

Observations on some mangrove-associated algae from the western Pacific (Guam, Chuuk, Kosrae, and Pohnpei)

John A. West^{1,*}, Mitsunobu Kamiya², Susan Loiseaux de Goër³, Ulf Karsten⁴ and Giuseppe C. Zuccarello⁵

¹School of Botany, University of Melbourne, Parkville, VIC 3010, Australia

²Department of Marine Bioscience, Faculty of Biotechnology, Fukui Prefectural University, 1-1 Gakuencho, Obama, Fukui 917-0003, Japan

³11 Rue des Moguerou, 29680 Roscoff, France

⁴Institute of Biological Sciences, University of Rostock, Albert-Einstein-Strasse 3, D-18057 Rostock, Germany

⁵School of Biological Sciences, Victoria University of Wellington, P. O. Box 600, Wellington 6140, New Zealand

The mangrove algal flora of Guam and the Federated States of Micronesia has been poorly explored. We add to our knowledge of this region by observations of collections from these regions. This paper presents new and additional records of: Rhodophyta-*Acrochaetium globosum*, *Colaconema* sp., *Caulacanthus indicus*, *Bostrychia moritziana* / *B. radicans*, *B. radicata*, *B. simpliciuscula*, *B. kelenensis* and *B. tenella*, *Murrayella pericladus*, and *Caloglossa ogasawaraensis*; Chlorophyta-*Boodileopsis carolinensis*; and Phaeophyceae-*Dictyota adnata*, *Dictyotopsis propagulifera*, and *Canistrocarpus cervicornis*. Most specimens were cultured to investigate their reproductive biology and many specimens were further identified using molecular data. Low molecular weight carbohydrates (dulcitol, sorbitol, and digeneaside) were identified in samples of *B. radicata* and *B. simpliciuscula*. We also present data on manganese-rich deposits found on *B. simpliciuscula* and *B. tenella* in culture, possibly formed by epiphytic bacteria.

Key Words: Chlorophyta; Chuuk; Guam; Kosrae; low-molecular-weight-carbohydrates; manganese-deposits; molecular phylogeny; Phaeophyceae; Pohnpei; Rhodophyta

INTRODUCTION

The region comprising Guam and the Federated States of Micronesia (including the islands of Chuuk, Kosrae, and Pohnpei) has abundant mangroves. Reports on the marine algae of the region focus mostly on algae from coral reefs (Hodgson and McDermid 2000, McDermid et al. 2002, Lobban and Tsuda 2003, Lobban and N'Yeurt 2006, Tsuda 2006, Tsuda et al. 2012). Consequently, algae associated with mangroves are not well recorded.

The two most well-known red algal genera associated

with mangroves are *Bostrychia* and *Caloglossa* (King and Puttock 1989, 1994). While the molecular phylogeny of these genera has been well studied, questions still remain into their taxonomy, especially in *Bostrychia* that contains several polyphyletic or cryptic species (Zuccarello and West 2003, 2006). Records of these algae are sparse in Guam and Micronesia. In Micronesia Tsuda (2006) listed no *Bostrychia* species, while Lobban and Tsuda (2003) reported *B. radicans* (based on Zuccarello et al. 1999b)

© This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/3.0/>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

Received June 9, 2013, Accepted August 9, 2013

*Corresponding Author

E-mail: jwest@unimelb.edu.au

Tel: +61-3-8344-8080, Fax: +61-3-9349-3268

and *B. tenella*. *Stictosiphonia kelasanensis* (Grunow ex Post) R. J. King et C. Puttock (now *B. kelasanensis* Grunow) was recorded by Lobban and Tsuda (2003) and Tsuda (2006) from Guam and Micronesia. This is in contrast with other island regions of the central and south Pacific. *Bostrychia moritziana* (Sonder ex Kützinger) J. Agardh, *B. simpliciuscula* Harvey ex J. Agardh and *B. tenella* (J. V. Lamouroux) J. Agardh have been reported from Fiji (South and Skelton 2003), and *B. moritziana* and *B. radicans* (Montagne) Montagne from New Caledonia (Zuccarello et al. 2006). West et al. (2008) provided extensive information on *B. moritziana*, *B. radicans*, *B. simpliciuscula*, and *B. tenella* from Vanuatu. *Bostrychia radicata* (Itono) J. A. West, G. C. Zuccarello et M. H. Hommersand, was reported from New Caledonia (West et al. 2006). Recently a new species, *B. anomala* (Itono) J. A. West, G. C. Zuccarello & M. H. Hommersand, was described from Guam and Kosrae (West et al. 2013). A few records of *Caloglossa* species have also been reported from the region: *C. ogasawaraensis* Okamura and *C. vieillardii* (Kützinger) Setchell from Vanuatu (West et al. 2008), *C. adnata* (Zanardini) De Toni (synonymous with *C. bengalensis* (G. Martens) King et Puttock) and *C. leprieurii* (Montagne) G. Martens from Guam and Micronesia (Lobban and Tsuda 2003).

To better explore the biodiversity of these poorly explored algal habitats we investigated the mangrove-associated algae from Guam, Chuuk, Kosrae, and Pohnpei including the collection of live specimens. Most collections were placed in laboratory culture to investigate their reproductive biology (patterns of sexual and asexual reproduction) and molecular phylogeny.

In some of our algal cultures microscopic brown deposits formed on the algae and glass. The preliminary findings on the chemical constitution on these brown deposits are reported here. The low molecular weight carbohydrates (LMWCs) of *B. radicata* and *B. simpliciuscula* from Guam and Micronesia were also analysed to compare with results for these species from other regions.

MATERIALS AND METHODS

Methods for collection, isolation and maintenance have been presented previously (West and Zuccarello 1999, West 2005). Collection information on the specimens is given in Table 1.

Molecular analyses

DNA was extracted using a Chelex extraction method

of Goff and Moon (1993). For *Caloglossa ogasawaraensis* polymerase chain reaction (PCR) conditions followed Kamiya et al. (2011). The sequence data of the three Micronesian strains of *C. ogasawaraensis* were deposited in the DNA Data Bank of Japan (AB728493–AB728495). For *Bostrychia* amplification of the RuBisCo spacer procedures and primers were from Zuccarello et al. (1999a). Sequences are deposited in Genbank (KF286619–KF286625). Phylogenetic methodologies followed Zuccarello et al. (2012). Maximum likelihood (ML) model for the *Caloglossa* data set was a TIM2 + I + G and for the *Bostrychia* data set was a GTR + I + G.

LMWC chromatographic analyses

The LMWCs dulcitol, sorbitol and digeneaside were qualitatively and quantitatively analyzed in the *Bostrychia* samples using high-performance liquid chromatography (HPLC) according the extraction and separation methods of Karsten et al. (1991, 2005).

Elemental scan analyses

In some cultures microscopic brown deposits were observed on the algae and glass surfaces. These specimens (air-dried algae with brown deposits and glass coverslips placed in culture dishes for 4–8 weeks also with brown deposits) were mounted on double sided carbon impregnated conducting tape prior to a conducting film of carbon (approx. 400 Å in thickness) being applied to the surface of the samples in a vacuum evaporative coating unit to avoid charging effects. The JEOL JSM35 scanning electron microscope (Jeol Ltd., Tokyo, Japan) is equipped with an EDAX windowless energy dispersive lithium drifted silicon detector capable of semi-quantitative analysis of elements. The image capture system is the “Image Slave” designed specifically for scanning electron microscopy (SEM) operation. Spectra are collected using an accelerating voltage ranging from 15 to 25 kV. The higher kV enables reliable identification of elements present by exciting associated higher energy peaks (K_{α} and K_{β}). Specimens were viewed in secondary and backscattered electrons and elemental X-ray data collected in specific areas of interest using an area scan mode for an average composition of a given area or spot mode that collects X-rays from approximately 1 μm^2 . A complete explanation for X-ray microanalysis is in Heinrich (1981).

Table 1. Marine and Freshwater Algae of Micronesia (FSM, Chuuk, Kosrae, Pohnpei) and Guam (GUM) collected February 2006

Species	Culture no.	Collection coordinates	Collection site and date	Substrate	Reproduction	Additional information
Rhodomelaceae <i>Bostrychia</i> <i>kelanensis</i>	3000	-	Pohnpei, UG; Aug 26, 1989	-	Tetraspores produced only females	RuBisCo spacer identification
	3075	13°20' N, 144°44' E	Talofofo R., GUM; Jul 8, 1990	-	Non-viable tetrasporangia or sporplings	RuBisCo spacer identification
	3076	13°20' N, 144°44' E	Talofofo R., GUM; Jul 8, 1990	-	Non-viable tetrasporangia or sporplings	RuBisCo spacer identification
	4589	13°15.371' N, 144°41.063' E	Achang Bay Resort, GUM; Feb 12, 2006	On <i>Avicennia</i>	Vegetative in field, sexual in culture	RuBisCo spacer identification
	4632	07°26.925' N, 151°53.313' E	Peniyak Village, Weno I., Chuuk; Feb 11, 2006	-	Tetrasporophyte in field and culture	RuBisCo spacer identification
	4635	07°26.925' N, 151°53.313' E	Peniyak Village, Weno I., Chuuk; Feb 11, 2006	-	Vegetative in field, sexual in culture	RuBisCo spacer identification
	4637	13°15.371' N, 144°41.063' E	Achang Bay Resort, GUM; Feb 12, 2006	On <i>Avicennia</i>	Female	RuBisCo spacer identification
	3001	-	Pohnpei; Aug 29, 1989	-	Asexual tetrasporophyte in culture	RuBisCo spacer lineage 2
	3003	-	Lelu Lagoon, Kosrae; Sep 2, 1989	On <i>Bruguiera</i>	Asexual tetrasporophyte in culture	RuBisCo spacer lineage 6
	4590	06°58.524' N, 158°13.366' E	Nett Point, Pohnpei; Feb 5, 2006	On <i>Sonneratia</i>	Asexual tetrasporophyte in culture	
<i>B. moritziana/radicans</i>	4591	06°58.524' N, 158°13.366' E	Nett Point, Pohnpei; Feb 5, 2006	On <i>Sonneratia</i>	Asexual tetrasporophyte in culture	RuBisCo spacer lineage 2
	4592	06°58.524' N, 158°13.366' E	Nett Point, Pohnpei; Feb 5, 2006	On <i>Sonneratia</i>	Abortive tetrasporangia in culture	
	4596	06°58.524' N, 158°13.366' E	Nett Point, Pohnpei; Feb 5, 2006	On <i>Sonneratia</i>	Sexual in culture	RuBisCo spacer lineage 2
	4597	06°58.607' N, 158°13.397' E	Nett Point, Pohnpei; Feb 5, 2006	On <i>Sonneratia</i>	Vegetative in field and culture	RuBisCo spacer lineage 6
	4607	05°21.406' N, 162°57.966' E	Okat Harbor, Kosrae; Feb 7, 2006	On <i>Sonneratia</i>	Female in field and culture	RuBisCo spacer lineage 2
	4608	05°21.406' N, 162°57.966' E	Near Okat Harbor, Kosrae; Feb 7, 2006	On <i>Sonneratia</i>	Sexual in culture	RuBisCo spacer lineage 2
	4609	05°21.406' N, 162°57.966' E	Near Okat Harbor, Kosrae; Feb 7, 2006	On <i>Sonneratia</i>	Asexual tetrasporophyte in culture	RuBisCo spacer lineage 2
	4611	05°21.406' N, 162°57.966' E	Near Okat Harbor, Kosrae; Feb 8, 2006	On <i>Sonneratia</i>	Female in culture	RuBisCo spacer lineage 7
	4616	05°21.406' N, 162°57.966' E	Near Okat Harbor, Kosrae; Feb 8, 2006	On <i>Sonneratia</i>	Asexual tetrasporophyte in culture	RuBisCo spacer lineage 2

Table 1. Continued

Species	Culture no.	Collection coordinates	Collection site and date	Substrate	Reproduction	Additional information
<i>B. moritziana/radicans</i>	4619	05°21.406' N, 162°57.966' E	Near Okat Harbor, Kosrae; Feb 8, 2006	On <i>Sonneratia</i>	Female in culture	RuBisCo spacer lineage 2
	4620	05°21.406' N, 162°57.966' E	Near Okat Harbor, Kosrae; Feb 8, 2006	On <i>Sonneratia</i>	Female in culture	RuBisCo spacer lineage 2
	4630	07°19.982' N, 151°50.339' E	Fefan I., Chuuk; Feb 10, 2006	-	Male in culture	RuBisCo spacer lineage 2
	4631	07°26.925' N, 151°53.313' E	Peniyak Village, Weno I., Chuuk; Feb 11, 2006	-	Asexual tetrasporophyte in culture	Monosiphonous in culture, RuBisCo spacer lineage 7
	4633	07°26.925' N, 151°53.313' E	Peniyak Village Weno I., Chuuk; Feb 11, 2006	-	Asexual tetrasporophyte in culture	Monosiphonous in culture, RuBisCo spacer lineage 2
<i>B. radicata</i>	4634	07°26.925' N, 151°53.313' E	Peniyak Village Weno I., Chuuk; Feb 11, 2006	-	Non-viable tetrasporan- gia	RuBisCo spacer lineage 2
	4614	05°21.406' N, 162°57.966' E	Okat Harbor, Kosrae; Feb 8, 2006	On <i>Sonneratia</i>	Sexual in culture	RuBisCo spacer identification
	4621	08°00' N, 147°00' E	Pacific Tree Lodge, Kosrae; Feb 8, 2006	On <i>Xylocarpus</i>	Sexual in culture	RuBisCo spacer identification
	4627	07°19.982' N, 151°50.339' E	Fefan I., Chuuk; Feb 10, 2006	-	Sexual in culture	RuBisCo spacer identification
	4650	05°21.406' N, 162°57.966' E	Near Okat Harbor, Kosrae; Feb 8, 2006	On <i>Sonneratia</i>	Sexual in culture	RuBisCo spacer identification
<i>B. simpliciuscula</i>	4663	07°26.925' N, 151°53.313' E	Peniyak Village Weno I., Chuuk; Feb 11, 2006	-	Asexual tetrasporophyte in culture	RuBisCo spacer identification
	4593	06°58.524' N, 158°13.366' E	Nett Point, Pohnpei; Feb 5, 2006	On <i>Sonneratia</i>	Vegetative in field and culture	H3 lineage
	4594	06°58.524' N, 158°13.366' E	Nett Point, Pohnpei; Feb 5, 2006	On <i>Sonneratia</i>	Vegetative in field and culture	H3 lineage
	4595	06°50' N, 158°20' E	Nan Madol, Pohnpei; Feb 5, 2006	On <i>Sonneratia</i>	Non-viable tetrasporan- gia	H3 lineage
	4600	06°48.939' N, 158°09.994' E	Lehn Mesi R., Pohnpei; Feb 4, 2006	On rock	Vegetative in field and culture	H3 lineage
	4603	06°58.572' N, 158°10.904' E	Sokehs Rock, Pohnpei; Feb 3, 2006	On <i>Avicennia</i>	Non-viable tetrasporan- gia	H3 lineage
	4610	05°21.406' N, 162°57.966' E	Near Okat Harbor, Kosrae; Feb 8, 2006	On <i>Sonneratia</i>	Vegetative in field and culture	H3 lineage
	4615	05°21.406' N, 162°57.966' E	Near Okat Harbor, Kosrae; Feb 8, 2006	On <i>Sonneratia</i>	Vegetative in field and culture	H3 lineage

