

***Phyllocladion robustum* (Setchell et Gardner) comb. nov.
(Siphonocladales, Chlorophyta),
a morphologically variable species
from the tropical Pacific coast of America**

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Abstract – The systematic positions of *Cladophoropsis robusta* and *Willeella mexicana*, both occurring along the tropical Pacific coast of America, have long been uncertain. *Willeella mexicana* has been generally accepted as a synonym of *C. robusta*, which is currently placed in the genus *Struveopsis*. Reinvestigation of type material and recently collected specimens from Mexico and Panama, including culture observations, along with a molecular phylogenetic analysis inferred from rDNA ITS1-5.8S-ITS2 sequences, confirm that both taxa belong to the same, morphologically variable species. Thallus architecture ranges from stipitate, *Struveopsis*-like blades (produced by apical cell divisions and formation of opposite and flabellate branches) to *Valoniopsis*-like plants, composed of coarse, cylindrical filaments with lateral branches initiated by lenticular cells. Additionally, DNA sequence data and morphological features such as branching pattern of blade filaments, presence of tenacular cells and crystalline cell inclusions provide evidence that *Struveopsis robusta* falls within the *Phyllocladion* clade and is most closely related with the Atlantic-Caribbean *Phyllocladion pulcherrimum*, with the Indo-Pacific *Phyllocladion orientale* forming a sister species. The binomial *Phyllocladion robustum* (Setchell et Gardner) comb. nov. is thus proposed.

Cladophorales / green algae / lenticular cell division / molecular phylogeny / morphological variability segregative cell division / tenacular cell

Résumé – *Phyllocladion robustum* (Setchell et Gardner) comb. nov. (Siphonocladales, Chlorophyta), espèce morphologiquement variable de la côte pacifique de l'Amérique tropicale. *Cladophoropsis robusta* and *Willeella mexicana* sont deux espèces de la côte Pacifique de l'Amérique tropicale dont la position systématique est longtemps demeurée incertaine. *Willeella mexicana* a généralement été considérée comme un synonyme de *C. robusta*, espèce à présent placée dans le genre *Struveopsis*. De nouvelles observations sur le matériel-type et sur des spécimens récemment récoltés au Mexique et au Panama, ainsi

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que des observations sur du matériel en culture et une analyse phylogénétique à partir des séquences du rADN ITS1-5.8S-ITS2, confirment que les deux taxons appartiennent à une seule espèce morphologiquement variable. L'architecture des thalles varie des frondes stipitées (semblables à *Struveopsis* et produites à partir de divisions des cellules apicales avec formation de branches opposées et flabellées) jusqu'à des plantes semblables à *Valoniopsis* et formées par des filaments cylindriques grossiers avec des branches latérales produites par les cellules lenticulaires. De plus, les séquences de l'ADN ainsi que des caractéristiques morphologiques telles que le mode de ramification des filaments des frondes, la présence de cellules ténaculaires et les inclusions cellulaires cristallines, montrent que *Struveopsis robusta* se place dans le clade du *Phyllocladon*. Le *Phyllocladon pulcherrimum*, espèce Atlantique-Caraïbe, représente l'espèce phylogénétiquement la plus proche, tandis que le *Phyllocladon orientale*, espèce Indo-Pacifique, constitue une espèce sœur. Par conséquent, le binôme *Phyllocladon robustum* (Setchell et Gardner) comb. nov. est proposé.

Algues vertes / cellules ténaculaires / Cladophorales / division cellulaire ségrégative / division des cellules lenticulaires / phylogénie moléculaire / variabilité morphologique

INTRODUCTION

Cladophoropsis robusta Setchell et Gardner (1924) and *Willeella mexicana* E.Y. Dawson (1950), both originally described from Baja California (Mexico), have a complex taxonomic history. Although Dawson (1950) was doubtful about the generic placement of his new species, he tentatively allied it with *Willeella ordinata* Børgesen based on the opposite, distichous branching pattern and the apparent lack of tenacular cells. Later, Dawson (1959) reduced *W. mexicana* to a synonym of *C. robusta*, considering the latter to be a juvenile stage of *W. mexicana*. *Cladophoropsis robusta* was later transferred to the newly circumscribed *Struveopsis* and *Pseudostruvea* by Rhyne & Robinson (1968) and Egerod (1975) respectively, apparently unaware of each others publications (Silva et al., 1996). Both genera were described to include stipitate, blade like-thalli and were distinguished from morphologically similar genera (*Struvea*, *Phyllocladon*) by the absence of tenacular cells. The nomenclatural history of *Struveopsis* and *Pseudostruvea* is rather complicated and at present a single genus, *Struveopsis* [type *S. siamensis* (Egerod) P.C. Silva], is recognized (Silva et al., 1996). Recently, morphological and molecular phylogenetic studies have suggested that different species of *Struveopsis* might merely represent growth forms of *Boodlea composita* (Harvey) F. Brand, *Cladophoropsis membranacea* (Hofman Bang ex C. Agardh) Børgesen or *Phyllocladon anastomosans* (Harvey) Kraft et M.J. Wynne (Wysor, 2002; Leliaert & Coppejans, 2007b; Leliaert et al., 2003, 2007a), and that the recognition of *Struveopsis*, based on a negative character (absence of tenacular cells) is probably untenable. With respect to *Willeella*, Leliaert et al. (2003) confirmed van den Hoek's (1982) notion that this genus falls within the paraphyletic genus *Cladophora*, and is unrelated to *Cladophoropsis* or *Struveopsis*. The single species included in the study of Leliaert et al. (2003), *W. ordinata*, was found to be closely allied to *Cladophora montagneana* Kützing.

Most authors have accepted the conspecificity of *Cladophoropsis robusta* and *Willeella mexicana*, as proposed by Dawson (1959), and the placement of the latter in *Struveopsis*, as suggested by Rhyne & Robinson (1968). However, the

synonymy of the two taxa was recently doubted by Leliaert & Coppejans (2006), who considered *Struveopsis robusta* (Setchell *et* Gardner) Rhyne *et* H. Robinson to be morphologically allied with *Valoniopsis*, based on thallus architecture and mode of cell division, which is via formation of lenticular cells.

