood tree of the Boodleaceae (*Chamaedoris* clade) inferred from the concatenated SSU+ITS+LSU rDNA dataset, showing the phylogenetic position of *Brilliantia kiribatiensis*. Clades in the *Boodlea* complex (Leliaert et al. 2009b) include various morphological forms that are traditionally regarded as different species and genera, including *Boodlea* spp. (clades 1, 7, 9, 10), *Cladophoropsis* spp. (clades 1, 5, 10, 11, 13), and *Phyllodictyon anastomosans* (clades 1, 7, 8, 10). ML (IQ-Tree) ultrafast bootstrap / nonparametric bootstrap / and MrBayes posterior probabilities are shown at branches. Black dots on branches indicate full support in all three analyses.

Fig. 3. *Brilliantia kiribatiensis* from the Southern Line Islands. In situ photographs from Millennium Atoll, showing bright green tufts of densely clustered filaments, growing on carbonate reef structures or dead coral skeletons (a-d), and details of fresh samples showing the irregularly branched rhizoids from which erect, unbranched, single-celled filaments develop (e-f). Arrows indicate filaments that have undergone segregative cell division, resulting in 5 to 6 cells of more or less equal length. Asterisks (*) indicate contracted protoplasts, likely as a response to mechanical damage by collecting. Scale bars = 1 cm (e, f).

Fig. 4. *Brilliantia kiribatiensis* from Orona Atoll, Phoenix Islands (a-c), and Rangiroa (d) and Takapoto (e, f), Tuamotus. In situ photographs, showing tufts of densely clustered filaments, growing on carbonate reef structures. Arrows (c) indicate filaments that are undergoing segregative cell division. Arrowheads (d, e) indicate empty cells in the upper part of the filaments, possibly after release of zoids. Double arrowheads (f) indicate filaments in which the cell walls of empty cells are degraded.

Fig. 5. *Brilliantia kiribatiensis*. Upright, single-celled filaments developing rhizoids in the middle and basal parts of the cell (a, b). Apical part of filaments (c), with chloroplast layer visible underneath the cell wall (d). Intertwining rhizoids (e), anastomosing by hapteroid protrusions (f, g). Drawing by Adi Khen. Scale bars = 5 mm (a, b), 2 mm (c, e), 200 µm (d), 100 µm (f, g).

Fig. 6. *Brilliantia kiribatiensis*. Segregative cell division, in which the protoplast in the distal end of the cell divides into several, rounded or elongated daughter protoplasts, which subsequently form new cell walls, resulting in 5 to 6 cells of more or less equal length (a-e). Cytoplasmic aggregation observed in some cells after segregative cell division (c-f), with some cells developing crater-like pores in the cell wall (Fig. 6f, arrowhead). Chloroplasts with a single pyrenoid, forming closed to open parietal reticulum (g, h). Scale bars = 2 mm (a, c, d), 1 mm (b, e), 200 µm (f), 10 µm (g), 50 µm (h).

Fig. 7. Schematic representation of the developmental stages of *Struvea* species (A-E) and *Brilliantia kiribatiensis* (A-B). A. Young thallus consisting of a single-celled stipe; B. Segregative cell division in the distal end of the stipe cell resulting in a uniseriate row of cels (later becoming the central axis in *Struvea*); C. Each cell producing a pair of equally developing opposite lateral branches which elongate and form the primary branch systems of the blade; D-E. Segregative cell division and formation of lateral branches repeated in the primary laterals and apical cell of the central axis. In some *Struvea* species, this process is repeated, resulting in branch systems up to the 4th or even 5th order.
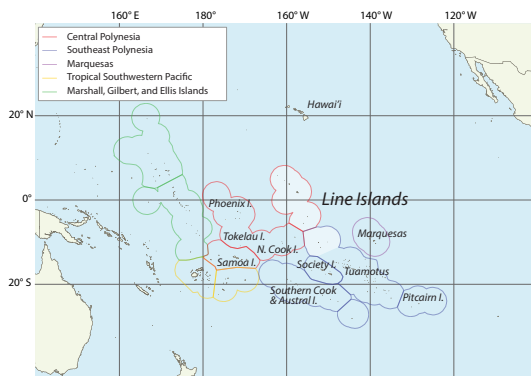
Fig. S1. Phylogeny of the Boodleaceae based on SSU (A) and LSU rDNA (B) single gene datasets. The Maximum Likelihood tree (IQ-Tree) is shown with ultrafast bootstrap supports at branches (>50 are shown). Each species name is followed by the isolate name, sequence accession, and site of isolation, separated by underscores, where available.