Table 1. Continued

Species	Culture no.	Collection coordinates	Collection site and date	Substrate	Reproduction	Additional information
<i>B. simpliciuscula</i>	4629	07°19.982' N, 151°50.339' E	Fefan I., Chuuk; Feb 10, 2006	-	Vegetative in field and culture	H3 lineage
	4636	13°21.704' N, 144°38.977' E	Old Taliatafak Bridge, GUM; Feb 12, 2006	On rock under bridge in river	Vegetative in field and culture	H3 lineage
	4638	13°21.704' N, 144°38.977' E	Ylig R., GUM; Feb 12, 2006	On mud	Vegetative in field and culture	H3 lineage
<i>B. tenella</i>	4602	06°48.939' N, 158°09.994' E	Lehn Mesi R., Pohnpei; Feb 4, 2006	On rock	Vegetative in culture + brown deposits	
	4617	05°21.406' N, 162°57.966' E	Near Okat Harbor, Kosrae; Feb 8, 2006	On <i>Sonneratia</i>	Unhealthy, many brown deposits	Terminated Aug 24, 2006
	4622	05°17.404' N, 163°01.711' E	Melam, Kosrae; Feb 7, 2006	On <i>Sonneratia</i>	Vegetative in culture	Terminated Oct 11, 2006
	4662	07°26.925' N, 151°53.313' E	Peniyak Village, Weno I., Chuuk; Feb 11, 2006	-	Female + procarys on shaker, many brown deposits	<i>B. tenella</i> molecular identification
	4664	07°26.925' N, 151°53.313' E	Peniyak Village, Weno I., Chuuk; Feb 11, 2006	-	Vegetative in culture	<i>B. tenella</i> molecular identification, terminate Dec 24, 2006
<i>Murrayella periclados</i>	4606	06°50' N, 158°20' E	Nan Madol Road, Pohnpei; Feb 4, 2006	On coral with <i>Boodleopsis</i> sp. and <i>Polysiphonia</i> sp.		
Delesseriaceae						
<i>Caloglossa ogasawaraensis</i>	4604	06°58.572' N, 158°10.904' E	Sokehs Rock, Pohnpei; Feb 3, 2006	On <i>Avicennia</i>	Tetraspores and non-reproductive sporelings	Molecular identification
	4612	05°21.406' N, 162°57.966' E	Near Okat Harbor, Kosrae; Feb 8, 2006	On <i>Sonneratia</i>	Vegetative in field and male in culture	
	4623	05°17.404' N, 163°01.711' E	Melam, Kosrae; Feb 7, 2006	On <i>Sonneratia</i>	Female, procarys, pseudo-cystocarps	Molecular identification
	4628	07°19.982' N, 151°50.339' E	Fefan I., Chuuk; Feb 10, 2006	-	Vegetative in field and culture	Molecular identification
Caulacanthaceae						
<i>Caulacanthus indicus</i>	4625	05°21.918' N, 162°58.853' E	Tanfusak, Kosrae; Feb 7, 2006	On <i>Sonneratia</i>	Vegetative in field, male in culture	
Acrochaetiales cf. <i>Acrochaetium globosum</i>	4626	05°21.918' N, 162°58.853' E	Tanfusak, Kosrae; Feb 7, 2006	On <i>Caulacanthus</i>	Monosporangia in field and laboratory	

Table 1. Continued

Species	Culture no.	Collection coordinates	Collection site and date	Substrate	Reproduction	Additional information
Colaconematales						
<i>Colaonema</i> sp.	4651	07°19.982' N, 151°50.339' E	Fefan L., Chuuk; Feb 10, 2006	On <i>Bostrychia sim- pliciuscula</i> (4629)	Monosporangia in field and laboratory	
Chlorophyta, Udoteaceae						
<i>Boodleopsis carolinensis</i>	4601	06°48.939' N, 158°09.994' E	Lehn Mesi R., Pohnpei; Feb 4, 2006	On rock	No reproduction	Terminated Oct 25, 2006
	4605	06°50' N, 158°20' E	Nan Madol road, Pohnpei; Feb 4, 2006	On <i>Sonneratia</i>	Sporangia in field and culture	Terminated May 30, 2007
	4618	05°21.406' N, 162°57.966' E	Near Okat Harbor, Kosrae; Feb 8, 2006	On <i>Sonneratia</i>	No reproduction	Terminated Oct 12, 2008
<i>B. carolinensis</i>	4624	05°16.256' N, 162°58.554' E	Uuwe, Kosrae; Feb 7, 2006	On <i>Sonneratia</i>	Sporangia in culture	
Phaeophyceae, Dictyotaceae						
<i>Canistrocarpus cervicornis</i>	-	06°58.524' N, 158°13.366' E	Nett Point, Pohnpei; Feb 5, 2006	On <i>Sonneratia</i>		
<i>Dictyota adnata</i>	-	06°58.524' N, 158°13.366' E	Nett Point, Pohnpei; Feb 5, 2006	On rock		
<i>Dictyotopsis propagulifera</i>	-	06°50' N, 158°20' E	Nan Madol road, Pohnpei; Feb 4, 2006	On <i>Sonneratia</i>		

Further information can be obtained from the Master Culture List (<http://www.botany.unimelb.edu.au/west>).

RESULTS

In Guam, Chuuk, Kosrae, and Pohnpei we collected a total of 15 taxa. These are presented below taxonomically and in Table 1.

Rhodophyta

Colaonematales, Colaonemataceae.

Colaonema sp.: Isolate 4651 developed a filamentous stoloniferous basal system wrapped around the branches of the host *Bostrychia simpliciuscula* (4629). Monospores were 10–12 μm in diameter and initially very amoeboid in motion, but later became spherical and showed active gliding. Spores germinated in a bipolar manner with a single erect shoot arising from one pole and from the other pole elongate basal filaments with cylindrical cells that were 5–6 μm wide and 17–18 μm long (Fig. 1A). This primary erect system had a fan-shaped appearance with dense multiple alternate branches in which the cells were 8–9 μm wide and 10–12 μm long appearing catenate (i.e., slight constrictions between the cells). The fully grown erect fan shaped shoots reached about 350 μm in length (Fig. 1A & E). Subglobose monosporangia about 10–12 μm wide were terminal on branch tips (arrows in Fig. 1A & D, arrowhead in Fig. 1B). Erect fan-shaped shoots also arose at variable intervals (5–10 cells apart) from the older elongate basal filaments (Fig. 1C & D). These had short clusters of monosporangia (Fig. 1D).

Preliminary molecular analysis showed this to be a *Colaonema* but species designation was not possible (Gary Saunders personal communication).

Acrochaetiales, Acrochaetiaceae.

Acrochaetium globosum Børgesen: Erect filaments were 7–8 μm in diameter, and cells of main filaments were 17–20 μm long (Fig. 2A & B). The single peripheral chloroplast had a centrally projecting pyrenoid (Fig. 2B). Branching of erect shoots was usually unilateral (Fig. 2A–C). Monosporangia were non-pedicellate, solitary and alternate along the filament or in lateral clusters (Fig. 2C). Sporangia were slightly elongate, 9–10 μm long and 7–9 μm wide. Free monospores were spherical and about 8 μm in diameter. Germinating monospores enlarged (10–12 μm) and appeared as empty cells producing a narrow elongate filament before branching (Fig. 1D). After 6 days the primary filament was 3 cells long and 8 μm diameter (Fig. 1D). At 14 days the sporelings were 7–10 cells long with 1–2 one-celled lateral branches. Monosporangia were the only reproductive structures seen. In laboratory

culture the basal system had branched filaments slightly narrower than the erect filaments. Individual plants grew well on glass becoming up to 1.5 mm overall (Fig. 2A).

Identification was based on the morphological description of the species in Børgesen (1915), but molecular evidence is clearly needed to verify its identification.

Gigartinales, Caulacanthaceae.

Caulacanthus indicus Weber-van Bosse: Morphological evidence placed our culture isolate (4625) as *C. indicus* (Weber-van Bosse 1921). Tentative molecular data also support this determination (Frederic Mineur unpublished observations). Mature male plants were up to 2 cm overall (Fig. 2E) with typical main axes about 100 μm in diameter and bearing lateral branches at variable intervals (1–4 mm) (Fig. 2E). Laterals often bore continuous encircling spermatangial sori (Fig. 2F). Cortical cells were spherical to polygonal along the axes (Fig. 2G).

Ceramiales, Rhodomelaceae.

Bostrychia: Zuccarello and West (2006) merged *Stictosiphonia* into *Bostrychia* based on molecular evidence. Tier cell formation (2 tier cells versus 3–5 tier cells per axial cell) was the morphological criterion previously used to distinguish the two genera (King and Puttock 1989). The attachment structures are usually peripherohaptera and cladohaptera. Peripherohaptera are composed of coalescent filaments arising from the tier cells often at branch nodes. These filaments may remain coalescent or diverge as they elongate. Cladohaptera are derived abaxially on lateral branches from the first or second axial cell and its tier cells.

Bostrychia kelanensis Grunow: *Bostrychia kelanensis* had three key characters: ecorticate, 3 tier cells per axial cell and cladohaptera (King and Puttock 1989, Zuccarello and West 2006). We obtained *B. kelanensis* from Pohnpei, Chuuk, and Guam (Table 1), although it probably is present elsewhere in this region. One Chuuk isolate (4635) had a *Polysiphonia*-type sexual life history. One Guam isolate (4637) was a female in the field and laboratory. The Pohnpei isolate (3000) was a tetrasporophyte and all the tetraspores developed into females. Isolates 3075, 3076, and 4589 from Guam and 4632 from Chuuk were tetrasporophytes that produced either abortive sporangia or viable sporelings with tetrasporangial stichidia and were considered to have an asexual life cycle. Reproductive status of isolates in the field and culture are also shown in Table 1.

Growth of all phases of isolates was good in culture with typical alternate or unilateral branching. All plants

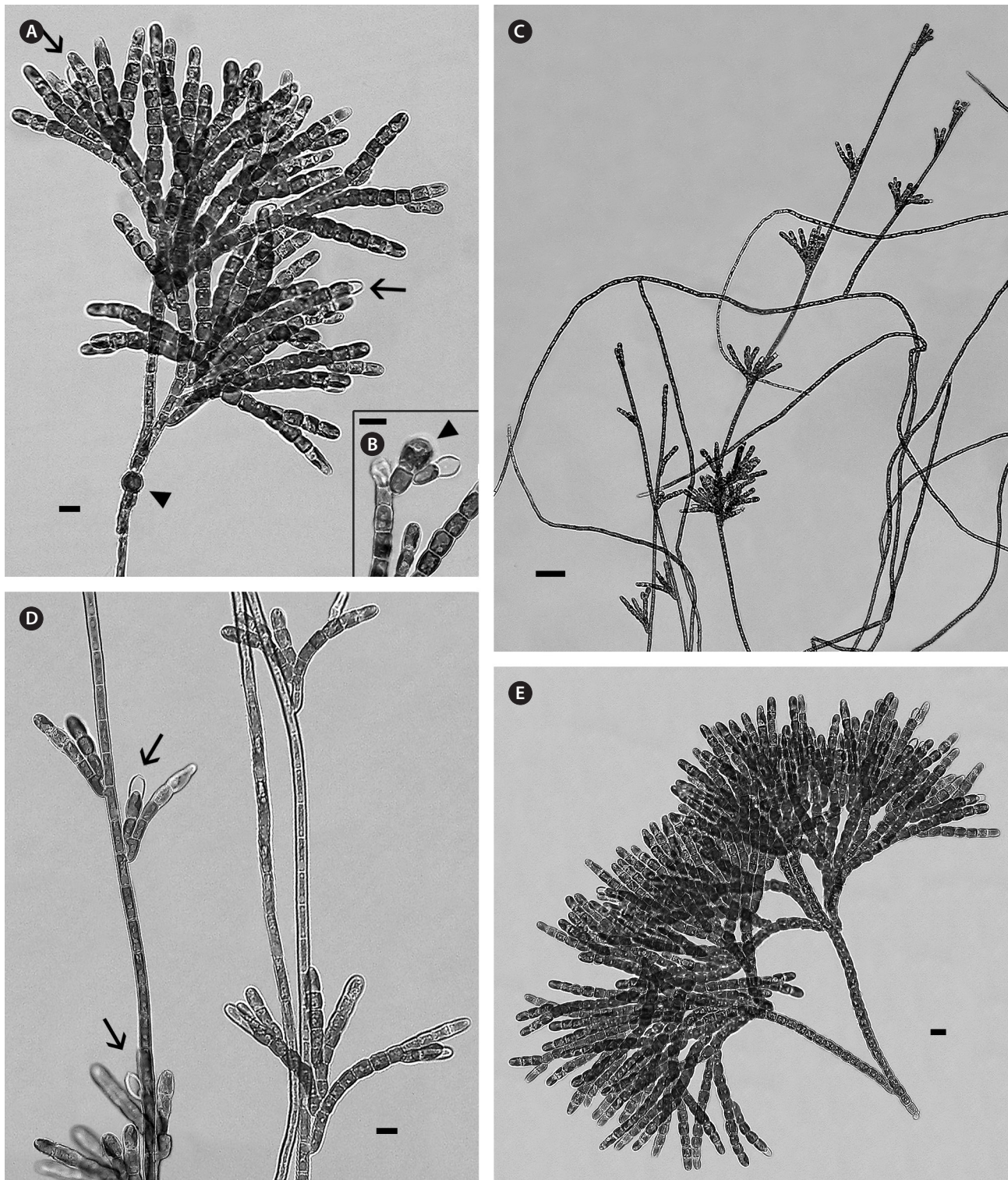


Fig. 1. *Colaconema* sp. 4651. (A) Original spore (arrowhead) with basal system shoot and erect shoot bearing fan shaped branches and some terminal sporangia (arrows). (B) Terminal sporangial cluster. Mature sporangium (arrowhead). (C) Habit view of thallus showing elongate branched horizontal filaments with erect flabelliform shoots. (D) Young flabelliform branches with terminal sporangia (arrows) on short laterals. (E) Well-developed mature flabelliform erect shoot separated from horizontal filament. Scale bars represent: A-E, 10 µm

