

## New records of algae from Efaté, Vanuatu

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**Abstract** – Collections of marine and freshwater algae were made on Efaté, Vanuatu on 14-16 June 2005. New records and cultures were obtained of the red algae *Actinotrichia fragilis*, *Acrochaetium corymbiferum*, *Bostrychia moritziana*, *B. radicans*, *B. simpliciuscula*, *B. tenella*, *Caloglossa vieillardii*, *C. ogasawaraensis*, *Chroodactylon ornatum*, *Colaconema* sp., *Compsopogon* sp., *Murrayella pericladus*, *Neosiphonia howei*, *Pulvinaster venetus*, *Stylonema alsidii*, *Thorea* sp., the green algae *Boodleopsis carolinensis* and *Derbesia tenuissima* prox. and the cryptomonad *Hemiselmis* sp.

**Acrochaetium / Actinotrichia / Boodleopsis / Bostrychia / Caloglossa / Chroodactylon / Colaconema / Compsopogon / Derbesia / Efaté / Hemiselmis / Neosiphonia / Pulvinaster / Stylonema / Vanuatu / culture / time lapse video-microscopy**

**Résumé** – Nouvelles récoltes d'algues d'Efaté, Vanuatu. Des algues marines et d'eau douce ont été collectées dans l'île d'Efaté, Vanuatu, du 14 au 16 juin 2005. Les algues rouges suivantes, nouvelles pour la région, ont été trouvées et mises en culture : *Actinotrichia fragilis*, *Acrochaetium corymbiferum*, *Bostrychia moritziana*, *B. radicans*, *B. simpliciuscula*, *B. tenella*, *Caloglossa vieillardii*, *C. ogasawaraensis*, *Chroodactylon ornatum*, *Colaconema* sp., *Compsopogon* sp., *Murrayella pericladus*, *Neosiphonia howei*, *Pulvinaster venetus*, *Stylonema alsidii*, *Thorea* sp., ainsi que les deux algues vertes, *Boodleopsis carolinensis*, *Derbesia tenuissima* prox. et la cryptomonade *Hemiselmis* sp.

**Acrochaetium / Actinotrichia / Boodleopsis / Bostrychia / Caloglossa / Chroodactylon / Colaconema / Compsopogon / Derbesia / Efaté / Hemiselmis / Neosiphonia / Pulvinaster / Stylonema / Vanuatu / culture / vidéomicroscopie**

## INTRODUCTION

Despite repeated visits by Europeans to the group of islands comprising Vanuatu since 1606, no taxonomic or floristic records of marine or freshwater algae have been published. Only recently phycologists from France, Australia and

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Japan have begun extensive collections around Vanuatu and neighbouring islands so additional new records are forthcoming. Two new *Myriogramme* species are recorded by N'Yeurt *et al.* (2007). N'Yeurt & Payri (2008a, b) described *Sebdenia cerebriformis* and *Chondria bullata* sp. nov. Dixon *et al.* (2007) have dealt with the crustose red algae of the Peyssonneliaceae from Vanuatu. West *et al.* (2007a, b) described the red alga *Pulvinaster venetus* (Compsopogonales) from the Eton River, Efaté.

In an effort to extend our understanding of the mangrove inhabiting algae in the Pacific Islands we collected around Efaté in June 2005. The following covers our collections of various taxa including morphological observations, culture, time lapse video-microscopy and molecular analyses.

## MATERIALS AND METHODS

Collections were made from 14-16 June 2005 except for *Bostrychia radicans* 3366 that was collected on 29 March 1993. Methods of collection and culture isolation are described in West & Zuccarello (1999). All cultures were maintained in Modified Provasoli's Medium (West, 2005) with a range of salinities, primarily 5 and 30 psu (practical salinity units). Culturing conditions were 18-23°C, 12:12 LD, 5-25  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  cool white fluorescent lighting. Growth was best on a rocking platform mixer at 17 rpm or a rotary shaker at 70 rpm. Cultures are available on request.

Observations were made with a Zeiss GFL bright field compound microscope and photography was done with a Zeiss MC 100 photomicrographic system using Ektachrome 200 colour film. The transparencies were scanned with an Epson FilmScan 200 and Photoshop 5.0 software on a Macintosh G4 computer. Low magnification photos were done with specimens arranged on microscope slides placed directly on the transparency carrier of the Epson 200.

**Time lapse video microscopy.** To induce spore release, acrochaetoid isolates 4547 and 4573 were immersed in fresh culture medium. Time lapse video-microscopy was used to observe spore motility as described in Pickett-Heaps *et al.* (2001). The specimens were placed in a drop of medium and then sealed under a coverslip with VALAP (equal weights vaseline, lanolin, paraffin wax melted together). This allowed a greater volume for spore release and vertical movement. All spore motility was recorded at room temperature (18-22°C) on a videodisc recorder. Velocities were also calculated by observing random spore travel up to 100  $\mu\text{m}$  in the two isolates. Spore motility in isolate 4573 was recorded at 3 second intervals (75x) and in isolate 4547 it was recorded at 5 second intervals (125x).

**Molecular analyses.** *Bostrychia moritziana* (Sonder ex Kützing) J. Agardh and *B. radicans* (Montagne) Montagne are part of a species complex, of seven major lineages, and only molecular examination can determine their evolutionary position within this complex (Zuccarello *et al.*, 1999; Zuccarello & West, 2003), methods for lineage determination followed procedures presented in Zuccarello & West (2003). *Bostrychia simpliciuscula* is a polyphyletic species (Zuccarello & West, 2006; Zuccarello *et al.*, 1999). Identification of lineages of *B. simpliciuscula* Harvey ex J. Agardh followed methods outlined in Zuccarello *et al.* (1999).

## RESULTS

### Phylum **Rhodophyta**

#### Class **Florideophyceae**

#### Order **Ceramiales**

#### Family **Rhodomelaceae**

Although molecular phylogenetic data show *Bostrychia radicans* and *B. moritziana* to be part of a genetic complex of seven clades (Zuccarello & West, 2003, 2006), here they are treated as two separate entities for convenience.

#### ***Bostrychia moritziana*** (Sonder ex Kützing) J. Agardh (Figs 1-7)

This species has polysiphonous main shoots with alternate laterals on every axial segment. Lateral main branches are also polysiphonous, usually with a cladohapteron at the base and secondary monosiphonous branches are evident further along the lateral axis. Some plants in the field and laboratory are completely monosiphonous with regular branching that is quite distinct and would not be recognized as *B. moritziana* without molecular evidence (Zuccarello & West, 2006). Most culture isolates have a typical *Polysiphonia*-type sexual life history but others from various regions produce tetrasporophytes having tetraspores or bispores that give rise to new tetrasporophytes (West & Zuccarello, 1999).