Fig. S2. Average percent cover of algae in the different island sites. Algal groups are identified to genus level for fleshy macroalgae or functional group for turf algae, branched red algae, crustose coralline algae, and cyanobacteria. Location of the sites is shown in Figure 1c. Data are available in Table S1.
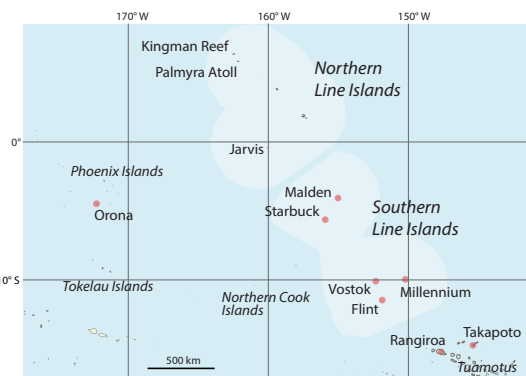
Table S1. Percent cover of different algal groups in 1 m$_2$ photoquadrats. Algal groups are identified to genus level for fleshy macroalgae or functional group for turf algae, branched red algae, crustose coralline algae, and cyanobacteria.

Table S2. GenBank accessions, sample isolate codes and sites of collection for sequences included in the concatenated phylogenetic data set in Figure 2
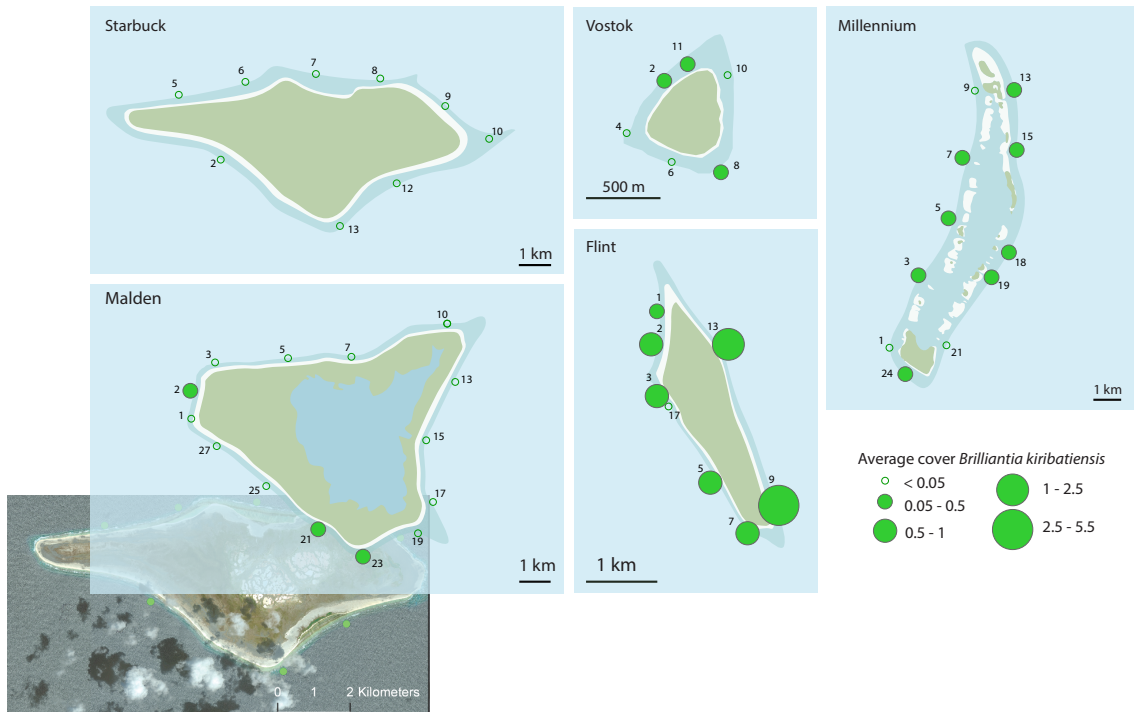
a