The specific status and the taxonomic position of *Struveopsis robusta* and *Willeella mexicana* remain doubtful based on the available data. Here we present a systematic re-assessment based on morphological data from type material and recently collected specimens, culture observations, and molecular data (rDNA internal transcribed spacer sequences).

MATERIAL AND METHODS

The holotype of *Cladophoropsis robusta* (Isla Tortuga, Baja California Sur, Mexico, leg. Ivan M. Johnston, June, no. 135, CAS in UC 1330) and the isotype of *Willeella mexicana* (Punta Colorado, adjacent to Bahia Bocochibampo, near Guaymas, Sonora, Baja California, Mexico, leg. E.Y. Dawson 1789, 16 May 1946, L 952078767, as “*Boodleya erecta* Dawson”) were received on loan from the University of California Herbarium (UC) and the National Herbarium of the Netherlands, Leiden University branch (L) respectively. Additionally, recent collections from Baja California (Mexico) and the Pacific coast of the Republic of Panama, and a culture (UTEX LB 2364) identified as *Valoniopsis pachynema* (G. Martens) Børgesen were studied (Table 1). Specimens were examined with a light microscope after samples were rehydrated in 5% formalin-seawater. Cells were also examined for crystalline inclusions, as the presence and morphologies of these structures have been found to be highly species-specific in siphonocladalean algae (Leliaert & Coppejans, 2004). Drawings were made with a camera lucida on a Leitz Diaplan bright field light microscope (Leica, Wetzlar, Germany). Photographs were taken with an Olympus-DP50 digital camera (Olympus, Tokyo, Japan) mounted on the light microscope or on a Leica Wild M10 stereo microscope (Leica, Wetzlar, Germany).

Algal cultures were grown in sterile 1x modified Provasoli enriched seawater (West & McBride, 1999) at 23°C, a 12:12h LD cycle and photon flux rate of 25-30 $\mu\text{E m}^{-2} \text{s}^{-1}$. Unialgal cultures were established from freshly collected specimens in the field by cutting clean apical cells, which were transferred and grown in fresh culture medium, as described by (Kawai *et al.*, 2005). Morphological observations were carried out on unialgal cultures that were grown for two months.

Molecular phylogenetic analyses were based on rDNA ITS1-5.8S-ITS2 sequences. Specimens used in the phylogenetic analyses are listed in Table 1. DNA was extracted using a DNeasy Plant Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions or using a standard CTAB-extraction method. PCR amplification with primers ITS1FL and Pana5FL and sequencing were performed as described in Leliaert *et al.* (2007a, b). The newly generated sequences were first compared with available sequences in Genbank, using a BLAST search (<http://www.ncbi.nlm.nih.gov/>), to assess their phylogenetic position in the Siphonocladales. The 5.8S rDNA sequences clearly blasted with *Boodlea*, *Phyllodictyon* and *Cladophoropsis*, corresponding to the *Chamaedoris* clade as defined by Leliaert *et al.* (2007a). The sequences, which ranged between 890 and 1055 bases in length, were then included in an alignment consisting of

Table 1. Specimens used in the phylogenetic analyses with collecting data (location, collector, date of collection and voucher information) and EMBL/GenBank accession numbers for the rDNA ITS1-5.8S-ITS2 sequences. For the specimens assigned to *Phyllocladon robustum*, a brief description of the thallus architecture is given along with a reference to a figure or description if available. Newly generated sequences are in bold.

<i>Species</i>	<i>Collecting data</i>	<i>Accession number</i>
<i>Apjohnia laetevirens</i> Harvey	Australia: Victoria, Melbourne, Barwon Heads (M. van Oppen, 1991, ApLB / Apj 1 / F273), culture	AM779624
<i>Boodlea composita</i> (Harvey) F. Brand	Tanzania: Matemwe, Zanzibar (F. Leliaert, 16.vii.2001, FL950 / F016, GENT)	AM779625
<i>Chamaedoris auriculata</i> Børgesen	Socotra: Nogid (F. Leliaert, 14.iii.1999, SOC395 / F010, GENT)	AM779627
<i>Chamaedoris delphinii</i> (Hariot) Feldmann <i>et</i> Børgesen	South Africa: KwaZulu-Natal, Sodwana Bay (E. Coppejans <i>et al.</i> , 8.viii.1999, KZN215 / F468, GENT)	AM779632
<i>Chamaedoris peniculum</i> (J. Ellis <i>et</i> Solander) Kuntze	Dominican Republic: Puerto Plata (O. Dargent, 8.ii.2002, HODRD2-02-20a / F333, GENT)	AM779637
<i>Cladophoropsis membranacea</i> (Hofman Bang <i>ex</i> C. Agardh) Børgesen	Syria: Latakia (unknown collector, 1991, CloMT / Csmem5 / CmMed SL / F295), culture	AY055880/ AY055947
<i>Cladophoropsis sundanensis</i> Reinbold	Tanzania: Mnazi Bay, Mtwara area (E. Coppejans <i>et al.</i> , 29.vii.2000, HEC12976 / F189, GENT)	AM779640
<i>Phyllocladon anastomosans</i> (Harvey) Kraft <i>et</i> M.J. Wynne	St Croix: Malta Baths (Kooistra, 1988, SaMB / Sana1 / F236), culture	AM779641
<i>Phyllocladon anastomosans</i>	Tanzania: Chwaka Bay, Zanzibar (F. Leliaert, 17.vii.2001, FL966 / F401, GENT)	AM779642
<i>Phyllocladon orientale</i> (A. Gepp <i>et</i> E.S. Gepp) Kraft <i>et</i> M.J. Wynne	Seychelles: Bird Island (E. Coppejans <i>et al.</i> , 20.xii.1992, SEY301 / F427, GENT)	AM778980
<i>Phyllocladon orientale</i>	Comoros: Grande Comoro I. (Earle, 1977, West1631 / Struv1 / F414), culture	AM779643
<i>Phyllocladon orientale</i>	Australia: Western Australia, Mermaid Reef, Rowley Shoals (J. Huisman, 15.ix.2006, 11 / F622, PERTH)	AM778981
<i>Phyllocladon orientale</i>	Australia: Western Australia, Scott Reef, Stn 38 (J. Huisman, 25.ix.2006, 17 / F624, PERTH)	AM778982
<i>Phyllocladon pulcherrimum</i> J.E. Gray	USA: Louisiana, northwest Gulf of Mexico (J. López-Bautista & S. Fredericq, 6-30-01 #6, LAF)	AM778983
<i>Phyllocladon robustum</i> (Setchell <i>et</i> Gardner) Leliaert <i>et</i> Wysor	Panama: W side of Iguana Island (W. Kooistra, BW657, LAF) – Natural morphology: Stipitate blades (Wysor 2004: fig. 7).	AM778979
<i>Phyllocladon robustum</i>	Panama: Santa Catalina, Perlas Islands (B. Wysor, BW1505, LAF) Natural morphology: Stipitate blades (Wysor 2004).	AM778978
<i>Phyllocladon robustum</i>	Mexico: Baja California, S of Bahía de Los Angeles, Punta Pescador (H. Verbruggen and F. Zechman, 15.viii.2007, HV1604 / F714, GENT) Natural morphology: Erect filaments with lateral branches initiated from lenticular cells and opposite branches in the apical part of the filaments (Figs 8-14). Culture: Entangled filaments with branches initiated from lenticular cells (Figs 24-33).	AM940055