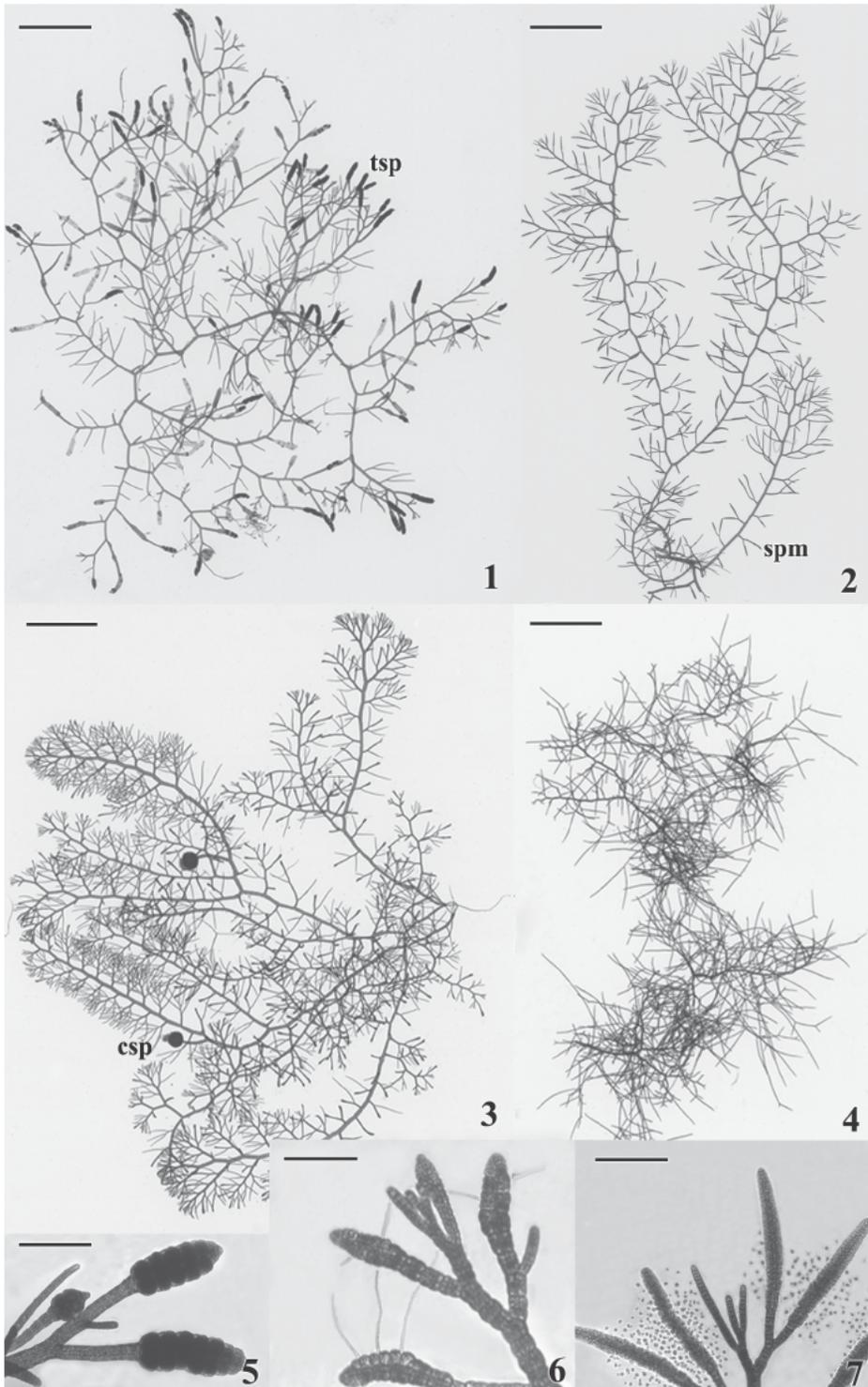
In mangroves around Efaté *B. moritziana* was relatively common. Isolate 4533 was collected from *Rhizophora* sp., near Port Havana (168° 16.911 E. 17° 32.757' S). It has a normal morphology and a *Polysiphonia*-type sexual life history. Molecular data placed this in lineage 1 along with isolates from western and eastern Australia, New Zealand, New Caledonia and South Africa (Zuccarello & West, 2003; Zuccarello *et al.*, 2006). The tetrasporophyte had typical tetrasporangial stichidia (Fig. 1). The male gametophytes generally were smaller and more fragile with finer branches (Fig. 2) than the robust larger females (Fig. 3). Isolate 4541 collected from *Avicennia* sp., near Erakor, showed normal morphology and sexual reproduction and is placed in lineage 1. The tetrasporangial stichidia (Fig. 5), procarps (Fig. 6) and spermatangial stichidia (Fig. 7) are similar to those described in most sexually reproducing isolates of *B. moritziana* (West & Zuccarello, 1999).

Isolates 4537 and 4538 were found on *Rhizophora* sp., past Batofatu, near the World War II Museum, (168° 21.193 E. 17° 48.038' S). Both were mostly monosiphonous with alternate to irregular branching and did not reproduce in culture (Fig. 4) but fragmented easily to form new plants. Occasionally they formed short polysiphonous shoots with normal branching. Both isolates are found in lineage 2 as were isolates from Sulawesi, Indonesia, New Caledonia and the Northern Territory, Australia (Zuccarello & West, 2003). Both show a similarity in lacking sporic reproduction, a reduction in polysiphonous main axes and an increased frequency of monosiphonous lateral branching.

#### ***Bostrychia radicans*** (Montagne) Montagne (Figs 8, 9)

Although some morphological inconsistencies (i.e. occasional monosiphonous lateral branch tips) are evident, this species has typical cladohaptera and alternate lateral polysiphonous branches throughout. Most isolates remain uniform in laboratory culture (Figs 8, 9).

Isolate 3366 was obtained in mangroves at the mouth of the Malatao River, (17° 33.01' S 168° 20.68' E) on 29 March 1993. It has been grown in



laboratory culture for 14 years and the tetrasporophyte consistently formed tetrasporangia from which spores germinated to form new tetrasporophytes. This nonsexual reproduction is quite common in *B. moritziana* (West & Zuccarello, 1999) but this is the only record of *B. radicans* with tetrasporophyte recycling although we have investigated the growth and reproduction of over 200 isolates in culture from around the world.

Isolate 4536 was collected from *Rhizophora* sp., near Port Havana (168° 16.911 E. 17° 32.757' S). It had normal *B. radicans* morphology and a typical non-reproductive behaviour in culture as seen in many tropical *B. radicans* isolates.

Isolate 4542 was tetrasporic in the field and was obtained from *Rhizophora*, near Eratap (17° 48.038' S 168° 21.193 E). In culture it grew well but remained vegetative. This isolate (4536) did not reproduce by vegetative fragmentation like isolates 4537 and 4538 of *B. moritziana*.

Although *B. radicans* is quite common in many other geographic regions it was uncommon, quite tiny and inconspicuous around Efaté. Molecular data placed isolates 3366, 4536 and 4542 in lineage 6 of the *B. moritziana/radicans* complex with isolates from Malaysia, Australia, New Caledonia, Costa Rica, Brazil and USA (Florida and Louisiana) (Zuccarello & West, 2003).

