

Revision of the genus *Ulvella* (Ulvellaceae, Ulvophyceae) based on morphology and *tufA* gene sequences of species in culture, with *Acrochaete* and *Pringsheimiella* placed in synonymy

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Microfilamentous green algae in the Ulvellaceae are notoriously difficult to identify and classify. We revised *Ulvella* based on the morphology of 46 unialgal culture isolates, including several from type localities, and we were guided by a phylogenetic reconstruction based on chloroplast-encoded *tufA* gene sequences. Species previously referred to *Acrochaete*, including the type species *A. repens*, formed a clade that included *Pringsheimiella scutata* and *Ulvella lens*, the type species of their respective genera. These species were placed in a single genus, and *Ulvella* had priority. The circumscription of the genus was emended to include microscopic species with branched filaments that may or may not form mono- and polystromatic disc-shaped thalli. Ten new species were described (viz. *U. aequicrassa*, *U. dasycala*, *U. gigas*, *U. glabra*, *U. globocaespitosa*, *U. inopinata*, *U. pachypes*, *U. pseudorepens*, *U. vacuospora*, and *U. waernii*), and two were resurrected (*U. parasitica*, previously considered a synonym of *A. repens*, and *U. porphyrae*, previously synonymised with *A. viridis*). *Ectochaete polymorpha* was placed in synonymy with *U. leptochaete* and *Acrochaete parasitica* f. *zosteriae* with *Ochlochaete hystrix*. Ten new combinations were proposed for species previously referred to *Acrochaete* (viz. *U. cingens*, *U. codicola*, *U. geniculata*, *U. inflata*, and *U. taylori*) or *Pringsheimiella* (viz. *U. gratulans*, *U. mauritiana*, *U. sanctae-luciae*, *U. striata*, and *U. udoteae*).

KEY WORDS: *Acrochaete*, Chloroplast-encoded *tufA* gene sequences, Culture studies, Microfilamentous green algae, *Ochlochaete*, *Pringsheimiella*, *Ulvella*, Ulvellaceae

INTRODUCTION

Microfilamentous green algae are reported from the tropics (e.g. Børgesen 1913) to subarctic regions (e.g. Rosenvinge 1893). They are common epibionts on a variety of solid substrata including wood, rock, pebbles, and plastic, and they sometimes grow into calcified material. They also occur on or in other organisms, and while most are considered harmless, a few have been reported as pathogens of other algae (Correa *et al.* 1994; Correa 1997) or corals (Goldberg *et al.* 1984). Projections of species richness among microfilamentous green algae are limited because the algae are hard to identify, and the number of researchers studying their diversity is small. Approximately 109 species of microfilamentous ulvophycean taxa are reported in Algaebase (Guiry & Guiry 2012), but the true number may be 200 species or more (R. Nielsen, unpublished data). Many species descriptions are based exclusively on observations from naturally occurring material, either as part of floristic studies (e.g. Setchell & Gardner 1920a, b, 1924; Printz 1926; Norris 2010) or investigations devoted particularly to these algae (e.g. Huber 1892a, b; Gardner 1909; Thivy 1942, 1943, 1945;

South 1974; Cribb 1995). A few descriptions were based on more detailed investigations of a single or a few species (e.g. Pringsheim 1862; Wille 1880).

Huber (1892a, b) published the first comprehensive account of the microfilamentous green algae. This study included both freshwater and marine species, and he referred them all to the Chaetophoraceae. Huber studied primarily the natural distribution of species and described several new taxa. He also examined the germination patterns for a few species in culture. Gradually, culture methods improved, and species descriptions based on culture studies slowly appeared. Thus, Kylin (1935) was able to follow the life history of shell-boring species and described a few species based on culture observations. Similarly, Moewus (1949) made culture studies on two epiphytic species. Culture studies identified important problems. Different species often grow in close proximity on tiny pieces of substratum, making it impossible to distinguish them visually. When initiating cultures from less than 1 mm² of substrate, it is often possible to identify and separate three to four different species not noticed in the original collection. Yarish (1976) studied cultures in response to different ambient conditions. Culture studies minimized the possibility that diagnostic characters were omitted; descriptions based only on field material led to incomplete species descriptions or erroneous descriptions based on

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several species [e.g. *Gomontia polyrhiza* (Lagerheim) Bornet & Flahault and *Eugomontia sacculata* Kornmann (Kornmann 1959, 1960); *Pringsheimiella scutata* (Reinke) Marchewianka and *Syncoryne reinkei* R. Nielsen & P. M. Pedersen (Nielsen & Pedersen 1977)]. Cultures are also important for revealing life-history patterns (e.g. Yarish 1975; Nielsen 1977, 1979, 1983, 1987, 1988; Nielsen & Pedersen 1977; O'Kelly & Yarish 1981; O'Kelly 1982a, 1983, 2004c; Goldberg *et al.* 1984; Nielsen & McLachlan 1986a, b; Correa *et al.* 1988), pigment composition (O'Kelly 1982b), and ultrastructural features (e.g. O'Kelly & Yarish 1980; Correa & McLachlan 1994). Within the last decade molecular work has also been based on plants in culture (e.g. Hayden & Waaland 2002; O'Kelly *et al.* 2004a, b, c).

O'Kelly and Floyd (1983) made an important step towards a modern classification when they resurrected and emended the family Ulvellaceae (Ulvales, Ulvophyceae) to include six genera: *Acrochaete*, *Endophyton*, *Entocladia*, *Ochlochaete*, *Pringsheimiella*, and *Ulvella*. *Entocladia viridis* Reinke was included based upon motile cell ultrastructure and earlier observations of life histories, sporangial development, and ultrastructure (Yarish 1975; Nielsen & Pedersen 1977; Nielsen 1979; O'Kelly & Yarish 1980, 1981; O'Kelly 1982a, 1983; Floyd & O'Kelly 1984). *Endophyton ramosum* N.L. Gardner was transferred to *Entocladia* based upon ultrastructural observations (Leonardi *et al.* 1997). O'Kelly and Floyd (1983) excluded several taxa from the Ulvellaceae. Ultrastructural (Chappell *et al.* 1990) and molecular (O'Kelly *et al.* 2004c) studies supported placement of *Phaeophila dendroides* P.L. Crouan & H.M. Crouan in its own family within Ulvales. Similarly, *Bolbocoleon piliferum* Pringsheim is a sister taxon to the remaining Ulvales. O'Kelly & Rinkel (in Brodie *et al.* 2007) formally established the Bolbocoleonaceae. Hayden and Waaland (2002) used molecular evidence to place *Pseudendoclonium fucicola* (Rosenvinge) R. Nielsen and *Tellamia contorta* Batters in the Kornmanniaceae. The mostly marine genera *Collinsiella*, *Eugomontia*, and *Gomontia* (O'Kelly *et al.* 2004c) and the freshwater *Planophila* (Friedl & O'Kelly 2002) have been referred to the Ulotrichales (Ulvophyceae) based on ultrastructural, life history, and molecular criteria. Finally, Sanchez-Puerta *et al.* (2006) used ultrastructural and gene-sequence data to refer the type species of *Pseudulvella* to the Chaetopeltidales (Chlorophyceae).

O'Kelly and Floyd's concept of Ulvellaceae has not survived intact. O'Kelly *et al.* (2004a) found that *Ochlochaete* and *Ruthnielsenia* occupied a phylogenetic position sister to the family Ulvaceae, and they removed *Ochlochaete* from the Ulvellaceae. This and similar analyses (O'Kelly *et al.* 2004b, c) supported Nielsen's merging of *Entocladia* within *Acrochaete* (Nielsen 1979). O'Kelly (in Gabrielson *et al.* 2006) transferred *E. ramosum* to *Acrochaete* [*A. ramosa* (N.L. Gardner) O'Kelly in Gabrielson *et al.*]. Of the six genera that O'Kelly and Floyd placed in Ulvellaceae, only *Acrochaete*, *Pringsheimiella*, and *Ulvella* remain. Comparative studies of species in these three genera are rare. Bown *et al.* (2003) compared species of *Acrochaete* analysing nuclear-encoded ITS2 rRNA and found that *A. viridis* (Reinke) Nielsen and *A. operculata* Correa & Nielsen were more closely related to each other than they were to *A. heteroclada* Correa & Nielsen. No DNA sequence data have been

available for the type or species of *Pringsheimiella* and *Ulvella*, and this precluded any test of the validity of the morphological characters that separate these genera and *Acrochaete*.

In this study, we focus on culture strains initially referred to *Acrochaete*, *Pringsheimiella*, and *Ulvella*. Many were isolated from type localities and some represent authentic strains used to describe new species. Morphological observations at the light microscopical level were combined with a phylogenetic inference based on chloroplast-encoded elongation factor *tufA* gene sequences providing the first detailed taxonomic revision of *Pringsheimiella*, *Ulvella*, and *Acrochaete*-like filamentous green algae.

MATERIAL AND METHODS

The strain of *Acrochaete endozoica* (Goldberg, Makemson & Collie) Wynne used in this study was obtained from the Culture Collection of Algae, University of Texas (UTEX B 2352), and three strains of *Ulvella lens* P. Crouan & H. Crouan were obtained (by CJO and BW) from abalone hatcheries in Australia and California. All other unialgal strains were established from crude cultures in Petri dishes initiated from host fragments bearing green epiphytic or endophytic microfilamentous algae or from scrapings from solid substrates like pebble and shells (Supplementary Table S1). All strains are available from the Scandinavian Culture Collection of Algae & Protozoa. Unialgal cultures were isolated using a hand-drawn glass pipette and washed several times in clean dishes containing MV30 medium (Christensen 1982). To prevent diatom growth 5 mg l⁻¹ of GeO₂ was added to crude cultures. Autoclaved seawater without additional nutrients was sometimes used to initiate hairs. Autoclaved calcite spar, fragments of oyster shells, or transparent bivalve shells were occasionally added to test the ability of the algae to grow into calcified material. The strains were maintained at either 15°C with a 16:8 light:dark cycle or at 20°C with a 12:12 light:dark cycle. The light intensity varied from 3 to 20 μmol photons m⁻² s⁻¹. Observations were made using actively growing plants transferred into fresh medium and grown in higher light. Photos were taken using Olympus digital camera DP-70 mounted on an Olympus AX-70 microscope; a saltwater immersion objective 20× was used to study prostrate plants growing in plastic Petri dishes.

When authentic material for a certain species did not include conserved plants but only iconographic lectotypes, we have designated epitypes in cases in which our material originated from type localities or nearby localities and were obtained from the same host-species or substrate as in the original descriptions. Even the best iconographic lectotype is ambiguous for the filamentous species in this group of algae because it can not reveal all diagnostic characters, and confusion of the dense growing species is very easy. The epitype support an ambiguous iconographic lectotype, per Article 9.7 of the International Code of Botanical Nomenclature (Vienna Code; McNeill *et al.* 2006) and also secure extraction of molecular material in future investigations. Herbaria abbreviations in the text follow Thiers (2010).

We included 50 accessions referred to *Acrochaete*, *Pringsheimiella*, and *Ulvella*. The *tufA* gene sequences from three *Acrochaete* species were retrieved from GenBank [viz. AY454407 (strain Ma1-2a1 = CCMP2331), AY454409 (strain NY1b = CCMP2382), and AY454410 (strain WA1-12A1)]. Some sequences were identical and then only one sequence was included in the data matrix. The outgroup taxa were *Ochlochaete hystrix* Thwaites (AY454406), *Percursaria percursa* (C. Agardh) Rosenvinge (AY454403), *Ruthnielsenia tenuis* (Kylin) O'Kelly, Wysor & Bellows (AY454404 and JQ302995), *Ulva californica* Wille (AY454401), *U. intestinalis* Linnaeus (AY454399), and *Ulvaria obscura* (Kützinger) P. Gayral ex Bliding (AY454402). Except for one sequence of *Ruthnielsenia*, the other sequences were from O'Kelly *et al.* (2004a). Thus, the final sequence data matrix included 48 sequences. Table S1 lists the origin of specimens and GenBank accession numbers.

Total genomic DNA was extracted from frozen cultures following the procedure of Doyle & Doyle (1987). Polymerase chain reaction amplifications were performed using primers *tufAF* and *tufAR* (Famà *et al.* 2002) at annealing temperatures between 40°C and 57°C (most frequently 52°C) using a standard polymerase (Ampliqon Taq DNA Polymerase, Ampliqon, Odense, Denmark) and the buffer supplied with the kit diluted to a final MgCl₂ concentration of 1.5 mM. The products were purified using the QIAquick PCR purification kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. Cycle sequencing was performed using the ABI PRISM Dye Terminator Cycle Ready Reaction kit with AmpliTaq DNA Polymerase, FS (Applied Biosystems, Wellesley, Massachusetts, USA), and the product was purified as above. DNA fragment were separated on an ABI 3130XL automated sequencer (Applied Biosystems), and sequence editing was done using Sequencher version 4.8 (Gene Codes Corporation, Ann Arbor, Michigan, USA). The *tufA* gene sequences were of equal length except for two sequences (GenBank no. JQ303000–JQ303001) which shared very long inserts (868 bp, 733 bp). These inserts were nonhomologous and had no significant similarity to GenBank sequences, and they were excluded.