Table 1. (continued)

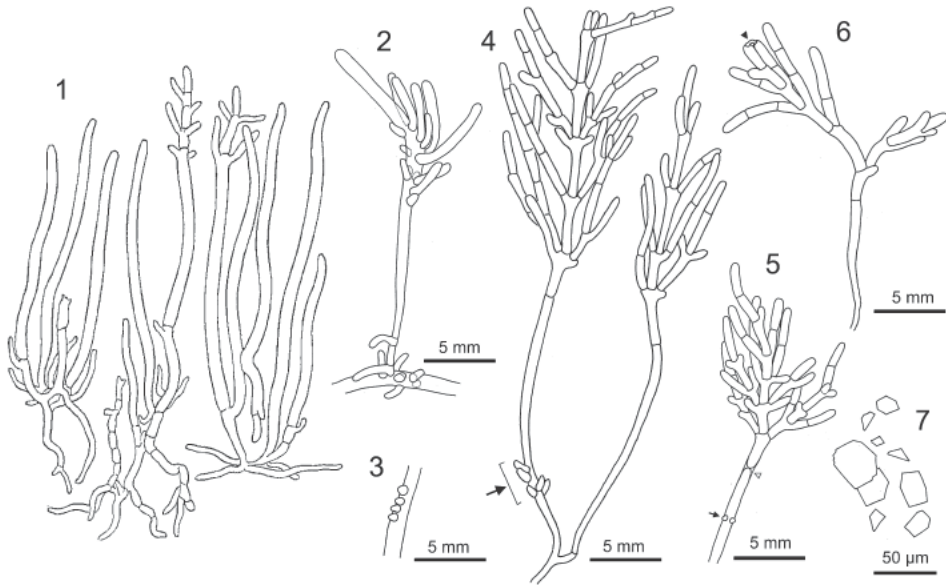
<i>Species</i>	<i>Collecting data</i>	<i>Accession number</i>
<i>Phyllocladon robustum</i>	Mexico: Baja California, Puerto Penasco (J.A. West, iii 1984, number 794, Vopsis4 / VPP794 / F245, as <i>Valoniopsis pachynema</i>) = UTEX LB 2364 (F736). Culture: Entangled filaments with branches initiated from lenticular cells. Some filaments with apical cell divisions and development of opposite branches (Figs 34-45).	AM940054 / AM943660
<i>Struvea elegans</i> Børgesen	Bahamas (J. West, SE1572 / Sele1 / F237), culture	AM779644
<i>Struvea gardineri</i> A. Gepp <i>et</i> E. Gepp	Seychelles: Plate Island (E. Coppejans <i>et al.</i> , 7.i.1993, SEY771a / F199, GENT)	AM779645
<i>Struvea okamurae</i> (Okamura <i>et</i> Higashi) Leliaert	Philippines: Bulusan (E. Coppejans, 22.iv.1998, HEC12301 / F104, GENT)	AM779646
<i>Struvea plumosa</i> Sonder	Australia: Western Australia (T. Schils, WA221 / F287, GENT)	AM779647
<i>S. thoracica</i> Kraft <i>et</i> A. Millar	New Caledonia: Ile aux Canards (Millar <i>et al.</i> , 13.ix.2002, NSW 610014)	AM779648
<i>Struveopsis siamensis</i> (Egerod) P.C. Silva	Tanzania: Mbudya Island (F. Leliaert & E. Coppejans, 11.vii.2001, FL 916 / F396, GENT)	AM850134
<i>Struveopsis</i> sp.	Taiwan: Magang Harbor (S. Fredericq & S.-M. Lin, 6.vii.1994, SF-7-6-94-1-14, LAF)	AF510119

21 exemplar taxa from this *Chamaedoris* clade, including species of *Apjohnia*, *Boodlea*, *Chamaedoris*, *Cladophoropsis*, *Phyllocladon* and *Struvea*. Sequences were aligned using Clustal W (Thompson *et al.*, 1994) with default settings implemented in BioEdit (Hall 1999). Bayesian phylogenetic 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 4.0b10/MrModeltest 1.0b (Swofford, 2002; Nylander, 2004). Two independent analyses were run for 3 million generations. Summary statistics and trees were generated using the last 2 million generations. The BI consensus tree was rooted based on Leliaert *et al.* (2007b). Uncorrected pairwise distances (p-distance) were calculated in PAUP.