***Bostrychia simpliciuscula* Harvey ex J. Agardh (Figs 10, 11)**

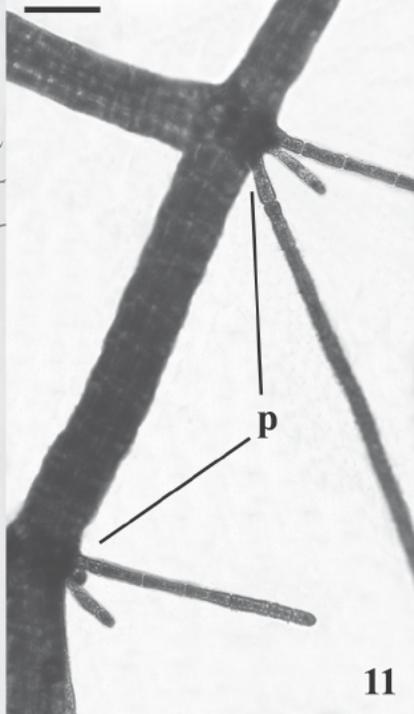
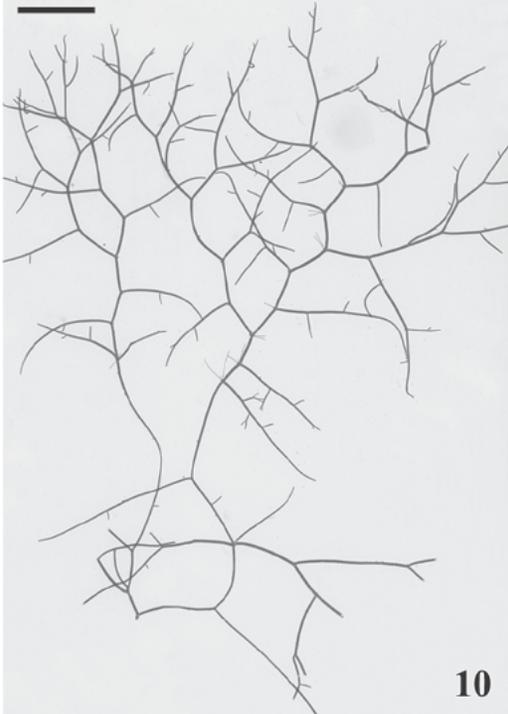
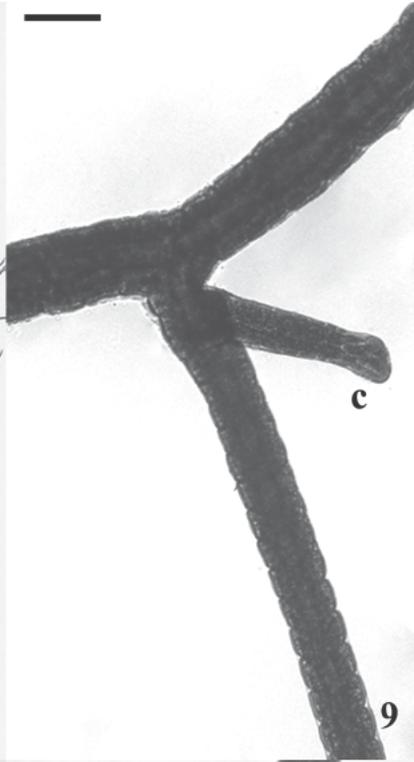
*Bostrychia simpliciuscula* is widespread in mangroves, estuarine mud flats and, occasionally, in freshwater streams of the Asia-Pacific region (King & Puttock 1989). It is now viewed as a polyphyletic species of three clades, and includes samples previously identified as *B. tenuissima* King et Puttock (Zuccarello & West, 2006). It is ecorticate, with peripherohaptera at nodes and with lateral, often monosiphonous, branches. The specimens found on a log in the freshwater of Eton River (< 2 psu), (168° 33.681 E. 17° 44.320' S) were mixed with *Caloglossa vieillardii* Setchell, *Thorea* sp. and *Pulvinaster venetus* J. West, G. Zuccarello et J. Scott (West et al., 2007a, b). Isolates 4526 and 4529 were not reproductive in the field. They grew well in laboratory culture at 5 to 30 psu salinity. No reproduction occurred and the branching was entirely polysiphonous with peripherohaptera regularly occurring at the branch nodes (Figs 10, 11). Both isolates are in the H3 clade of *B. simpliciuscula* (Zuccarello et al., 1999; Zuccarello and West, 2006).

***Bostrychia tenella* (Lamouroux) J. Agardh (Fig. 12)**

This species is widespread in tropical marine and estuarine habitats (King & Puttock 1989). We have two isolates from Efaté: 4528 was collected on the base of the mangrove *Bruguiera* sp. on the open coast at Eton Beach

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Figs 1-7. *Bostrychia moritziana*. **1.** Isolate 4533, habit view of tetrasporophyte with many tetrasporangial stichidia. Scale bar = 3 mm. **2.** Isolate 4533, habit view of male gametophyte with numerous spermatangial stichidia (spm). Scale bar = 3 mm. **3.** Isolate 4533, habit view of female gametophyte with two carposporophytes. (csp). Scale bar = 3 mm. **4.** Isolate 4538, main shoots with mostly monosiphonous branching, without reproduction except by fragmentation of filaments. Scale bar = 4 mm. **5.** Isolate 4541, short tetrasporangial stichidia. Scale bar = 200 µm. **6.** Isolate 4541, higher magnification of female branches bearing procarps with elongate trichogynes in Fig. 2. Scale bar = 125 µm. **7.** Isolate 4541, higher magnification of spermatangial stichidia showing discharge of numerous spermatia. Scale bar = 100 µm.



(168° 33.681 E. 17° 44.320' S); 4543 was collected on the mangrove *Rhizophora* sp. near Eratap (168° 21.193 E. 17° 48.038' S). Isolate 4528 was not reproductive in the field and occasionally tetrasporangia developed in laboratory culture. The branching pattern with peripherohaptera opposite lateral indeterminate branches is shown in Fig. 12. Isolate 4534 was a tetrasporophyte in the field but remained vegetative in laboratory culture.

***Murrayella pericladus* (C. Agardh) F. Schmitz (Fig. 13)**

Isolate 4534 was the only specimen of *M. pericladus* collected from Efaté. It was growing on *Avicenia* sp. near Port Havana (168° 16.911 E. 17° 32.757' S). In the field it was vegetative and in the laboratory it occasionally developed tetrasporangia (Fig. 13). The tetraspore germlings developed into male and female gametophytes that showed a normal sexual cycle. The characteristic spirally arranged monosiphonous laterals were often less frequent in culture than in most field-collected plants but usually were spaced about 120-150 µm apart (Fig. 13).

***Neosiphonia howei* (Hollenberg in W.R. Taylor) Skelton et South (Figs 14, 15)**

Isolate 4532 was found on rocks by *Avicennia* sp., near Port Havana (168° 16.911 E. 17° 32.757' S), 16 June 2005. Plants were vegetative with a prostrate stolons and erect shoots in the field, had 8-9 pericentral cells per axial segment (Fig. 14), radial branching was spaced 2-3 axial segments apart (Figs 15, 16), trichoblasts were infrequent, and rhizoid bases cut off from pericentral cells. These same characters persisted in culture. Occasionally one tetrasporangium was formed per axial cell in the fertile branches. Without other reproductive structures and careful molecular analysis it was very difficult to ascertain the correct species name. Identification was based on descriptions in Abbott (1999) and Skelton & South (2007).

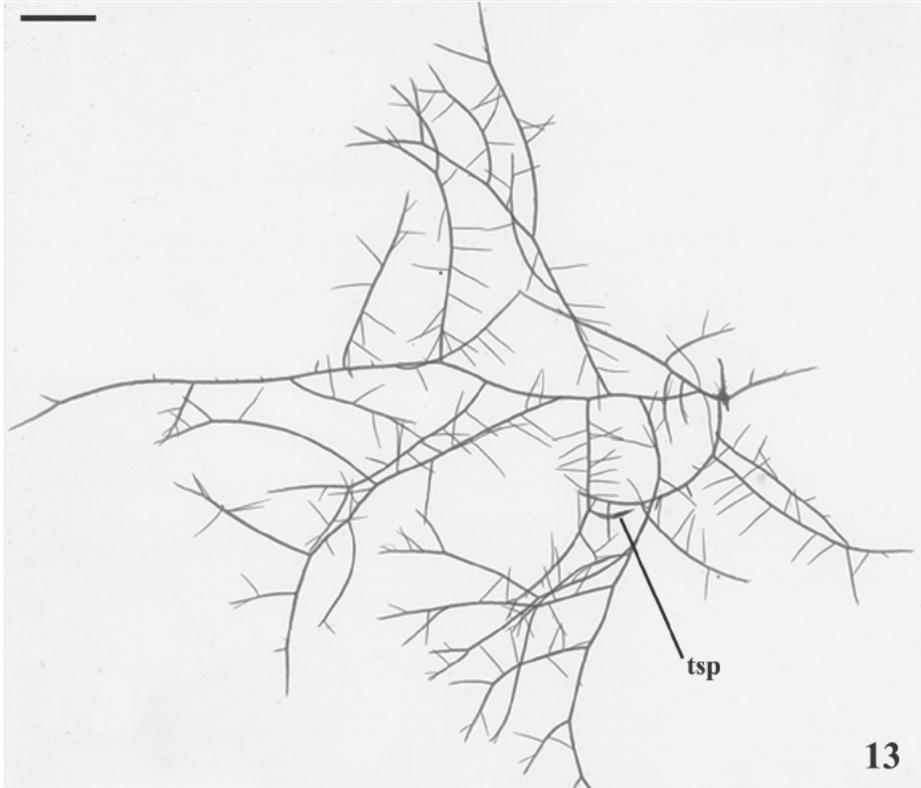
Family **Delesseriaceae**

***Caloglossa vieillardii* (Kützinger) Setchell (Figs 17-20)**

This species is restricted to mangroves, salt marshes, estuaries and freshwater rivers of the western Pacific region (Kamiya *et al.* 2003, 2004). It is distinguished from *C. leprieurii* (Montagne) G. Martens which has 2 or more cell rows from the first axial cell above the node opposite the lateral branch, whereas *C. vieillardii* has a single cell row. Isolate 4525 was obtained on a log from the freshwater Eton River (< 2 psu), mixed with *Bostrychia simpliciuscula* and *Thorea* sp. at Eton Beach. Isolate 4525 was collected as a tetrasporophyte (Figs 17, 18) and showed a normal *Polysiphonia*-type sexual life history in culture with female gametophytes having typical curled blade tips bearing numerous procarps with long trichogynes (Figs 19, 20). Growth and reproduction were very good at 5 psu and 30 psu. Isolate 4527 was a female blade, collected on the base of the mangrove



