Phylogenetic position of *Boodlea vanbosseae* (Siphonocladales, Chlorophyta)

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Abstract — The marine green alga *Boodlea vanbosseae* is widely distributed in the tropical Indo-West Pacific region. The phylogenetic position of this somewhat unusual member of the genus is assessed based on morphological examination and phylogenetic analysis of nuclear-encoded partial large subunit rDNA sequences. *Boodlea vanbosseae* differs from other species of the genus by its coarse, irregularly branching filaments, the typical rhizoids developing from the proximal or apical poles of cells and the absence of calcium oxalate crystalline cell inclusions. These apparent morphological differences are confirmed by the molecular data, which reveal that *B. vanbosseae* is distantly related to other species of *Boodlea* (*B. composita* and *B. montagnei*), but instead clusters in another siphonocladalean clade that includes *Anadyomene*, *Microdictyon*, *Cladophora liebetruthii* and *C. catenata*. Within this clade, *B. vanbosseae* is most closely related to *C. catenata*, with which it shares several morphological features, such as a cushion-like habit composed of coarse filaments, and the presence of similar rhizoidal cells with terminal haptera. At this stage we restrain ourselves from making taxonomic changes because recent molecular phylogenetic studies have demonstrated that a complete rearrangement of the classification in the Siphonocladales and Cladophorales is inevitable. Remarkably, the cells in both taxa are infected by marine, ascomycotan fungi, which grow either on the distal face of the cross walls in *B. vanbosseae*, or on the longitudinal cell wall in *C. catenata*.

***Anadyomene* / *Cladophora catenata* / Cladophorales / Cladophorophyceae / marine fungi / *Microdictyon* / molecular phylogeny / tenacular cell**

INTRODUCTION

Boodlea Murray *et* De Toni is a common benthic marine green alga in the tropics. The genus was created by Murray & De Toni (*in* Murray 1889: 245) to receive *Cladophora coacta* Dickie, collected along the coast of Japan during the "Challenger" expedition. The original circumscription of the genus – sponge-like thalli composed of a three-dimensional reticulum, reinforced by anastomosis by tenacular cells – is still generally accepted today. The genus was distinguished from *Struvea* by the lack of a stipe and the irregular, three-dimensional branching, and from *Microdictyon* by the presence of small tenacular cells. To date, nine species have been described or transferred to *Boodlea* (Silva & Moe, 1999). Both Feldmann (1938) and Egerod (1952) classified *Boodlea* in the Siphonocladales and considered the genus to be closely related to *Microdictyon*, *Struvea* and *Cladophoropsis*, based on similarities in reticulum formation. Phylogenetic analyses based on ribosomal DNA sequences have confirmed the placement of *Boodlea* in the Siphonocladales and have demonstrated that the species *B. composita* (Harvey) F. Brand, *B. montagnei* (Harvey ex J.E. Gray) Egerod and *B. siamensis* Reinbold (which is morphologically very similar to the type of *Boodlea*) fall within a clade including the genera *Apjohnia*, *Chamaedoris*, *Cladophoropsis*, *Phyllodictyon*, *Struvea* and *Struveopsis* (Leliaert *et al.*, 2003; Leliaert *et al.*, submitted). No sequence data is yet available for the type of *Boodlea*, *B. coacta*, but this taxon is morphologically very close to *B. siamensis* (Leliaert & Coppejans, 2007).

Boodlea vanbosseae, originally described from Indonesia by Reinbold (1905: 148), was included in the genus based on its cushion-like habit and the presence of tenacular cells. The species is a somewhat unusual member of the genus as it has very coarse, irregularly branching filaments, rhizoids developing from the proximal or apical poles of cells, and lacks calcium oxalate crystalline cell inclusions (Reinbold, 1905; Weber-van Bosse, 1913; Leliaert & Coppejans, 2004). These apparent morphological differences relative to other species of *Boodlea* raise questions about its systematic position.

The present study aims to assess the phylogenetic position of *Boodlea vanbosseae* based on morphological examination of type material and recent collections in the Indo-west Pacific region, and by molecular phylogenetic analysis based on nuclear-encoded partial large subunit rDNA sequences.

MATERIALS AND METHODS

Specimens examined and morphology

Various localities in Indonesia (including Rotti reef and Lucipara Island) were indicated in the original prologue of *Boodlea vanbosseae* (Reinbold, 1905: 148). Only a single specimen, collected from Lucipara Island, Indonesia by Weber-van Bosse during the Siboga expedition, and identified by Reinbold and labelled in his hand "*Boodlea van Bossei* Rbd", was found in M (Herbarium Reinbold no. 1915) and is here indicated as lectotype. Other specimens examined are listed below. Herbarium abbreviations follow Holmgren *et al*. (1990).