RESULTS

Morphology

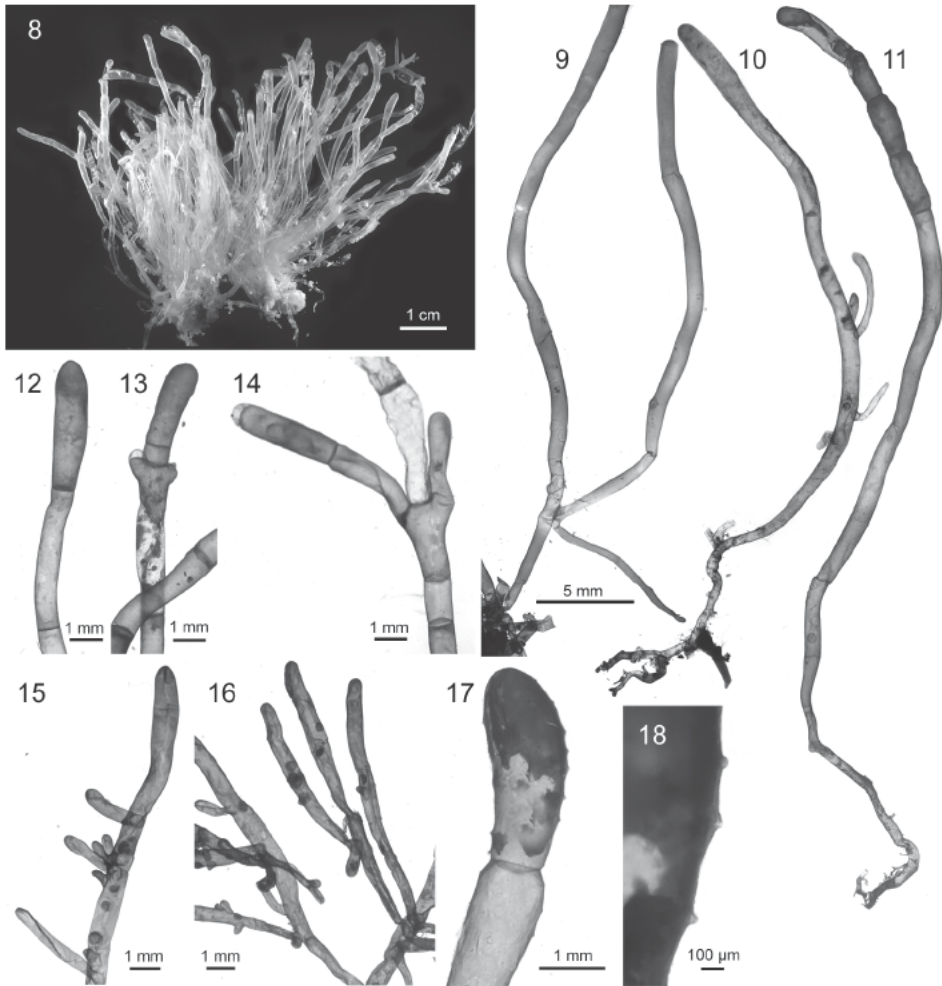
The holotype of *Cladophoropsis robusta* (Figs 2-3, 15-16) consists of a cushion-like thallus with erect filaments with branches that are initiated exclusively by lenticular cells, resulting in a *Valoniopsis*-like thallus architecture.



Figs 1-7. *Phyllocladon robustum*. **1.** Original drawing of the type material of *Cladophoropsis robusta* showing erect filaments with a few opposite branches in the apical part of the thallus (reproduced from Setchell & Gardner, 1924: pl. 13: fig. 16). **2, 3.** Holotype of *Cladophoropsis robusta* (CAS in UC 1330), prostrate and erect filaments with branches initiating from lenticular cells. **4.** Holotype of *Willella mexicana* (redrawn from Dawson, 1950: fig. 11), stipitate blades composed filaments with opposite or flabellate branches; arrow indicates short branches on the stipe, initiated from lenticular cells. **5-7.** Isotype of *Willella mexicana* (L 952 078 767). **5-6.** Stipitate blades composed of oppositely branching filaments, arrow indicates short branches on the stipe, initiated from lenticular cells, black arrowhead shows a tenacular cell, formed at the tip of an apical cell. **7.** Calcium oxalate crystalline cell inclusions.

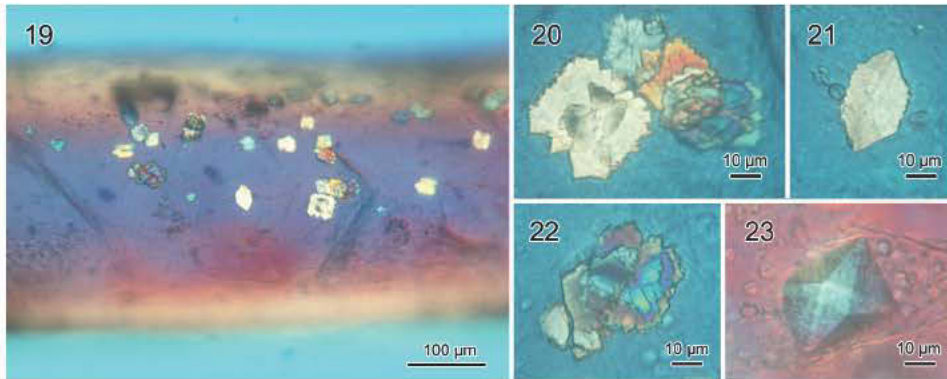
The original illustration (Fig 1, reproduced from Setchell & Gardner, 1924) does not depict lenticular cells but instead shows the apical part of a thallus with a few opposite branches. Conversely, the isotype of *Willella mexicana* is characterized by plants forming clusters of erect stipitate *Struveopsis*-like blades with opposite and flabellate branches (Figs 4-6). A few branches along the stipe are, like in the type of *C. robusta*, also initiated by lenticular cells.