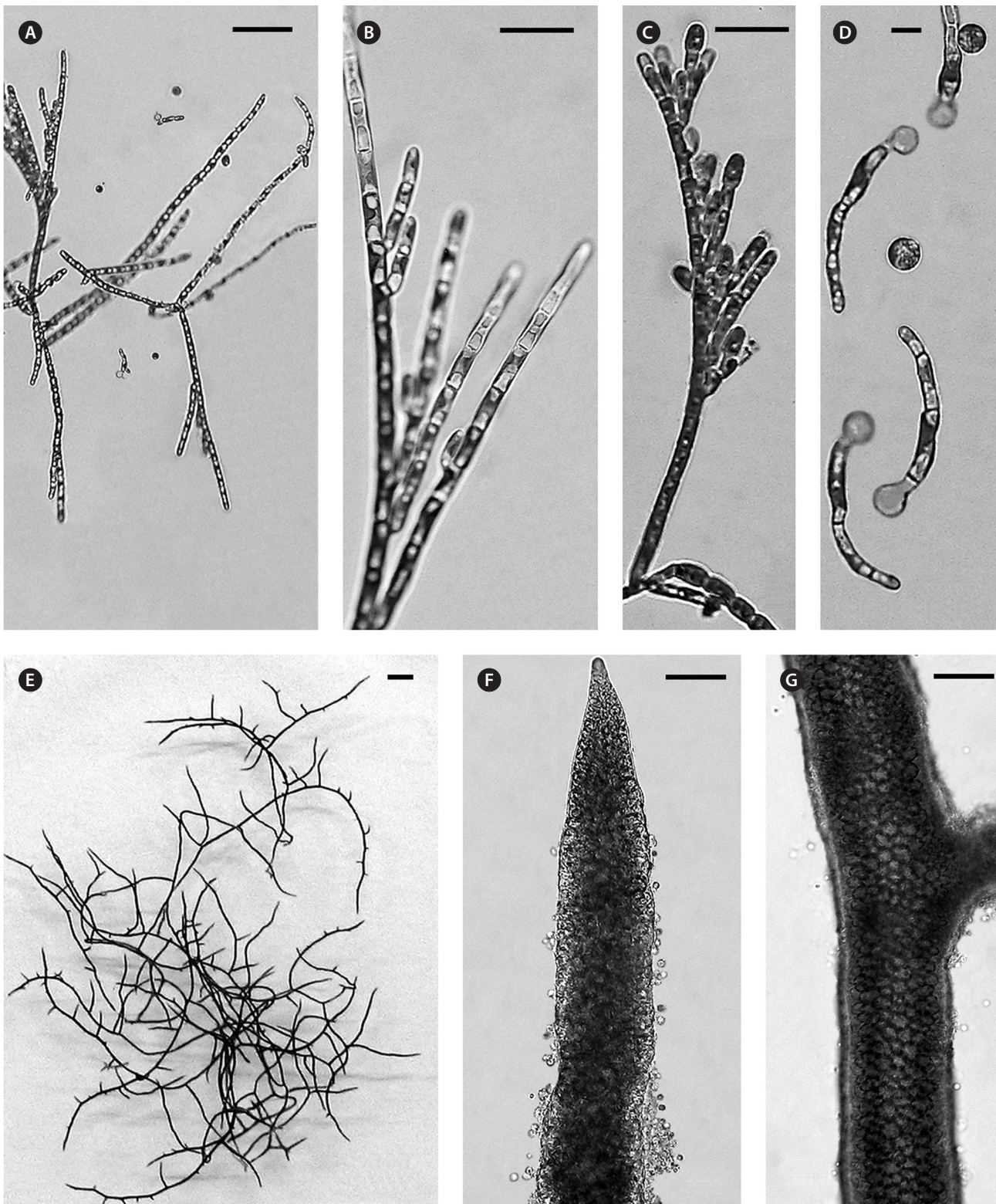


Fig. 2. *Acrochaetium globosum* 4626 (A-D) and *Caulacanthus indicus* 4625 (E-G). (A) Mature thallus with basal system and erect shoots usually bearing unilateral branches and sporangia. (B) Cells showing single parietal chloroplast with a centrally projecting pyrenoid. (C) Small clusters of sporangia (8 μ m diam. \times 12 μ m long) borne on short unilateral branches. (D) Six-day-old sporelings, 3 cells long with empty spores at base. (E) Habit showing the horizontal stolons and short erect shoots. (F) Erect shoot apex with single apical cell, spermatangial sorus surrounding the branch. (G) Small polygonal cortical cells of mature branch. Scale bars represent: A, 80 μ m; B & C, 40 μ m; D, 10 μ m; E, 1 mm; F & G, 50 μ m.

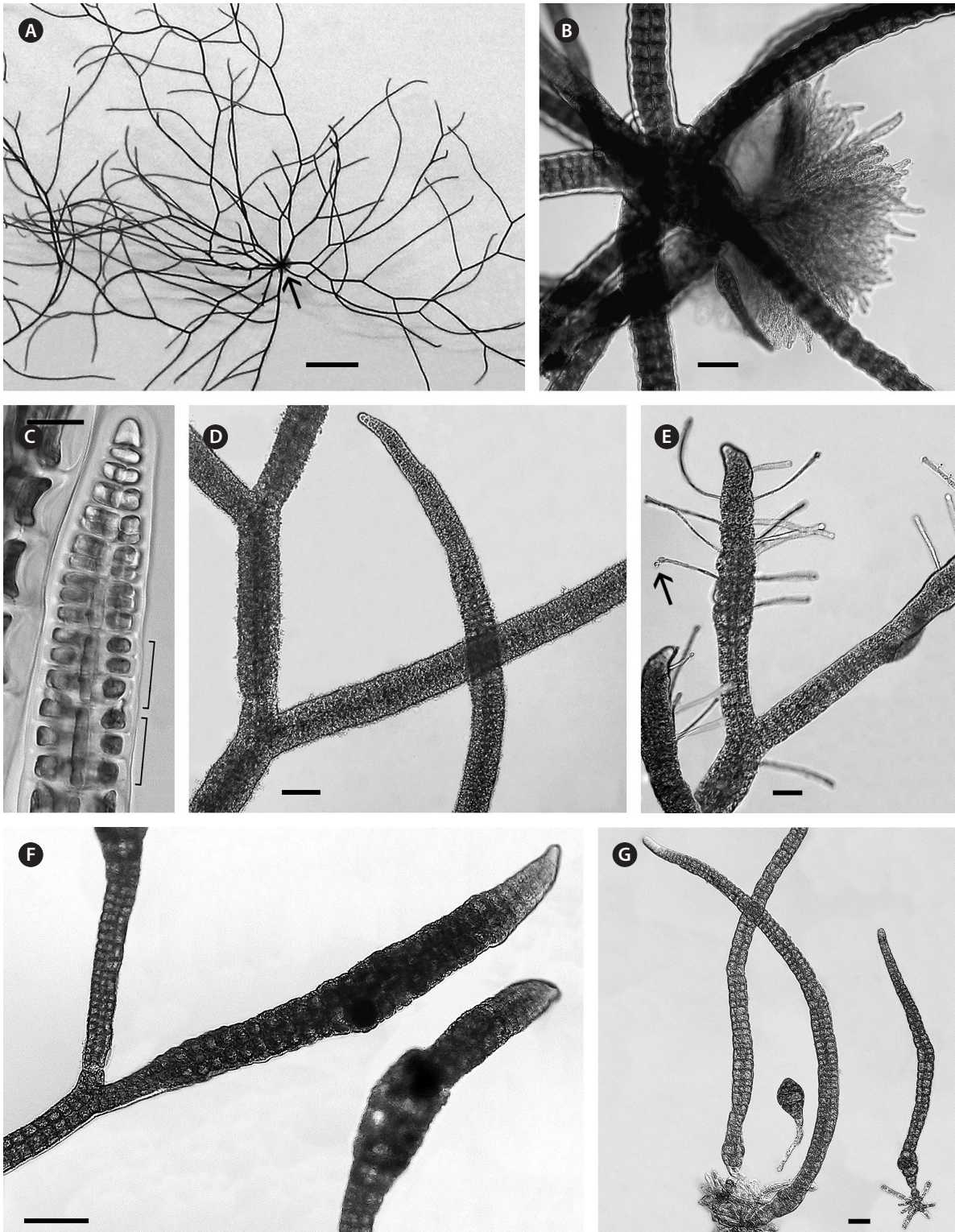


Fig. 3. *Bostrychia kelanensis* 3075 (A-C), 4589 (D), and 4632 (E-G). (A) Habit of non-reproductive tetrasporophyte with alternate lateral branching and an attachment disc (arrow). (B) Multiple new shoots arising from cladohaptera attachment disc. (C) Microwave-treated branch showing 3-tier cells per pericentral cell. (D) Male gametophyte with continuous spermatangial sorus on upper branches. (E) Female gametophyte with short lateral branches bearing whorls of procarys. Spermatium attached to tip of carpogonium (arrow). (F) Tetrasporangial stichidia. (G) Tetraspore germlings with an initial single rhizoid developing into multiple branched rhizoids. Scale bars represent: A, 2 mm; B, 100 µm; C, 50 µm; D, 75 µm; E, 85 µm; F, 120 µm; G, 60 µm.

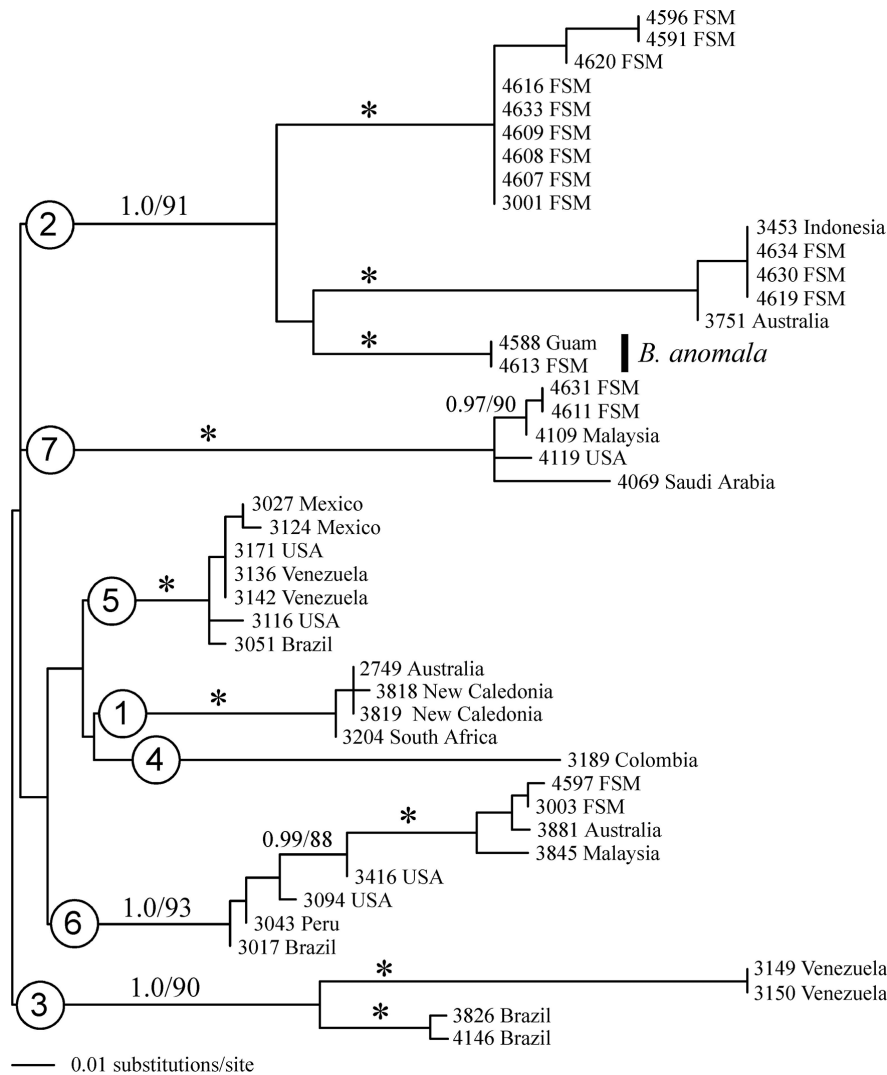


Fig. 4. Maximum-likelihood phylogeny of the RuBisCo spacer data of select *Bostrychia moritziana* / *B. radicans* isolates. Seven lineages marked (after Zuccarello and West 2003). Isolates from Micronesia (FSM) found in lineages 2, 6, and 7. * indicates PP values ≥ 0.95 and maximum likelihood bootstrap (ML BP) values $\geq 95\%$. Otherwise values presented as PP/ML BP.

grew to 2-3 cm overall and only occasionally producing cladohaptera (Fig. 3A & B). Three tier cells were produced per axial cell in all stages (Fig. 3C). Males of isolate 4589 produced compoundly branched spermatangial stichidia, 70-75 μm wide, along the upper shoots (Fig. 3D). The female shoots of 4632 were 85-100 μm diameter. Branching was usually alternate at 0.5-1 mm intervals (Fig. 3A). Procarps were borne in irregular whorls along upper shoots and laterals (Fig. 3E) with trichogynes up to 220 μm long and about 8 μm wide.

Tetrasporangial stichidia of 4632 were variable in length and had irregular numbers of tetrasporangia (Fig. 3F). Sporelings were about 60-70 μm wide reaching about 0.5-1.0 mm in length before branching (Fig. 3G). As

sporelings grew their bases produced numerous coherent, branched, and uniseriate rhizoids (Fig. 3G).

Bostrychia moritziana / *B. radicans* species complex: This species complex is comprised of seven molecular lineages (Zuccarello and West 2003, 2006, Zuccarello et al. 2006). These genetic lineages are not reproductively inter-compatible (Zuccarello and West 1995, 1997, Zuccarello et al. 1999b, 2011). Morphologically *B. moritziana* has been primarily distinguished by abundant compound monosiphonous lateral branches, whereas *B. radicans* had mostly polysiphonous laterals. Both 'species' produce cladohaptera from the basal cell of the lateral branches. In Micronesia isolates from three lineages (2, 6, 7) are present (Fig. 4).

B. moritziana / *B. radicans* was especially common (Table 1). A Kosrae isolate of lineage 2 (4607) and others formed typical polysiphonous shoots with some monosiphonous laterals in culture (Fig. 5A & B). A *Polysiphonia*-type sexual life history is evident in 4607 and other strains (Table 1). Mature tetrasporophytes were up to 2-3 cm long with tetrasporangial stichidia variable in abundance (Fig. 5B).

Males had lateral branches with single spermatangial stichidia (Fig. 5C), or some had compound-branched lateral stichidia. Females bore procarys in series along the apices of polysiphonous lateral branches (Fig. 5D). Self-fertilization was typical in culture with many spermatia attaching to trichogynes (Fig. 5D, arrow in center) and well developed cystocarys (Fig. 5D) releasing carpospores that germinated to form tetrasporophytes.