Indian Ocean: Diego Garcia. unknown locality, (B 09457, B 09453); **Seychelles.** Bird Island, East coast, epilithic on coral boulders, (leg. Coppejans, Kooistra & Audiffred, 20.xii.1992, SEY 221, GENT); Plate Island, NW side, subtidal, 15 m depth, (leg. Coppejans, Kooistra & Audiffred, 7.i.1993, SEY 752, GENT); Poivre Island, NW side, epilithic on shallow limestone platform, (leg. Coppejans, Kooistra & Audiffred, 31.xii.1992, SEY 603, GENT); St. Joseph Atoll, subtidal, 2-10 m depth, (leg. Coppejans, Kooistra & Audiffred, 26.xii.1992, SEY 409; 28.xii.1992, SEY 460; 29.xii.1992, SEY 501, GENT); **Tanzania.** Vitongoji, E coast of Pemba Island, epilithic on horizontal coral substrate, infralittoral fringe; strongly exposed to surf (leg. Coppejans & De Clerck, 23.i.1996, HEC 11411); **Australia:** Scott Reef, Western Australia, epilithic on the reef flat, exposed at low tide, (leg. Huisman, 15.ii.2006, TR1, PERTH); **Pacific Ocean: Indonesia.** Banda reef, (leg. Weber-van Bosse, Siboga expedition s.n., L 936 181 362); E of Melolo, Sumba, intertidal reef flat, (leg. Coppejans, Prud'homme van Reine & Heijs, 14.ix.1984, Snellius-II 10442, GENT); Kaledupa reef, Tukang Besi Islands, Banda Sea, subtidal, (leg. Coppejans, Prud'homme van Reine & Heijs, 9.ix.1984, Snellius-II 10260; 10.ix.1984, Snellius-II 10292, GENT); Kawa Ceram, (leg. Weber-van Bosse, Siboga expedition s.n., 3.ix.1899, L 936 181 363); Liroeng, (leg. Weber-van Bosse, Siboga expedition s.n., L 936 181 350); Maisel Island, Banda Sea, (leg. Coppejans, Prud'homme van Reine & Heijs, 5.ix.1984, Snellius-II 10421; 7.ix.1984, Snellius-II 10117, GENT); Maisel Island, Banda Sea, intertidal reef flat, (leg. Coppejans, Prud'homme van Reine & Heijs, 5.ix.1984, Snellius-II 10404; 7.ix.1984, Snellius-II 10383; Snellius-II 10390, GENT); Maisel Island, Banda Sea, low intertidal, (leg. Coppejans, Prud'homme van Reine & Heijs, 7.ix.1984, Snellius-II 10113, GENT); NE Taka Bone Rate, S of Tarupa Kecil, in seagrass meadow, (leg. Coppejans, Prud'homme van Reine & Heijs, 17.x.1984, Snellius-II 11216, GENT); Rotti reef, (leg. Weber-van Bosse, Siboga expedition s.n., L 936 181 364); **Solomon Islands.** New Georgia, Matiu Island, mid intertidal, (leg. Womersley & Bailey 436, 30.viii.1965, L 211507).

Portions of liquid-preserved material and rehydrated herbarium specimens were stained with 1% methylene blue, mounted on glass microscopic slides, and examined with a light microscope. Drawings were made with a camera lucida on a Leitz-Diaplan bright field light microscope (Leitz, Wetzlar, Germany). Photographs were taken with an Olympus-DP50 digital camera (Olympus, Tokyo, Japan) mounted on the microscope, and arranged for publication in Adobe Photoshop CS2.

Molecular phylogeny

We analyzed partial large subunit (LSU) rDNA sequences of two specimens of *Boodlea vanbosseae*: SEY 460 from St. Joseph Atoll, Seychelles (EMBL accession number AM504044) and TR1 from Scott Reef, Western Australia (EMBL accession number AM504045).

The DNA was extracted from herbarium material or from material preserved in 95% v/v ethanol. Total genomic DNA was extracted using a standard CTAB-extraction method and subsequent purification with a Wizard® DNA Clean-Up System (Promega) following the manufacturer's protocol. The partial LSU rDNA gene (the first part of the gene, corresponding to position 26-633 of the *Chlorella ellipsoidea* LSU rDNA sequence: Genbank accession number D17810) was amplified as two overlapping products using primers C1FL (forward: 5'-ACCCGCTGAACTTAAGCATATC-3') and D2FL (reverse: 5'-GGTCCGTGTTTCAAGACGG-3'). PCR conditions consisted of an initial denaturation step of 94°C for 3min, followed by 35 cycles of 94°C for 30s, 55°C for 30s, 72°C for 30s, followed by a final extension of 3min at 72°C. Excess primer and dNTP were removed with ExoSAP-IT® (USB Corporation) for 15min at 37°C, followed by 15min at 80°C to inactivate the enzymes. The resulting products were used for cycle sequencing with the primers of the initial PCR reactions using an ABI Prism Dye Terminator Cycle Sequencing Ready Reaction kit following the manufacturer's instructions. Sequencing products were analysed with an ABI 3100 Prism Genetic Analyzer (PE Applied Biosystems). Sequences were edited and assembled with Sequencher v4.0.5 software (Gene Codes).