b

c

Starbuck

Vostok

Millennium

Malden

Flint

Average cover *Brilliantia kiribatiensis*

○ < 0.05   ● 1 - 2.5

● 0.05 - 0.5   ● 2.5 - 5.5

● 0.5 - 1

98/100/1   *Boodlea* complex clade 8

71/68/.99   *Boodlea* complex clade 7

81/67/.98   *Boodlea* complex clade 1

82/82/1   *Boodlea* complex clade 5

87/79/.96   *Boodlea* complex clade 10

Boodlea complex clade 9

Boodlea complex clade 11

Boodlea complex clade 13

98/95/1

50/47/.58   *Struvea okamurae*

93/89/1   *Struvea thoracica*

Struvea plumosa

97/92/1   *Struvea gardineri*

Boodleaceae

79/61/.96

Struvea elegans

76/48/.61   **Brilliantia kiribatiensis**

89/77/.97

98/97/.99   *Chamaedoris auriculata*

Chamaedoris delphinii

Chamaedoris peniculum

99/98/1

87/80/.97   *Phyllodictyon pulcherrimum*

Phyllodictyon robustum

Phyllodictyon orientale

93/84/.90   *Apjohnia laetevirens*

75/74/.98

Valoniopsis pachynema

Dictyosphaeria cavernosa

Valonia ventricosa

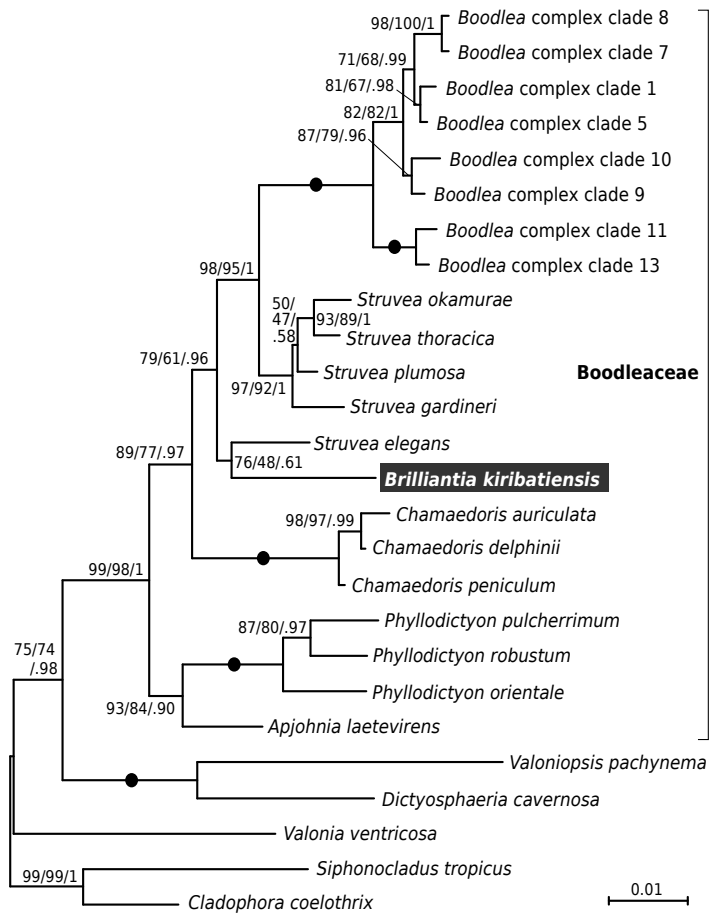99/99/1   *Siphonocladus tropicus*

Cladophora coelothrix

0.01

Fig. 3. Brilliantia kiribatiensis from the Southern Line Islands. In situ photographs from Millennium Atoll, showing bright green tufts of densely clustered filaments, growing on carbonate reef structures or dead coral skeletons (a-d), and details of fresh samples showing the irregularly branched rhizoids from which erect, unbranched, single-celled filaments develop (e-f). Arrows indicate filaments that have undergone segregative cell division, resulting in 5 to 6 cells of more or less equal length. Asterisks (*) indicate contracted protoplasts, likely as a response to mechanical damage by collecting. Scale bars = 1 cm (e, f).