Cladophaptera were typical on most isolates in culture, although in 4607 the cladophaptera usually had free rhizoids and new shoot also arising from the tips of the cladophaptera (Fig. 5E). We do not know if this occurs only in culture.

Another isolate of lineage 2 (4619) also had typical polysiphonous shoots, monosiphonous secondary laterals and procarys with elongate trichogynes (Fig. 6A).

By contrast, isolate 3001, also from lineage 2, has produced for 24 years successive generations of asexual tetrasporophytes with monosiphonous filaments that begin to form short polysiphonous terminal tetrasporangial stichidia (Fig. 6B). These stichidia developing from monosiphonous shoots are similar to tetrasporangial stichidia of *Bostrychia anomala* J. A. West, de Goër and Zuccarello (West et al. 2013), but *B. anomala* is in a different subclade (Fig. 4) and has a sexual life history.

Another lineage 2 isolate (4591) produced successive generations of branched monosiphonous filaments bearing terminal segments with asexual tetrasporangial stichidia (Fig. 6C) for seven years. Isolate 4596 from lineage 2 was also primarily monosiphonous, but when reproductive, developed terminal polysiphonous segments with procarys bearing elongate trichogynes (arrows in Fig. 6D). Isolate 3003 from lineage 6 produced successive polysiphonous generations of asexual tetrasporophytes for 19 years.

Two isolates from lineage 7 were quite different from each other in morphology and reproduction. Isolate 4611 was a polysiphonous tetrasporophyte from which spores produced polysiphonous females, whereas 4631 was primarily monosiphonous, producing polysiphonous shoot tips with tetrasporangial stichidia and spores resulting in successive generations of tetrasporophytes. All these iso-

lates are identical in RuBisCo spacer sequences.

Reproductive patterns of the remaining isolates are shown in Table 1. Some isolates (e.g., 4597) lacked any form of sporic reproduction, producing new thalli by fragmentation.

***Bostrychia radicata*:** This species originally was described as *Rhodolachne radicata* Itono from Fiji (Itono 1985). Based on morphological evidence, it belongs to the genus *Bostrychia*. Isolates 4614, 4621, 4627, and 4650 had *Polysiphonia*-type sexual life histories and 4663 had a tetrasporophyte recycling asexual life history. After one year all the isolates reverted to only vegetative growth. The general habit in culture was long horizontal shoots showing at variable intervals short (1-2 mm), erect branches having slightly recurved tips and sometimes branched once or twice (Fig. 7A). The attachment structures were different from peripherophaptera and cladophaptera seen in other *Bostrychia* species, being one or more individual multicellular uniseriate rhizoids arising from the tier cells at the branch nodes from which one or more erect shoots developed as well (Fig. 7B). Each rhizoidal cell was uninucleate and had multiple small plastids (Fig. 7C). Tetrasporangial stichidia arose at shoot tips, were up to 300 µm long and usually bear 1-2 tetrasporangia per segment (Fig. 7D). Male gametophytes had terminal spermatangial stichidia up to 500 µm long with a recurved tip (Fig. 7E). Female gametophytes had procarys with elongate trichogynes along the upper part of a shoot tip (Fig. 7F). Mature carposporophytes were approximately 150 µm wide and often arose terminally or subapically (Fig. 7F). Carpospore germlings had typical bipolar germination (Fig. 7G).

In all isolates the LMWC analyses showed sorbitol and digeneaside, but no dulcitol (Table 2).

***Bostrychia simpliciuscula*:** Based on molecular evidence, Zuccarello and West (2006) merged *B. tenuissima* R. King et Puttock with *B. simpliciuscula*. All the Micronesian isolates were from the H3 lineage as were those from northern Australia and Singapore (Zuccarello et al. 1999a).

Alternate lateral branches arose at variable intervals of 2-10 axial cells (Fig. 8A). Pericentral cells and tier cells arose at the 5th-6th axial cell (Fig. 8B). Plants were ecorticate with whorls of 4-7 pericentral cells each having 2 tier cells. Peripherophaptera usually developed at the nodes of branches or without opposite branches (Fig. 8C). The lateral branches were polysiphonous or partially monosiphonous in field specimens.

The H3 lineage of *B. simpliciuscula* was also characterized by the presence of the LMWC's sorbitol, dulcitol

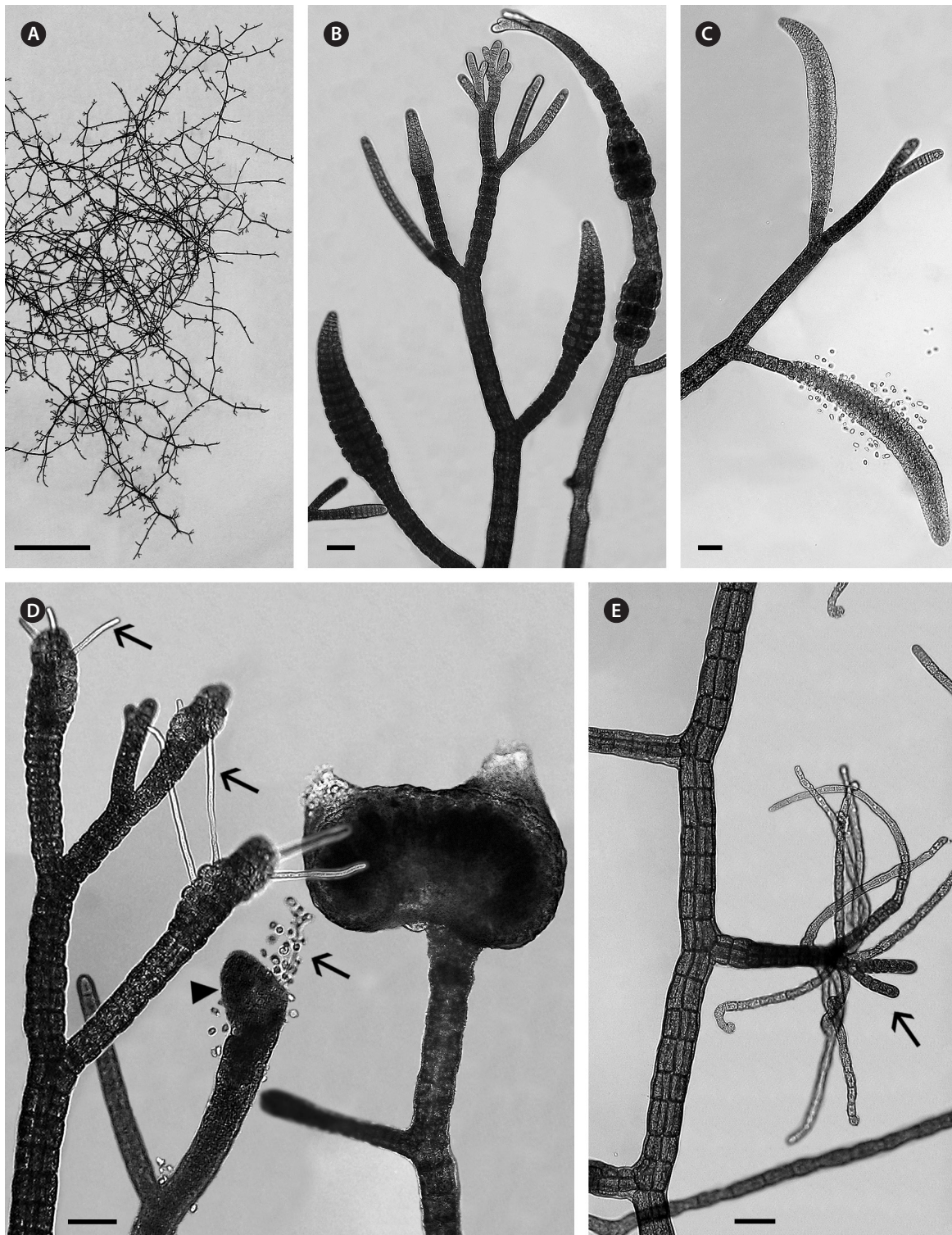


Fig. 5. *Bostrychia moritziana* / *B. radicans*, lineage 2, isolate 4607. (A) Habit of cultured specimen. (B) Tetrasporophyte with sporangial stichidia on a cultured specimen. (C) Male gametophyte with spermatangial stichidia, lower one releasing spermatia. (D) Female gametophytes. On the left are unfertilized procars with trichogynes (arrows). Center branch has fertilized procarp with spermatia around the trichogyne, 2-week-old cystocarp (arrowhead) developing a pericarp. On the right is branch with two 5-week-old cystocarps showing the darkened carposporangial masses. (E) Cladophaptera often developed differently from most *B. moritziana* / *B. radicans* isolates. Individual pericentral cells at tip become dissociated instead of remaining firmly coalesced. The lateral branch tip has a new indeterminate compound branch (arrow). Scale bars represent: A, 5 mm; B, D & E, 80 μ m; C, 70 μ m.

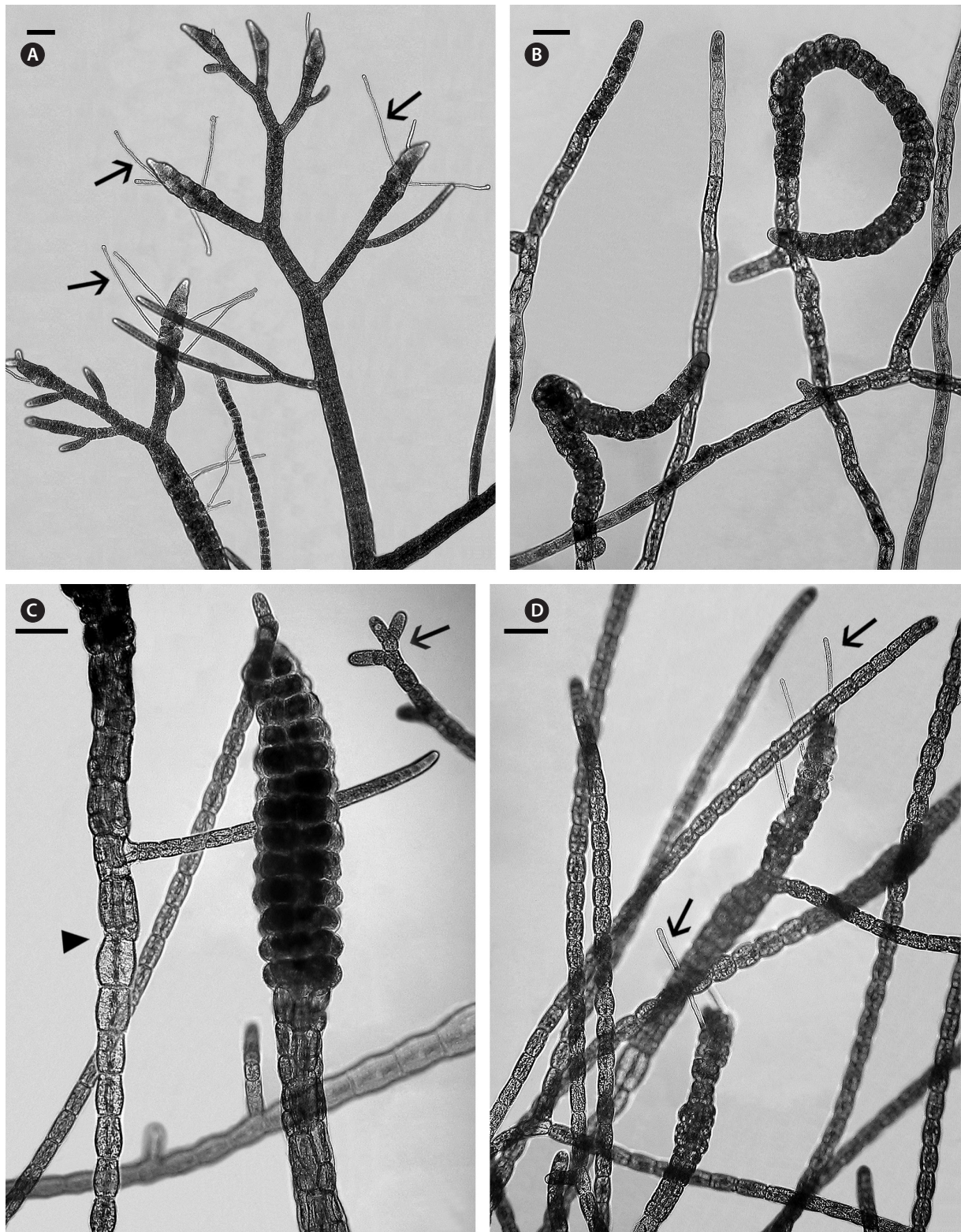


Fig. 6. *Bostrychia moritziana* / *B. radicans*, lineage 2. (A) Isolate 4619. Female with typical polysiphonous branches and procarps (arrows show trichogynes). (B) Isolate 3001. Monosiphonous and polysiphonous branches of asexual tetrasporophyte. (C) Isolate 4591. Asexual tetrasporophyte, apical branching of monosiphonous shoots. Arrowhead indicates transition from mono- to poly-siphonous sector, arrow indicates apical meristem of branched monosiphonous shoot. (D) Isolate 4596. Monosiphonous branches and terminal polysiphonous sectors with procarps (arrows show trichogynes). Scale bars represent: A & B, 75 μ m; C & D, 70 μ m.