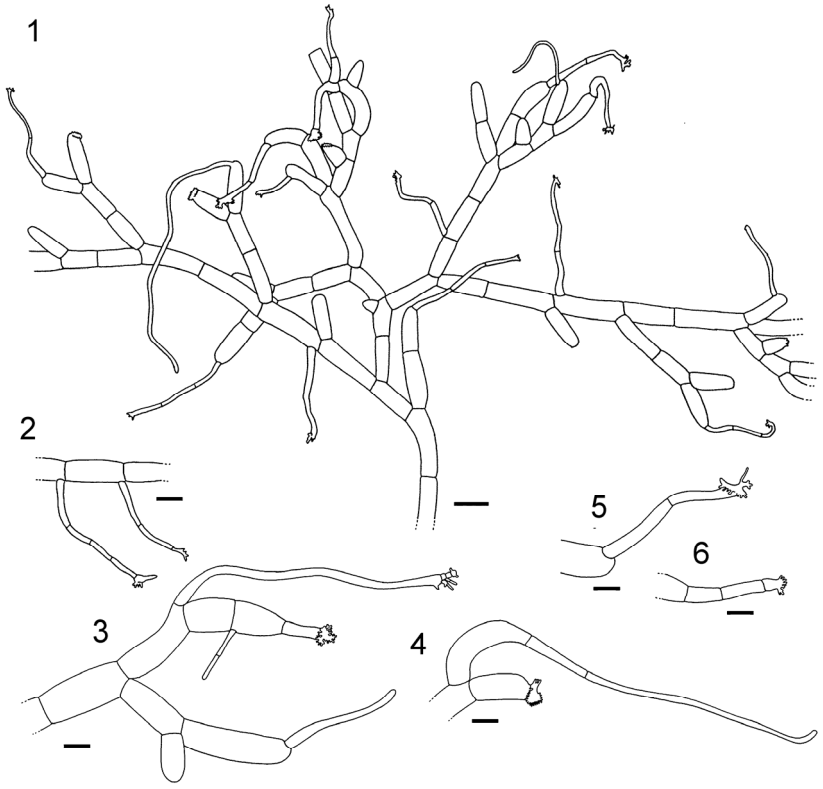
The *B. vanbosseae* sequences were first compared with available sequences in Genbank, using a BLAST search (http://www.ncbi.nlm.nih.gov/), to ensure its position in the Siphonocladales lineage. Both sequences were then included in an alignment consisting of all 169 available siphonocladalean partial LSU sequences and an initial neighbor-joining (NJ)

analysis was performed in PAUP* 4.0b10 (Swofford, 2002) to evaluate the phylogenetic position of *B. vanbosseae* in the Siphonocladales. This tree was then used to assemble a reduced dataset of 25 ingroup and four outgroup sequences for the subsequent phylogenetic analyses.

Bayesian inference (BI) was performed with MrBayes v3.1.2 (Ronquist & Huelsenbeck, 2003) under a general time-reversible model with rate variation across sites and a proportion of invariable sites (GTR+I+Γ), as estimated by PAUP* and MrModeltest 1.0b (Nylander, 2004). Two independent, simultaneous analyses were run for $3 \cdot 10^5$ generations, each starting from different random trees and sampled every 1000th generation. The stationary distribution of the runs was confirmed by plotting the ln likelihood values of the cold chain against generation numbers, and by evaluation of the average standard deviations of split frequencies between the two analyses, which approached zero (0.008) after no more than $9 \cdot 10^4$ generations, reflecting the fact that the two tree samples became increasingly similar. Summary statistics and trees were then generated using the last $2 \cdot 10^5$ generations.

Maximum parsimony (MP) analyses were performed using PAUP* with a heuristic search, consisting of 1000 random sequence addition replicates and Tree Bisection Reconnection (TBR) with the option MULTREES and branches being collapsed if it was possible for them to have zero length. Robustness of the inferred MP tree was tested using nonparametric bootstrapping (Felsenstein, 1985) with 1000 pseudoreplicates.

Secondary structures of the partial LSU rRNA sequences of *Boodlea vanbosseae* were constructed by using the MFOLD software (http://www.bioinfo.rpi.edu/, Zuker 2003) and compared with the common secondary structure of the Cladophorophyceae as determined by Leliaert *et al*. (2003). The RNA secondary structure diagrams were created by using RnaViz (De Rijk *et al*. 2003) in conjunction with DCSE v. 2.60 (De Rijk & De Wachter 1993).

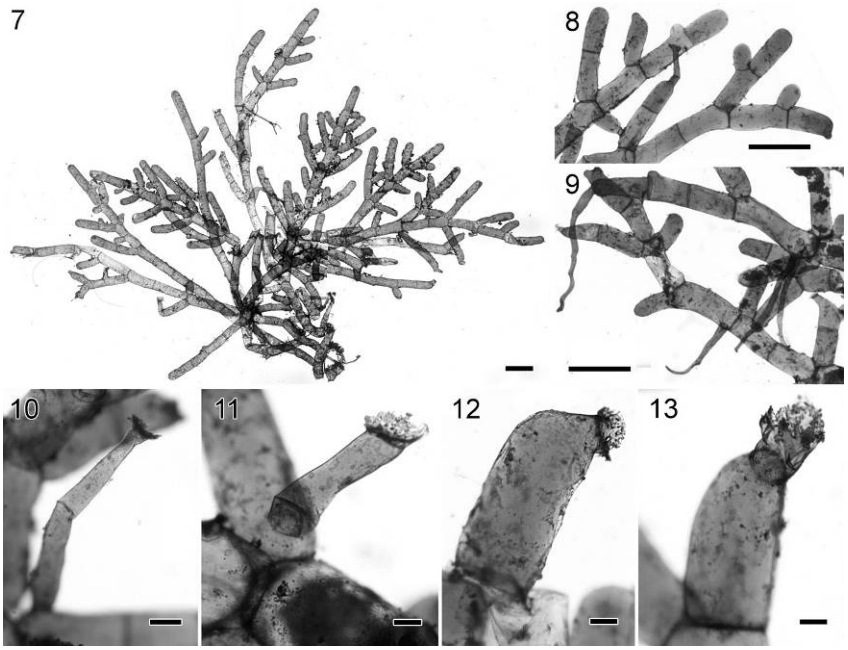


Figs 1-6. Lectotype of *Boodlea vanbosseae* from Lucipara Island, Indonesia (M). **1**. Irregularly branched filaments with numerous tenacular cells and cells producing rhizoids at their proximal or distal poles. **2**. Rhizoids developing from the proximal pole of cells. **3-6**. Details of tenacular cells and rhizoids developing from the proximal or distal poles of cells. Scales: 1, 2 = 500 µm; 3-6 = 200 µm.