Morphological observations of recently collected specimens from Mexico and Panama, including culture observations, reveal a high degree of morphological variation within a single putative species. Plants are medium to dark green with thallus architecture ranging from clusters of erect stipitate blades (Figs 4-6) to cushions of erect filaments producing lateral branches (Figs 2, 9-11, 15, 16). Thalli may form 2-8 cm high clusters of erect stipes, which are branched or unbranched, with blades that are 1-2 cm high and ca. 1 cm broad and elliptical to irregular in outline. Thalli often remain as the stipes only, with or without a few apically formed, opposite branches (Figs 1, 8, 12-14). Formation of blades is initiated by division of the stipe cell resulting in filaments of 3 to 6 cells. These cells produce opposite branches at their apical poles, which in turn divide by centripetal invagination of the cell wall. Growth of the blades is achieved by a



Figs 8-18. *Phyllocladon robustum* (Figs 8-14, 17-18. Plant from Baja California, Mexico, HV1604; Figs 15-16. Holotype of *Cladophoropsis robusta*, CAS in UC 1330). **8.** Cushion composed of erect filaments developing from prostrate rhizoids. **9.** Erect filament with a lateral branch producing a rhizoid at its basal pole. **10.** Erect filament with lateral branches formed by lenticular cells. **11.** Erect filament with a row of four apical cells transformed into reproductive cells. **12-14.** Apical cell division and formation of opposite branches in the erect filaments. **15-16.** Formation of lateral branches by lenticular cell divisions. **17-18.** Detail of reproductive cells with raised pores.

repetitive process of cell division of the terminal cells (by centripetal wall invagination) and branch formation; each newly formed cell produces a pair of opposite branches at its distal pole (Figs 12-14); older cells may produce a second pair of branches under the first pair and in the same plane, resulting in flabellate branches (Figs 4, 5). Formation of cross walls at the base of the branches is delayed, resulting in branches of up to 2500 µm long that remain in open



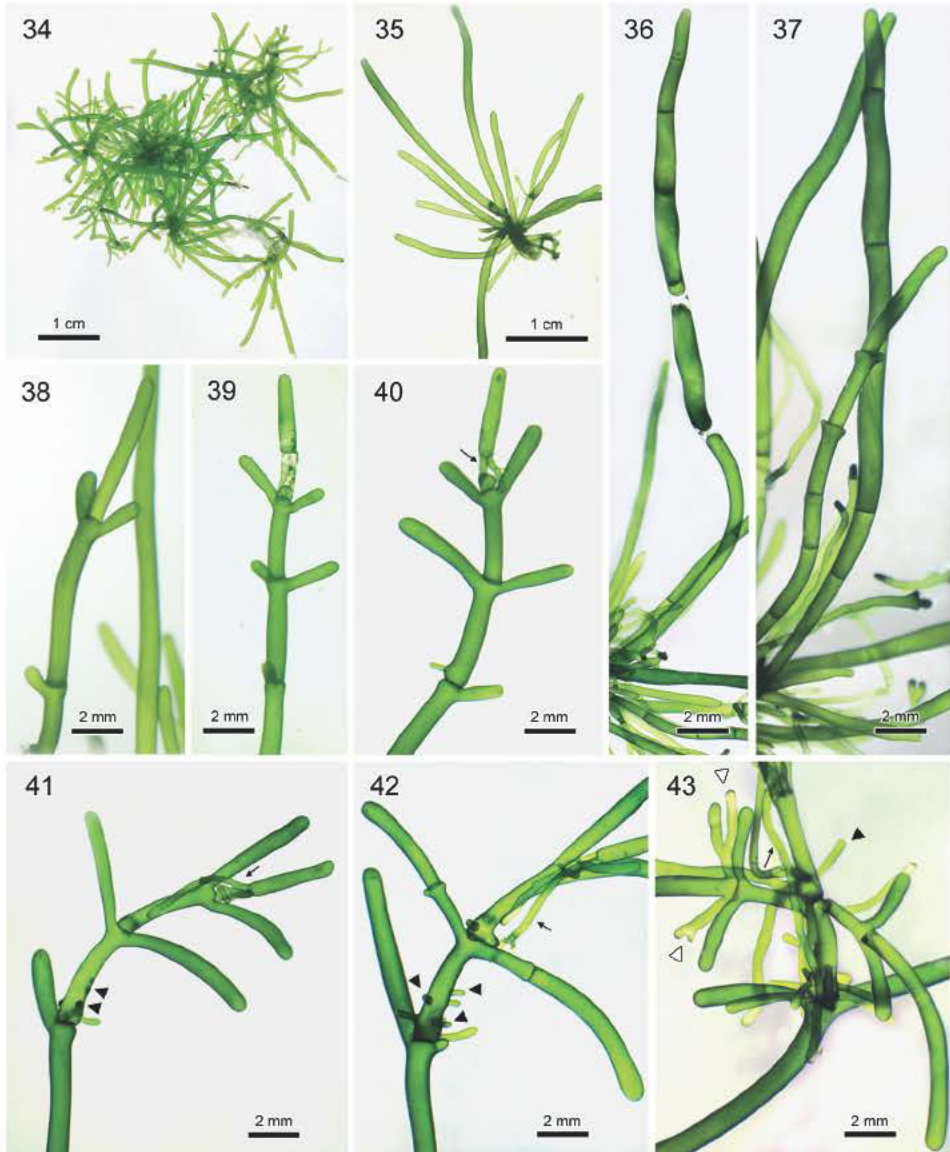
Figs 19-23. Calcium oxalate crystalline cell inclusions. **19-22.** Rectangular or polygonal crystals, with dentate margins, often aggregated (Figs 20, 22) (HV1604). **23.** Octahedral crystal (CAS in UC 1330).

connection with the mother cell. Branching is up to the 2nd or occasionally 3rd order. A second mode of branch formation is by lenticular cell division along the cylindrical axes or stipes. These lateral branches, which are often clustered (Figs 4 and 5 arrow, 10, 15), generally form rhizoids at their proximal pole and grow into new erect axes (Figs 1, 9, 16). Attachment and reinforcement of the thallus occurs by entanglement of these rhizoids and attachment by minute tenacular cells formed on them (Figs 29, 31). Plants are also attached by rhizoids developing from the base of the erect filaments (Figs 1, 8-11). Limited reinforcement of the blade is also achieved by infrequent tenacular cells, produced at the tips of apical cells (Fig. 6, arrowhead; fig. 7 in Wysor, 2004). Diameter of main axes and stipe cells increases towards the apex from ca. 370 to 900 (-1300) μm . Apical cells of the blade filaments are cylindrical with rounded tips, 950-1120 μm in diameter, 1.8-3.2 mm long, length/width ratio 2-3. Cell walls are 15-25 μm thick in the blade cells, 30-65 μm thick in the stipe cells. Reproductive cells with numerous raised pores develop from rows of 2-4 slightly swollen apical cells of the main axes (Figs 11, 17-18). Calcium oxalate crystals are present in most cells, rectangular, polygonal or irregular in outline, with dentate margins, often aggregated, 8-40 (-60) μm across (Fig. 19-22); octahedral crystals rare (Fig. 23).