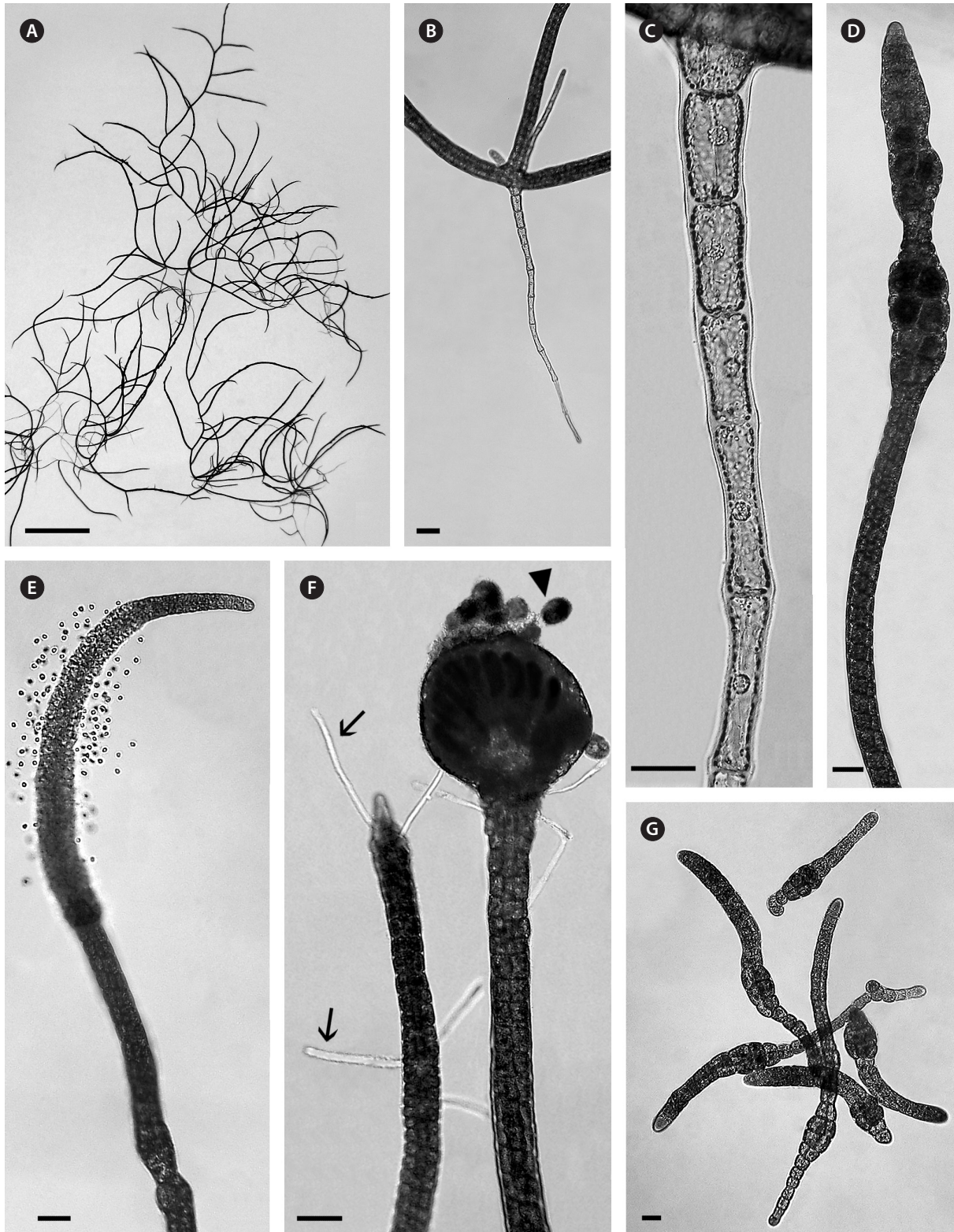


Fig. 7. *Bostrychia radicata*. (A) Habit view of isolate 4663 with lax branching of long horizontal filaments. (B) Isolate 4614 node with 3 rhizoids and single erect shoot. (C) Isolate 4614. Rhizoid clearly showing central nucleus and peripheral plastids. (D) Isolate 4621 terminal tetrasporangial stichidium. (E) Isolate 4621 male with terminal spermatangial stichidium and discharged spermatia. (F) Isolate 4621 female with terminal cystocarp and released carpospores. Procarys with trichogynes (arrows) along a lateral branch. (G) Isolate 4621. Carpospore germlings with rhizoid and erect shoot. Scale bars represent: A, 3 mm; B, 42 μ m; C, 24 μ m; D, 50 μ m; E, 40 μ m; F & G, 45 μ m.

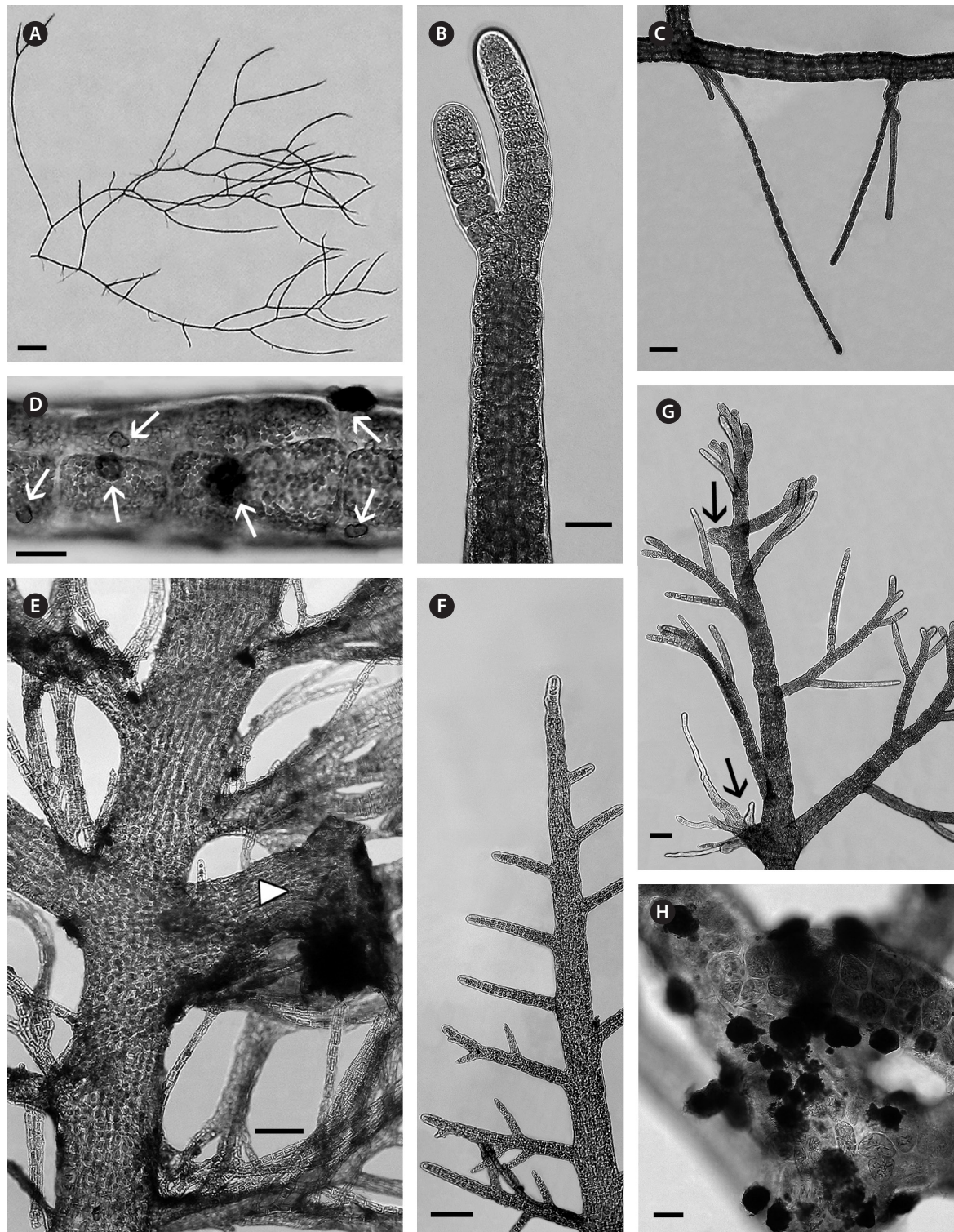


Fig. 8. (A-D) *Bostrychia simpliciuscula* isolate 4636. (E-H) *B. tenella* Isolate 4622 (E & F), isolate 4662 (G & H). Isolate 4622 (E & F), isolate 4662 (G & H). (A) Habit showing alternate / bifurcate branching and nodal and intercalary peripherohaptera. (B) Branch apex and subapical branching. Pericentral and tier cells developing at the fifth-sixth axial cell. (C) Nodal and intercalary peripherohaptera both showing coalescent cells from several tier cells becoming free as individual rhizoids elongate. (D) Manganese deposits (arrows) on cell surfaces. (E) Field specimen, heavily corticated and branched, some monosiphonous laterals, large peripherohaptera (arrowhead). (F) Field specimen, narrow, reduced branching, light cortication, no peripherohaptera. (G) Grown on shaker with bright light, showing good cortication, monosiphonous laterals, peripherohaptera (arrows). (H) Small manganese deposits on branches. Scale bars represent: A, 1 mm; B & C, 60 μ m; D, 20 μ m; E & F, 100 μ m; G, 24 μ m; H, 18 μ m.

and digeneaside (Zuccarello et al. 1999a, Zuccarello and West 2011). Most isolates from Micronesia had the LM-WC's dulcitol, sorbitol and digeneaside except for isolate 4636 in which digeneaside was not detected (Table 2).

B. simpliciuscula appeared to be one of the more common species in Micronesia. Most field-specimens were not reproductive, but three were tetrasporangiate (Table 1). In culture all the isolates grew well and showed typical morphology (Fig. 8A-C) in moderate light ($>5 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) but in lower light ($<2 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) had reduced branching with more peripherohaptera that developed extensive branched rhizoids and new shoots. No isolates in culture showed monosiphonous laterals.

Most isolates remained vegetative in culture. Only two (4595 and 4603) developed tetrasporangial stichidia in which most sporangia were abortive. A few spores were released, and sporelings developed normally, but their final reproductive status was not determined.

SEM elemental analyses were done on microscopic dark brown deposits that frequently occurred on the alga and on glass surfaces in cultures of 4636 (Fig. 8D). These dark brown bodies contained manganese, sulphur, magnesium, aluminium, chlorine, potassium, and bromine (Fig. 9B). Branch surfaces of *B. simpliciuscula* 4636 free of brown deposits contained potassium, sulphur, chlorine, magnesium, manganese, and potassium with no detectable aluminium or bromine (Fig. 9A).

***Bostrychia tenella*:** Plants usually robust with heavily

corticated main axes and partly monosiphonous laterals, 5-8 pericentral cells per axial cell, two tier cells per pericentral cell and conspicuous peripherohaptera at some branch nodes. Molecular evidence showed *B. tenella* is comprised of several well-defined clades (Zuccarello and West 2006).

Field specimens of 4622 showed two distinct morphologies: 1) wide, densely branched with some monosiphonous laterals, heavy cortication and frequent peripherohaptera (Fig. 8E), and 2) narrow, reduced branching, light cortication and absence of peripherohaptera (Fig. 8F). The more lax branching and reduced cortication may be due to lower light levels from self-shading and sediment cover, but this needs to be investigated further.

A similar pattern of variable morphology was visible in other specimens (e.g., 4662). Plants grown in stationary culture with low light ($<3 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) had reduced and somewhat irregular branching, light cortication and sparse small peripherohaptera. In shaker culture (50-60 rpm) and brighter light ($6-8 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) more frequent branching with heavier cortication, monosiphonous terminal segments, robust peripherohaptera (Fig. 8G) and occasional procarps were observed. No other reproduction was seen. The other four isolates were not reproductive in culture.

Microscopic brown deposits similar to those on *B. simpliciuscula* (4636) were frequent on the branches of *B. tenella* (4602, 4617, 4662) in stationary culture (Fig. 8H).

Table 2. Low molecular weight carbohydrates in select species of *Bostrychia*

Species	Culture no.	Sorbitol ($\mu\text{mol}^{-1} \text{g DW}$)	Dulcitol ($\mu\text{mol}^{-1} \text{g DW}$)	Digeneaside ($\mu\text{mol}^{-1} \text{g DW}$)
<i>B. radicata</i>	4614	25 \pm 6	n.t.	391 \pm 21
	4621	39 \pm 0	n.t.	967 \pm 670
	4627	28 \pm 0	n.t.	727 \pm 0
	4650	25 \pm 11	n.t.	459 \pm 78
	4663	21 \pm 4	n.t.	477 \pm 2
<i>B. simpliciuscula</i>	4593	280 \pm 44	192 \pm 45	190 \pm 7
	4594	226 \pm 80	108 \pm 43	235 \pm 59
	4595	13 \pm 72	141 \pm 89	123 \pm 14
	4600	363 \pm 0	151 \pm 0	292 \pm 0
	4603	214 \pm 9	140 \pm 5	122 \pm 9
	4610	296 \pm 0	122 \pm 0	133 \pm 0
	4615	425 \pm 57	278 \pm 21	304 \pm 3
	4629	157 \pm 1	95 \pm 7	116 \pm 7
	4636	325 \pm 0	160 \pm 0	n.t.
	4638	173 \pm 0	74 \pm 0	92 \pm 0

n.t., no trace.

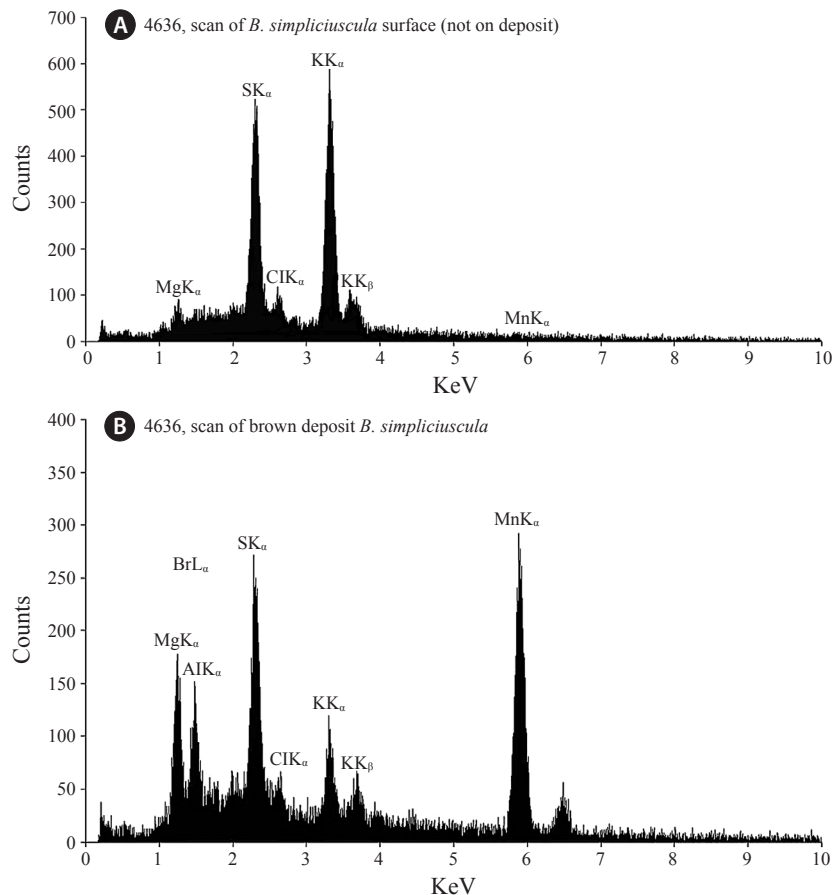


Fig. 9. *Bostrychia simpliciuscula* 4636. (A) Scanning electron microscopy (SEM) elemental analyses of algal cell surface. Sulphur and potassium levels very high, manganese is not evident. (B) SEM elemental analyses of dark brown deposit with highest peak of manganese and somewhat lower peaks of sulphur, aluminium, magnesium, potassium, chlorine, and bromine. Note the comparative levels of the different elements on the algal cell surface and the brown deposit. K_{α} and K_{β} designate different energy states for each element.

Analyses showed results similar to those of *B. simpliciuscula*. Deposits were sparse on plants in shaker culture.