RESULTS

Morphology and habitat of *Boodlea vanbosseae*

Thalli of *Boodlea vanbosseae* are medium to dark green, forming dense, matted cushions up to 8 cm across (Fig. 14), composed of densely branched, entangled filaments (Figs 1, 7, 15). The plants are attached by rhizoids and tenacular cells, produced by cells in any part of the thallus. Rhizoids are unbranched, septate or aseptate and develop from the distal poles of apical and intercalary cells or from the proximal pole of intercalary cells (Figs 1-6, 8-10, 17, 18). Two types of tenacular cells can be distinguished: by far the most abundant type consists of normal sized apical cells with distally

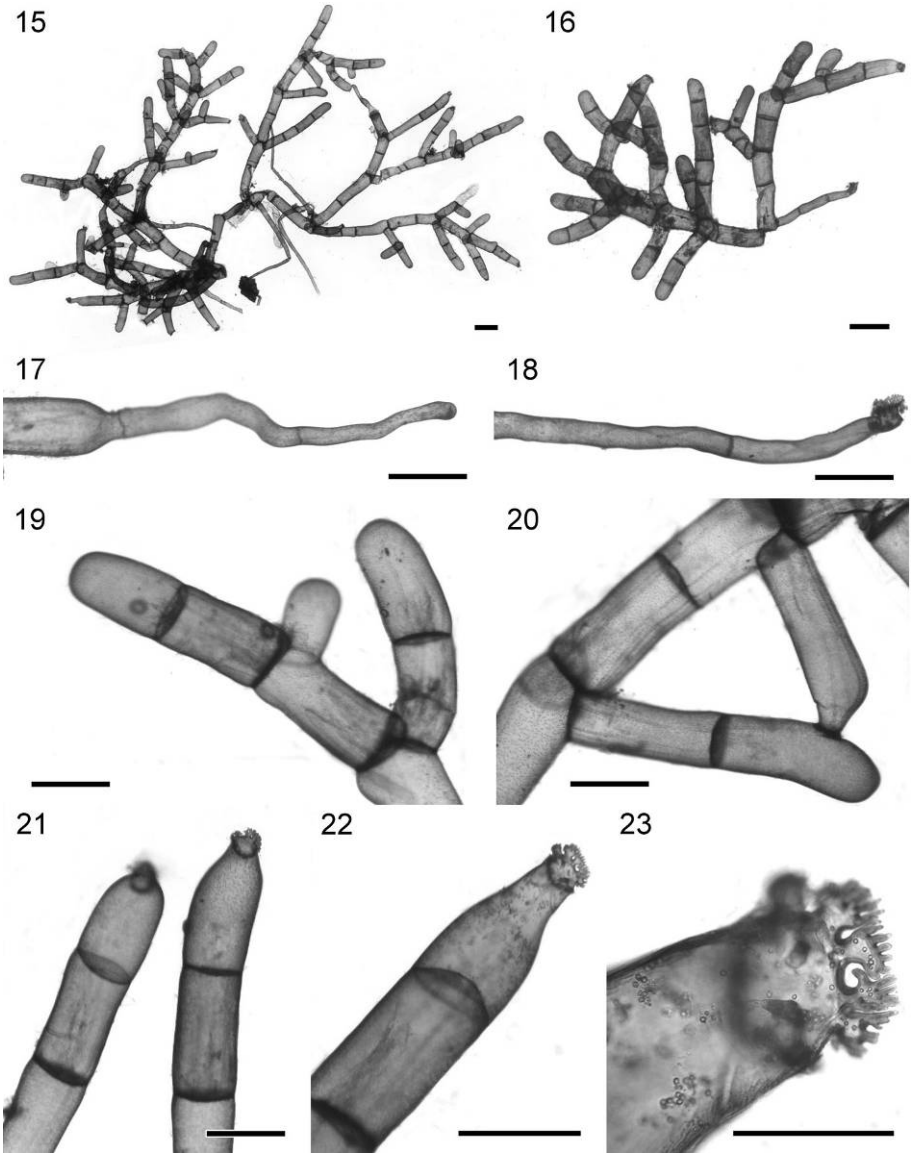


Figs 7-13. *Boodlea vanbosseae* from the Seychelles. **7**. Thallus composed of irregular to opposite branch systems (filaments spread out on a microscopic slide) (SEY 401). **8, 9**. Detail of terminal branch systems with rhizoids produced at the proximal or distal poles of cells (SEY 221). **10, 11**. Detail of rhizoidal cells with crenulated apical ends (SEY 221). **12**. Detail of a tenacular cell consisting of a normal sized apical cell with distally formed crenulated adhesion pads (type-I) (SEY 221). **13**. Detail of a tenacular cell consisting of a small cell produced distally on an apical cell (type-III) (SEY 221). Scales: 7-9 = 1 mm; 10-13 = 100 µm.