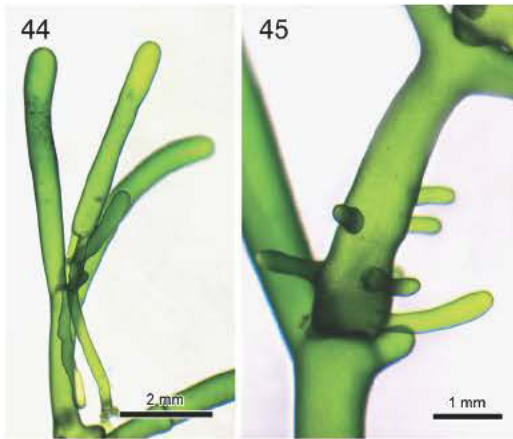
In culture conditions, the plants formed irregular cushions, composed of entangling filaments (Figs 24, 34). Isolate HV1604 failed to form blade-like structures or undergo apical cell divisions and only produced lateral branches initiated by lenticular cell division (Figs 25-27, 32-33). These branches later formed rhizoids at their basal pole (Figs 28-31), which in turn produced minute tenacular cells that attach to adjacent cells (Figs 29, 31). Initially, isolate UTEX LB 2364 also only produced branches by lenticular cell division. However, after several weeks at 23°C, the cells of this culture started to undergo segregative cell division (Fig. 36), resulting in filaments of up to 6 cells (Fig. 37), followed by the formation of opposite branches (Figs 37-41). Two weeks later, these branches re-divided by centripetal wall invagination and re-branched (Figs 42-43). On a single occasion, a second pair of opposite branches was formed above the first pair (Fig. 43, white arrowheads). This is somewhat strange, since in nature (Figs 4, 5) a second pair of



Figs 24-33. Culture of HV1604. **24.** Entangling filaments forming irregular masses. **25-27.** Filaments with lateral branches initiated by lenticular cells (arrows). **28-30.** Rhizoids (arrows) developing from the base of the lateral branches; small tenacular cells (arrowheads) formed on the rhizoids. **31.** Small tenacular cells formed on rhizoids. **32, 33.** Abundant lateral branch formation.



Figs 34-43. Culture UTEX LB 2364. **34, 35.** Filaments forming irregular masses. **36-37.** Segregative cell division of a stipe cell, resulting in a filament of 6 cells. **38-43.** Development of opposite branches in the course of 8 weeks; arrows indicate formation of rhizoids after cell damage; black arrowheads indicate additional lenticular cell divisions; white arrowheads indicate formation of a second pair of opposite branches above the first pair.



Figs 44-45. Culture UTEX LB 2364. **44.** Detail of rhizoids that are formed at the base of cell after cell damage. **45.** Detail of branches initiating from lenticular cell.

opposite branches is formed beneath the first pair. Apart from opposite branch formation, this isolate also produced numerous lateral branches initiated from lenticular cells (Figs 41-42, black arrowheads; Fig. 45). Cell damage of an apical cell resulted in the formation of rhizoids from the basal pole of this cell (Figs 40-43, arrows; Fig. 44).

Molecular phylogeny

The rDNA ITS1-5.8S-ITS2 alignment consists of 1076 sites, 763 of which were phylogenetically informative. The BI analysis yielded a well resolved tree (Fig. 46) with three main clades (*Boodlea*-complex and

Cladophoropsis sundanensis; *Chamaedoris*; *Apjohnia* and *Phyllocladon*) and a grade of *Struvea* species. The specimens with a *Cladophoropsis robusta* (HV1604) or *Willeella mexicana*-like morphology (BW657, BW1505) grouped in a single clade together with a culture specimen (VPP794 = UTEX LB 2364) identified as *Valoniopsis pachynema*. This clade of closely related sequences is preceded by a relatively long, well-supported branch, suggestive of a single phylogenetic species. This species was found to be most closely related to the Atlantic-Caribbean *P. pulcherrimum*, with *P. orientale* from the Indian Ocean forming a sister species. Sequence divergence between these three species ranged between 0.13 and 0.26, while within species divergence was always lower than 0.04 (Table 2). Within the Pacific clade, two ITS ribotypes are evident that differ by 17 nucleotides and

Table 2. Pairwise ITS1-ITS2 sequence divergence between (in bold) and within the three *Phyllocladon* species, calculated as uncorrected pairwise distances (5.8S rDNA region excluded in the analysis).

	<i>P. robustum</i>					<i>P. orientale</i>			
	1	2	3	4	5	6	7	8	9
1 <i>P. robustum</i> BW1505									
2 <i>P. robustum</i> BW657	0.00								
3 <i>P. robustum</i> HV1604	0.02	0.02							
4 <i>P. robustum</i> VPP794	0.02	0.02	0.00						
5 <i>P. robustum</i> UTEX LB2364	0.02	0.02	0.00	0.00					
6 <i>P. orientale</i> West1631	0.22	0.22	0.23	0.23	0.23				
7 <i>P. orientale</i> SEY301	0.25	0.25	0.25	0.25	0.25	0.04			
8 <i>P. orientale</i> F622	0.24	0.24	0.24	0.24	0.24	0.02	0.04		
9 <i>P. orientale</i> F624	0.25	0.25	0.25	0.26	0.26	0.02	0.04	0.00	
10 <i>P. pulcherrimum</i>	0.14	0.14	0.13	0.14	0.14	0.21	0.23	0.22	0.23