Parsimony analyses were performed using PAUP* vers. 4.0b8 (Swofford 2001) and the following options: heuristic search, 100 random addition sequences, five trees held each step, and TBR swapping. PAUP* v. 4.0b8 has been preferred to the most recent version 10 (Swofford 2002) because the latter outputs erroneous tree lengths and an excessive number of tree islands. Uninformative characters were excluded and all characters were treated as equally weighted and nonadditive. Bootstrap support was calculated using PAUP* v. 4.0b8 running 1000 replicates. In each replicate we ran 10 random addition sequences saving no more than 500 trees per replicate.

MrBayes (v. 3.2, Ronquist & Huelsenbeck 2003) was used to perform Bayesian analysis with a general time reversible substitution model. We used partitioning of the codon positions for the protein-encoded chloroplast gene and ran two independent Markov Chain Monte Carlo (MCMC) (each comprising 1 cold and 3 heated chains) with 4×10^6 generations. Parameter values and trees were sampled and saved every 500th generation. The numbers of substitution types allowed were two for first and second codon positions

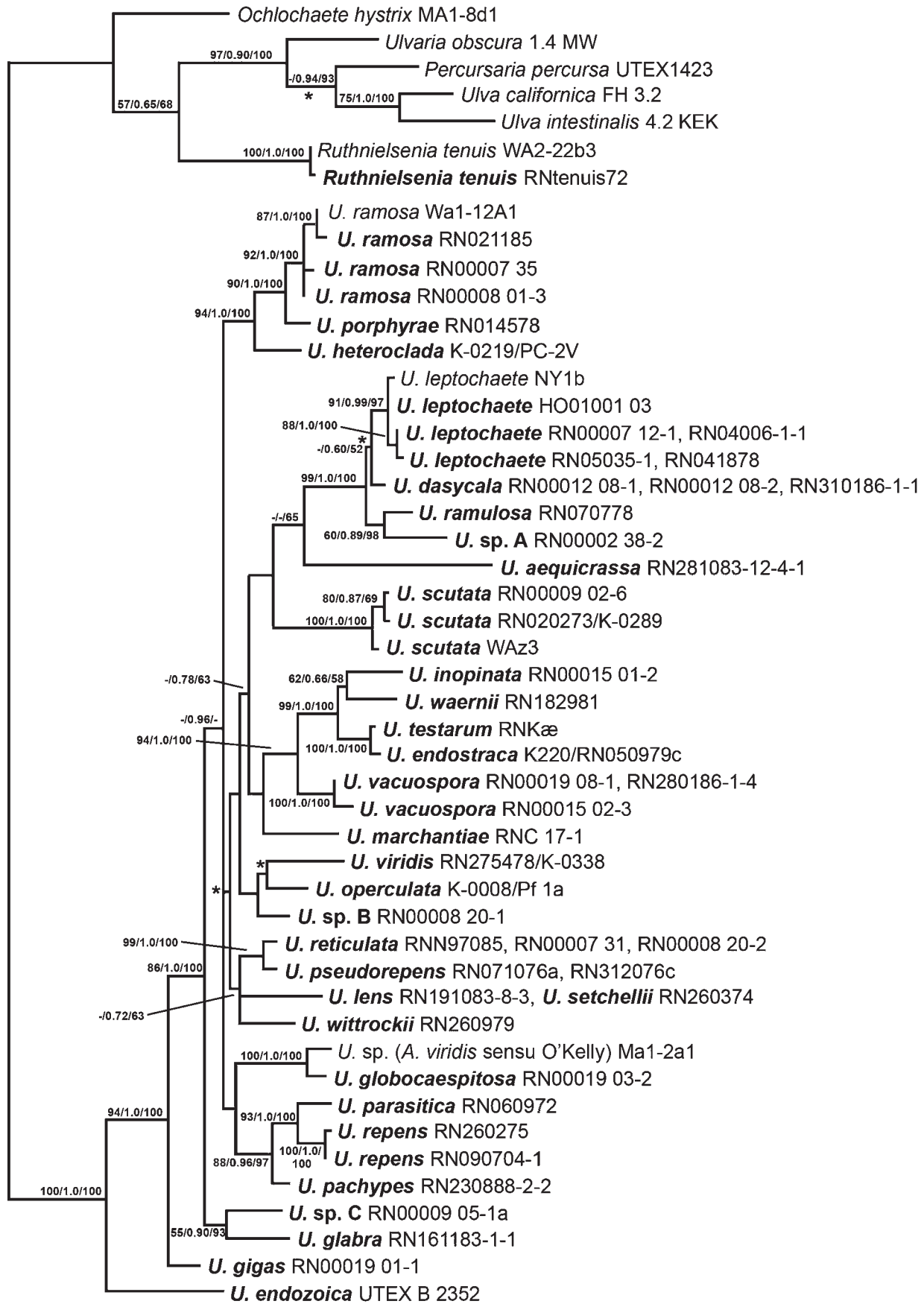
(Iset = 2) and six types for third codon positions (Iset = 6). We assumed that all of the model parameters were unlinked and rate multipliers were variable across partitions. Using Microsoft Excel we plotted the log likelihood values as a function of generations. The lnL values converged at *c.* 5745 after 40,500 generations leaving 7920 trees. These were imported into PAUP* to produce a 50% majority rule consensus tree. Posterior probabilities (pp) were also obtained from the 7920 trees and the values added to the tree topology shown as Fig. 1. The Bayesian tree is shown in Fig. S1.

We also performed a maximum likelihood analysis using PhyML (Guindon *et al.* 2010). For this we used the parameter settings suggested by Modeltest (v. 3.7, Posada & Crandall 1998). We used 1000 bootstrap replications in maximum likelihood to evaluate the robustness of the tree topology. PhyML was run via the online version available on the Montpellier bioinformatics platform at <http://www.atgc-montpellier.fr/phyml>.

RESULTS

The data matrix included 894 characters of which 226 were phylogenetically informative. Parsimony analysis resulted in 48 equally parsimonious trees of length 726 (consistency index = 0.51, retention index = 0.71). One tree randomly selected among the 48 parsimonious trees is shown (Fig. 1); bootstrap proportions (BS) and the branches collapsed in the strict consensus tree indicated. Bayesian inference resulted in a 50% majority rule consensus tree largely congruent with the strict consensus tree of the most parsimony trees (Fig. S1) and the posterior probabilities (pp) of individual branches were shown (Fig. 1). The ingroup remained monophyletic (BS = 100%; pp = 1.0) and included *Acrochaete* species as well as species from *Ectochaete*, *Entocladia*, *Endophyton*, *Pringsheimiella*, *Pseudodictyon*, and *Ulvella*. These small satellite genera were embedded within part of *Acrochaete*, where two individual species [viz. *U. endozoica* (R. Nielsen) R. Nielsen, C.J. O'Kelly & B. Wysor *comb. nov.* and *U. gigas* R. Nielsen *sp. nov.*] formed highly supported sister groups (BS = 94%, pp = 1.0, BS = 100 and BS = 86%, pp = 1.0, BS = 100, respectively).

In order to maintain monophyly of the already described genera, we extended the concept of *Ulvella* P.L. Crouan & H.M. Crouan 1859 as circumscribed by Nielsen *et al.* in Brodie *et al.* (2007). This genus has nomenclatural priority, antedating *Acrochaete* N. Pringsheim 1863 ('1862') and all other generic names in the family. The following growth forms were distinguished: (1). Filaments all alike with a gradual transition from a distal part of cylindrical cells into rounded or polygonal cells in the middle part of plants and any cell able to become a sporangium [e.g. *U. viridis* (Reinke) R. Nielsen, C.J. O'Kelly & B. Wysor *comb. nov.*]. Substrate attached pseudoparenchyma occurred as a basal layer. (2). Heterotrichous, some filaments relatively broad and formed tufts of upright branches from narrow long-celled filaments, only cells of the broad filaments able to become sporangia [e.g. *U. repens* (Pringsheim) R. Nielsen, C.J. O'Kelly & B. Wysor *comb. nov.*]. Substrate attached pseudoparenchyma



occurred as a basal layer. (3). Rosettes, substrate attached pseudoparenchyma of radiating filaments with mutually free filaments at the margin [e.g. *U. marchantiae* (Setchell & N.L. Gardner) R. Nielsen, C.J. O'Kelly & B. Wysor comb.]. (4). Discs, confluent filaments with bifurcate marginal cells, monostromatic [e.g. *U. scutata* (Reinke) R. Nielsen, C.J. O'Kelly & B. Wysor comb. nov.] or polystromatic (e.g. *U. lens*); (5). Flossy, openly branched filaments with rather long cylindrical or irregularly shaped cells [e.g. *U. ramosa* (N.L. Gardner) R. Nielsen, C.J. O'Kelly & B. Wysor comb. nov.]. (6). Shell boring, can grow into calcified material such as mollusc shells [e.g. *U. testarum* (Kylin) R. Nielsen, C.J. O'Kelly & B. Wysor comb. nov.].

Vegetative cells contain a parietal lobed chloroplast with a few perforations or almost reticulate and have one, one to a few, or several pyrenoids. *Acrochaete*-type hairs, sometimes termed 'setae' following Huber 1892b ('soies'), consist of hyaline nonseptate merocytic extensions from a more or less bulbous base, which may be separated by a wall from the vegetative cell below (Nielsen 1979; Christensen 1994). Hairs usually form at the apical end or on protuberances from intercalary cells of broad filaments in heterotrichous plants; in plants with a different morphology they often form on intercalary cells. They usually develop when actively growing plants were transferred to medium without addition of nutrients or when maintained under bright light conditions. *Acrochaete*-type hairs are a very characteristic feature of the genus although not observed in *Ulvella glabra* R. Nielsen sp. nov., *U. ramosa*, or *U. testarum*. These species were exposed to the same conditions as the rest, so we doubt that lack of observation reflects lack of attention. Sporangia develop from the same kind of cells as those supporting hairs. The shape reflects that of the vegetative cells with addition of a conical top or an exit tube. Sporangia from intercalary cells often become bottle-shaped and sporangia from apical cells get an elongate cylindrical to linear shape. The exit papilla of mature sporangia are closed by a mucilage plug, which dissolves before the swarmers are released (Leonardi *et al.* 1997). Swarmers form after sequential division of the cytoplasm (O'Kelly & Yarish 1980). They are pyriform and may be bi-, tri-, or quadriflagellate zoospores or biflagellate gametes (Moewus 1949; O'Kelly 1982a; Kornmann 1993). Copulation between small, pale and larger, green anisogametes has been reported by Moewus (1949), O'Kelly (1982a), and Kornmann (1993). The swarmers are pyriform with the flagella inserted in an apical papilla (O'Kelly & Floyd 1983; Leonardi *et al.* 1997). The life history comprises alternation of isomorph generations. Spores germinate unilateral, the spore remain part of the developing plants in some species, while an evacuated spore-wall and germination tube occur in others. Table 1 summarizes characters for identification of the species.

Revision

Ulvella aequicrassa R. Nielsen sp. nov.

Figs 2–5

Fila aequicrassa aperte et alterne ad angulum 90° ramificata e cellulis cylindricis, 5.75–7.5 µm latis, latitudine sua duplo vel triplo longioribus, 1 pyrenoide foveatibus. Pili generi Acrochaete peculiare e cellulis apicalibus crescent. Fila facultate in substantiam calcariam penetrandi donata. Multiplicatio carie cellularum intercalarium.

HOLOTYPE: Dried sample of isolate RN281083-12-4-1 maintained at Botanical Museum Copenhagen, Denmark as no. CAT 2441.

TYPE LOCALITY: Chile, Puerto Aldea in mollusc shell.

ETYMOLOGY: Named from Latin, *aequicrassus* = even thickness.

Plants in culture consisted of loosely entangled, open alternately branched filaments of a uniform thickness. Branches were often set off at a 90° angle with the first cell wall at some distance from the branching point (Figs 2, 3). The cylindrical cells were 6–7.5 µm in diameter and 2–3 times as long, each of them contained a parietal chloroplast and one prominent pyrenoid (Fig. 4). *Acrochaete*-type hairs were observed at the apical end or on protuberances from intercalary cells (Fig. 5). The plants easily propagated by fragmentation when intercalary cells decayed and broke, so small parts separated from the rest of the plants and continued growth (Fig. 3). Sporangia and germlings were not observed. The alga grew into calcified material such as mollusc shells.

Ulvella dasycala R. Nielsen sp. nov.

Figs 6–10

Fila aperte et alterne ramificata e cellulis cylindricis, 10–13 µm latis, latitudine sua duplo vel triplo longioribus, ad maturitatem crassioribus-rotundis 20–30 µm latis, 1–6 pyrenoides foveatibus. Pili generi Acrochaete peculiare (usque ad 6 merocyticas projecturas praebentes) e cellulis intercalariis crescent. Sporangia exitibus tubularibus armata e cellulis rotundatis formata. Sporae dilatatae unilateraliter germinant et manent partem plantae juvenilis.

HOLOTYPE: Dried sample of strain RN310186-1-1 maintained at Botanical Museum Copenhagen, Denmark as no. CAT 2437.

TYPE LOCALITY: Canary Islands, Lanzarote, Arrieta, north of town; epiphyte on *Champia parvula* (C. Agardh) Harvey.