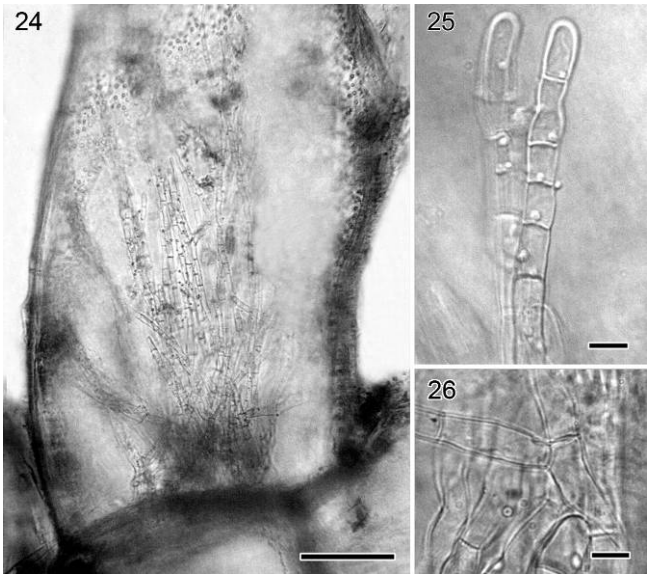
formed crenulated adhesion pads (Figs 12, 20-23); a second type of tenacular cells was only observed on a few occasions and consists of a small cell produced distally on apical cells (Fig. 13). Growth of the thallus occurs by cell divisions of both apical and intercalary cells, resulting in irregular branch systems. Newly formed cells generally produce a single lateral branch at their apical poles (Fig. 19); older cells may produce a second lateral, often opposite to the first one (Figs 7, 15). Cross wall formation occurs almost immediately after the initiation of a lateral branch (Fig. 8). The branches are laterally inserted with a steeply inclined cross wall cutting it from the mother cell (Figs 19, 20). The lower adaxial face of the basal cell remains in contact, and later fuses, with the cross wall of the cell distal to the mother cell (Fig. 19, 20). The angle of ramification is generally broad, in the range of 40°-90°. Apical cells are cylindrical with rounded tips, 150-240 µm in diameter, with a l/w ratio of 2-4. Cells of the main axes are 190-340 µm in diameter, with a l/w ratio of 1.5-4. Rhizoidal cells are 70-100 µm in diameter and up to 4200 µm long. Cell walls are relatively thick, ca. 5 µm thick in the apical cells, up to 16 µm thick in the main axes, giving the thallus a rigid texture. Chloroplasts are angular, 5-8 µm in diameter and interconnected by delicate strands to form a parietal network. Each chloroplast has a single pyrenoid, 4-5 µm in diameter. No crystalline cell inclusions were observed. The cells were found to be infected with a fungus (Ascomycota), growing inside the cell, on the distal face of the cross walls (Fig. 24). Fungal thalli were up to 800 µm high, composed of branched hyphae 8-12 µm in diameter (Figs 25-26).

Fig. 14. *Boodlea vanbosseae* from Scott Reef, Western Australia (TR1), forming matted cushions on reef flats exposed at low tide (colour photograph is available at http://www.algaebase.org/).

Boodlea vanbosseae grows epilithic on reef flats, from the mid intertidal to subtidal, down to 10 m depth. At Scott Reef it sometimes formed extensive mats on reef flats exposed at low tide.



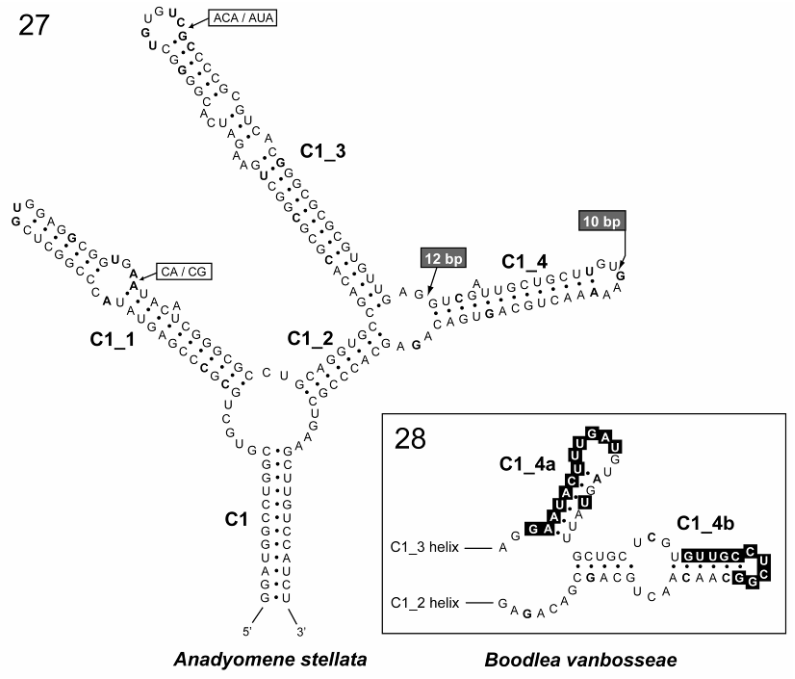
Figs 15-23. *Boodlea vanbosseae* from Scott Reef, Western Australia (TR1). **15, 16**. Thallus composed of irregular to opposite branch systems (filaments spread out on a microscopic slide). **17, 18**. Detail of rhizoidal cells without or with crenulated apical ends. **19**. Detail of a terminal branch system with newly formed lateral branches; cross wall formation in the youngest branch is not yet completed. **20**. Attachment of a tenacular cell to a neighbouring cell. **21-23**. Detail of a tenacular cell consisting of a normal sized apical cell with distally formed crenulated adhesion pads (type-I). Scales: 14, 15 = 1mm; 16-22 = 500 µm.



Figs 24-26. Endophytic fungus (Ascomycota) in the cells of *Boodlea vanbosseae* (Snellius-II 10117). **24**. Fungal hyphae attached on the distal face of a cross-wall of *B. vanbosseae*. **25**. Distal ends of hyphae. **26**. Basal branches of hyphae. Scales: 23 = 100 µm; 24, 25 = 10 µm.