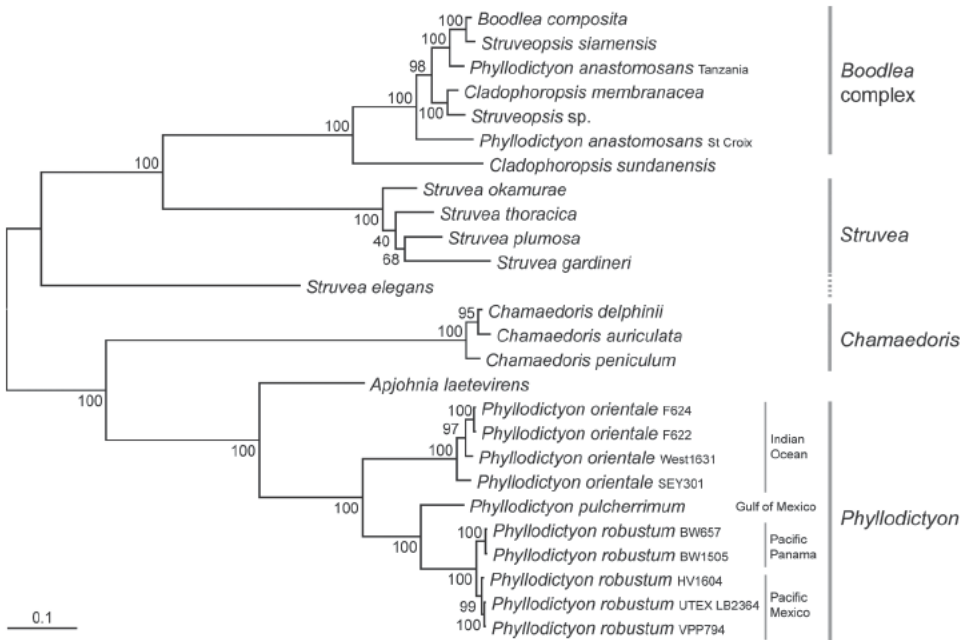


Fig. 46. Bayesian inference tree inferred from rDNA ITS1-5.8S-ITS2 sequence data, analyzed under a GTR+I+ Γ model. Values at the branches indicate Bayesian posterior probabilities. Scale represents the number of expected substitutions per site.

4 indels, corresponding with an uncorrected distance of 0.02. The first ITS type includes the sequences from Panama (BW657, BW1505), the other one consists of the Mexican sequences (HV1604 and VPP794). *Phyllocladon anastomosans* (including plants from the western Indian Ocean and the Caribbean Sea) is unrelated to the other *Phyllocladon* species but instead falls in a clade together with species of *Boodlea*, *Cladophoropsis* and *Struveopsis*.

DISCUSSION

The nomenclatural history of *Cladophoropsis robusta* and *Willeella mexicana* is rather complex. Most authors have accepted the conspecificity of the two taxa, as proposed by Dawson (1959), and the placement of *C. robusta* in *Struveopsis*, as suggested by Rhyne & Robinson (1968). However, Leliaert & Coppejans (2006) observed that Setchell and Gardner's illustration of *Cladophoropsis robusta* did not completely correspond with the holotype specimen. They also reported a pattern of cell division that was consistent with *Valoniopsis pachynema*, but made no formal proposal to invalidate the name *Cladophoropsis robusta* because of incongruence between the type material and its illustration. These observations are now reconciled with a more comprehensive concept based on culture studies and molecular systematics. We

recognize a single species, *Struveopsis robusta*, to encompass a range of morphologies including stipitate, flabellate plants (= "*Willeella mexicana*" and recent collections from Panama), filaments with reduced blades (= original illustration of *Cladophoropsis robusta* and the new collections from Mexico) and *Valoniopsis* like plants (= fraction of the holotype of *C. robusta* and cultures identified as *Valoniopsis pachynema*). These plants have closely related ITS sequences that form a clade preceded by a relatively long, well supported branch, suggestive of a single phylogenetic species. Interestingly, two distinct ITS ribotypes were found within this clade; the first one including the two specimens from Panama, characterized by stipitate blades; the second one consisting of the Mexican plants, with reduced blade development (Fig. 8) or lacking blades altogether (*Valoniopsis*-like morphology). More samples are needed to examine whether the two ribotypes are correlated with morphological variation or geographical distribution.

We assessed the systematic position of the species by means of phylogenetic analysis of rDNA ITS sequence data, along with detailed morphological examinations, including culture observations. *Struveopsis robusta* shares a set of unique morphological characters with two *Phyllocladon* species, *P. pulcherrimum* (the type of the genus) and *P. orientale*. These taxa are characterized by erect stipitate blades with opposite branching in the young parts of the blade and flabellate branches in the older, main filaments. This flabellate branching pattern is accomplished by the formation of a second (and sometimes third) pair of opposite branches, under and in the same plane as the first pair (Kraft & Wynne, 1996; Leliaert & Coppejans, 2007a). Structural reinforcement of the blade is accomplished by attachment of adjacent filaments by tenacular cells, which are formed at the tips of apical cells. Tenacular cells were originally not observed in *C. robusta* and *W. mexicana* and, based on this negative character, the species have been placed in *Cladophoropsis* (Setchell & Gardner, 1924) and *Willeella* (Dawson, 1950), and later in *Struveopsis* and *Pseudostruvea* by Rhyne & Robinson (1968) and Egerod (1975) respectively. However, re-examination of the type material and plants from Panama did reveal the presence of a few tenacular cells at the tips of apical cells, which enables anastomoses between cells of the blade. Tenacular cells were also abundantly formed on rhizoidal cells of plants in culture. *Phyllocladon pulcherrimum* and *P. orientale* are characterized by a second type of tenacular cells, which are produced at the proximal poles of the basal cells of the main axes and attach to the cell below (Leliaert & Coppejans, 2007a). However, this type of tenacular cell has not been observed in *S. robusta*. The Pacific species further differs from the two *Phyllocladon* species by its smaller habit and the coarser filaments (Table 3). A highly unusual feature of *S. robusta* is the formation of lateral branches along the stipe or erect axes, initiated by lenticular cell division, a mode of cell division that is characteristic for the genera *Valonia* and *Valoniopsis*. This mode of cell division does not occur in any *Phyllocladon* species and has so far not been observed in any of the related genera. Unlike in *Valonia* and *Valoniopsis*, these lateral branches form typical rhizoidal branches at their proximal pole (Figs 1, 9, 16, 28-31) which in their turn produce small tenacular cells (Figs 29, 31). *Struveopsis robusta* and the two *Phyllocladon* species also have similar calcium oxalate crystals, which are diamond-shaped or polygonal. In *S. robusta*, these crystals generally have dentate margins and are often aggregated, a feature that is shared with *Cladophoropsis magna* Womersley, a species with uncertain systematic affinity (Leliaert & Coppejans, 2004, 2006). Interestingly, octahedral calcium oxalate crystals were also observed in *S. robusta*. This type of crystals has not yet been observed in

Table 3. Morphological comparison of *Phyllocladion pulcherrimum*, *P. orientale* and *P. robustum*.