Figs 8-11. **8.** *Bostrychia radicans*, isolate 4542. Habit view showing branching pattern. Scale bar = 2 mm. **9.** *Bostrychia radicans*, isolate 4542. Main axis with cladohapteron (c) borne at base of lateral branch. Scale bar = 80 µm. **10.** *Bostrychia simpliciuscula*, isolate 4526. Habit view showing branching pattern. Scale bar = 2 mm. **11.** *Bostrychia simpliciuscula*, isolate 4526. Main axis with erect shoots and peripherohaptera (p) at nodes. Scale bar = 150 µm.



*Bruguiera* sp. in the open coast at Eton Beach, (168° 33.681 E. 17° 44.320' S). It was grown in culture at 30 psu and continued to form procarps near the blade tips.

***Caloglossa ogasawaraensis* Okamura (Figs 21-22)**

This species is pantropical and pantemperate in mangroves, salt marshes, estuaries and freshwater rivers and is distinguished by narrow blades with adventitious branching at the nodes (Kamiya *et al.*, 2003). In culture it often has a *Polysiphonia*-type sexual life history (West *et al.*, 2001).

Isolate 4539 was in a channel containing *Rhizophora* sp., past Batofatu, near the World War II Museum, (168° 21.193 E. 17° 48.038' S). The tetrasporophyte and gametophytes are typical of this species (Figs 21, 22). This was our only collection of this species on Efaté.

Order **Acrochaetiales**

Family **Acrochaetiaceae**

***Acrochaetium corymbiferum* (Thuret) Batters (Figs 23-26)**

Isolate 4547 was growing on *Actinotrichia fragilis* (Forsskål) Børgesen in a *Rhizophora* dominated channel near the World War II Museum. In culture it had an extensively branched creeping basal system of long slender filaments with cells 20-26 × 3-4 µm that contain a long spiral chloroplast and parietal pyrenoid. The erect shoots were straight and up to 3-5 mm tall, lightly and alternately branched, with cells 12-30 × 5-7 µm and chloroplast forming a thick parietal band with a small pyrenoid less than 2 µm in diameter. Monosporangia were ovoid, 12- 14 × 6-7 µm, lateral on the erect shoots, sometimes solitary and sessile or more commonly with a pedicel and formed in pectinate rows or in lateral clusters of 3-10 (Fig. 23). Successive spores were often formed in each sporangium. Upon germination the spore became empty and the germ tube (Fig. 24) developed into the long branched filaments that coalesced in parallel and adhered closely to the substratum (Fig. 25). Free spores were 8-9 µm in diameter and often were amoeboid with one or two pseudopodia that extended and retracted quickly within 5-10 seconds (Fig. 26). The spore velocities ranged between 0.03-0.04 µm min<sup>-1</sup>.

Order **Colaconematales**

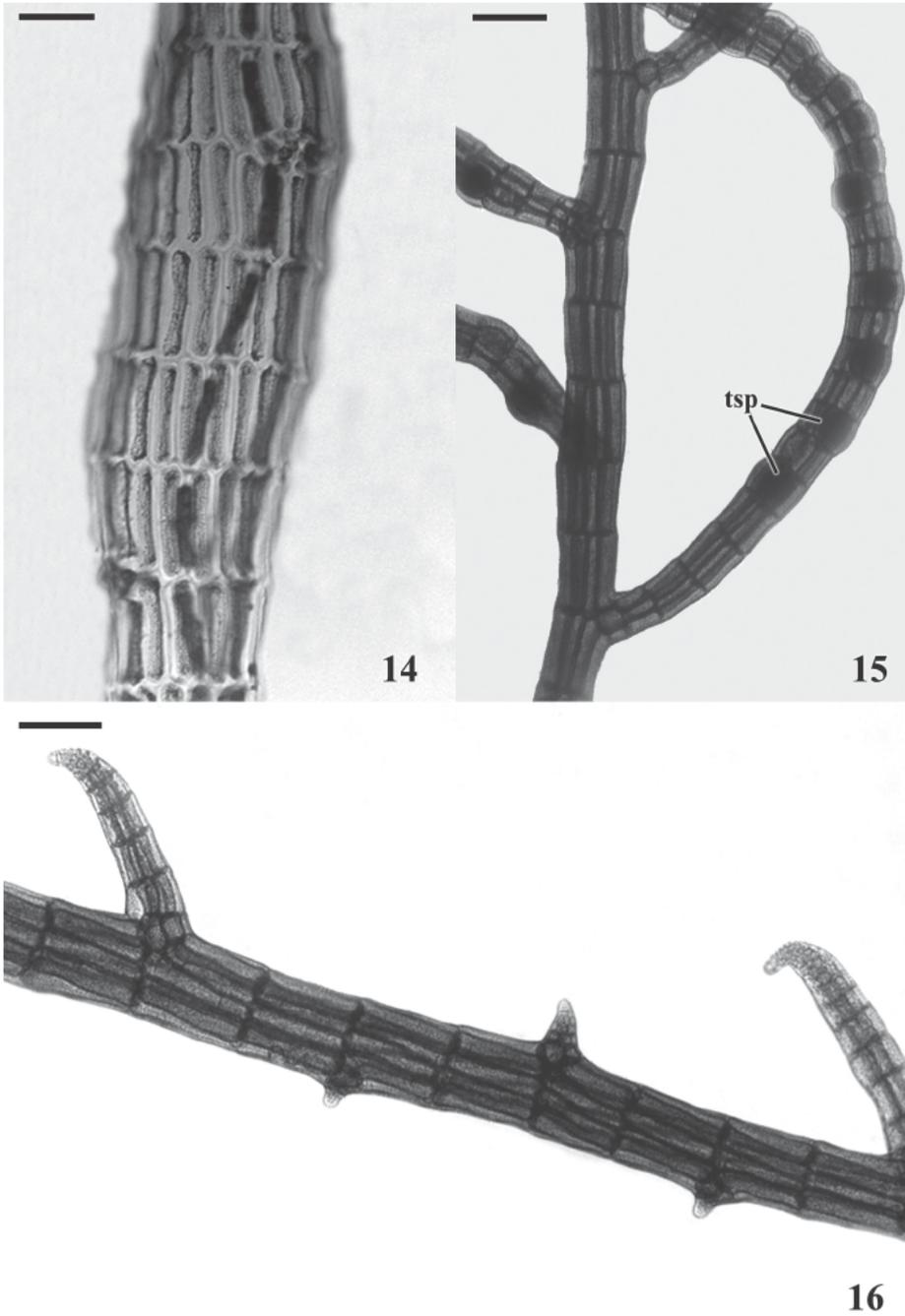
Family **Colaconemataceae**

***Colaconema* sp. (Figs 27-30)**

Isolate 4573 was growing on *Caloglossa vieillardii* in the freshwater stream at the Eton Bridge. In culture it grew and reproduced vigorously at 30 psu. The erect filaments were up to 2 mm and slightly falcate (Fig. 27). The vegetative

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Figs 12-13. **12.** *Bostrychia tenella*, isolate 4528. Habit view of branching pattern typical of plants in the field and laboratory culture. Peripherohaptera (p) evident opposite lateral indeterminate branches. Scale bar = 2.2 mm. **13.** *Murrayella pericladus*, isolate 4534. Monosiphonous lateral branches are less frequent in laboratory culture. Solitary tetrasporangial branches (tsp) arose sometimes. Scale bar = 1.25 mm.