Molecular phylogeny

The partial LSU rDNA sequences of *Boodlea vanbosseae* from the Seychelles and Western Australia were both 602 nucleotides long and differed from each other only in a single position. A BLAST search of the partial LSU rDNA sequences revealed that *Boodlea vanbosseae* belongs to the Siphonocladales. The sequences of *B. vanbosseae* were easily aligned with other siphonocladalean sequences and no sites were removed in the phylogenetic analyses. Total length of the alignment was 621 sites. Compared with other siphonocladalean partial LSU sequences, the sequences of *B. vanbosseae* differed mainly by two insertions of 12 (position 529-540) and 10 bases (position 558-567) respectively, which resulted in a slightly altered secondary structure of the RNA molecule. The C-helices of the LSU rRNA (De Rijk *et al*., 1999) have a conserved structure in the Siphonocladales, consisting of a basal stem (C1), two central loops connected by a central stem (C1-2), and three peripheral helices with terminal loops (C1_1, C1_3 and C1_4). This structure is illustrated in Fig. 27 for *Anadyomene stellata* (AJ544746). The insertions in *B. vanbosseae* resulted in the division of the conserved C1_4 helix in two different stems (Fig. 28).



Figs 27, 28. Secondary structure models of the C-helices of the LSU rRNA molecule (De Rijk *et al.* 1999), presented in a two-dimensional format. **27**. Secondary structure for *Anadyomene stellata* (EMBL accession number AM503410), illustrating the conserved model in the Siphonocladales lineage. Variable sites within the *Anadyomene* clade are indicated in bold. White boxes with arrows indicate inserts in *Microdictyon* sequences; grey boxes with arrows indicate inserts in the *B. vanbosseae* sequences. **28**. Secondary structure of the C1_4 helix for *B. vanbosseae* (EMBL accession number AM504045) showing the split of the conserved helix into two stems. Sites in black boxes indicate the two inserts.

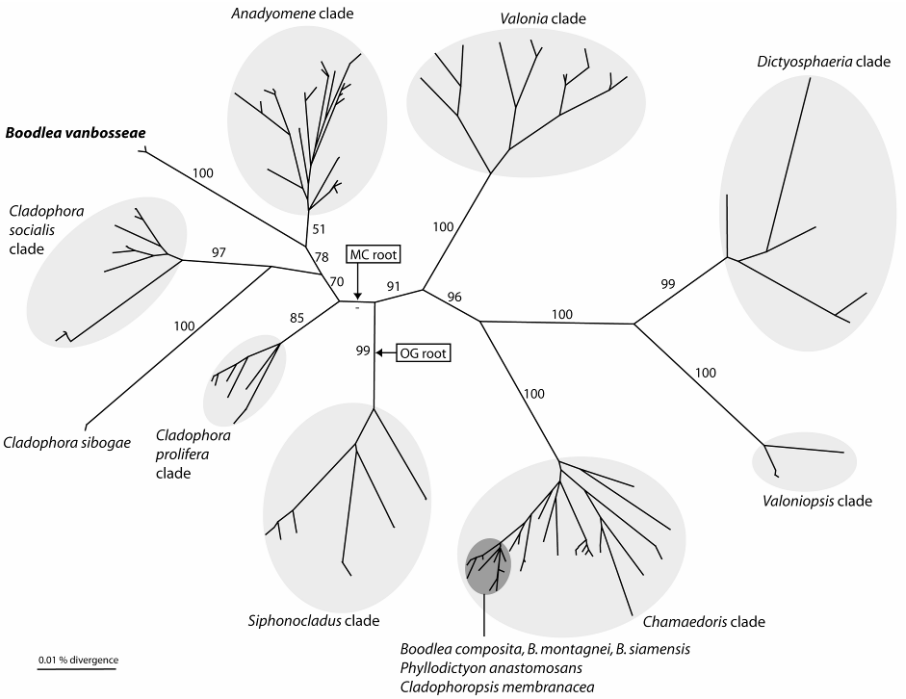
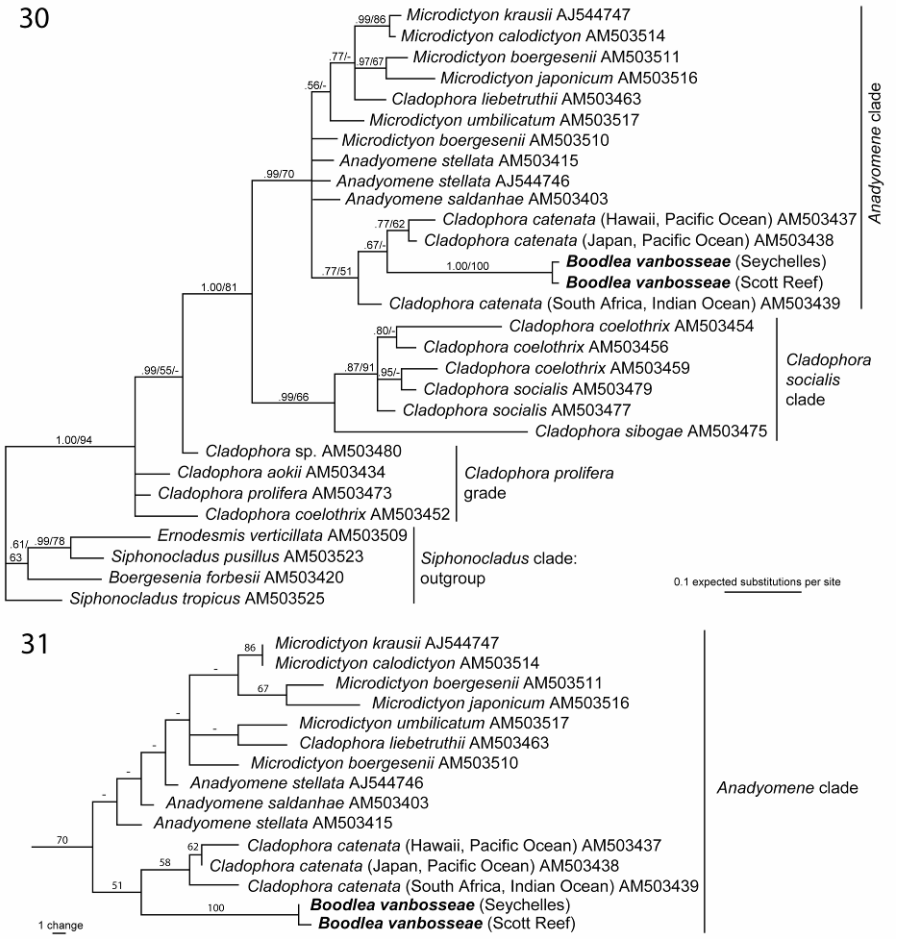