	<i>P. pulcherrimum</i>	<i>P. orientale</i>	<i>P. robustum</i>
Thallus height	up to 36 cm	up to 7 cm	up to 5 cm
Maximum number of branches per cell	6	6	4
Tenacular cells	Abundant at tips of apical cells; also at the base of cells of the main axes.	Abundant at tips of apical cells; also at the base of cells of the main axes.	Rare at tips of apical cells.
Lenticular cells	Absent	Absent	Present
Apical cell diameter	200-380 µm	(70-) 90-170 (-200) µm	950-1120 µm
Calcium oxalate crystalline cell inclusions	Diamond-shaped or broad hexagonal.	Diamond-shaped, sometimes triangular.	Rectangular or polygonal, with dentate margins. Octahedral crystals rare.

Phyllocladion or related genera but are known in *Valoniopsis pachynema*, *Cladophora dotyana* Gilbert and *Siphonocladus tropicus* (P. Crouan et H. Crouan) J. Agardh (Leliaert & Coppejans, 2004).

The putative relationship of *Cladophoropsis robusta* with the two *Phyllocladion* species, based on the above mentioned shared morphological characters is confirmed by the present phylogeny inferred from rDNA ITS sequences, which distinctly places *S. robusta* in the *Phyllocladion* clade. *Struveopsis robusta* is most closely related to the Atlantic-Caribbean species *P. pulcherrimum*, and *P. orientale* from the Indian Ocean is sister to this clade. Additionally, the close relationship between *S. robusta* and *P. pulcherrimum* has also been demonstrated (although less convincingly) on the basis of small subunit rDNA sequence data (Wysor, 2002). Thus, both the morphological and molecular data are concordant in allying *S. robusta* with *Phyllocladion pulcherrimum* and *P. orientale*. Hence, the following binomial is effectuated:

Phyllocladion robustum (Setchell et Gardner) Leliaert et Wysor, comb. nov.

Basionym: *Cladophoropsis robusta* Setchell et Gardner, *Proc. Calif. Acad. Sc.* ser. 4, 12: 714, pl. 13: fig. 16 (1924).

Nomenclatural synonyms: *Pseudostruvea robusta* (Setchell et Gardner) Egerod, *Bot. Mar.* 18: 47 (1975); *Struveopsis robusta* (Setchell et Gardner) Rhyne et H. Robinson, *Phytologia* 17: 470 (1968).

Taxonomic synonym: *Willeella mexicana* E.Y. Dawson, *Am. J. Bot.* 37: 151, fig. 11 (1950).

The geographical distribution of *P. robustum* is restricted to the tropical eastern Pacific Ocean, where it has been recorded from Baja California (Mexico) and the Pacific coast of Mexico (Dawson, 1950; Taylor, 1945), El Salvador (Dawson, 1961) and Panama (Wysor, 2004). The only record of *P. robustum* outside the eastern Pacific (Islam 1976: Bangladesh) represents an unidentifiable *Cladophora* species (Leliaert & Coppejans, 2006). Based on literature data, *P. robustum* is especially common in Mexico where it grows in intertidal to shallow subtidal habitats (down to 7 m depth), occurring all year round (Aguilar-Rosas et al., 2000, 2002; Mateo-Cid et al., 1993, 2006).

In contrast to the morphologically variable *P. robustum*, the sister species *P. pulcherrimum* and *P. orientale* have a relatively constant thallus architecture, characterized by large stipitate, reticulate blades (Kraft & Wynne, 1996; Leliaert & Coppejans, 2007a). Interestingly, *P. pulcherrimum* and *P. orientale* both grow in deep waters, down to 55 m or more, as opposed to *P. robustum*, which is only known from the intertidal to shallow subtidal. The large blades are possibly an adaptation to low light intensities in deep water habitats. In addition, a correlation between morphological variability and ecological distribution could be suggested. Intertidal and shallow subtidal habitats are more variable and unpredictable in terms of physical stress and biological interactions than deep water habitats. Such variable environments could induce a selective pressure favoring individuals that show phenotypic plastic responses (Via *et al.*, 2005), or morphological variability within a species or population could be acquired through maintenance of genetic variation (polymorphism).

In a taxonomic re-assessment of *Phyllocladon* and *Struvea*, Kraft & Wynne (1996) distinguished the two genera on the basis of the different modes of cell division: *Struvea*, including those species in which cells divide by a process of segregative cell division (SCD), and *Phyllocladon*, encompassing taxa in which cells only divide by centripetal invagination of the cell wall (CI). Phylogenetic analyses based on rDNA ITS data (this study) and large subunit rDNA sequences (Leliaert & Coppejans, 2007a) only partly confirm this generic separation. Monophyly of *Struvea* could not be confirmed in the present study and polyphyly is clearly shown in *Phyllocladon*, with *P. anastomosans* in the *Boodlea* clade being unrelated to the *Phyllocladon* clade including *Phyllocladon robustum*, *P. orientale* and *P. pulcherrimum*. These “true” *Phyllocladon* species differ morphologically from *P. anastomosans* by the flabellate branching pattern, the presence of small tenacular cells formed at the basal poles of the cells and attaching to the cell below (not observed in *P. robustum*), and the diamond-shaped or polygonal crystalline cell inclusions. Although in all three *Phyllocladon* species, cell division in the blade takes place by CI, the initial division of the stipe cell in *P. robustum* is segregative. Similarly, in the closely related genus *Chamaedoris* the stipes first divide by SCD, followed by CI in the capitulum filaments (Leliaert *et al.*, 2007b). Also in *Struvea gardineri* SCD occurs in the initial stages of blade formation while CI takes place in older blades (Leliaert & Coppejans 2007a).

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