Figs 14-16. *Neosiphonia howei*, isolate 4532. 14. Fixed and squashed specimen with 8-9 periaxial cells per axial cell. Scale bar = 50  $\mu\text{m}$ . 15. One tetrasporangium (tsp) per axial cell in fertile branch. Scale bar = 125  $\mu\text{m}$ . 16. Reduced branching pattern in laboratory culture. Scale bar = 100  $\mu\text{m}$ .

cells were also uniformly shorter, 12-14 × 5-6 µm, than in *A. corymbiferum* (4547). Sporangia (10-11 × 7-8 µm) were terminal and solitary or lateral and in small clusters. Free spores were 7-8 µm in diameter, amoeboid and active for over one hour. The spore velocities ranged between 0.04-0.05 µm min<sup>-1</sup>. Spores germinated via a single shoot leaving an empty spore case (Fig. 28). Each cell has a single peripheral chloroplast with a pyrenoid (Fig. 29). The basal system was multicellular and formed numerous erect filaments (Fig. 30).

Initially it was identified as *Acrochaetium robustum* Børgesen based primarily on Abbott (1999) but molecular data (Gary Saunders & Susan Slayden, personal communication, see also Harper & Saunders, 2002) placed this in the genus *Colaconema* and the species was not determined.

### Class **Compsopogonophyceae**

#### Order **Composopogonales**

#### Family **Compsopogonaceae**

#### ***Pulvinaster venetus*** J.A. West, G.C. Zuccarello *et* J.L. Scott

This is a small bluegreen coloured alga forming prostrate cushions. It was described in West *et al.* (2007a) as *Pulvinus veneticus*, although this name was not acceptable because it coincided with a Latin technical term used in morphology (Art. 20.2 International Code of Botanical Nomenclature). The genus and species name were corrected in West *et al.* (2007b). Isolate 4571 was epiphytic on *Caloglossa vieillardii* growing on a log in the freshwater Eton River (< 2 psu) at Eton Beach (168° 33.681 E. 17° 44.320' S). A second isolate (4579) was obtained on *Bostrychia tenella* from the open coast near the Eton Bridge on 14 June 2005.

#### ***Compsopogon* sp.**

The genus *Compsopogon* is distributed worldwide and has perhaps 4 currently recognized species (Guiry & Guiry, 2007). *Compsopogon* was seen in several freshwater streams around Efaté. We obtained two isolates. Isolate 4530 was collected mixed with *Spirogyra* and filamentous cyanobacteria from Cressionnières Cascades, a rocky waterfall, 2 km north of Eton Beach. Isolate 4531 was found in a small waterfalls, 15 km north of Eton Beach. Both isolates grew well in 5 psu medium, produced monosporangia and sporelings that developed into plants typical of the genus (Kumano, 2002).