Fig 29. Unrooted NJ tree based on 169 partial LSU rDNA sequences of Siphonocladales, indicating the nine main siphonocladalean clades as well as the position of *Boodlea vanbosseae* and the other *Boodlea* species (grey ellipse in the *Chamaedoris* clade). White boxes and arrows indicate the position of the root as determined by outgroup rooting (OG root) and molecular clock rooting (MC root) in Leliaert *et al*. (submitted). Values at the branches indicate bootstrap values.

An initial NJ analysis, based on all 169 available siphonocladalean partial LSU sequences in genbank, revealed that *B. vanbosseae* was most closely related to the *Anadyomene* clade, which includes the genera *Anadyomene*, *Microdictyon* and the *Cladophora* species, *C. catenata* and *C. liebetruthii* (Fig. 29). Moreover, *B. vanbosseae* was found to be only distantly related to the other *Boodlea* species (*B. composita*, *B. montagnei* and *B. siamensis*), which are all located in the *Chamaedoris* clade with the genera *Apjohnia*, *Chamaedoris*, *Cladophoropsis*, *Phyllodictyon*, *Struvea* and *Struveopsis*. Within this clade, the *Boodlea* species were closely related to *Cladophoropsis membranacea*, *Phyllodictyon anastomosans* and *Struveopsis siamensis*. Based on this NJ tree, a reduced dataset of 25 ingroup taxa was assembled for the subsequent phylogenetic analyses, including sequences from the *Anadyomene*, *C. socialis* and *C. prolifera* clades, along with one *C. sibogae* and both *B. vanbosseae* sequences. Based on the placement of the root of the Siphonocladales phylogeny (Leliaert *et al*., submitted) (Fig. 29), four outgroup taxa were selected from the *Siphonocladus* clade, as these sequences are the closest and less divergent relatives of the ingroup. In this reduced alignment of 621 nucleotide positions, 85 were variable and 68 parsimony-informative.

Phylogenetic trees constructed with BI and MP methods were similar in topology and concordant in placing *Boodlea vanbosseae* in the *Anadyomene* clade with moderate to high support (Figs 30, 31). The results of both analyses suggested a close relationship with *Cladophora catenata*, although with low support. In the BI tree, *B. vanbosseae* was placed within the *C. catenata* clade, sister to the Pacific isolates from Japan and Hawaii (Fig. 30), while in the MP analysis, *B. vanbosseae* was positioned sister to the *C. catenata* clade (including both Pacific and Indian Ocean plants) (Fig. 31).



Figs 30, 31. Reconstructed phylogenies of the *Anadyomene* / *Cladophora* clades based on partial LSU rDNA data. **30**. BI 50% majority-rule consensus tree inferred from partial LSU rDNA data, analyzed under a GTR+I+Γ model (estimated marginal likelihood of the model: -lnL harmonic mean = 2198.47). Values above the branches indicate Bayesian posterior probabilities (left) and MP bootstrap values (right). **31**. Relationships within the *Anadyomene* clade as determined by MP (one of the 18 MP trees, tree length = 216, CI = 0.55, RI = 0.73). Values above the branches indicate bootstrap values.

DISCUSSION

The collections of *Boodlea vanbosseae* from various parts of the Indian and Pacific oceans correspond very well with the lectotype from Indonesia. Distinctive morphological characters are the stiff, cushion-like thalli composed of irregularly branching filaments, and the presence of rhizoidal and tenacular cells.

Based on the distribution data of the specimens examined in the present study, and on verifiable literature data, *B. vanbosseae* is probably widely distributed in the tropical Indo-West Pacific. In the Pacific Ocean it is known from

Indonesia (Reinbold, 1905: 148; Weber-van Bosse, 1913: 70-71, fig. 12; Leliaert *et al*., 1998; this study), Queensland, Australia (Cribb, 1960: 15), Ogasawara Islands (Tsuda, 1968: 209), Caroline Islands (Dawson, 1956: 29, fig. 6; Trono, 1968: 161), Samoa (Setchell, 1924: 176) and Solomon Islands (Womersley & Bailey, 1970: 270; this study). In the Indian Ocean *B. vanbosseae* has been reported from Tanzania (Leliaert & Coppejans, 2004; this study), Sri Lanka (Børgesen, 1936: 63), the Seychelles, Cargados Carajos, Diego Garcia (Chagos Archipelago) and Mauritius (Gepp & Gepp, 1908: 165; Rhyne, 1971: 49; this study) and Scott Reef, Western Australia (this study). Based on the description and illustration of Chang *et al*. (1975: 40, 59, fig. 12), the Chinese record of *B. vanbosseae* is referable to *Cladophoropsis membranacea* (Hofman Bang *ex* C. Agardh) Børgesen. Some records from the Indian Ocean (Somalia: Sartoni, 1992: 307, fig. 7E and Thailand: Egerod, 1975: 52, figs 16-18) and the Pacific Ocean (Fiji: N'Yeurt, 2001: 689-869, fig. 28) are misapplied names for *Phyllodictyon anastomosans* (Harvey) Kraft *et* M.J. Wynne.