ETYMOLOGY: Named from Greek *dasys* = thick haired and *calos* = beautiful to characterise the beautiful plants with many hairs.

Young plants consisted of alternately branched filaments of cylindrical cells 10–13 µm in width and 2–3 times as long. Cells in the middle part of older plants became rounded or polygonal, 20–30 µm broad. Prostrate plants developed in contact with a solid substratum (Fig. 6). Vegetative cells had

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Fig. 1. One of 48 equally parsimonious trees based on chloroplast-encoded *tufA* sequences from species of *Ulvella* (Ulvellaceae). Branches collapsing in the strict consensus tree are marked with an asterisk (*). Bootstrap values ($\geq 50\%$) from parsimony analyses and posterior probabilities (≥ 0.50) from Bayesian analyses are given for each node. Values below 50 are indicated by -. *Ochlochaete*, *Percursaria*, *Ruthnielsenia*, two species of *Ulva* and *Ulvaria* (Ulvaceae) comprised the outgroup. The sequences determined in this study are in bold. Scale bar = 0.05 substitutions/site.

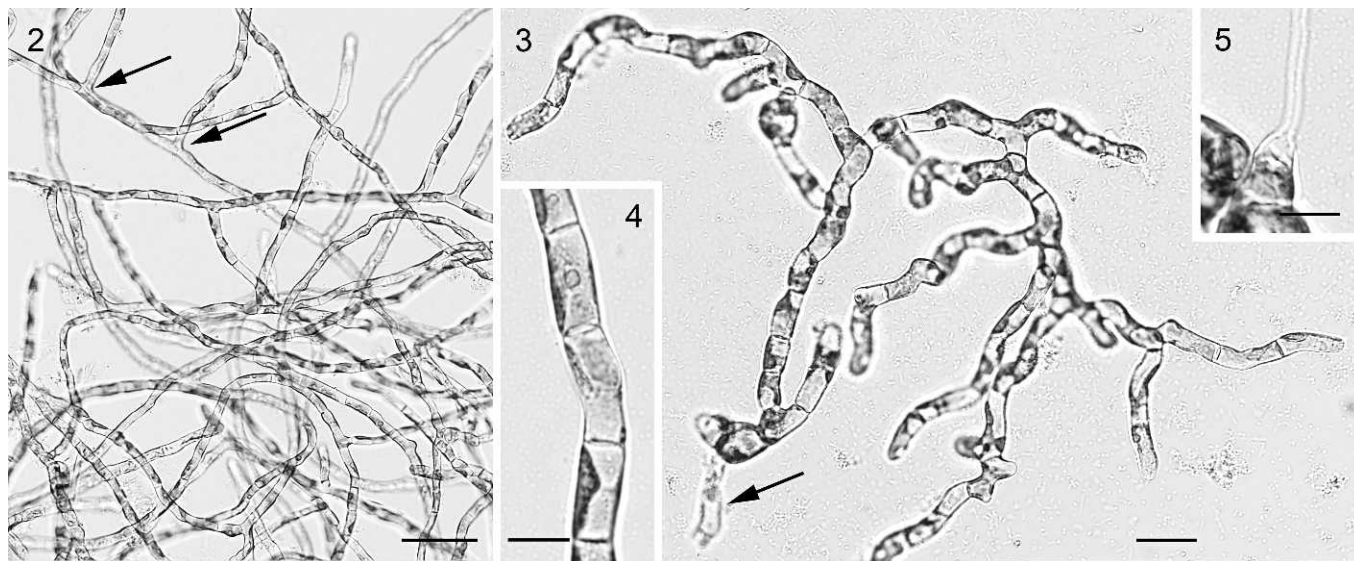
Table 1. Key identification characters for *Ulvella* species in culture: most important characters are in bold.

Species	Growth form ¹	Number of pyrenoids	Width of distal cell (µm)	Length:width ratio of distal cells	Width of middle cells (µm)	Hair position	Sporangia	Spore wall evacuated after germination
<i>U. glabra</i>	a	1	3-3.5	3-8	9-13	no hair	egg- or bottle-shaped	-
<i>U. globocarpitosa</i>	a	1	4.5-9	1.5-3	10-16	distal	bottle-shaped, apical	-
<i>U. wittrockii</i>	a	1	7-10.5		10-20	intercalary	irregular-bottle-shaped	-
<i>U. ramulosa</i>	a	1	4.5-7	2-3	10.5-14	several extensions	bottle-shaped, intercalary rounded cells	-
<i>U. sp. B</i>	a	1	6-8	3-4	15-19	?	?	?
<i>U. pachypes</i>	a	1 big	4.5-5	3-5	12.5-22	intercalary	intercalary bottle-shaped, long neck	-
<i>U. viridis</i>	a	1-2	4.5-5.5	2-4(-8)	10.5-16	intercalary	bottle-shaped, intercalary rounded cells	-
<i>U. vacuospora</i>	a	1-2	5-6	3-5	13-17	intercalary	rounded cells, long exit tube	+
<i>U. endozaica</i>	a	1-2	5-6	3-10(-20)	13-17	intercalary	rounded cells	?
<i>U. leptochaete</i>	a	1-3(-4)	7.5-10.5	2.5(-8)	13-22	several extensions	bottle-shaped, intercalary rounded cells	-
<i>U. sp. A</i>	a	1-3	5-8	4-20	13-16	several extensions	?	?
<i>U. parasitica</i>	a	1-3	7.5-8	3-6	19-23	apical	bottle-shaped, apical	-
<i>U. dasykala</i>	a	1-6	10-13	2-3	20-30	several extensions	intercalary rounded cells, long exit tube	-
<i>U. reticulata</i>	a	2-6	10-14	1.5-3	20-27	distal, coarse	rounded to bottle-shaped exit tube	-
<i>U. porphyrae</i>	b	1	3-4	NA ²	8.5-9.5³	apical	apical, elongate-cylindrical to linear	-
<i>U. inopinata</i>	b	1-2	5-8.5	NA ²	12-17 ³	apical / intercalary	intercalary long elongate-exit tube	-
<i>U. waemii</i>	b	1-2	6-8.5	NA ²	11-16.5 ³	apical	apical/semi apical, elongate-cylindrical	-
<i>U. heteroclada</i>	b	1-3	4-6.5	NA ²	6.5-10(-18) ³	apical	apical cylindrical	-
<i>U. repens</i>	b	1-4	7.5-10	NA ²	17-20³	apical	apical, elongate linear	-
<i>U. pseudorepens</i>	b	3-7	6	NA ²	11-15³	apical	apical, elongate-cylindrical to linear	-
<i>U. marchantiae</i>	c	1-2	4-5.5	1-3	7-9.5	intercalary	bottle-shaped from intercalary cells	-
<i>U. scutata</i>	d	1	5-7	2-3	12-20	central cells	intercalary cells in monostromatic plants	-
<i>U. lens</i>	d	1	3-5	2-4	5-10	central cells	apical in multilayered middle part	-
<i>U. setchellii</i>	d	1	4	2-10	5-8	central cells	apical in multilayered middle part	-
<i>U. sp. C</i>	e	1	7-8		12-15	?	?	?
<i>U. ramosa</i>	e	1-2	3-6	5-15	7.5-9	no hair	intercalary and apical cells	+
<i>U. operculata</i>	e	1-4	3-5	-20	5-11	apical	subcylindrical with a lid	+
<i>U. gigas</i>	e	3-9	10-12	8-10	10-12	apical	apical, elongate-cylindrical	+
<i>U. acquitrassa</i>	f	1	6-7.5	2-3	6-7.5	apical	?	?
<i>U. endostraca</i>	f	1-2	6-10	4-10	12-20	intercalary	intercalary globular cells with exit tube	-
<i>U. testarum</i>	f	1-3(-4)	3-3.5	4-10	9.5-15	no hair	intercalary irregular cells long exit tube	-

¹a = filaments similar, gradual transition from distal, cylindrical cells to rounded or polygonal mid-filament cells; b = heterotrichous; c = rosettes; d = discs; e = flossy (openly branched filaments with long or irregularly shaped cells); f = grows into calcified material.

²Not applicable.

³Width of broad filaments.



Figs 2–5. *Ulvella aequicrassa* strain RN281083-12-4-1.

Fig. 2. Loosely entangled filaments with 90° branch-angles (arrows). Scale bar = 50 µm.

Fig. 3. Fragment from a larger thallus showing continued growth and remnants of decayed cell (arrow). Scale bar = 20 µm.

Fig. 4. Vegetative cells showing the parietal chloroplast and single pyrenoid. Scale bar = 10 µm.

Fig. 5. *Acrochaete*-type hair. Scale bar = 10 µm.

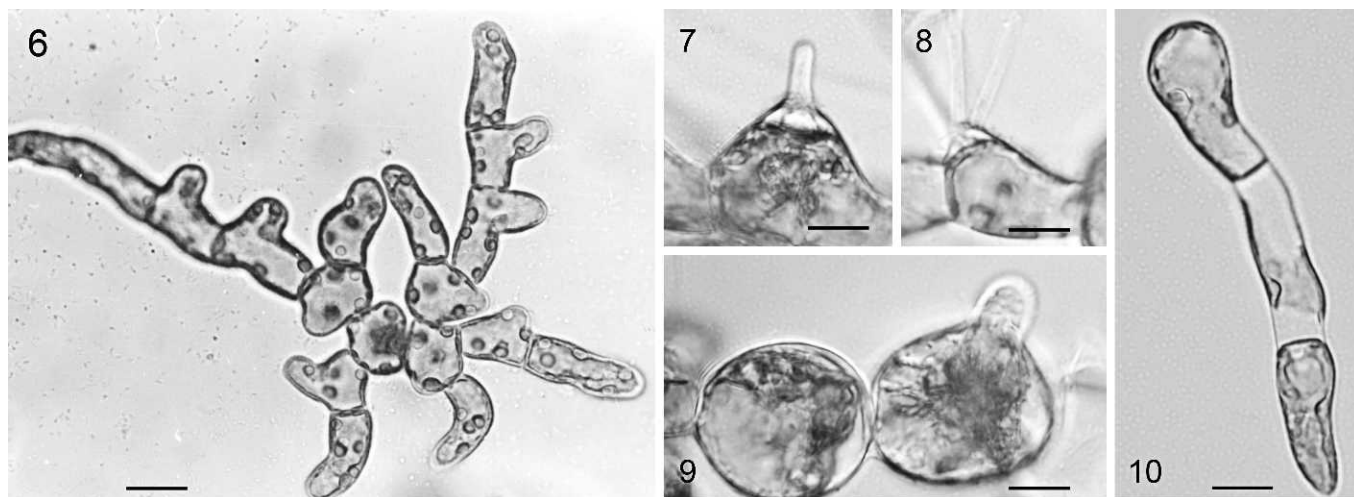
a parietal lobed chloroplast with few perforations and (1–)3–6 pyrenoids. *Acrochaete*-type hairs developed on intercalary cells often with several (up to six) merocytic projections from a single bulbous base (Figs 7, 8). Sporangia developed from any of the intercalary cells; they became slightly larger, 23–32 µm broad, and formed a long exit tube. Otherwise they had the same shape as in the vegetative condition (Fig. 9). Sporangia developed in a unicellular condition when growth was fast. Swarmers developed after sequential divisions. Spores germinated unilaterally after a slight enlargement and remained part of the developing plants (Fig. 10). Also

recorded from Japan, strains (RN00012 08–1 and RN00012 08–2).

***Ulvella gigas* R. Nielsen sp. nov.**

Figs 11–15

Fila aequicrassa aperte et alterne ad angulum 90° ramificata e cellulis cylindricis, 10–12 µm latis, latitudine sua 8plo vel 10plo longioribus, 3–9 pyrenoides foventibus. Pili generi Acrochaete peculiare e cellulis apicalibus crescent. Sporangia cylindracea e cellulis apicalibus formata. Sporangia unilateraliter germinant et evacuantur.



Figs 6–10. *Ulvella dasycala* strain RN310186-1-1.