### Class **Stylonematophyceae**

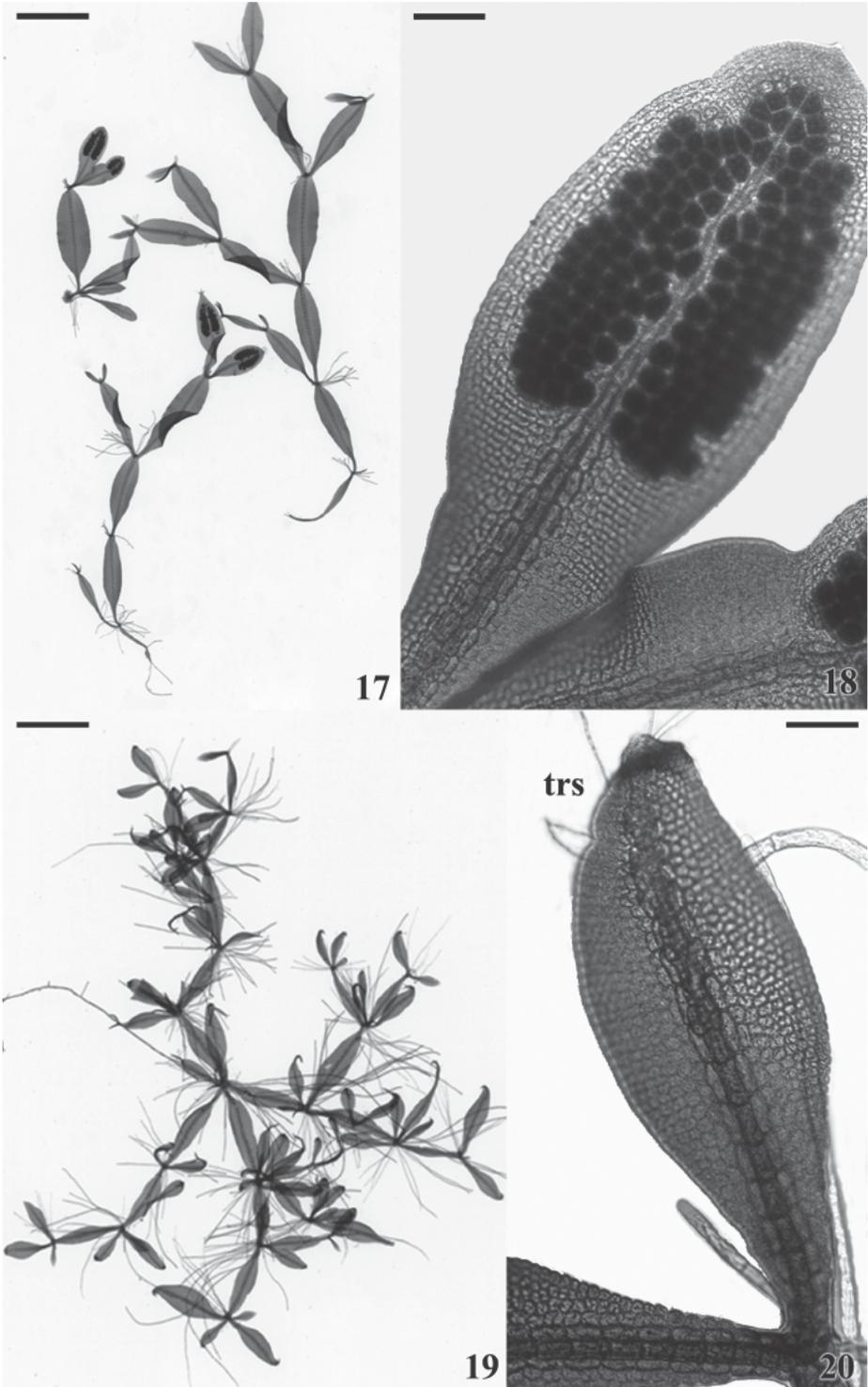
#### Order **Stylonematales**

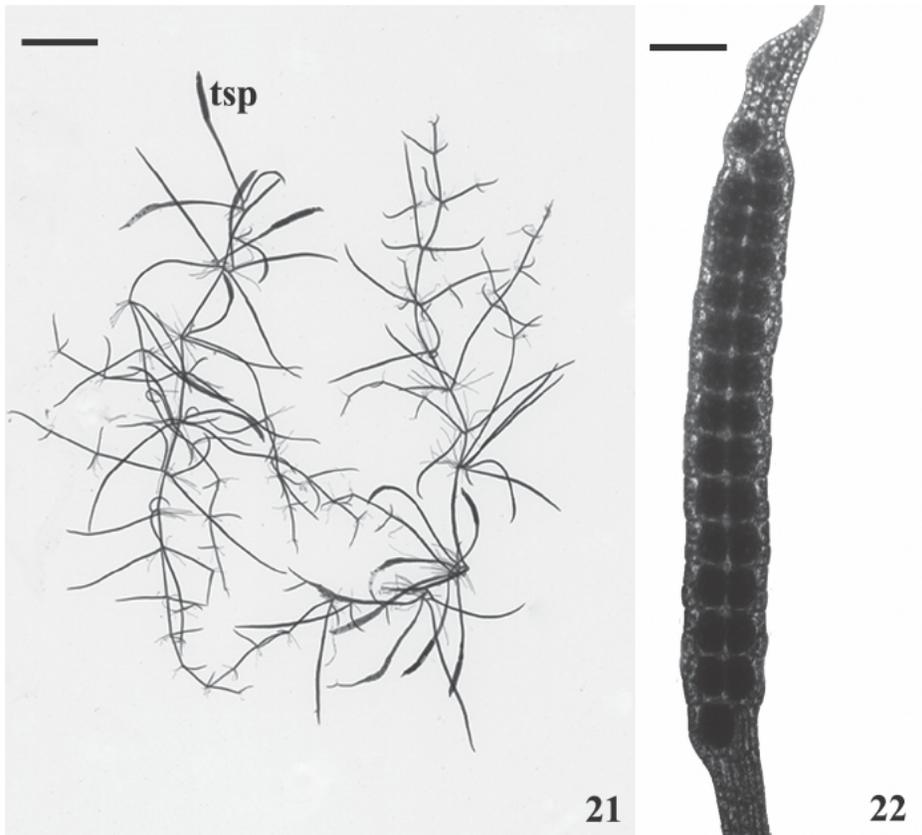
#### Family **Stylonemataceae**

The order/class has been investigated phylogenetically using multiple DNA markers. These data indicate that most species/genera are well supported (Zuccarello *et al.*, 2008)

#### ***Chroodactylon ornatum*** (C. Agardh) Basson **(Fig. 31)**

*Chroodactylon ornatum* occurs as an epiphyte and is global in distribution. It has uniseriate branched filaments with a clear mucilaginous matrix; each cell has a distinct stellate blue-green chloroplast with a central pyrenoid.





Figs 21-22. *Caloglossa ogasawaraensis*, isolate 4539. **21.** Tetrasporophyte with tetrasporangial branches (tsp). Scale bar = 1mm. **22.** Tetrasporangial branch with two rows of tetrasporangia. Scale bar = 100  $\mu$ m.

Reproduction is only by monospores that are vegetative cells released from the matrix. Isolate 4550 occurred on *Neosiphonia howei* on *Avicennia*, near Port Havana (168° 16.911 E. 17° 32.757' S). It grew well in culture showing the typical features (Fig. 31) of the species (Brodie & Irvine, 2003).

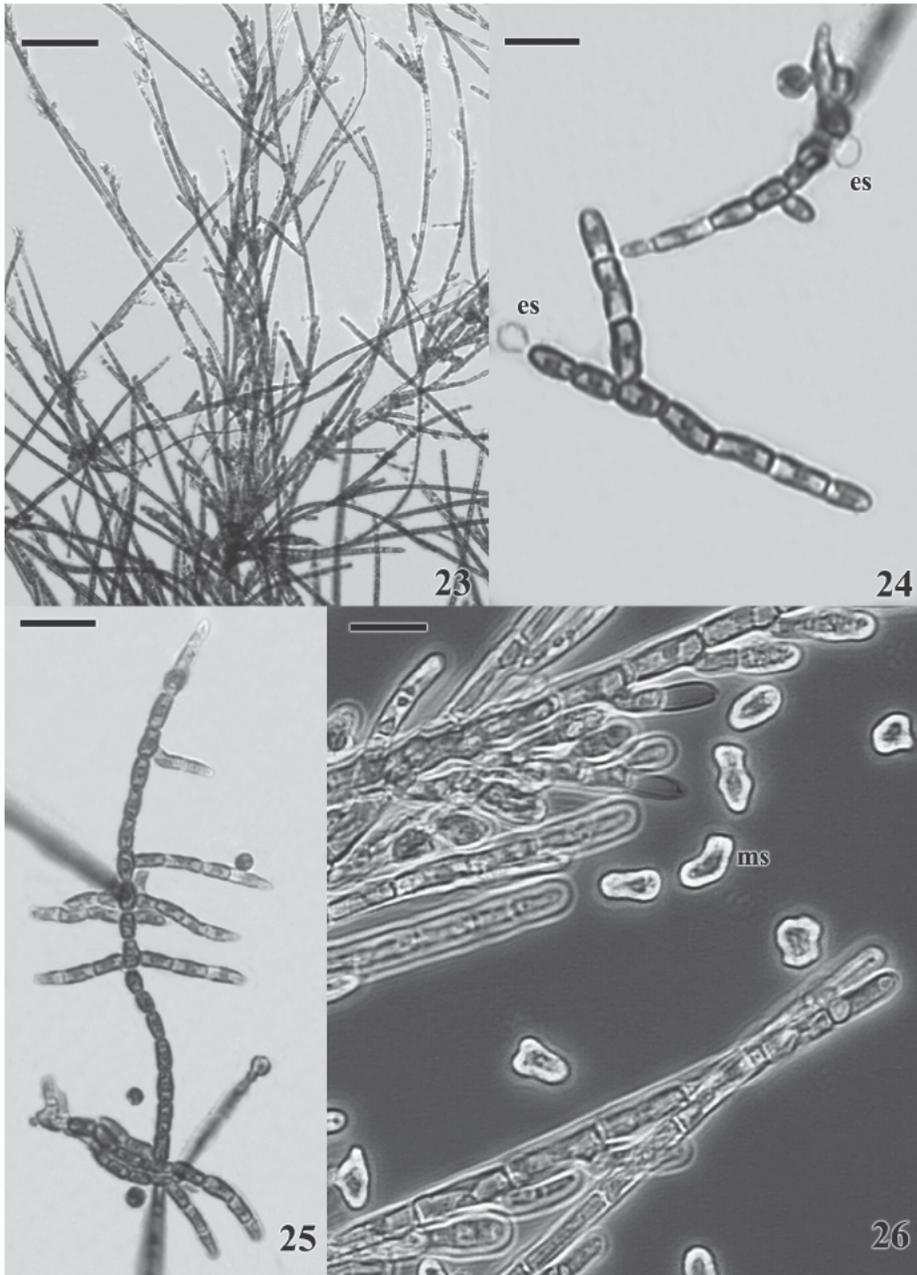
***Stylonema alsidii* (Zanardini) K.M. Drew**