174x204mm (72 x 72 DPI)

Fig. 4. Brilliantia kiribatiensis from Orona Atoll, Phoenix Islands (a-c), and Rangiroa (d) and Takapoto (e, f), Tuamotus. In situ photographs, showing tufts of densely clustered filaments, growing on carbonate reef structures. Arrows (c) indicate filaments that are undergoing segregative cell division. Arrowheads (d, e) indicate empty cells in the upper part of the filaments, possibly after release of zoids. Double arrowheads (f) indicate filaments in which the cell walls of empty cells are degraded.

174x123mm (72 x 72 DPI)
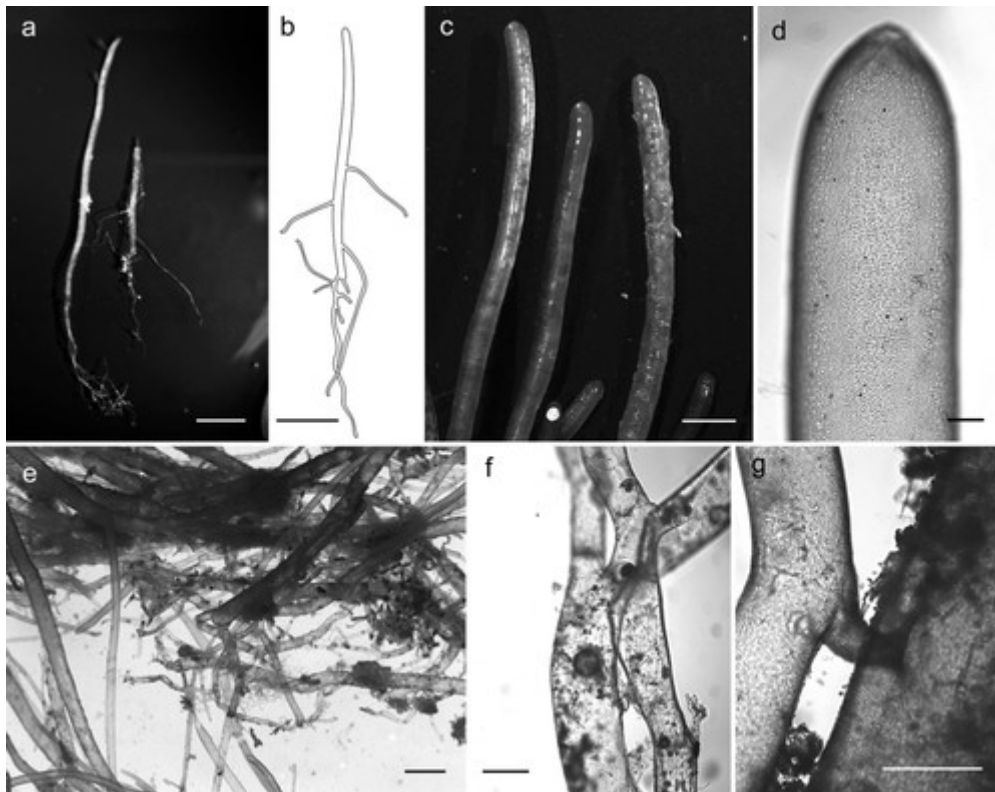
Fig. 5. Brilliantia kiribatiensis. Upright, single-celled filaments developing rhizoids in the middle and basal parts of the cell (a, b). Apical part of filaments (c), with chloroplast layer visible underneath the cell wall (d). Intertwining rhizoids (e), anastomosing by hapteroid protrusions (f, g). Drawing by Adi Khen. Scale bars = 5 mm (a, b), 2 mm (c, e), 200 µm (d), 100 µm (f, g).
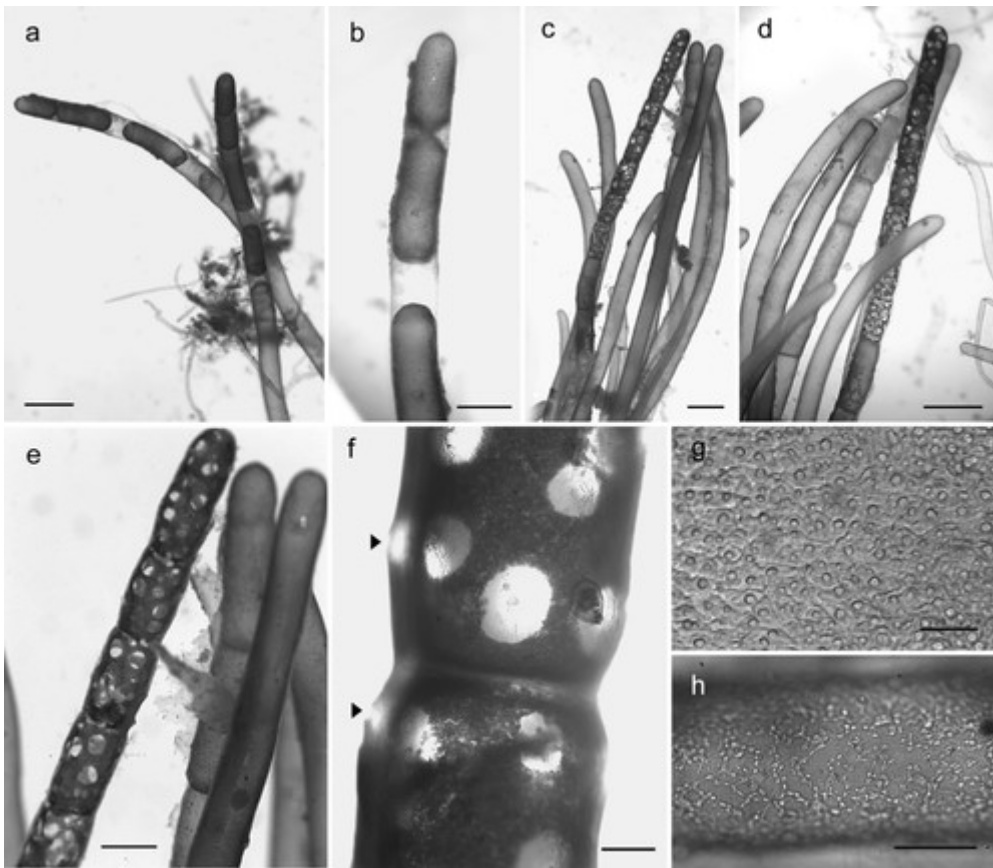
174x138mm (72 x 72 DPI)