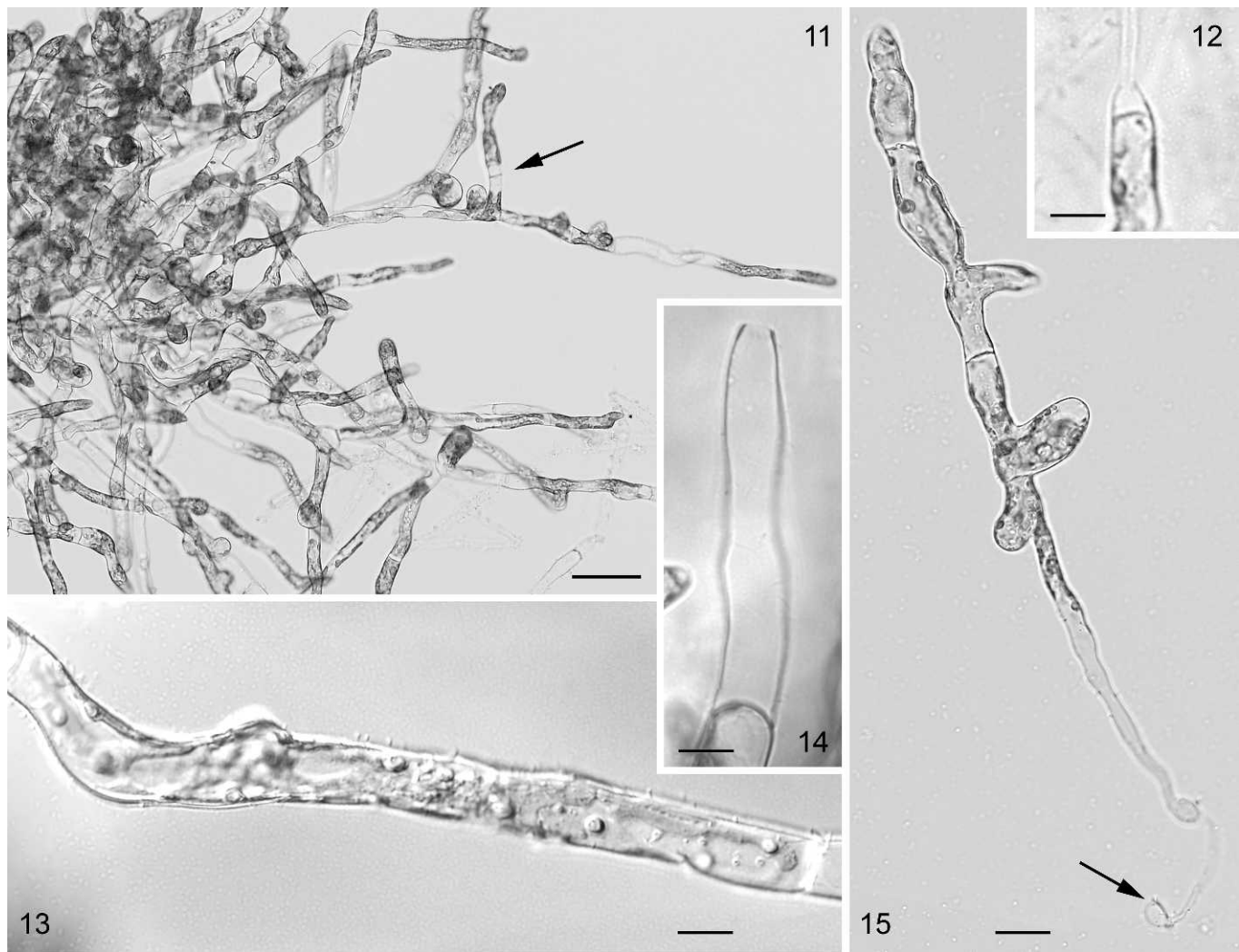
Fig. 6. Prostrate vegetative plant. Vegetative cells each with a parietal chloroplast and several pyrenoids. Scale bar = 20 µm.

Fig. 7. Young *Acrochaete*-type hair. Scale bar = 10 µm.

Fig. 8. *Acrochaete*-type hair with three merocytic extensions. Scale bar = 10 µm.

Fig. 9. Young intercalary sporangium. Scale bar = 10 µm.

Fig. 10. Spore remains part of the three celled young plant. Scale bar = 10 µm.



Figs 11–15. *Ulvella gigas* strain RN00019 01–1.

Fig. 11. Part of a vegetative plant forming loosely entangled filaments of a uniform thickness. Note the first cell walls at some distance from branching points (arrow). Scale bar = 50 μ m.

Fig. 12. Apical *Acrochaete*-type hair. Scale bar = 10 μ m.

Fig. 13. Almost reticulate chloroplast with several pyrenoids in a vegetative cell. Scale bar = 10 μ m.

Figs 14. Empty sporangium. Scale bar = 10 μ m.

Fig. 15. Few-celled young plant with an evacuated spore-wall and germination tube (arrow). Scale bar = 20 μ m.

HOLOTYPE: Dried sample of strain RN00019 01–1 maintained at Botanical Museum Copenhagen, Denmark as no. CAT 2442.

TYPE LOCALITY: Japan, Shizuoka Prefecture, Nabeta Bay, Ohra, Shimoda. Endophytic in *Sargassum ringoldianum* Harvey.

ETYMOLOGY: Named from Latin *gigas* = a giant.

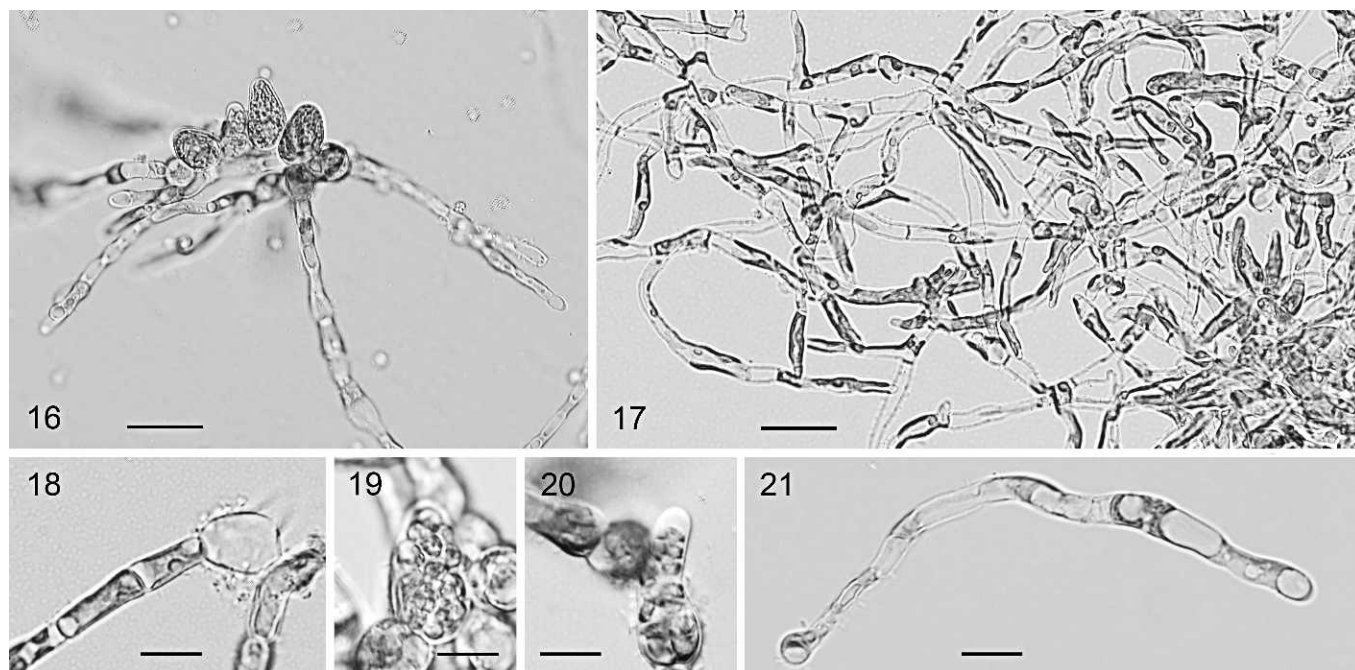
Plants in culture formed loosely entangled, alternately branched filaments of a uniform thickness (Fig. 11). The cylindrical cells measured 10–12 μ m in width and were 8–10 times as long. The first cell wall of a branch often developed at some distance from the branching point thus many cells had long protuberances and looked L- or T-shaped in optical section (Fig. 11). *Acrochaete*-type hairs were apical or on protuberances from intercalary cells (Fig. 12). Vegetative cells contained a parietal lobed and perforated to almost

reticulate chloroplast with three to nine pyrenoids (Fig. 13). Sporangia developed from apical cells, they retained the shape apart from a long conical top (Fig. 14). At maturity a prominent ‘mucilaginous’ plug occurred at the apex and after sequential division of the cytoplasm 16 quadriflagellate zoospores were formed. The settled spores germinated unilaterally; the evacuated spore-wall and a germination tube were present in young plants (Fig. 15).

***Ulvella glabra* R. Nielsen sp. nov.**

Figs 16–21

Fila aperte et alterne ramificata e cellulis cylindricis, 3–3.5 μ m latis, latitudine sua triplo vel 8plo longioribus, ad maturitatem crassioribus-rotundis 9–13 μ m latis, 1 pyrenoiden foventibus. Sporangia oviformia e cellulis rotundatis formata. Sporae unilateraliter germinant et manent partem plantae juvenilis.



Figs 16–21. *Ulvella glabra* strain RN161183-1-1.

Fig. 16. Morphology of a small almost mature plant. Middle cells young egg-shaped sporangia. Scale bar = 20 μm .

Fig. 17. Part of a vegetative plant of alternately branched filaments of cylindrical cells. Scale bar = 10 μm .

Fig. 18. Vegetative cells showing the parietal chloroplast and single pyrenoid. Scale bar = 10 μm .

Figs 19, 20. Mature egg and bottle-shaped sporangia. Scale bars = 10 μm .

Fig. 21. Young plant. The original spore part of the plant. Scale bar = 10 μm .

HOLOTYPE: Dried sample of strain RN161183-1-1 maintained at Botanical Museum Copenhagen, Denmark as no. CAT 2443.

TYPE LOCALITY: Chile, Coquimbo, Bahia Herradura de Guayacan, low littoral on pebble.

ETYMOLOGY: Named from Latin *glaber* = glabrous.

Plants in culture formed bushes of openly branched, uniseriate filaments. The branching was irregularly alternate, in some plants sparse, and these plants became mature at an early stage (Fig. 16), other plants grew large before maturation (Fig. 17). Young plants and distal branches of older plants had cylindrical cells 3–3.5 μm wide and 3–8 times as long. The cells in the middle part of older plants were rounded to globular, 9–13 μm across. Vegetative cells contained a parietal, slightly lobed chloroplast with a single pyrenoid (Fig. 18). Sporangia formed from the rounded cells; they became egg-shaped or bottle-shaped and were slightly larger than in the vegetative condition (Figs 19, 20). Pyriform zoospores with four flagella, a red eyespot, and a basal chloroplast have been observed, they measured 3 by 5 μm . Spores germinated unilaterally, and remained part of the developing plants (Fig. 21). Many young plants often settled around older plants resulting in big aggregations forming a dense mat and resembled a pseudoparenchyma. Hairs have not been observed. Unusually large cells with several pyrenoids were supposed to be a culture artefact.

***Ulvella globocaespitosa* R. Nielsen sp. nov.**

Figs 22–24

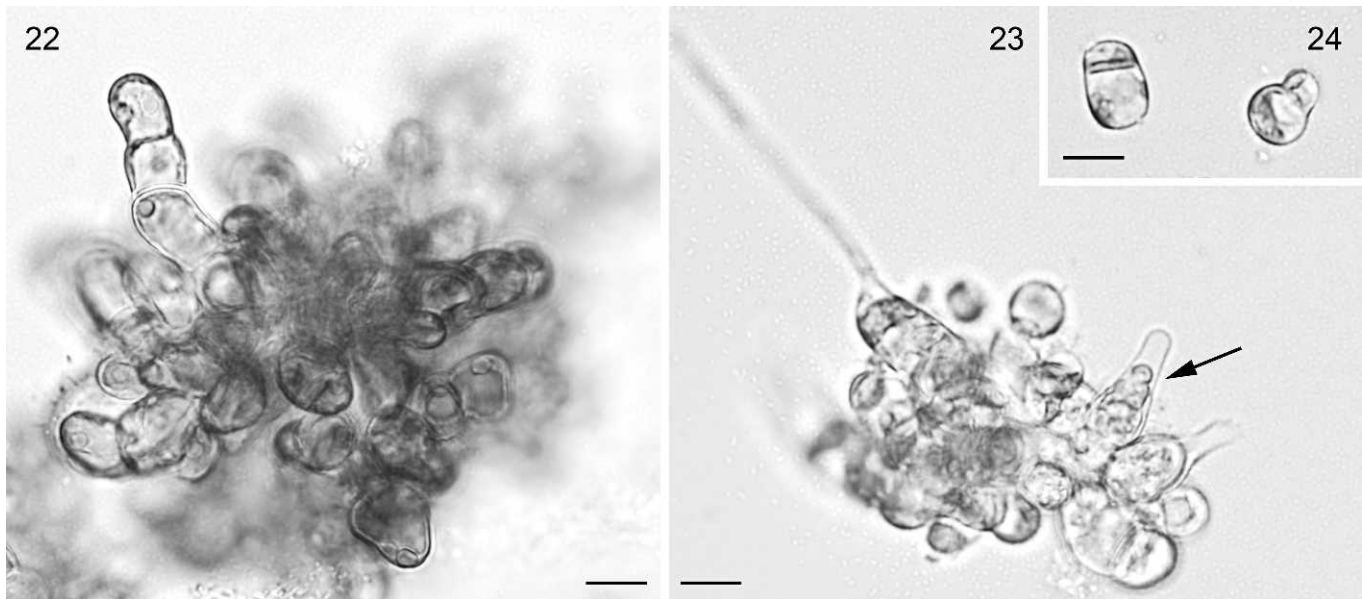
Thallus est caespes e cellulis cylindricis, 10–16 μm latis, latitudine sua uno vel duplo longioribus dense compositus. Cellula 1 pyrenoiden fovens. Pili generi Acrochaete peculiare e cellulis apicalibus crescent. Sporangia exitibus conoideis armata e cellulis apicalibus formantur. Sporae unilateraliter germinant et manent partem plantae juvenilis.