**(Fig. 32)**

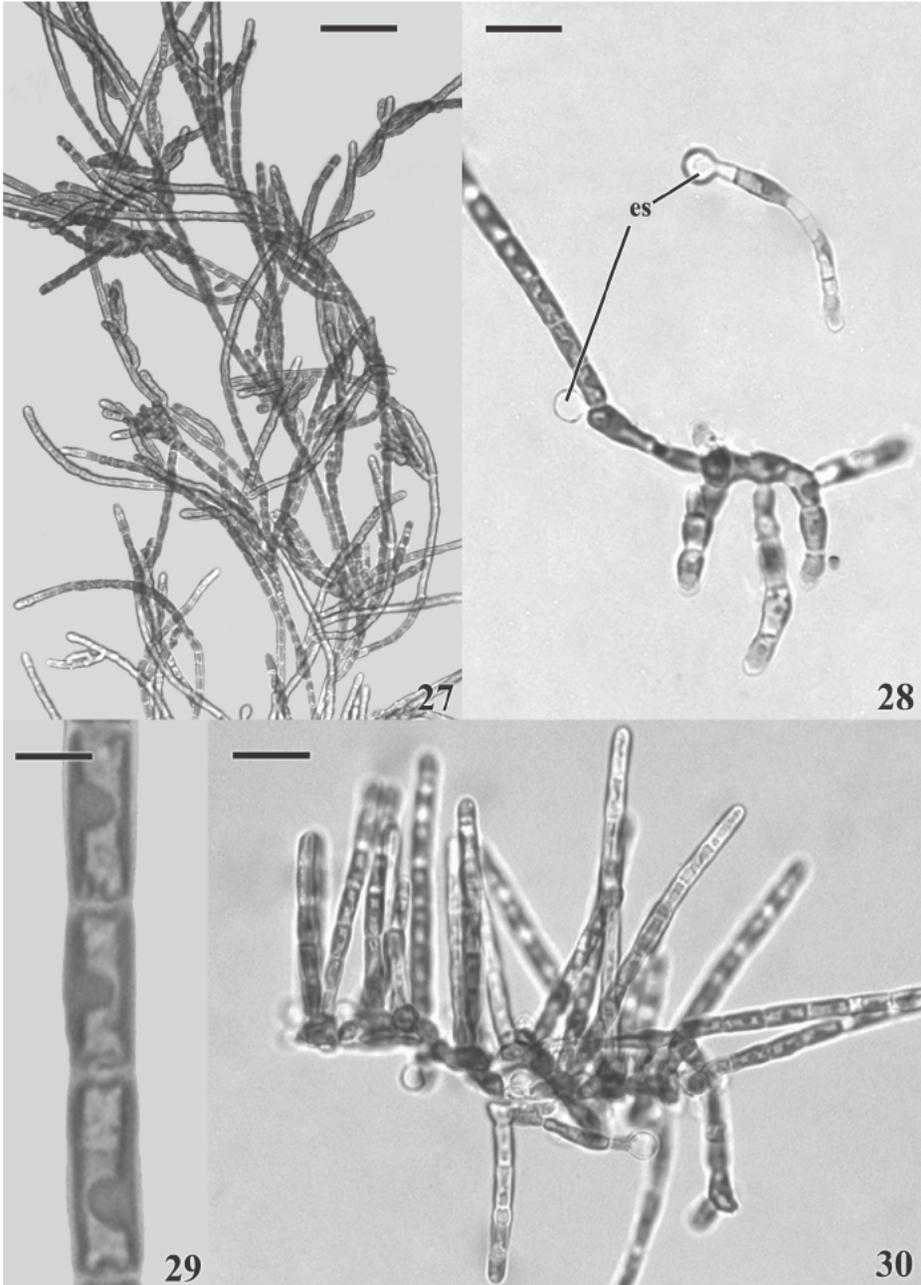
*Stylonema alsidii* is a widespread epiphyte in temperate and tropical marine habitats. It has branched uni- to multiseriate filaments with a clear mucilaginous matrix and basal pad. Each cell has a stellate red to purple chloroplast with a conspicuous pyrenoid. Monospores are the only known



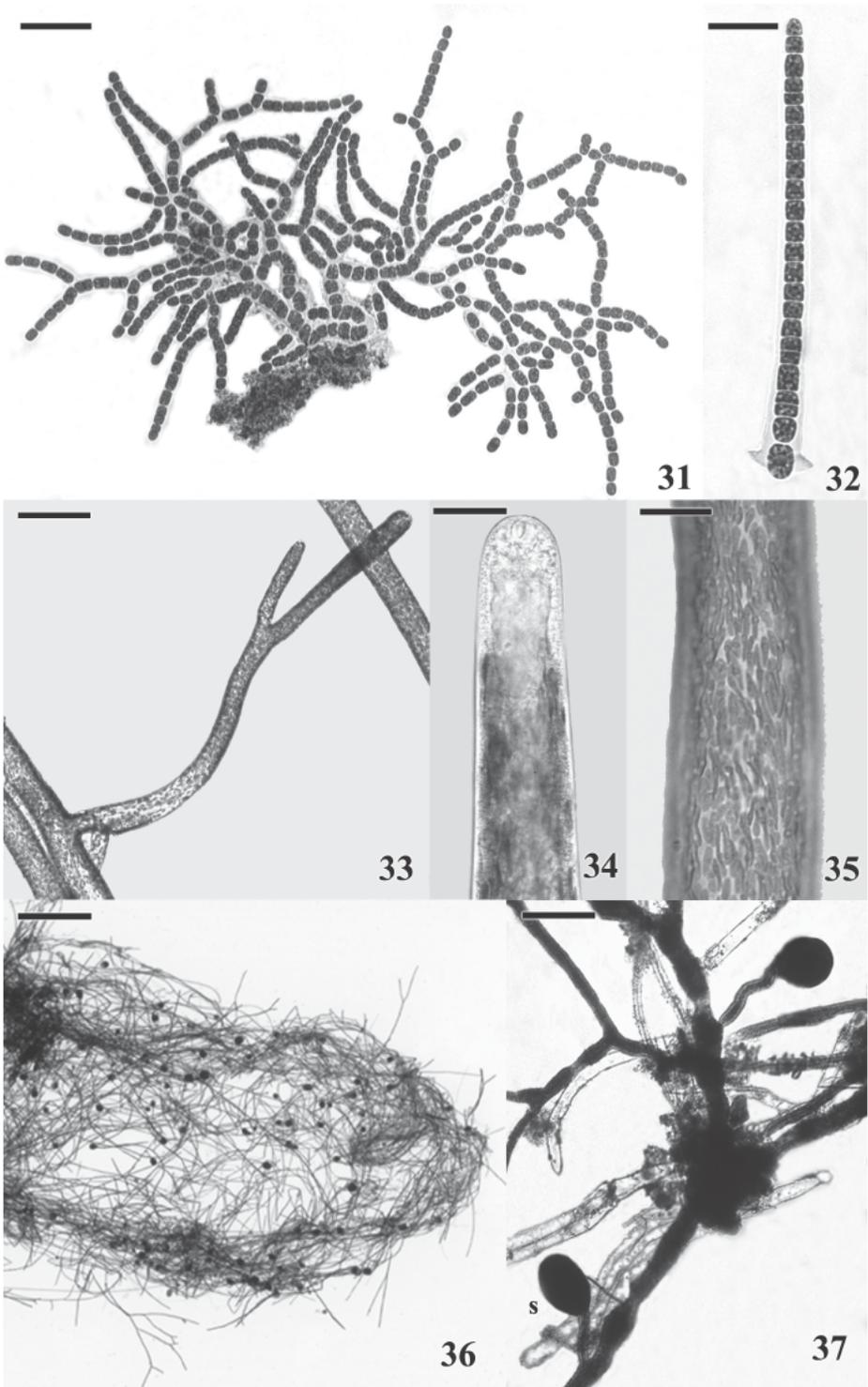
Figs 17-20. *Caloglossa vieillardii*, isolate 4525. **17.** Habit view of tetrasporophyte Scale bar = 1 mm. **18.** Tetrasporangial sorus Scale bar = 200  $\mu$ m. **19.** Habit view of female gametophyte with curled blade tips bearing procarps. Scale bar = 1.5 mm. **20.** Higher magnification view of female blade tip bearing procarps and trichogynes (trs). Scale bar = 200  $\mu$ m.



Figs 23-26. *Acrochaetium corymbiferum*, isolate 4547. **23.** Habit view of erect filaments with short lateral branches bearing monosporangia, sometimes solitary or more commonly with in pectinate rows. Scale bar = 100  $\mu\text{m}$ . **24.** Spore germination results in an empty spore(es) and prostrate filament that quickly branches. Scale bar = 18  $\mu\text{m}$ . **25.** Older sporeling with prostrate filament showing free and coalescing lateral branches. Scale bar = 30  $\mu\text{m}$ . **26.** Monosporangia releasing amoeboid monospores (ms). Scale bar = 15  $\mu\text{m}$ .