***Murrayella pericladus* (C. Agardh) F. Schmitz:** This species is frequently observed in mangroves and coral habitats. We obtained one tetrasporophyte (4606) in Pohnpei that grew well in culture but did not reproduce and this isolate is available as a culture from Kobe University Marine Algal Culture Collection (<http://www.research.kobe-u.ac.jp/rcis-ku-macc/E.index.html>).

Ceramiales, Delesseriaceae.

***Caloglossa ogasawaraensis* Okamura:** This is the first record for this species in Chuuk, Kosrae, and Pohnpei (Table 1), although it is widespread in the tropics (Zucarello et al. 2012, Guiry and Guiry 2013).

The blades (0.9-3.0 mm long and 50-250 μ m wide) of cultured specimens were usually not constricted at the nodes (Fig. 10A). Secondary adventitious branches

frequently arose from the marginal cells in the thallus plane, and additional adventitious branches developed from these secondary branches in the same way (Fig. 10B). Endogenous branches were not observed. Single rhizoidal filaments, 20-30 μ m in diameter and up to 800 μ m long, were derived from the nodal pericentral cells or the adjacent pericentral cells immediately above and below the node. Although the number of internodal cell rows was up to six in the field-collected specimens, the cultured specimens tended to be more slender and usually had one or two internodal cell rows (Fig. 10B). The axial cells around the node produced one cell row to both sides (Fig. 10B).

Reproductive structures were observed in culture. Formation of tetrasporangia took place in acropetal succession and from the lateral pericentral cells toward the margins of the blade (Fig. 10C). Sporangial sori (150-800 μ m long, 110-150 μ m wide) were produced on

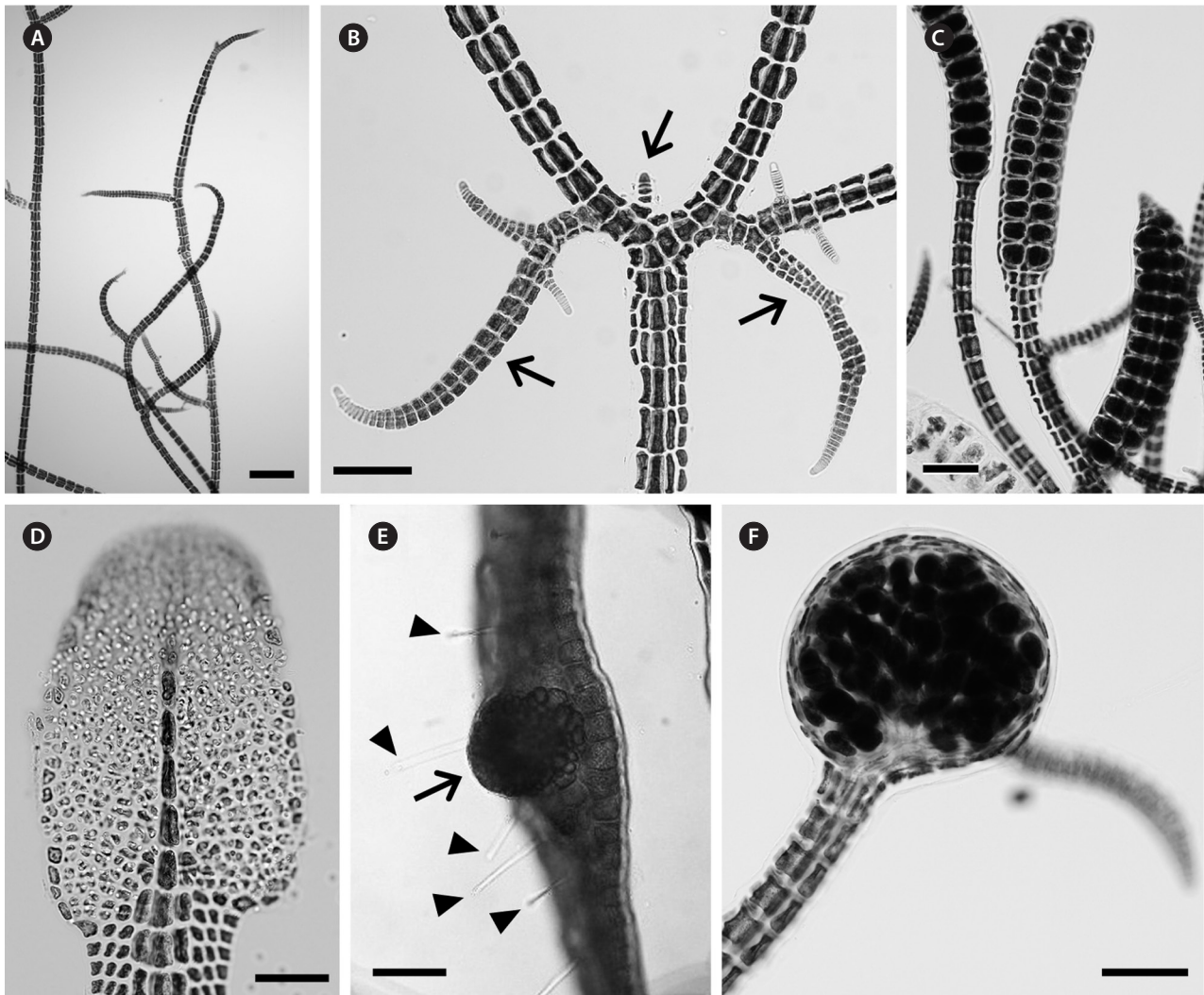


Fig. 10. *Caloglossa ogasawaraensis*. (A) Habit photograph of cultured specimen (4628). (B) Enlarged view of node (4628) showing secondary adventitious branches (arrows) derived from lateral pericentral cells. (C) Branches of tetrasporophyte (4604) bearing long rows of tetrasporangia along the central axis. (D) Branches of male gametophyte (4604) bearing spermatangial sorus. (E) Pseudocystocarp (arrow) on a female (4623). Many trichogynes are visible (arrowheads). (F) Mature cystocarp on a female (4604). Scale bars represent: A, 200 µm; B, C & F, 100 µm; D & E, 50 µm.

both sides of the midrib in the upper part of the blades. Mature tetrasporangia, 40 to 55 µm in diameter, were divided cruciate-decussately or tetrahedrally. Tetraspores germinated into male and female gametophytes, and several females developed carposporophytes. Spermatangial sori were 200–550 µm long and 140–220 µm wide and were found on both sides of the midrib at the upper and middle parts of the blades (Fig. 10D). Spermatangial mother cells (3.8–7.5 µm in diameter) cut off three to five spherical spermatangia (2.5–5.0 µm in diameter) toward the outer surface by anticlinal divisions. Many carpogonial branches with elongate trichogynes were produced in a line along a central axis (Fig. 10E).

Cystocarps were oblate-ovate, 230–350 µm in height, 230–390 µm in diameter with a narrow ostiole and contained many carposporangia, 50–110 µm in diameter (Fig. 10F). Sometimes pseudocystocarps with a pericarp but lacking a gonimoblast occurred in the absence of males (Fig. 10E).

In the large subunit (LSU) rRNA gene tree, the entities of *C. ogasawaraensis* including three FSM strains were resolved as monophyletic with other *C. ogasawaraensis* from around the world (Fig. 11). The strains from Kosrae (4612) and Chuuk (4628) showed identical LSU sequences and were closely related to the Australian entities. However, there was a 13 bp difference between these two strains and the strain from Pohnpei (4604).

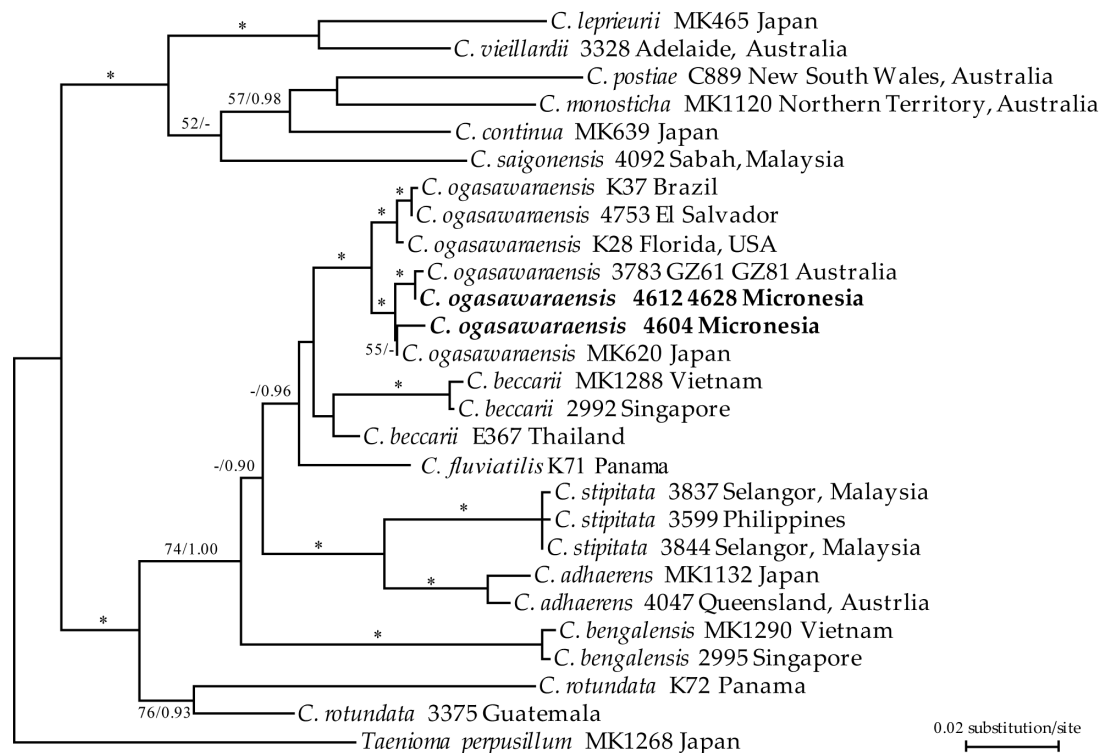


Fig. 11. Maximum-likelihood (ML) phylogeny of *Caloglossa* inferred from the partial large subunit rRNA gene sequences using *Taenioma perpusillum* as an outgroup. * ML bootstrap (BP) values $\geq 95\%$ and posterior probabilities (PP) for Bayesian inference ≥ 0.95 . Otherwise ML BP ($> 50\%$; left) and PP (≥ 0.80 ; right) are presented for each branch. Strain numbers and localities are shown with species epithets.

Chlorophyta

Ulvophyceae, Bryopsidales, Udoteaceae.

***Boodleopsis carolinensis* Trono:** This species was described by Trono (1971) from Palau and was recorded in Micronesia by Lobban and Tsuda (2003) but the specific island is not mentioned. Our field samples formed turfs on various substrates around Pohnpei and Kosrae (Table 1). Isolates 4601 and 4618 grew slowly with reduced branching but did not reproduce and gradually died after 8 months of culture, possibly related to the numerous microscopic brown deposits on the branches. Isolates 4605 and 4624 lacked microscopic brown deposits seen on 4601 and 4618, grew very well and formed long stolons bearing numerous erect dichotomous to polychotomous branches with slightly constricted basal nodes (Fig. 12A). Filaments varied from 20 to 32 μm in diameter and contained numerous small (5–7 μm long) elliptical chloroplasts without pyrenoids and colourless amyloplasts lining the large central vacuole (Fig. 12B). Sporangia were

usually single along the filaments, ovoid (100–130 by 180–200 μm), each with a stalk up to 100 μm long densely packed with amyloplasts and chloroplasts (Fig. 12B). Spore discharge was never observed.

Phaeophyceae

Dictyotales, Dictyotaceae.

Numerous algal records for Micronesia by Hodgson and McDermid (2000), Lobban and Tsuda (2003), Lobban and N'Yeurt (2006), McDermid et al. (2002) and Tsuda (2006) include various *Dictyota* species. Specimens listed below were identified by De Clerck et al. (2006) using molecular and morphological evidence.

***Canistrocarpus cervicornis* (Kützinger) De Paula et De Clerck (= *Dictyota cervicornis*):** This species is recorded from Fiji (South and Skelton 2003) and in Micronesia (Lobban and Tsuda 2003) although the specific localities were not indicated. Our specimens were from Nett Point, Pohnpei (Table 1).

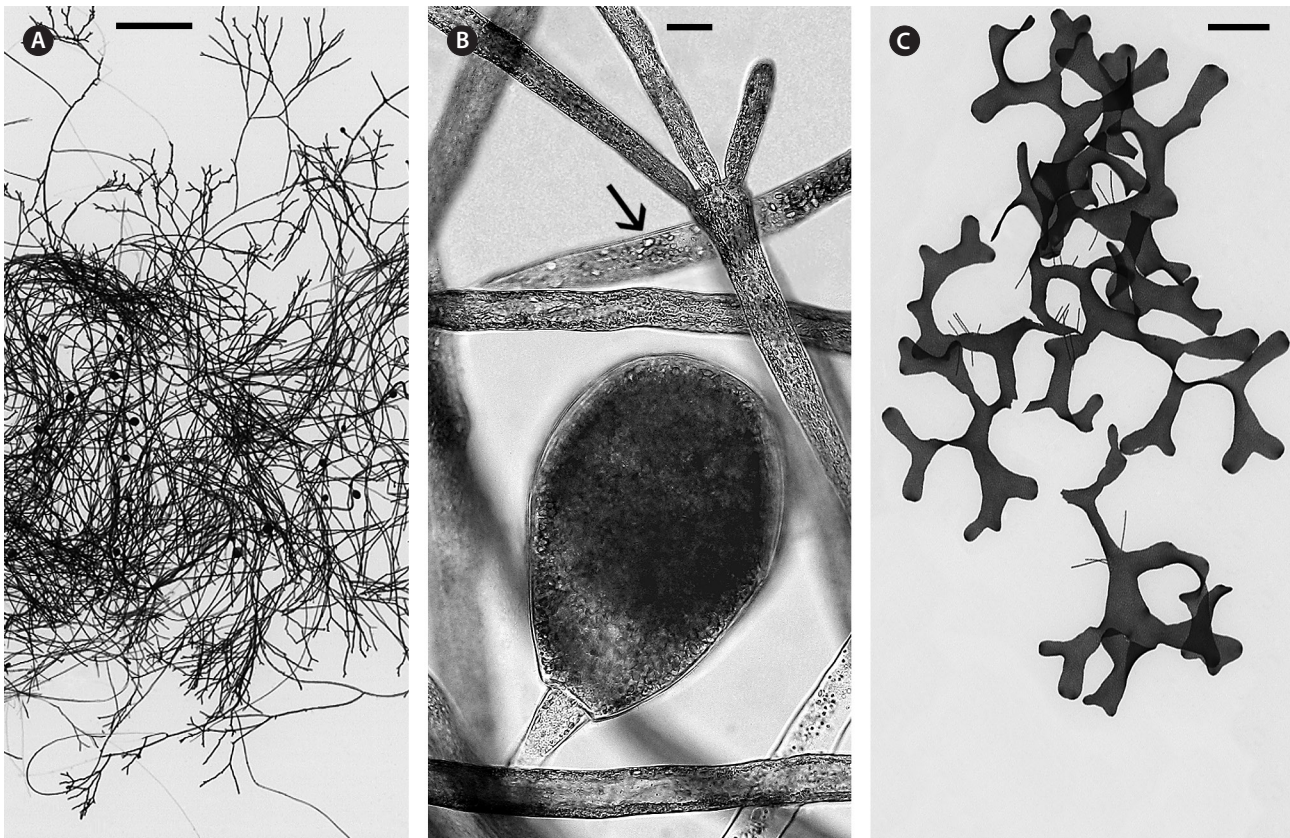


Fig. 12. (A) *Boodleopsis carolinensis* isolate 4605. Habit photo showing dichotomous to polychotomous branching and scattered sporangia. (B) *Boodleopsis carolinensis* isolate 4624. Sporangium (130 × 200 µm) with partially collapsed stalk and some spores visible around the periphery. Colorless refractive starch containing amyloplasts (arrow) visible in filament. (C) *Dictyotopsis propagulifera*, Lehn Mesi River, Pohnpei. Habit photo showing typical branching. Scale bars represent: A & C, 1 mm; B, 25 µm.