HOLOTYPE: Dried sample of strain RN00019 03–2 maintained at Botanical Museum Copenhagen, Denmark as no. CAT 2444.

TYPE LOCALITY: Japan, Ohra, Nabeta Bay, Shimoda, Shizuoka Prefecture associated with *Padina aborescens* Holmes.

ETYMOLOGY: Named from Latin *globus* = ball and *caespes* = tuft.

The plants had an almost globular to ball-shaped external morphology and consisted of few-celled, radiating filaments (Figs 22, 23). The cells were short, cylindrical, or rounded to globular and measured 10–16 μm across. A few irregularly alternately branched narrow filaments developed especially when the plants were not transferred to fresh medium. The cylindrical cells of these filaments measured 4.5–9 μm in diameter and were 1.5–3 times as long. Vegetative cells contained a parietal chloroplast with one pyrenoid (Fig. 22). *Acrochaete*-type hairs developed on apical cells (Fig. 23). Sporangia became bottle-shaped with a conical top or a short exit tube (Fig. 23). The settled spores germinated unilaterally after slight enlargement and remained part of the developing plant (Fig. 24).



Figs 22–24. *Ulvella globocaespitosa* strain RN00019 03–2.

Fig. 22. Vegetative plant almost globular in shape with radiating filaments of short vegetative cells, with single pyrenoid. Scale bar = 10 μ m.

Fig. 23. Plant with *Acrochaete*-type hair and bottle-shaped sporangium (arrow). Scale bar = 10 μ m.

Fig. 24. Germlings. Scale bar = 10 μ m.

***Ulvella inopinata* R. Nielsen sp. nov.**

Figs 25–32

Fila aperte alterne et ramificata e cellulis cylindricis, 5–8.5 μ m latis, latitudine sua duplo vel quatuor longioribus, ad maturitatem crassioribus-rotundis 12–17 μ m latis, caespites densos formatis. Cellula 1–2 pyrenoides fovens. Pili generi Acrochaete peculiare e cellulis apicalibus et intercalariis crescent. Sporangia exitibus longis conoideis armata e cellulis latis formantur. Sporae unilateraliter germinant et manent partem plantae juvenilis.

HOLOTYPE: Dried sample of strain RN00015 01–2 maintained at Botanical Museum Copenhagen, Denmark as no. CAT 2445.

TYPE LOCALITY: Japan, Awaji Island, Yura. Associated with *Chondrus ocellatus* Holmes.

ETYMOLOGY: Named from Latin, *inopinatus* = unexpected, refers to the unexpectedly irregularly placed tufts of broad filaments and the *Acrochaete*-type hairs developing on all kinds of vegetative cell.

Young plants in culture were openly branched filaments of cylindrical cells 5–8.5 μ m in width and 2–4 times as long. The alternate branches had the first cell wall at some distance from the branching point (Fig. 25). The middle cells of older plants became rounded, 12–17 μ m broad (Fig. 26). Similar cells developed at irregular intervals in larger plants and divided to form tufts of few-celled, broad filaments or pseudoparenchymatous cell masses (Figs 27, 28). Vegetative cells had a parietal lobed chloroplast with one to two pyrenoids (Figs 25, 26). *Acrochaete*-type hairs were observed both on apical and intercalary cells (Figs 29–31). Sporangia developed from cells of the broad filaments; they obtained an elongated shape with a long exit tube (Fig. 32). Spores germinated unilaterally and remained part of the developing plants.

***Ulvella pachypes* R. Nielsen sp. nov.**

Figs 33–39

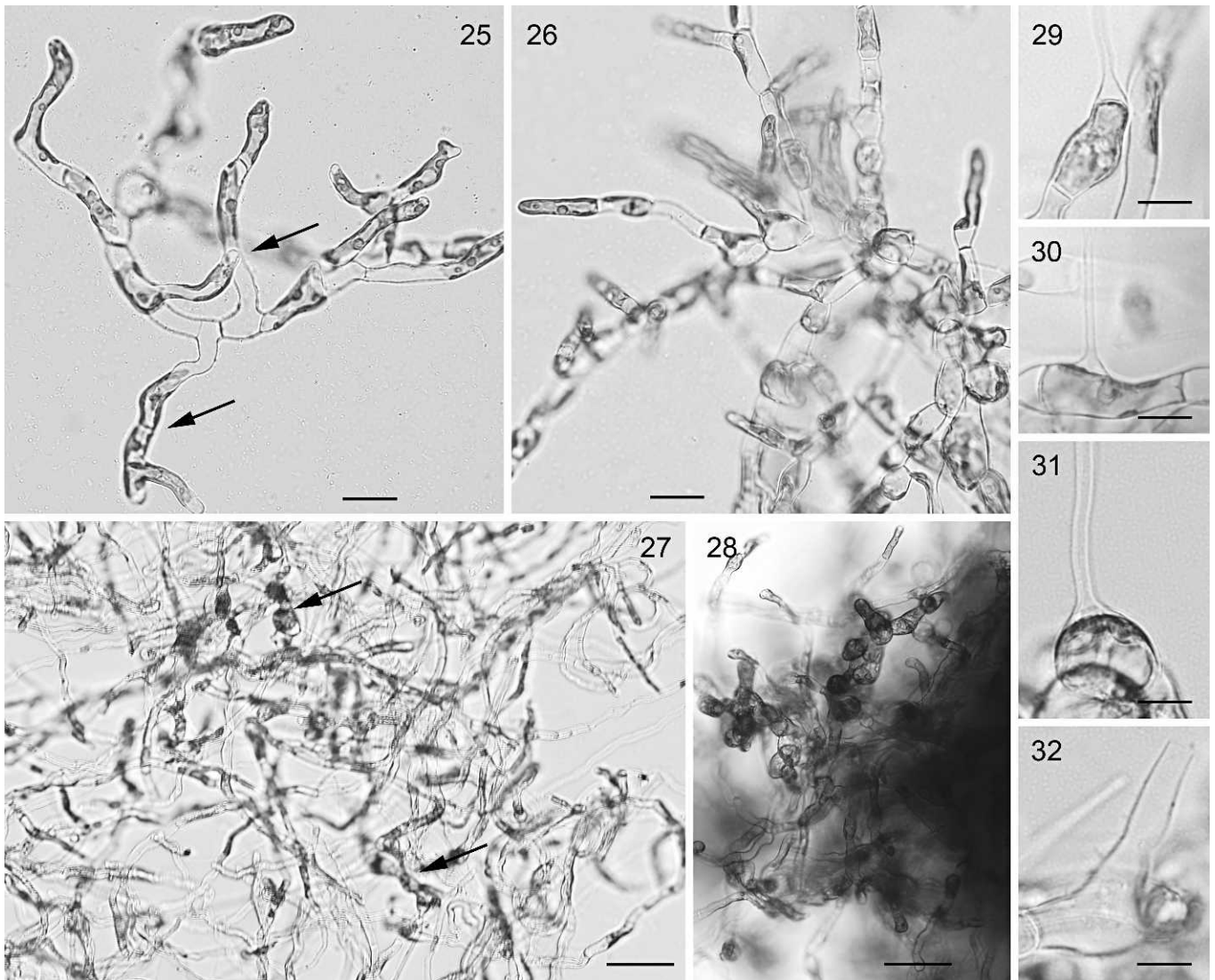
Fila aperte et alterne ramificata e cellulis cylindricis, 4.5–5 μ m latis, latitudine triplo vel 4plo longioribus, ad maturitatem crassioribus-rotundis 12.5–22 μ m latis, unam magnam pyrenoidem foventibus. Pili generi Acrochaete peculiare cum bulbo basali sua latitudine elatiori, e cellulis intercalariis crescent. Sporangia exitibus tubularibus armata e cellulis rotundatis formata. Sporae dilatatae unilateraliter germinant et manent partem plantae juvenilis.

HOLOTYPE: Dried sample of strain 230888-2-2 maintained at Botanical Museum Copenhagen, Denmark as no. CAT 2440.

TYPE LOCALITY: Denmark, Endelave, on pebble at 11-m depth.

ETYMOLOGY: Named from Greek *pachys* = thick, *pes* = foot, refers to the large bulbous base of the hairs.

Plants in culture formed openly branched bushes (Fig. 33). Branches were often set off at 90° angle with the first cell wall at some distance from the branching point (Fig. 34). The cylindrical cells were 4.5–5 μ m in diameter and 3–5 times as long. The middle cells became rounded 12.5–22 μ m across or irregular in shape when two or more branches initiated from a single cell (Fig. 34). Vegetative cells contained a parietal slightly lobed chloroplast with small perforations and 1(–2) unusual large pyrenoids 3–4 μ m in diameter (Figs 35, 36). *Acrochaete*-type hairs developed from intercalary cells. The large basal swellings were 7–8 μ m tall and 5–5.5 μ m broad, it looked as if they stood on the vegetative cells (Figs 36, 37). Sporangia developed from intercalary rounded cells; they became bottle-shaped with a long neck and contained quadriflagellate zoospores at maturity (Fig. 38). The settled swimmers germinated unilaterally and remained part of the developing plants (Fig. 39).



Figs 25–32. *Ulvella inopinata* strain RN00015 01–2.

Fig. 25. Vegetative young plant the cells showing the parietal chloroplast and single pyrenoid. The first cell wall at some distance from branching point (arrows). Scale bar = 20 μ m.

Fig. 26. Slightly older plant, the middle cells rounded. Scale bar = 20 μ m.

Fig. 27. Large openly branched plant with few arbitrary placed clusters of rounded cells (arrows). Scale bar = 50 μ m.

Fig. 28. Middle part of a large plant very dense and consists of round broad cells. Scale bar = 50 μ m.

Figs 29–31. *Acrochaete*-type hairs, developed from apical, intercalary cylindrical or rounded cells. Scale bars = 10 μ m.

Fig. 32. Empty sporangium. Scale bar = 10 μ m.

***Ulvella pseudorepens* R. Nielsen sp. nov.**

Figs 40–44

Morphologia et reproductio Ulvella repens similis. Fila caespitum 11–15 μ m lata, cellulae filorum 3–6 pyrenoides foventes.

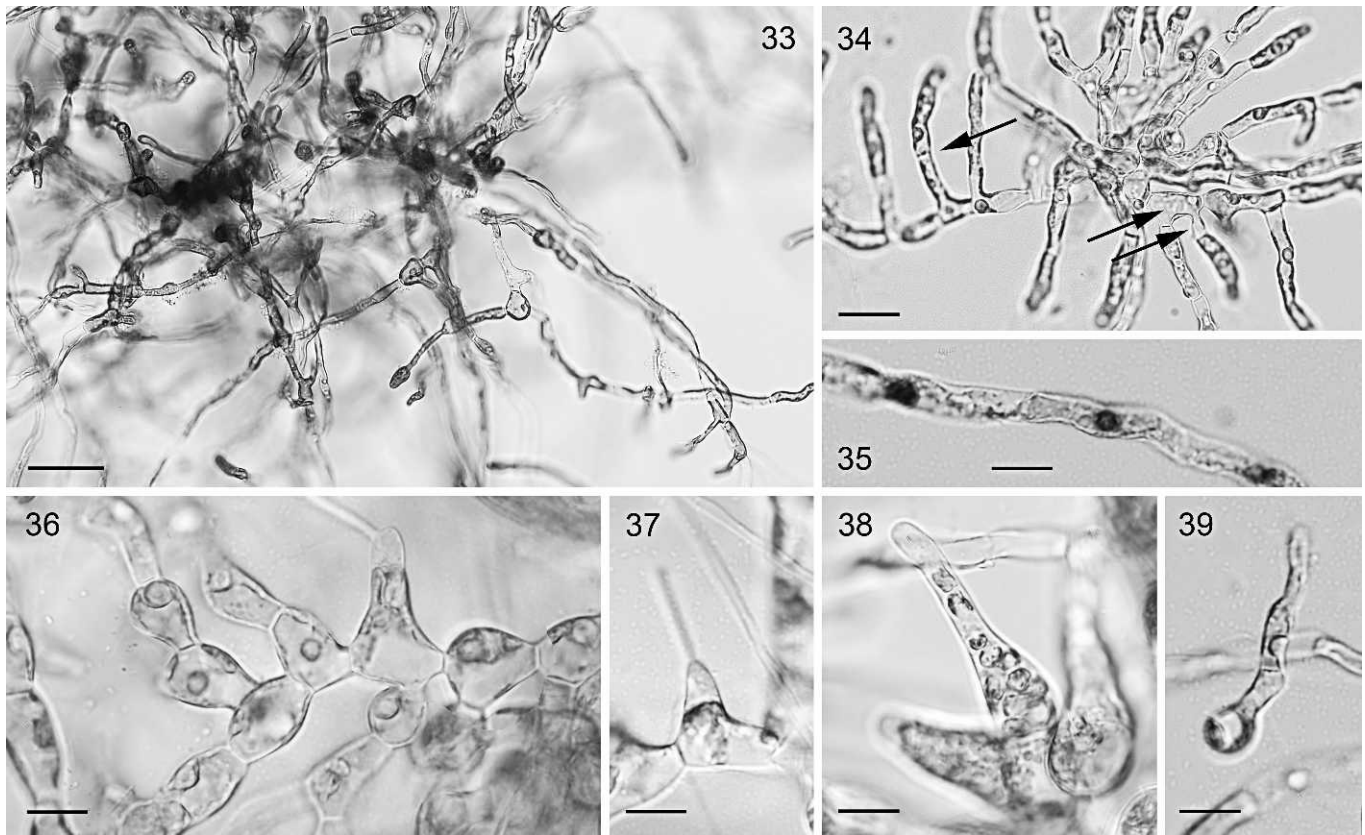
HOLOTYPE: Dried sample of strain RN071076a maintained at Botanical Museum Copenhagen, Denmark as no. CAT 2446.