Figs 27-30. *Colaçonema* sp., isolate 4573. **27.** Slightly falcate erect filaments are up to 2 mm with variable branching Scale bar = 50  $\mu$ m. **28.** Spore germination showing empty spore(es) and prostrate filament with lateral branches. Scale bar = 25  $\mu$ m. **29.** Each cell contains a peripheral chloroplast with an inward projecting pyrenoid. Scale bar = 7  $\mu$ m. **30.** Prostrate basal system forms many erect filaments. Scale bar = 25  $\mu$ m.



reproduction and are produced by release of vegetative cells. Spores were round and showed gliding motility as described in Pickett-Heaps *et al.* (2001). Spore germination resulted in uniseriate filaments with a basal mucilage pad (Fig. 32). Isolate 4544 developed on *Actinotrichia fragilis* collected in a *Rhizophora* dominated channel near the World War II Museum. It grew well in culture with typical characters of the species.

## Phylum **Chlorophyta**

### Order **Bryopsidales**

#### Family **Derbesiaceae**

*Derbesia* cfr. *tenuissima* (Moris *et* De Notaris) P.L. Crouan *et* H.M. Crouan

(Figs 33-35)

Currently *Derbesia* has 21 recognized species (Guiry & Guiry 2007) and is characterised by its diphasic life history: The branched filamentous phase is a diploid sporophyte with pyriform sporangia and haploid multiflagellate zoospores giving rise to the gametophyte of large ovate (5-10 mm) coenocytic dioecious vesicles (*Halicystis* phase) producing biflagellate sperm and biflagellate eggs with zygotes developing into new sporophytes.

This small specimen (isolate 4549) was obtained on *Actinotrichia fragilis* in a *Rhizophora* dominated channel near the World War II Museum. It grew very well in culture but no reproduction was observed in two years. The filaments could be cut with micro-scissors and transferred by forceps to a new culture. Wound recovery was very rapid like that observed in other isolates of *D. tenuissima* by Martin *et al.* (2006).

Filaments exhibit typical *Derbesia* branching (Fig. 33) and apices have a typical clear protoplast (Fig. 34). Chloroplasts in some cells were aggregated, oval (3-5 × 5-10 µm) with no visible pyrenoids but in normal cells with active cytoplasmic streaming the plastids were elongate and spindle-shaped (3-5 × 15-40 µm) with a small but conspicuous pyrenoid (± 2 µm) (Fig. 35). Filaments are 50-70 µm in diameter with infrequent branching and sometimes with a series of septa. The growth pattern, filament diameter, chloroplast shape and pyrenoid presence indicate this is *Derbesia tenuissima* prox.

DNA molecular analyses (Heroen Verbruggen, personal communication) reveal this is closest to a *D. tenuissima* isolate (2773) from the Philippines (West and Calumpong, 1990; Martin *et al.*, 2006) although it is distant from other isolates of *D. tenuissima* from other regions. Clearly the systematics of the genus requires much further work.



Figs 31-37. **31.** *Chroodactylon ramosum*, isolate 4550. Well developed thallus with pseudodichotomous branching of uniseriate filaments. Scale bar = 200 µm. **32.** *Stylonema alsidii*, isolate 4544. A young sporeling showing the basal attachment pad and uniseriate growth with each cell having stellate chloroplast and pyrenoid. Scale bar = 50 µm. **33.** *Derbesia tenuissima*, isolate 4549 with sub-dichotomous lateral branches. Scale bar = 250 µm. **34.** *Derbesia tenuissima*, isolate 4549 branch apex with clear meristematic zone. Scale bar = 30 µm. **35.** *Derbesia tenuissima*, isolate 4549 elliptical chloroplasts with pyrenoids. Scale bar = 25 µm. **36.** *Boodleopsis carolinensis*, isolate 4548. Frequent sporangia along the filaments in culture. Scale bar = 1.5 mm. **37.** *Boodleopsis carolinensis*, isolate 4548 Ovate sporangium(s) on stalk from horizontal stolon and adjacent erect shoot with constrictions and dichotomous branches. Scale bar = 200 µm.

**Family Udoteaceae*****Boodleopsis carolinensis* Trono****(Figs 36, 37)**

This genus is widespread in the tropical mangrove habitats with seven currently recognized species (Guiry & Guiry, 2007). It is characterized by slender coenocytic filaments with distinct constrictions at the nodes and dichotomous branching. Chloroplasts and amyloplasts are present. Often the mass of filaments form compact bright green cushions in nature. Subspherical zoosporangia with a short stipe and cross wall are formed laterally. It is very difficult to identify the species with plants in liquid culture because the vegetative and reproductive features change compared to field specimens.

The culture isolate 4548 was obtained from rocks near *Avicenia* sp. near Erakor. It had characteristic long siphonous horizontal stolons with erect shoots (1-4 mm tall) that were dichotomously branched with constrictions at variable intervals (Fig. 36). The erect shoots were spaced from 100-500 µm apart. Stolons and erect shoots were 20-30 µm in diameter. Cytoplasmic streaming was very active in transporting chloroplasts and amyloplasts throughout the whole system. In laboratory culture ovate sporangia 150-180 diam. µm × 180-200 µm long were borne on stalks 150-300 µm long from stolons (Figs 36, 37). They are usually interspersed between the erect shoots. No discharged spores were seen. Reproduction in culture was entirely by fragmentation of filaments.

Filament dimensions and sporangial shape were similar to *B. carolinensis* Trono that was recorded from the Caroline Islands (Trono, 1972) and from Singapore (West, 1992). However, the filament diameter of 15-21 µm is somewhat smaller than in our isolate and the sporangia are somewhat smaller as well but this may be caused by laboratory culture conditions. *Boodleopsis pusilla* (Collins) Taylor, Joly *et* Bernatowicz is widely distributed, with filaments 23-45 µm in diameter (Taylor *et al.*, 1953; Calderon-Saenz & Schnetter, 1989) and is also similar to the *Boodleopsis* we have isolated. Clearly a great deal of molecular phylogeny research is necessary to resolve species patterns in *Boodleopsis*.

**Phylum Cryptophyta****Order Cryptomonadales****Family Hemiselmidaceae*****Hemiselmis* sp.**

*Hemiselmis* has seven currently recognized species (Guiry & Guiry, 2007). The genus is characterised by small (4-9 µm long) bean-shaped cells with two laterally inserted flagella, a single bluegreen chloroplast, single pyrenoid (Butcher, 1967). Isolate 4599 arose as a contaminant in a primary culture of *Bostrychia moritziana* isolate 4541. It grew well in dual culture with *B. moritziana* and with *Caloglossa ogasawaraensis* from the same locality. In unialgal culture *Hemiselmis* did not grow well.

**DISCUSSION**

The presence of the algae discussed above indicate that Vanuatu has various genera and species (*Bostrychia moritziana*, *B. radicans*, *B. simpliciuscula*, *B. tenella*, *Caloglossa vieillardii*, *C. ogasawaraensis*) typical of mangrove habitats

in the Asia-Pacific region. The apparent absence of other *Bostrychia* species such as *B. radicata* (Itono) J.A. West, G.C. Zuccarello et M.H. Hommersand, *B. flagellifera* Post, *B. kelanensis* Grunow [= *Stictosiphonia kelanensis* (Grunow ex E. Post) R.J. King et Puttock], *Caloglossa stipitata* E. Post and others is puzzling since these are recorded in other regions of the South Pacific (King & Puttock, 1989, 1994; West et al., 2006).

Various ships may have introduced algae on their hulls or in ballast water to Vanuatu. During World War II (1941-1945) numerous military and cargo ships from the USA, Australia and many other countries had Espiritu Santo as a principal port of call for offloading and loading materials. Many of these ships required routine dry-docking for hulls to be scraped to remove growth of marine algae and invertebrates and to be repainted with anti-fouling paint (Stafford, 1984; Doscher, 1994). Most US Navy wooden hull subchasers were constructed in the NE USA and stopped at numerous naval ports such as Miami for resupply and training before passage through the Panama Canal to various south Pacific ports including Espiritu Santo and Port Vila (Treadwell, 2000).

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