Apart from its cushion-like habit, *B. vanbosseae* lacks many of the morphological features typical of the genus *Boodlea*. Branching in *B. vanbosseae* is much more irregular than in the other *Boodlea* taxa (*B. coacta*, *B. composita*, *B. montagnei* and *B. siamensis*), which are generally characterized by regular, opposite branch systems. In *B. vanbosseae*, cross walls at the base of lateral branches are formed almost immediately after their initiation, while in other *Boodlea* species cross wall formation is markedly delayed, resulting in lateral branches in open connection with the mother cell. In *B. vanbosseae*, rhizoidal cells develop from the basal or apical poles of the cells, while in the other *Boodlea* species rhizoids are formed only occasionally at the distal poles of apical cells. Tenacular cells are characteristic for several genera in the Siphonocladales, including *Boodlea*, *Dictyosphaeria*, *Microdictyon*, *Phyllodictyon*, *Struvea* and *Valonia*. Four different types are distinguished (Olsen-Stojkovich, 1986), two of which also occur in the different *Boodlea* species, including *B. vanbosseae*. The first type, unspecialized cells with crenulated apices (type-I in Olsen-Stojkovich, 1986), is by far the most abundant type in *B. vanbosseae*, but is more rare in the other *Boodlea* species. Conversely, the second type of tenacular cells, comprising small hapteroid cells formed at the distal ends of apical cells (type-III in Olsen-Stojkovich, 1986), only occurs sporadically in *B. vanbosseae* but is very common in the other *Boodlea* species. Different types of crystalline cell inclusions have recently been described in various members of the Siphonocladales (Leliaert & Coppejans, 2004). Prismatic calcium oxalate crystals were found in the cells of all *Boodlea* taxa, except in those of *B. vanbosseae*.

Boodlea vanbosseae shares a number of morphological characters with some cushion-forming *Cladophora* species in the siphonocladalean lineage (Leliaert *et al*., 2003). The rhizoidal cells, developing from the basal and apical poles of the cells, are very similar to these found in *C. coelothrix* Kützing (van den Hoek, 1963: figs 72-77) and *C. liebetruthii* Grunow (Leliaert & Coppejans, 2003: fig. 5). The tenacular cells closely resemble those of *C. liebetruthii* (van den Hoek, 1963: figs 128-129) and *C. catenata* (Linnaeus) Kützing (van den Hoek, 1982: figs 47-49). *Boodlea vanbosseae* and *C. catenata* are also similar in forming cushion-like thalli, composed of stiff, entangled filaments.

The partial LSU rDNA sequences of *B. vanbosseae* from the Seychelles and Western Australia differed only in a single nucleotide position, corresponding with an uncorrected p-distance of 0.002. This level of sequence divergence is within the range of other species (genotypic clusters) found in the Siphonocladales (Leliaert *et al*., submitted). In comparison, sequence divergence within *C. catenata* was found to be much higher (maximum p-distance of 0.016), possibly indicating the presence of two cryptic species, each with a restricted geographical distribution (Indian and Pacific Ocean). The present molecular phylogeny echoes the morphological observations and clearly demonstrates that *B. vanbosseae* is distantly related to the other *Boodlea* species (*B. composita*, *B. montagnei* and *B. siamensis*, which are morphologically closely allied with the type of *Boodlea*), but instead is closely allied with *C. catenata*, in the *Anadyomene*-clade. At this stage we restrain ourselves from making taxonomic changes because previous molecular phylogenetic studies have demonstrated that a complete rearrangement of the classification in the Siphonocladales is inevitable (Bakker *et al*., 1994; Hanyuda *et al*., 2002; Leliaert *et al*., 2003; Leliaert *et al*., submitted). For the representatives of the *Anadyomene*-clade, this will either involve the recognition of a single genus (*Anadyomene*), or the delineation of two genera with the *C. catenata*-*B. vanbosseae* clade representing a new genus.

Interestingly, in all examined specimens of *B. vanbosseae*, cells were infected by an endoparasitic or endosymbiotic, ascomycotan fungus, growing inside the cell, on the distal face of the cross walls. The fungus, which was composed of branched hyphae, could not be identified as it lacked fruiting bodies. Remarkably, *C. catenata* is also known to be invariably infected with a ascomycotan fungus, *Blodgettiomyces borneti* (E.P. Wright) Feldmann. Unlike in *B. vanbosseae*, the fungus here infects the inner cell wall and differs in morphology by forming a network of anastomosing hyphae, bearing branches composed of swollen, rounded cells (van den Hoek, 1982: 60, figs 48, 49, 54, 56, 64; van den Hoek & Chihara, 2000: 46, fig. 18D). About 40 marine parasitic or symbiotic fungi have been described in seaweeds, mainly in brown algae, but endoparasitic or endosymbiotic fungi in green algae are relatively poorly known (Kohlmeyer & Kohlmeyer, 1979; Hyde & Pointing, 2000).

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