TYPE LOCALITY: Denmark, Northern Kattegat, Lyngså Strand. Endophyte among paraphyses of drift *Chorda filum* (Linnaeus) Stackhouse.

ETYMOLOGY: Name reflects the similarity to *U. repens*.

Plants in culture formed almost ball-shaped bushes of radiating, uniseriate filaments or tufts of upright branches

from prostrate filaments (Fig. 40). The cylindrical cells of broad filaments were 11–15 μ m in width and 3–6 times as long, cells in the middle basal part of plants obtained an irregular rounded shape and measured 25–40 μ m across (Fig. 41). Filaments about 6 μ m wide developed at the base of the tufts as runners with broad, upright branches and slender filaments in the opposite directions (Fig. 42). Vegetative cells of upright and narrow filaments contained a parietal lobed chloroplast with small perforations and three to seven pyrenoids (Fig. 43). *Acrochaete*-type hairs developed at the apical end of upright filaments (Fig. 44) or on protuberances from broad intercalary cells. Sporangia formed from similar cells and became elongate linear or looked L-shaped in optical section when formed from intercalary cells. At



Figs 33–39. *Ulvella pachypes* strain RN230888-2-2.

Fig. 33. Part of a large vegetative plant. Scale bar = 50 μ m.

Fig. 34. Part of a young vegetative plant almost 90° branch angles and first cell wall at some distance from branching point (arrow). Middle cells with an irregular shape (two arrows). Scale bar = 20 μ m.

Figs 35, 36. Vegetative cells with one large pyrenoid per cell, cells in Fig. 35 stained with iodine. Scale bars = 10 μ m.

Fig. 37. Young *Acrochaete*-type hair. Note the unusually tall bulbous base. Scale bar = 10 μ m.

Fig. 38. Bottle-shaped sporangium with a long neck. Scale bar = 10 μ m.

Fig. 39. Few-celled plant, original spore part of the plant. Scale bar = 10 μ m.

maturity they contained biflagellate, pyriform swimmers, either small, pale ones (3 by 3.5 μ m) or larger, green ones (6–6.5 by 6.5–7 μ m). Both size classes had a red eyespot. Plants of the strains studied have previously been referred to *Acrochaete repens*, e.g. strain RN071076a illustrated by Nielsen (1979, figs 1E, 1J).

The species can be distinguished from *U. repens* by the larger number of pyrenoids per cell, and the different *tufA* gene sequences. *Ulvella pseudorepens* formed a well-supported clade with *U. reticulata* (Printz) R. Nielsen, C.J. O’Kelly & B. Wysor *comb. nov.* (BS = 99%, pp = 1.0) and *U. repens* another well-supported clade with *U. parasitica* (Oltmanns) R. Nielsen, C.J. O’Kelly & B. Wysor *comb. nov.* (BS = 93%, pp = 1.0) (Fig. 1).

***Ulvella vacuospora* R. Nielsen sp. nov.**

Figs 45–51

Fila aperte et alterne ramificata e cellulis cylindricis, 5–6 μ m latis, latitudine duplo vel 4plo longioribus, ad maturitatem crassioribus rotundis 13–17 μ m latis, 1–2 pyrenoides foventibus. Pili generi Acrochaete peculiare e cellulis intercalariis crescent. Sporangia

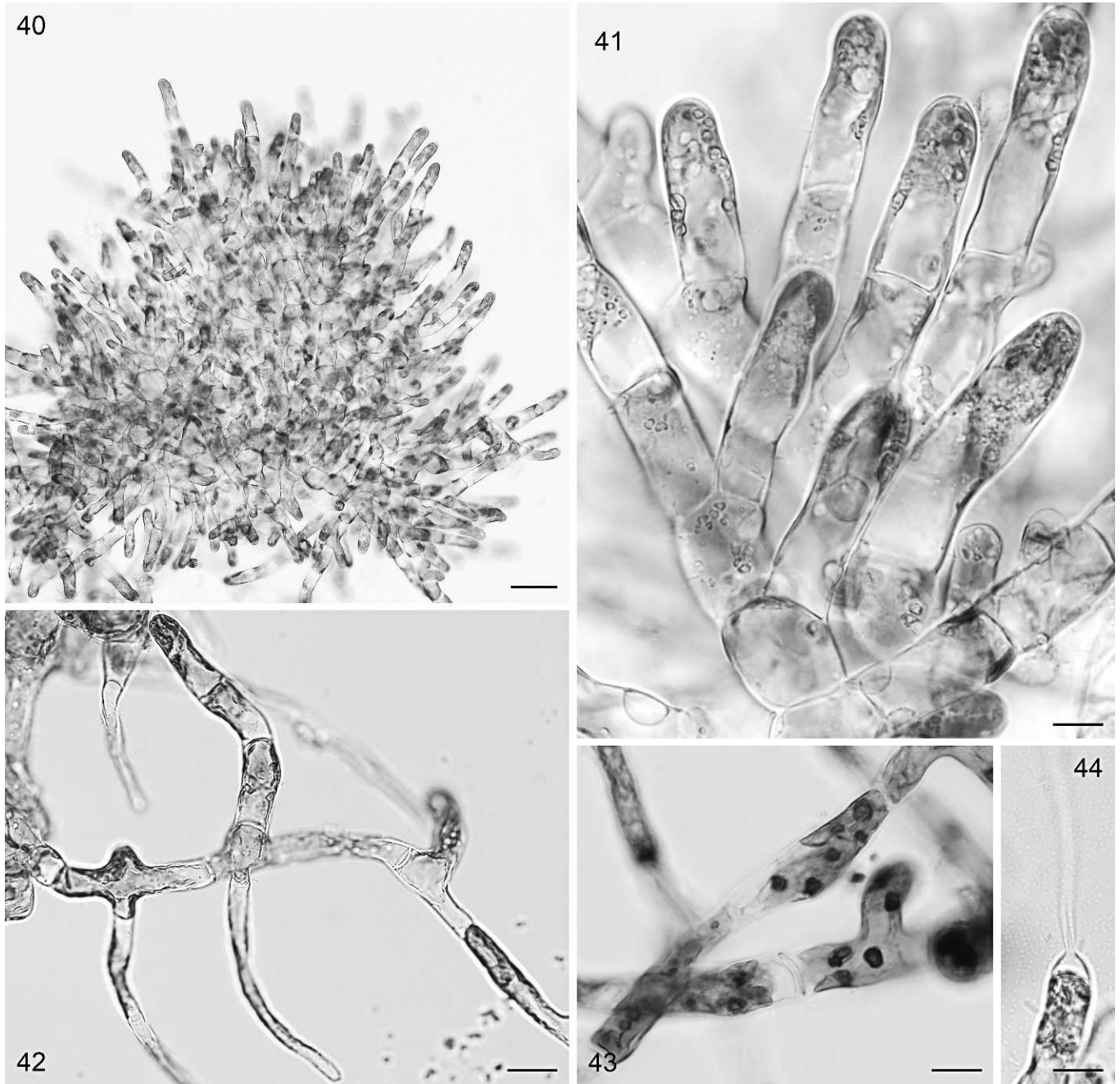
exitibus tubularibus armata e cellulis intercalariis formantur. Sporae unilateraliter germinant et evacuantur.

HOLOTYPE: Dried sample of isolate RN280186-1-4 maintained at Botanical Museum Copenhagen, Denmark as no. CAT 2447.

TYPE LOCALITY: Canary Islands, Lanzarote, Arrieta, on the shell of an intertidal snail.

ETYMOLOGY: Named from Latin, *vacuus* = empty and *spora* = spore referring to the evacuated spore wall seen in young plants.

Plants in culture consisted of irregularly, alternately branched filaments. The distal ones were openly branched and had cylindrical cells 5–6 μ m in width and 3–5 times as long (Fig. 45). The middle part of plants was denser; cells became rounded to almost globular and measured 13–17 μ m across. A pseudoparenchymatous basal layer formed in contact with a solid substratum (Fig. 46, 47). *Acrochaete*-type hairs occurred on short intercalary cells (Fig. 48). Vegetative cells had a parietal lobed chloroplast with some perforations and one pyrenoid (Fig. 45). Sporangia developed from any apical or intercalary cell and kept the same shape and size apart from an exit tube (Figs 49, 50). Spores



Figs 40–44. *Ulvella pseudorepens* strain RN071076a.

Fig. 40. Large vegetative plant with a tufted almost ball-shaped morphology. Scale bar = 50 μm .

Fig. 41. Cells in distal part of a tuft showing the parietal chloroplast with several pyrenoids. Scale bar = 10 μm .

Fig. 42. Runner with upright broad branches and slender ones in opposite direction. Scale bar = 20 μm .

Fig. 43. Vegetative cells with many pyrenoids, stained with iodine. Scale bar = 10 μm .

Fig. 44. Apical *Acrochaete*-type hair. Scale bar = 10 μm .

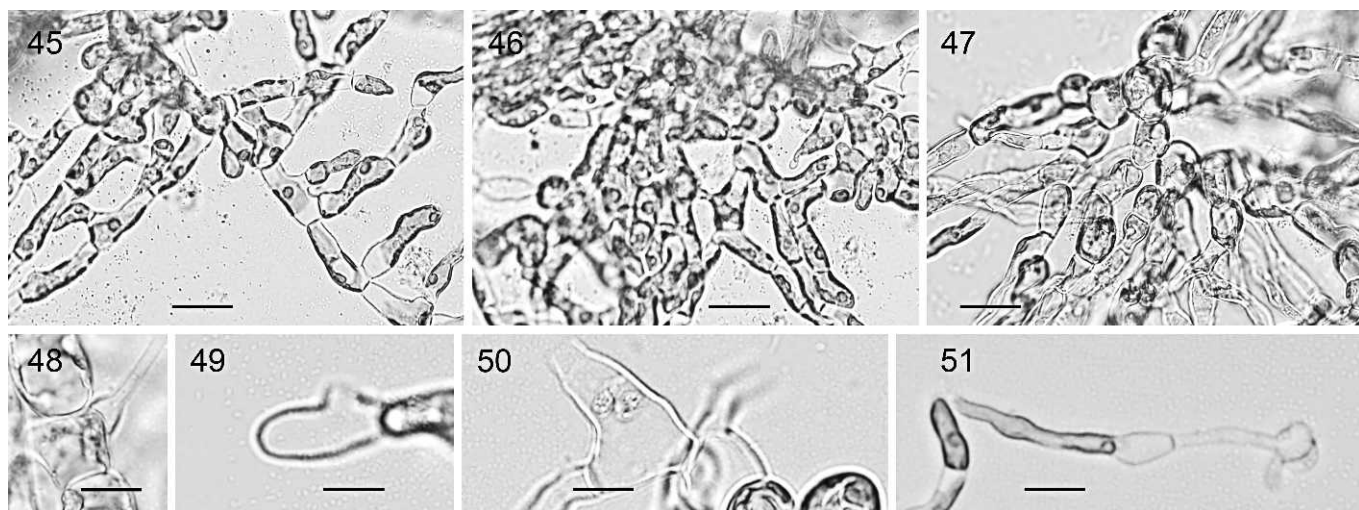
germinated unilaterally, an evacuated spore-wall and germination tube were present in young plants (Fig. 51).

The *tufA* gene sequences were identical in plants of the strains RN280186-1-4 and RN00019 08-1 but the one for RN00015 02-3 differed *c.* 0. 4%. The middle cells of the latter strain were more rounded than in the other two strains (Fig. 47). We considered this intraspecific variation.