***Dictyota adnata* Zanardini:** This is the first record for *D. adnata* from Micronesia (Pohnpei) although it is recorded in Fiji (South and Skelton 2003) and American Samoa (Skelton and South 2004) (Table 1).

***Dictyotopsis propagulifera* Troll:** This is the first record of this monotypic genus from Micronesia (Table 1). The branching of the cultured specimen from Pohnpei is shown (Fig. 12C). No reproduction was observed.

DISCUSSION

This report expands information on species diversity in the islands of Chuuk, Pohnpei, and Kosrae in Micronesia and neighbouring Guam. While extensive field work has been done in these areas (e.g., Lobban and Tsuda 2003, Tsuda 2006), the small algae associated with mangroves are often overlooked. These algae are common in mangroves around the world and add to the diversity and ecology of these ecosystems. While some of these algae

are probably opportunistic epiphytes (*Colaenema* sp. and *Acrochaetium globosum*) or typical shallow water algae (e.g., *Canistrocarpus cervicornis*), and are not specific to mangroves, some are frequently associated with mangroves (*Bostrychia* and *Caloglossa*).

The taxonomy and molecular phylogeny of *Bostrychia*, while well studied, is still not completely resolved. Within the morphological species corresponding to *B. moritziana* and *B. radicans* there are several evolutionary lineages (seven, plus other species) (Zuccarello and West 2006, West et al. 2013), of these, three lineages are found in this study (lineage 2, 6, 7). These lineage are widespread, with lineage 2 limited to the Indo-Pacific Ocean. Lineage 7 occurs in Florida-USA, Malaysia, Micronesia, New Caledonia, and Saudi Arabia whereas lineage 6 occurs in both the eastern and western Pacific (Zuccarello and West 2003, this paper). This indicates that, at least, historical dispersal is common in these algae, possibly travelling on dislodged mangrove wood or other substrates. It is interesting that one of the more common lineages in Australia

and New Caledonia, lineage 1 (Zuccarello and West 2003, Zuccarello et al. 2006) was not found in these collections. Whether it is restricted to the more western Pacific Ocean will need further sampling.

New Records / Geographic range extensions

Although other *Acrochaetium* species were recorded by Lobban and Tsuda (2003) this is the first report of *A. globosum* from Kosrae. Currently it is known only from the Americas (Guiry and Guiry 2013). The species designation is uncertain and awaits molecular definition.

This is the first report of a *Colaconema* species from Chuuk but definitive molecular data is needed to assign a species name. *Colaconema gracile* (Børgesen) Atweberhan et Prud'homme van Reine and *C. hypneae* (Børgesen) Santos et Moura are recorded in Micronesia (Guiry and Guiry 2013).

Caulacanthus indicus is not previously recorded in Micronesia but molecular analyses are required to resolve its relationship with *Caulacanthus ustulatus*, known from Pohnpei (McDermid et al. 2002) and Hawaii (Abbott 1999).

B. moritziana was previously recorded for Micronesia by Zuccarello and West (2006). *B. radicans* and *B. tenella* are reported around Micronesia by Lobban and Tsuda (2003). We have added new collections around Chuuk, Kosrae and Pohnpei for the *B. moritziana/B. radicans* complex.

B. radicata is recorded in Australia, Fiji, Malaysia, New Caledonia, Thailand, and Madagascar (West et al. 2006) but it was not seen in Vanuatu (West et al. 2008). The new collections from Chuuk and Kosrae extend the range of distribution approximately 3,000 km from the closest previously recorded population in New Caledonia.

Surprisingly, *B. simpliciuscula* was not recorded previously even though it is quite common around Guam, Chuuk, Kosrae, and Pohnpei. Perhaps it can be confused with *B. moritziana* / *B. radicans*. The closest previous record is in Vanuatu (West et al. 2008) about 2,600 km from Kosrae.

Murrayella pericladus has not been reported in Micronesia (Tsuda 2006) but Lobban and Tsuda (2003) reported it from Guam. Zuccarello et al. (2002) carried out extensive breeding and molecular analyses with an isolate from Guam and other tropical regions showing clearly that *M. pericladus* is sexually compatible and genetically uniform throughout the tropics. We found one isolate (4606) in Pohnpei, the first record for Micronesia.

C. ogasawaraensis is a new record for Micronesia

(Chuuk, Kosrae, Pohnpei) although the closest record is known in Fiji, about 3,500 km from Pohnpei and 4,200 km from Chuuk (South and Skelton 2003). Other *Caloglossa* species seem widespread in the western Pacific: Australia (King and Puttock 1994, Kamiya et al. 2003, 2004), Guam (Tsuda 2003), Japan (Yoshida 1998), Micronesia (Lobban and Tsuda 2003), New Caledonia (Garrigue and Tsuda 1988, Millar and Prud'homme van Reine 2005), New Zealand (Adams 1994), Papua New Guinea (King 1990), Philippines (Silva et al. 1987), Samoa (Skelton and South 2002, 2007), Solomon Islands (Womersley and Bailey 1970), Tonga (Kamiya et al. 2003), and Vanuatu (West et al. 2008). However, they appear to be absent in French Polynesia (Payri et al. 2000) and Hawaii (Abbott 1999). More extensive collections are necessary.

Dictyota adnata is new to Micronesia (Pohnpei), about 3,500 km from Fiji, the closest previous record (South and Skelton 2003). *Dictyotopsis propagulifera* is recorded in Indonesia, Malaysia, Singapore, Australia and Fiji (Guiry and Guiry 2013). The closest known population to Pohnpei is on the Annan River, Queensland, Australia (isolate 4382, Master Culture List, <http://www.botany.unimelb.edu.au/west>), a distance of about 2,900 km.

Culturing

Culturing isolates from the field collections is necessary for most critical observations on growth and reproduction. Even though standard culture conditions are usually satisfactory and we have tested various levels of light, temperature, salinity, water motion and nutrients it is not possible to simulate the daily tidal and salinity patterns of typical mangrove habitats. It is quite possible that these ecological factors affect growth, reproduction and many biochemical patterns.

Reproductive patterns in *Bostrychia* species and *Caloglossa ogasawaraensis*

Thirty-one isolates of *B. kelanensis* from Australia, Guam, India, Indonesia, Japan, Malaysia, Micronesia, Singapore and Thailand (see Master Culture List). Twenty-three were sexual and eight were asexual. Tetraspores in most sexual tetrasporophytes produced male and female gametophytes but in several tetrasporophytes the tetraspores only developed into females. The tetrasporophytes of asexual isolates recycled through many generations. *B. kelanensis* is the only species of the genus that exhibited the curious pattern of only females produced by the tetraspores. How do populations without males

survive?

For the *B. moritziana*/*B. radicans* complex a total of 415 isolates from around the world have been cultured. Approximately 55% had sexual cycles, 28% had asexual recycling of tetrasporophytes, 11% showed only vegetative growth, and 9% were undefined (see Master Culture List). Asexual reproduction of *B. moritziana*/*B. radicans* is recorded mostly in northern latitudes (above 37° S) of Australia and along the west coast of New Caledonia whereas sexual reproduction is frequent along the southern coast of Australia, Brazil, El Salvador, Fiji, Guatemala, the east coast of New Caledonia, India, Indonesia, Madagascar, Malaysia, Mexico, New Zealand, Peru, Philippines, South Africa, USA, Vanuatu, and Venezuela (West et al. 1992, West and Zuccarello 1999, Zuccarello et al. 1999b, 2006, Zuccarello and West 2003, 2011). Asexual and sexual reproduction are intermixed within Fiji, India, Indonesia, Malaysia Madagascar, South Africa, and Vanuatu. From Micronesia ten isolates were asexual, seven were sexual and one was vegetative.

In the six *Bostrychia radicata* isolates from other regions reproduction was either of a typical sexual *Polysiphonia*-type pattern or asexual by recycling of tetrasporophytes although two only showed vegetative growth without reproduction (West et al. 2006). In the five Micronesia isolates four were sexual and one was asexual. The factors controlling these different reproductive patterns are not known.

B. simpliciuscula isolates from Singapore and Japan were investigated by West (1992) and Kamiya et al. (1994) and showed a *Polysiphonia*-type sexual life history with unisexual or bisexual gametophytes. Of the ninety *B. simpliciuscula* isolates we investigated forty-six did not reproduce, twenty-seven were sexual with a *Polysiphonia*-type life history and seventeen were tetrasporophytes with abortive sporangia (see Master Culture List). From Guam and Micronesia eight isolates were non-reproductive and two produced abortive tetrasporangia.

Of the seventy-eight *B. tenella* isolates obtained around the world, thirty-nine were sexual with a *Polysiphonia*-type life history, eighteen were tetrasporophytes with abortive sporangia or produced asexual sporelings and 21 only had vegetative growth in culture. Four Micronesia isolates were vegetative and one was a female (see Master Culture List), generally growth was not satisfactory possibly because of the numerous brown deposits.

We have thirty-eight *C. ogasawaraensis* isolates from around the world. Twenty-one have *Polysiphonia*-type sexual life history, five have an asexual life history with

tetrasporophyte recycling, five are vegetative and seven undefined (see Master Culture List). One isolate from Chuuk is vegetative, one is male and one is female from Kosrae and one from Pohnpei is a tetrasporophyte that produces tetraspores and sporelings that are non-reproductive.

Low molecular weight carbohydrates

We obtained seven isolates of *Bostrychia kelenensis* in Guam, Chuuk and Pohnpei but no analyses were done. Other specimens from Australia contained only sorbitol and digeneaside (Karsten et al. 1992, 1995).

B. moritziana / *B. radicans* specimens from Australia, Brazil, Georgia and Florida (USA), Mexico, Micronesia, Peru, and Venezuela have sorbitol and dulcitol but populations from the North Carolina to Connecticut (USA) have sorbitol and lack dulcitol (Karsten et al. 1992, 1993, 1994, 1995, Pedroche et al. 1995). No analyses were done with the nineteen Micronesian isolates.

B. radicata isolates from Micronesia have sorbitol and digeneaside but dulcitol was absent (Table 2) whereas isolates from other regions (Australia, Madagascar, Malaysia, New Caledonia, and Thailand) had high levels of digeneaside and low levels of sorbitol and dulcitol (West et al. 2006). At present there are no other characters we recognize that suggest a taxonomic difference between the two groups.

B. simpliciuscula is geographically very widespread and has 3 distinct molecular lineages, H1, H2, and H3 that have different polyol patterns (Karsten et al. 1992, 1995, Zuccarello et al. 1999a). All those populations (H2 and H3) north of 34° S have sorbitol, dulcitol and digeneaside and those (H1) south of 34° S have sorbitol and digeneaside. The ten isolates from Guam, Chuuk, Kosrae and Pohnpei all are H3 and contain sorbitol, dulcitol and digeneaside except 4636 that lacks digeneaside (Table 2).

B. tenella is also very widespread geographically and occupies varied intertidal habitats (mangroves, coral reefs, rocks) even some freshwater and low salinity habitats (e.g., Marbo Caves with freshwater seepage and adjacent to the intertidal zone, Guam). All specimens previously analysed from Australia, Belize, Brazil, Fiji, Indonesia, Madagascar, Panama, Philippines, Puerto Rico, South Africa, Samoa, Thailand, USA, Vanuatu, and Venezuela have both sorbitol and dulcitol (Kremer 1976, Karsten et al. 1992, 1995). The five isolates from Guam and Micronesia were not analysed.

Manganese deposits

Microscopic dark brown deposits are seen in some cultures from their initial establishment and could not be removed with bacterial antibiotics (Penicillin G, Ciprofloxacin, Rifampin, and Rocephin). These brown deposits are found in greater abundance in cultures that are slow growing. Occasionally they appear to impair the growth of the alga but usually do not. We used electron-scatter SEM microscopy to determine the elemental composition of these brown deposits that have high levels of manganese as well as sulphur, magnesium, aluminium, chlorine, potassium, and bromine in lesser amounts, whereas branch surfaces of *B. simpliciuscula* 4636 and *B. tenella* 4662 have high levels of potassium sulphur with low levels of chlorine and magnesium. The levels are clearly much different in the brown deposits than on the algal surfaces. Similar brown structures were evident on isolates of many other red, green, and brown marine algae (West et al. 2010, in this paper see the results on *Boodleopsis*). Considering the very low level of manganese as a trace element in seawater these brown deposits are surprising. It is possible that the deposits are associated with manganese-depositing bacteria (Spiro et al. 2010, Geszvain et al. 2012). Manganese deposits have not been previously recorded on marine algae. While this is a preliminary study the high accumulation of manganese (and other metals) warrants further investigation into the causative agent of these deposits.

Manganese deposits were reported on the basal stalks of the freshwater Eustigmatophycean alga *Pseudochlorocopsis* (Wujek 2012), on loricas of freshwater chrysophytes (Dunlap et al. 1987), on the lorica of the euglenoid *Trachelomonas* (Moss and Gibbs 1979).

ACKNOWLEDGEMENTS

This research is partially supported by Australian Research Council grants SG0935526 (1994), S19812824 (1998), S19917056 (1999-2001), S0005005 (2000), a grant from the Australian Biological Resources Study (2002-2005) and a grant from the Hermon Slade Foundation (2005-2007). Bill Rainey and Kim O'Connor collected several samples on Pohnpei, Kosrae and Guam. Olivier De Clerck provided much help on the identification of the Dictyotales we collected. Frederic Mineur gave us current information about the status of *Caulacanthus indicus*. Gary Saunders provided the molecular evidence for identification of our *Colaenema* culture isolate. Pat Kelly,

GeoTrack International, did the elemental scan analyses.