Fig. 6. Brilliantia kiribatiensis. Segregative cell division, in which the protoplast in the distal end of the cell divides into several, rounded or elongated daughter protoplasts, which subsequently form new cell walls, resulting in 5 to 6 cells of more or less equal length (a-e). Cytoplasmic aggregation observed in some cells after segregative cell division (c-f), with some cells developing crater-like pores in the cell wall (Fig. 6f, arrowhead). Chloroplasts with a single pyrenoid, forming closed to open parietal reticulum (g, h). Scale bars = 2 mm (a, c, d), 1 mm (b, e), 200 µm (f), 10 µm (g), 50 µm (h).
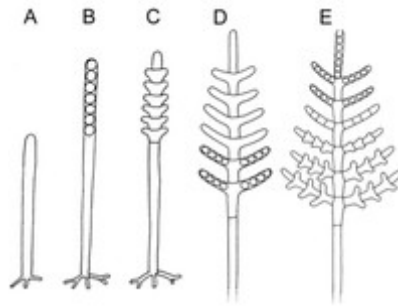
174x152mm (72 x 72 DPI)

Fig. 7. Schematic representation of the developmental stages of Struvea species (A-E) and Brilliantia kiribatiensis (A-B). A. Young thallus consisting of a single-celled stipe; B. Segregative cell division in the distal end of the stipe cell resulting in a uniseriate row of cells (later becoming the central axis in Struvea); C. Each cell producing a pair of equally developing opposite lateral branches which elongate and form the primary branch systems of the blade; D-E. Segregative cell division and formation of lateral branches repeated in the primary laterals and apical cell of the central axis. In some Struvea species, this process is repeated, resulting in branch systems up to the 4th or even 5th order. 69x54mm (72 x 72 DPI)