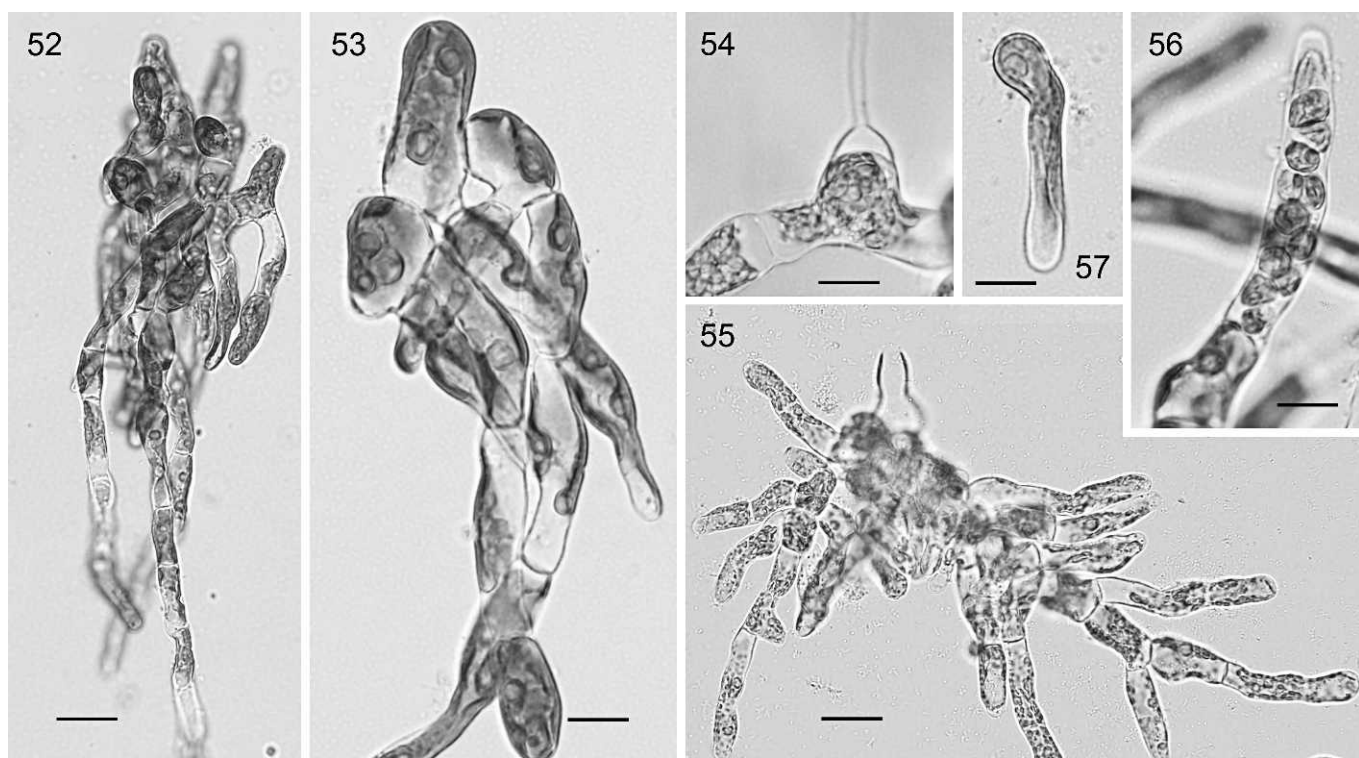
***Ulvella waernii* R. Nielsen sp. nov.**

Figs 52–57

Thallus heterotrichus, e caespitis densis e cellulis cylindricis, 11–16.5 μm latis, latitudine sua uno vel duplo longioribus constructis, et filis e basi caespitum orientibus, aperte alterne ramificatis, cellulis cylindricis, 6–8.5 μm latis latitudine sua 5plo vel 10plo longioribus junctis, compositus. Cellula 1–2 (–3) pyrenoides fovens. Pili generi



Figs 45–51. *Ulvella vacuospora* Figs 45, 46, 49–51 strain RN280186-1-4. Fig. 47. Strain RN00015 02–3.
 Fig. 45. Distal part of a large vegetative plant cells showing the parietal chloroplast with single pyrenoid. Scale bar = 20 μ m.
 Fig. 46. Middle part of a vegetative plant form prostrate pseudoparenchyma. Scale bar = 20 μ m.
 Fig. 47. Vegetative plant with rounded cells in middle part. Scale bar = 10 μ m.
 Fig. 48. *Acrochaete*-type hair on a short intercalary cell. Scale bar = 10 μ m.
 Fig. 49. Empty apical sporangium. Scale bar = 10 μ m.
 Fig. 50. Empty intercalary sporangia. Scale bar = 10 μ m.
 Fig. 51. Germling, an evacuated spore-wall and germ tube present. Scale bar = 10 μ m.



Figs 52–57. *Ulvella waernii* strain RN182981.
 Fig. 52. Vegetative plant consists of a tuft of broad upright branches and slender filaments growing down. Scale bar = 20 μ m.
 Fig. 53. Young plant vegetative cells showing the parietal chloroplast and one to three pyrenoids. Scale bar = 10 μ m.
 Fig. 54. *Acrochaete*-type hair on a protuberance of an intercalary broad cell. Scale bar = 10 μ m.
 Fig. 55. Prostrate plant with an empty sporangium. Scale bar = 20 μ m.
 Fig. 56. Mature sporangium. Scale bar = 10 μ m.
 Fig. 57. Germling. Scale bar = 10 μ m.

Acrochaete peculiare e *cellulis apicalibus crescent*. Sporangia exitibus longis conoideis armata e *cellulis apicalis* formantur. Sporae unilateraliter germinant et manent partem plantae juvenilis.

HOLOTYPE: Dried sample of isolate RN182981 maintained at Botanical Museum Copenhagen, Denmark as no. CAT 2448.

TYPE LOCALITY: Finland, Tvärminne, Shore at Zoological Station. Epiphyte on lower leaf sheath of *Phragmites australis* (A.J. Cavillier) Trin.

ETYMOLOGY: Named in honour of Mats Wærn. He turned (RN's) attention to this species and passed on his enthusiasm and careful studies of microfilamentous algae of the Baltic Sea to colleagues in the Nordic countries.

The middle part of plants formed tufts of sparsely branched, broad filaments, surrounded by irregularly alternately branched narrow filaments. The cylindrical cells of the broad filaments were 11–16.5 µm in width and 1.5–2 times as long, the narrow filaments were 6–8.5 µm in width and the cells were 5–10 times as long (Figs 52–53). *Acrochaete*-type hairs developed at the apical end of broad filaments or on protuberances of broad cells (Fig. 54). A pseudoparenchymatous basal layer developed in contact with a solid substratum (Fig. 55), with broad as well as narrow filaments similar to those of other plants. Vegetative cells had a parietal chloroplast with 1–2(–3) pyrenoids (Fig. 53). Sporangia developed from middle cells in attached plants (Fig. 55) or they were located at the apical end of broad filaments and got an elongated almost cylindrical shape (Fig. 56). Spores germinated unilaterally and remained part of the developing plants (Fig. 57).

We examined 18 previously described species as part of this study, and our observations are presented in the supplemental materials (supplemental text, Figs S2–S66). Three taxa were referred to as *Ulvella* sp. A, *Ulvella* sp. B, and *Ulvella* sp. C (see supplemental text, Figs S67–S71). When a holotype was not designated in the original publication, and no subsequent lectotype or neotype was designated, we designated a lectotype from the original material. Because the lectotype could not be analyzed using molecular methods, we further designated an epitype that was based upon material used to collect our molecular data. The following nomenclatural changes are proposed:

***Ulvella endostraca* (R. Nielsen) R. Nielsen, C.J. O'Kelly & B. Wysor comb. nov.**

Figs S2–S5

BASIONYM: *Acrochaete endostraca* R. Nielsen (in *New Zealand Journal of Botany* 25: 426. 1987).

***Ulvella endozoica* (Goldberg, Makemson & Colley) R. Nielsen, C.J. O'Kelly & B. Wysor comb. nov.**

Fig. 26

BASIONYM: *Entocladia endozoica* Goldberg, Makemson & Colley (in *Biological Bulletin* 166: 379. 1984).

NOMENCLATORIAL SYNONYM: *Acrochaete endozoica* (Goldberg, Makemson & Colley) Wynne 1986.

***Ulvella heteroclada* (Correa & R. Nielsen) R. Nielsen, C.J. O'Kelly & B. Wysor comb. nov.**

BASIONYM: *Acrochaete heteroclada* Correa & R. Nielsen (in Correa et al. in *Journal of Phycology* 24: 529. 1988).

***Ulvella lens* P.L. Crouan & H.M. Crouan**

Figs S6–S7

This taxon was described by Crouan and Crouan (1859) and our observations were reported in the supplemental materials.

***Ulvella leptochaete* (Huber) R. Nielsen, C.J. O'Kelly & B. Wysor comb. nov.**

Figs S8–S15

BASIONYM: *Endoderma leptochaete* Huber (in *Annales des Sciences Naturelles, Botanique* ser. 7, 16: 319. 1892a).

NOMENCLATORIAL SYNONYMS: *Ectochaete leptochaete* (Huber) Wille 1909. Type of *Ectochaete* acc. to Hoek 1965. *Acrochaete leptochaete* (Huber) R. Nielsen 1983. *Phaeophila leptochaete* (Huber) R. Nielsen 1972.

LECTOTYPE: Original illustration by Huber (1892a, p. XV, figs 1–9) selected by Burrows (1991, p. 108) is ambiguous because it illustrates several plants of different origin.

EPITYPE DESIGNATED HERE: Dried material of strain RN041878 maintained at Botanical Museum Copenhagen, Denmark as no. CAT 2439.

HETEROTYPIC SYNONYM: *Acrochaete polymorpha* (L. Moewus) Nielsen 1988.

BASIONYM: *Ectochaete polymorpha* L. Moewus (in *Botaniska Notiser* 1949: 303. 1949).

NOMENCLATORIAL SYNONYMS: *Phaeophila polymorpha* (L. Moewus) R. Nielsen 1972. *Entocladia moewusiae* ('moewusae') (L. Moewus) O'Kelly & Yarish 1981.

LECTOTYPE DESIGNATED HERE: Original illustration by Moewus (1949, fig. 22).

***Ulvella marchantiae* (Setchell & N.L. Gardner) R. Nielsen, C.J. O'Kelly & B. Wysor comb. nov.**

Figs S16–S22

BASIONYM: *Pringsheimia marchantiae* Setchell & N.L. Gardner (in *Proceedings of the California Academy of Sciences*. Ser. 4, 12: 720. 1924).

NOMENCLATORIAL SYNONYMS: *Pringsheimiella marchantiae* (Setchell & N.L. Gardner) Schmidt & Petrak in Schmidt 1935. *Acrochaete marchantiae* (Setchell & N.L. Gardner) R. Nielsen & McLachlan 1986a.

HOLOTYPE: UC #221049 (see Setchell & N.L. Gardner 1924).

***Ulvella operculata* (Correa & R. Nielsen) R. Nielsen, C.J. O'Kelly & B. Wysor comb. nov.**

BASIONYM: *Acrochaete operculata* Correa & R. Nielsen (in Correa et al. (in *Journal of Phycology* 24: 531. 1988).

***Ulvella parasitica* (Oltmanns) R. Nielsen, C.J. O’Kelly & B. Wysor comb. nov.**
Figs S23–S29

BASIONYM: *Acrochaete parasitica* Oltmanns (in *Botanische Zeitung*. Berlin 52: 208. 1894).

LECTOTYPE DESIGNATED HERE: Original illustration by Oltmanns (1894, fig. 6).

EPITYPE DESIGNATED HERE: Dried material of strain RN060972 maintained at Botanical Museum Copenhagen, Denmark as no. CAT 2451.

Original material (Svedelius 1901) of *Acrochaete parasitica* f. *zosteræ* Svedelius hardly exists; it has not been possible to trace it in the herbaria at Lund (LD) (P. Lassen, personal communication, 1985) or Uppsala (UPS) (S. Ryman, personal communication, 1985). The sporangia illustrated and mentioned in the text have long exit tubes; this and the hairs with coarse merocytic extensions from rounded to egg-shaped (probably vegetative) cells are both characteristic features for *Ochlochaete hystrix* (Nielsen 1978). This species is a very common epiphyte in the Baltic Sea, and recorded in association with *Zostera marina* Linnaeus (Wærn 1952, Nielsen 1988). We therefore have no hesitation to consider *A. parasitica* f. *zosteræ* a synonym of *O. hystrix*.

***Ulvella porphyrae* (Feldmann) R. Nielsen, C.J. O’Kelly & B. Wysor comb. nov.**
Figs S30–S37

BASIONYM: *Pseudodictyon* (?) *porphyrae* Feldmann (in *Bulletin de la Société d’Histoire Naturelle de l’Afrique du Nord* 22: 200. 1931).

LECTOTYPE DESIGNATED HERE: Slide preparation maintained at PC, number PC0719071.

***Ulvella ramosa* (N.L. Gardner) R. Nielsen, C.J. O’Kelly & B. Wysor comb. nov.**
Figs S38–S44

BASIONYM: *Endophyton ramosum* N.L. Gardner (in *University of California Publications in Botany*. Berkeley, Calif. 3: 372. 1909.) Type of the genus *Endophyton*.

NOMENCLATORIAL SYNONYM: *Acrochaete ramosa* (N.L. Gardner) O’Kelly in Gabrielson *et al.* 2006

HOLOTYPE: UC 207136 (see O’Kelly 1982a).

***Ulvella ramulosa* (L. Moewus) R. Nielsen, C.J. O’Kelly & B. Wysor comb. nov.**
Figs S45–S49

BASIONYM: *Ectochaete ramulosa* L. Moewus in *Botaniska Notiser* 1949: 311. 1949)

NOMENCLATORIAL SYNONYM: *Phaeophila ramulosa* (L. Moewus) R. Nielsen 1972.

LECTOTYPE DESIGNATED HERE: Original illustration by Moewus (1949, fig. 28).

EPITYPE DESIGNATED HERE: Dried material of isolate RN070778 maintained at Botanical Museum Copenhagen, Denmark as no. CAT 2453.

***Ulvella repens* (Pringsheim) R. Nielsen, C.J. O’Kelly & B. Wysor comb. nov.**
Figs S50–S54

BASIONYM: *Acrochaete repens* Pringsheim (in *Königlichen Akademie der Wissenschaften zu Berlin Reprint*: 8. 1862). Type of the genus *Acrochaete*.