REFERENCES

- Abbott, I. A. 1999. *Marine red algae of the Hawaiian Islands*. Bishop Museum Press, Honolulu, HI, 477 pp.
- Adams, N. M. 1994. *Seaweeds of New Zealand: an illustrated guide*. Canterbury University Press, Christchurch, 360 pp.
- Børgesen, F. 1915. The marine algae of the Danish West Indies. Part 3. Rhodophyceae (1). Dansk. Bot. Arkiv. 3:1-80.
- De Clerck, O., Leliaert, F., Verbruggen, H., Lane, C. E., De Paula, J. C., Payo, D. A. & Coppejans, E. 2006. A revised classification of the Dictyotales (Dictyotales, Phaeophyceae) based on *rbcL* and 26S ribosomal DNA sequence data analyses. J. Phycol. 42:1271-1288.
- Dunlap, J. R., Walne, P. L. & Preisig, H. R. 1987. Manganese mineralization in chrysophyte loric. Phycologia 26:394-396.
- Garrigue, C. & Tsuda, R. T. 1988. Catalog of marine benthic algae from New Caledonia. Micronesica 21:53-70.
- Geszvain, K., Butterfield, C., Davis, R. E., Madison, A. S., Lee, S. -W., Parker, D. L., Soldatova, A., Spiro, T. G., Luther, G. W. & Tebo, B. M. 2012. The molecular biogeochemistry of manganese (II) oxidation. Biochem. Soc. Trans. 40:1244-1248.
- Goff, L. J. & Moon, D. A. 1993. PCR amplification of nuclear and plastid genes from algal herbarium specimens and algal spores. J. Phycol. 29:381-384.
- Guiry, M. D. & Guiry, G. M. 2013. AlgaeBase. World-wide electronic publication, National University of Ireland, Galway. Available from: <http://www.algaebase.org>. Accessed May 28, 2013.
- Heinrich, K. F. 1981. *Electron beam x-ray microanalysis*. Van Nostrand Reinhold Co., New York, NY, 588 pp.
- Hodgson, L. M. & McDermid, K. J. 2000. Marine plants of Pohnpei and Ant Atoll: Chlorophyta, Phaeophyta and Magnoliophyta. Micronesica 32:289-307.
- Itono, H. 1985. *Rhodolachne radicata*, a new species of red alga (Rhodomelaceae, Ceramiales) from Fiji and southern parts of Japan. Kagoshima Univ. Res. Center S. Pac. Occas. Pap. 5:53-64.
- Kamiya, M., West, J. A. & Hara, Y. 1994. Reproductive structures of *Bostrychia simpliciuscula* (Ceramiales, Rhodophyceae) in the field and culture. Jpn. J. Phycol. 42:165-174.
- Kamiya, M., West, J. A. & Hara, Y. 2011. Induction of apomixis by outcrossing between genetically divergent entities of *Caloglossa leprieurii* (Ceramiales, Rhodophyta) and

- evidence of hybrid apomicts in nature. *J. Phycol.* 47:753-762.
- Kamiya, M., Zuccarello, G. C. & West, J. A. 2003. Evolutionary relationships of the genus *Caloglossa* (Delesseriaceae, Rhodophyta) inferred from large-subunit ribosomal RNA gene sequences, morphological evidence and reproductive compatibility, with description of a new species from Guatemala. *Phycologia* 42:478-497.
- Kamiya, M., Zuccarello, G. C. & West, J. A. 2004. Phylogeography of *Caloglossa leprieurii* and related species (Delesseriaceae, Rhodophyta) based on the *rbcL* gene sequences. *Jpn. J. Phycol.* 52(Suppl):147-151.
- Karsten, U., Bock, C. & West, J. A. 1995. ^{13}C -NMR spectroscopy as a tool to study organic osmolytes in the mangrove red algal genera *Bostrychia* and *Stictosiphonia* (Ceramiales). *Phycol. Res.* 43:241-247.
- Karsten, U., Michalik, D., Michalik, M. & West, J. A. 2005. A new unusual low molecular weight carbohydrate in the red algal genus *Hypoglossum* (Delesseriaceae, Ceramiales) and its possible function as an osmolyte. *Planta* 222:319-326.
- Karsten, U., Thomas, D. N., Weykam, G., Daniel, C. & Kirst, G. O. 1991. A simple and rapid method for extraction and separation of low molecular weight carbohydrates from macroalgae using high-performance liquid chromatography. *Plant Physiol. Biochem.* 29:373-378.
- Karsten, U., West, J. A. & Ganesan, E. K. 1993. Comparative physiological ecology of *Bostrychia moritziana* (Ceramiales, Rhodophyta) from freshwater and marine habitats. *Phycologia* 32:401-409.
- Karsten, U., West, J. A. & Zuccarello, G. 1992. Polyol content of *Bostrychia* and *Stictosiphonia* (Rhodomelaceae, Rhodophyta) from field and culture. *Bot. Mar.* 35:11-19.
- Karsten, U., West, J. A., Zuccarello, G. & Kirst, G. O. 1994. Physiological ecotypes in the marine alga *Bostrychia radicans* (Ceramiales, Rhodophyta) from the east coast of the U.S.A. *J. Phycol.* 30:174-182.
- King, R. J. 1990. Macroalgae associated with the mangrove vegetation of Papua New Guinea. *Bot. Mar.* 33:55-62.
- King, R. J. & Puttock, C. F. 1989. Morphology and taxonomy of *Bostrychia* and *Stictosiphonia* (Rhodomelaceae/Rhodophyta). *Aust. Syst. Bot.* 2:1-73.
- King, R. J. & Puttock, C. F. 1994. Morphology and taxonomy of *Caloglossa* (Delesseriaceae, Rhodophyta). *Aust. Syst. Bot.* 7:89-124.
- Kremer, B. P. 1976. Distribution of alditols in the genus *Bostrychia*. *Biochem. Syst. Ecol.* 4:139-141.
- Lobban, C. S. & N'Yeurt, A. D. R. 2006. Provisional keys to the genera of seaweeds of Micronesia, with new records for Guam and Yap. *Micronesica* 39:73-105.
- Lobban, C. S. & Tsuda, R. T. 2003. Revised checklist of benthic marine macroalgae and seagrasses of Guam and Micronesia. *Micronesica* 35/36:54-99.
- McDermid, K. J., Hodgson, L. M. & Abbott, I. A. 2002. Marine plants of Pohnpei and Ant Atoll: Rhodophyta, with biogeographic comparisons to other Pacific atolls and island groups. *Micronesica* 34:113-140.
- Millar, A. J. K. & Prud'homme van Reine, W. F. 2005. Marine benthic macroalgae collected by Vieillard from New Caledonia and described as new species by Kützing. *Phycologia* 44:536-549.
- Moss, M. O. & Gibbs, G. 1979. A comparison of the levels of manganese and iron in the tests of *Trachelomonas* Ehrenb. in Surrey rivers. *Br. Phycol. J.* 14:255-262.
- Payri, C. E., N'Yeurt, A. D. R. & Orepuller, J. 2000. *Algues de Polynésie française [Algae of French Polynesia]*. Au Vent des Iles Editions, Tahiti, 320 pp.
- Pedroche, F. E., West, J. A., Zuccarello, G. C., Senties, A. G. & Karsten, U. 1995. Marine red algae of the mangroves in south Pacific Mexico and Pacific Guatemala. *Bot. Mar.* 38:111-119.
- Silva, P. C., Meñez, E. G. & Moe, R. L. 1987. Catalog of the benthic marine algae of the Philippines. *Smithson. Contrib. Mar. Sci.* 27:1-179.
- Skelton, P. A. & South, G. R. 2002. Mangrove-associated algae from Samoa, South Pacific. Available from: http://ucjeps.berkeley.edu/constancea/83/skelton_south/skelton_south.html. Accessed May 28, 2013.
- Skelton, P. A. & South, G. R. 2004. New records and notes on marine benthic algae of American Samoa: Chlorophyta and Phaeophyta. *Cryptogam. Algal.* 25:291-312.
- Skelton, P. A. & South, G. R. 2007. The benthic marine algae of the Samoan Archipelago, South Pacific, with emphasis on the Apia District. *Nova Hedwigia Beih.* 132:1-350.
- South, G. R. & Skelton, P. A. 2003. Catalogue of the marine benthic macroalgae of the Fiji Islands, South Pacific. *Aust. Syst. Bot.* 16:699-758.
- Spiro, T. G., Bargar, J. R., Sposito, G. & Tebo, B. M. 2010. Bacteriogenic manganese oxides. *Acc. Chem. Res.* 43:2-9.
- Trono, G. C. Jr. 1971. Some new species of marine benthic algae from the Caroline Islands, western-central Pacific. *Micronesica* 7:45-77.
- Tsuda, R. T. 2003. Checklist and bibliography of the marine benthic algae from the Mariana Islands (Guam and CNMI). *Tech. Rep. Univ. Guam Mar. Lab.* 107:1-49.
- Tsuda, R. T. 2006. *Checklist and bibliography of the marine benthic algae within Chuuk, Pohnpei, and Kosrae States, Federated States of Micronesia. Bishop Museum Technical Report No. 34.* Bishop Museum Press, Honolulu, HI, 43 pp.

- Tsuda, R. T., Vroom, P. S. & Page-Albins, K. N. 2012. New marine algal records from the Polynesia-Micronesia region of the Pacific Ocean. *Mar. Biodivers. Rec.* 5:e18.
- Weber-van Bosse, A. 1921. Liste des algues du Siboga. II. Rhodophyceae. Première partie. Protoflorideae, Nemalionales, Cryptonemiales. *Siboga Exped. Monogr.* 59b:187-310.
- West, J. A. 1992. New algal records from the Singapore mangroves. *Singapore Gardens' Bull.* 43:19-21.
- West, J. A. 2005. Long term macroalgal culture maintenance. Chapter 11. *In* Andersen, R. A. (Ed.) *Algal Culturing Techniques*. Academic Press, New York, NY, pp. 157-163.
- West, J. A., Loiseaux de Goër, S. & Zuccarello, G. C. 2013. Monosiphonous growth and cell-death in an unusual *Bostrychia* (Rhodomelaceae, Rhodophyta): *B. anomala* sp. nov. *Algae* 28:161-171.
- West, J. A. & Zuccarello, G. C. 1999. Biogeography of sexual and asexual reproduction in *Bostrychia moritziana* (Rhodomelaceae, Rhodophyta). *Phycol. Res.* 47:115-123.
- West, J. A., Zuccarello, G. & Calumpong, H. P. 1992. *Bostrychia bisporea* sp. nov. (Rhodomelaceae, Rhodophyta), an apomictic species from Darwin, Australia: reproduction and development in culture. *Phycologia* 31:37-52.
- West, J. A., Zuccarello, G. C., Hommersand, M., Karsten, U. & Görs, S. 2006. Observations on *Bostrychia radicata* comb. nov. (Rhodomelaceae, Rhodophyta). *Phycol. Res.* 54:1-14.
- West, J., Zuccarello, G. & Kelly, P. 2010. Manganese and iron oxidizing bacteria on marine macroalgae in laboratory culture. 20th International Seaweed Symposium Program. Abstract p. 106. Available from: <http://www.botany.unimelb.edu.au/West>. Accessed May 28, 2013.
- West, J. A., Zuccarello, G. C., West, K. A. & Loiseaux de Goër, S. 2008. New records of algae from Efate, Vanuatu. *Cryptogam. Algal.* 29:235-254.
- Womersley, H. B. S. & Bailey, A. 1970. Marine algae of the Solomon Islands. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 259:257-352.
- Wujek, D. E. 2012. Biomineralization on the stalk of the eustigmatophyte *Pseudocharaciopsis* (Eustigmatophyceae). *Algae* 27:135-137.
- Yoshida, T. 1998. *Marine algae of Japan*. Uchida Rokakuho Publishing Co., Ltd., Tokyo, 1222 pp.
- Zuccarello, G. C., Buchanan, J., West, J. A. & Pedroche, F. F. 2011. Genetic diversity of the mangrove-associated alga *Bostrychia radicans*/*Bostrychia moritziana* (Ceramiales, Rhodophyta) from southern Central America. *Phycol. Res.* 58:98-104.
- Zuccarello, G. C., Kamiya, M., Ootsuki, R., Loiseaux de Goër, S., Pedroche, F. F. & West, J. A. 2012. New records of red algae from mangroves in El Salvador and Pacific Mexico, combining culture and molecular observations. *Bot. Mar.* 55:101-111.
- Zuccarello, G. C., Sandercock, B. & West, J. A. 2002. Diversity within red algal species: variation in world-wide samples of *Spyridia filamentosa* (Ceramiales) and *Murrayella pericladus* (Rhodomelaceae) using DNA markers and breeding studies. *Eur. J. Phycol.* 37:403-417.
- Zuccarello, G. C. & West, J. A. 1995. Hybridization studies in *Bostrychia*. I: *B. radicans* (Rhodomelaceae, Rhodophyta) from Pacific and Atlantic North America. *Phycol. Res.* 43:233-240.
- Zuccarello, G. C. & West, J. A. 1997. Hybridization studies in *Bostrychia*: 2. correlation of crossing data and plastid DNA sequence data within *B. radicans* and *B. moritziana* (Ceramiales, Rhodophyta). *Phycologia* 36:293-304.
- Zuccarello, G. C. & West, J. A. 2003. Multiple cryptic species: molecular diversity and reproductive isolation in the *Bostrychia radicans*/*B. moritziana* complex (Rhodomelaceae, Rhodophyta) with focus on North American isolates. *J. Phycol.* 39:948-959.
- Zuccarello, G. C. & West, J. A. 2006. Molecular phylogeny of the subfamily Bostrychioideae (Ceramiales, Rhodophyta): subsuming *Stictosiphonia* and highlighting polyphyly in species of *Bostrychia*. *Phycologia* 45:24-36.
- Zuccarello, G. C. & West, J. A. 2011. Insights into the evolution and speciation in the red alga *Bostrychia*: 15 years of research. *Algae* 26:21-32.
- Zuccarello, G. C., West, J. A., Karsten, U. & King, R. J. 1999a. Molecular relationships within *Bostrychia tenuissima* (Rhodomelaceae, Rhodophyta). *Phycol. Res.* 47:81-85.
- Zuccarello, G. C., West, J. A. & King, R. J. 1999b. Evolutionary divergence in the *Bostrychia moritziana*/*B. radicans* complex (Rhodomelaceae, Rhodophyta): molecular and hybridization data. *Phycologia* 38:234-244.
- Zuccarello, G. C., West, J. A. & Loiseaux de Goër, S. 2006. Diversity of the *Bostrychia radicans*/*Bostrychia moritziana* species complex (Rhodomelaceae, Rhodophyta) in the mangroves of New Caledonia. *Cryptogam. Algal.* 27:245-254.