LECTOTYPE: Original illustration by Pringsheim (1862, pl. XIX reprint pl. II) selected by Burrows (1991, p. 102).

EPITYPE DESIGNATED HERE: Dried material of isolate RN090704-1 maintained at Botanical Museum Copenhagen, Denmark as no. CAT 2450 here designated.

HETEROTYPIC SYNONYM: *Pilinia endophytica* Collins, 1908 acc. to Nielsen & McLachlan 1986b.

***Ulvella reticulata* (Printz) R. Nielsen, C.J. O’Kelly & B. Wysor comb. nov.**
Figs S55–S60

BASIONYM: *Endoderma* [*Entoderma*] *reticulata* Printz [in *Skrifter utgitt av Det Norske Videnskaps-Akademi i Oslo. I. Matem.-Naturvidensk. Klasse* 1926 (5): 240. 1926].

NOMENCLATORIAL SYNONYM: *Entocladia reticulata* (Printz) Levring 1937.

LECTOTYPE DESIGNATED HERE: Original illustration by Printz (1926, fig. 104).

EPITYPE DESIGNATED HERE: Dried material of isolate RNN97085 maintained at Botanical Museum Copenhagen, Denmark as no. CAT 2452.

***Ulvella scutata* (Reinke) R. Nielsen, C.J. O’Kelly & B. Wysor comb. nov.**

BASIONYM: *Pringsheimia scutata* Reinke (in *Berichte der Deutschen Botanischen Gesellschaft Berlin*. 6: 241. 1888).

NOMENCLATORIAL SYNONYM: *Pringsheimiella scutata* (Reinke) Marchewianka 1924. Type of the genus *Pringsheimiella*.

LECTOTYPE: Original illustration by Reinke (1889, pl. 25) selected by Burrows (1991, p. 122).

EPITYPE DESIGNATED HERE: Dried material of isolate RN020273 maintained at Botanical Museum Copenhagen, Denmark as no. CAT 2455.

***Ulvella setchellii* Dangeard**

This taxon was described by Dangeard (1931), and our observations are reported in the supplemental materials.

LECTOTYPE DESIGNATED HERE: Original illustration by Dangeard (1931, pl. I).

EPITYPE DESIGNATED HERE: Dried material of isolate RN260374 maintained at Botanical Museum Copenhagen, Denmark as no. CAT 2454.

***Ulvella testarum* (Kylin) R. Nielsen, C.J. O’Kelly & B. Wysor comb. nov.**
Figs S61–S65

BASIONYM: *Entocladia testarum* Kylin (in *Kungl. Fysiografiska Sällskapet i Lund Förhandlingar*. Lund 5(19): 12. 1935).

NOMENCLATRURAL SYNONYM: *Epicladia testarum* (Kylin) R. Nielsen 1980.

LECTOTYPE DESIGNATED HERE: Original illustration by Kylin (1935, fig. 5).

EPITYPE DESIGNATED HERE: Dried material of isolate RNKæ maintained at Botanical Museum Copenhagen, Denmark as no. CAT 2438.

***Ulvella viridis* (Reinke) R. Nielsen, C.J. O’Kelly & B. Wysor comb. nov.**

Fig. S66

BASIONYM: *Entocladia viridis* Reinke (in *Botanische Zeitung* 37: 476. 1879). Type of the genus *Entocladia*.

NOMENCLATRURAL SYNONYMS: *Endoderma viridis* (Reinke) Lagerheim 1883. *Acrochaete viridis* (Reinke) R. Nielsen, 1979.

LECTOTYPE: Original illustration by Reinke (1879, pl. 17) selected by Burrows (1991, p. 113).

EPITYPE DESIGNATED HERE: Dried material of isolate RN275478 maintained at Botanical Museum Copenhagen, Denmark as no. CAT 2456.

***Ulvella wittrockii* (Wille) R. Nielsen, C.J. O’Kelly & B. Wysor comb. nov.**

BASIONYM: *Entocladia wittrockii* Wille (in *Skrifter udgivne af Videnskabs-Selskabet i Christiania. Matematisk-Naturvidenskabelig Klasse. Christiania* 4: 3. 1880).

NOMENCLATRURAL SYNONYMS: *Ectochoete wittrockii* (Wille) Kylin 1938. *Phaeophila wittrockii* (Wille) R. Nielsen 1972. *Acrochaete wittrockii* (Wille) R. Nielsen 1983.

LECTOTYPE: Original illustration by Wille (1880, pl. 1) selected by Nielsen in Brodie *et al.* (2007).

HETEROTYPIC SYNONYM: *Chlorofilum ephemerum* Dangeard 1965, acc. to Nielsen 1983. Type of the genus *Chlorofilum*.

Pringsheimiella became synonymised with *Ulvella* by the transfer of the type species *P. scutata* into *Ulvella*. Therefore the following new combinations are introduced for species referred to *Pringsheimiella* by Nielsen & McLachlan (1985) or described later.

***Ulvella gratulans* (Weber-Van-Bosse) R. Nielsen, C.J. O’Kelly & B. Wysor comb. nov.**

BASIONYM: *Ochlochaete gratulans* Weber-Van-Bosse (in *Annales du Jardin Botanique de Buitenzorg Supplement* 2: 1. 1898).

***Ulvella mauritiana* (Børgesen) R. Nielsen, C.J. O’Kelly & B. Wysor comb. nov.**

BASIONYM: *Pringsheimiella mauritiana* Børgesen [in *Det Kgl. Danske Videnskabernes Selskab Biologiske Meddelelser* 20(6): 6. 1946].

***Ulvella santae-luciae* (R. Nielsen & McLachlan) R. Nielsen, C.J. O’Kelly & B. Wysor comb. nov.**

BASIONYM: *Pringsheimiella santae-luciae* R. Nielsen & McLachlan (in *Nordic Journal of Botany* 5: 515. 1985).

***Ulvella striata* (Cribb) R. Nielsen, C.J. O’Kelly & B. Wysor comb. nov.**

BASIONYM: *Pringsheimiella striata* Cribb (in *Proceedings of the Royal Society of Queensland* 105: 27. 1995).

***Ulvella udoteae* (Børgesen) R. Nielsen, C.J. O’Kelly & B. Wysor comb. nov.**

BASIONYM: *Pringsheimia(?) udoteae* Børgesen (in *Dansk Botanisk Arkiv* 1 (4): 11. 1913).

***Ulvella crenulata* Lami**

This taxon was treated as a species of *Pringsheimiella* by Nielsen & McLachlan (1985); it was originally described by Lami (1935).

In addition to the species dealt with in the present study 10 species are referred to *Acrochaete* by Guiry & Guiry (2012). Preliminary data suggest that one of the species (*Epicladia flustrae* Reinke) belongs in its own genus. We have insufficient information to make taxonomic judgments for *A. apiculata* (Setchell & Gardner) C.J. O’Kelly, *A. cladophorae* (Hornby) R. Nielsen, *A. major* (Feldmann) Perret-Boudouresque & Seridi, and *A. pterosiphoniae* (Nagai) Zhigadlova, so these must remain ‘*species inquirendae*’ for now. The remaining five we here transfer to *Ulvella* based on our interpretation of prior work (O’Kelly & Yarish 1981, O’Kelly 1983).

***Ulvella cingens* (Setchell & N.L. Gardner) R. Nielsen, C.J. O’Kelly & B. Wysor comb. nov.**

BASIONYM: *Entocladia cingens* Setchell & N.L. Gardner (in *University of California Publications in Botany* 7: 292. 1920).

NOMENCLATRURAL SYNONYM: *Acrochaete cingens* (Setchell & N.L. Gardner) C.J. O’Kelly (in Gabrielson *et al.* 2006, p.30).

***Ulvella codicola* (Setchell & N.L. Gardner) R. Nielsen, C.J. O’Kelly & B. Wysor comb. nov.**

BASIONYM: *Entocladia codicola* Setchell & N.L. Gardner (in *University of California Publications in Botany* 7: 293. 1920).

NOMENCLATRURAL SYNONYM: *Acrochaete codicola* (Setchell & N.L. Gardner) C.J. O’Kelly (in Gabrielson *et al.* 2006, p. 29).

***Ulvella geniculata* (N.L. Gardner) C.J. O’Kelly & B. Wysor comb. nov.**

BASIONYM: *Pseudodictyon geniculatum* N.L. Gardner (in *University of California Publications in Botany*. 3: 374. 1909).

NOMENCLATRURAL SYNONYM: *Acrochaete geniculata* (N.L. Gardner) C.J. O’Kelly 1983 p. 14.

***Ulvella inflata* (A. Ercegovic) R. Nielsen, C.J. O’Kelly & B. Wysor comb. nov.**

BASIONYM: *Pseudodictyon inflatum* Ercegovic [in *Acta Adriatica Institut za Oceanografiju i Ribarstvo – Split FNR Jugoslavija*. 8 (8): 22. 1957].

NOMENCLATRURAL SYNONYM: *Acrochaete inflata* (Ercegovic) Gallardo *et al.* 1993.

***Ulvella taylorii* (Thivy) R. Nielsen, C.J. O'Kelly & B. Wysor
comb. nov.**

BASEONYM: *Ectochaete taylorii* Thivy (in *Biological Bulletin of the Marine Biological Laboratory, Woods Hole, Mass. Boston* 83: 98. 1942).

NOMENCLATURAL SYNONYMS: *Acrochaete taylori* (Thivy) C.J. O'Kelly (in Gabrielson *et al.* 2006, p. 31). *Entocladia taylorii* (Thivy) O'Kelly & Yarish (1981). *Phaeophila taylorii* (Thivy) R. Nielsen 1972.

DISCUSSION

A previously published study of related microfilamentous marine green algae, including species of *Ulvella* (as *Acrochaete* and *Endophyton*), revealed that tree topologies based on *tufA* were congruent to those based on nuclear-encoded SSU rDNA (O'Kelly *et al.* 2004a). Carlile *et al.* (2011) obtained similar results, and their study incorporated SSU rDNA sequences from *U. lens*. Saunders and Kucera (2010), moreover, have argued that *tufA* is the most suitable 'barcode' gene for most marine green algae. We therefore think that this gene, interpreted in the context of the morphological information obtained from the cultures, provides sufficient robustness both for identifying species within *Ulvella* and for establishing phylogenetic relationships among the species. The short branch lengths among the species of *Ulvella* indicate that they have diverged within the same evolutionary time frame, given the apparently reasonable assumption that evolutionary rates have remained similar in all *Ulvella* lineages, and that these divergences are relatively recent. We think that additional gene-sequence data will support the findings of this study, and will also provide the data needed to expand our analyses further, to place the Ulvellaceae in a wider phylogenetic context.

The satellite genera are clearly closely related to clades of *Acrochaete* species, and the type species of *Ectochaete* (*E. leptochaete*), *Entocladia* (*E. viridis*), *Endophyton* (*E. ramusum*), and *Pseudodictyon* (*P. geniculatum*) have previously been transferred to *Acrochaete* based on morphological observations (Nielsen 1979, 1983; O'Kelly 1983; O'Kelly in Gabrielson *et al.* 2006). These transfers are confirmed as all type species are within our ingroup, apart from *A. geniculata* which was not included in the study. At the generic level disc-shaped morphology previously has been considered strong evidence for separating *Pringsheimiella* and *Ulvella*. However, species of both genera (*P. scutata*, *U. lens*, and *U. setchellii*) are found in two individual subclades, deeply embedded within clades of filamentous species, indicating parallel evolution not monophyly. As many individual species are characterized by a mosaic of the morphological characters, it is impossible to extend these to circumscription of genera, yet another reason for combining all taxa into *Ulvella*.

Comparison of the morphological characters with the results of the phylogenetic analyses (Figs 1, S1) shows the characters to be almost arbitrarily distributed on the tree. Thus, the ability to penetrate calcified material occurs in two different clades (*U. aequicrassa* versus *U. testarum*, *U. endostraca*), and does not define a single group. Addition-

ally, endophytes often develop a flossy morphology in culture, which is observed in *U. gigas*, *U. operculata*, *U. ramosa*, and *Ulvella* sp. C all in different subclades on the tree (Fig. 1, S1). An exception to this is the well-supported subclade including *U. leptochaete* in which several merocytic extensions from a single basal swelling have been observed in all species, but not in other species included in this investigation. We expect that future investigations will raise the number of species referred to *Ulvella*, thus species retained in *Acrochaete*, *Ectochaete*, *Entocladia*, and *Pseudodictyon* may belong into *Ulvella*. It is likely that taxa previously referred to *Endophyton* include several new species. Morphological observations for the three species referred to as *Uvella* sp. A., *Uvella* sp. B., and *Uvella* sp. C (Figs 1, S1, S67–71) can be found in the supplemental material while a detailed systematic treatment awaits future observations of sporangia, germlings, and possibly hairs. The species referred to *A. viridis* sensu O'Kelly (Fig. 1) also demands more attention to document the proper identity. It was associated with *Phycodryis rubens* (Linnaeus) Batters and similar to algae from the same host referred to *A. viridis* by Nielsen (1979).

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SUPPLEMENTARY DATA

Supplementary data associated with this article can be found online at <http://dx.doi.org/10.2216/11-067.1.s1>.

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