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European Journal of Phycology

Publication details, including instructions for authors and subscription information:
<http://www.tandfonline.com/loi/tejp20>

Description of *Ulvella elegans* sp. nov. and *U. islandica* sp. nov. (Ulvellaceae, Ulvophyceae) from Iceland - a study based on morphology of species in culture and *tufA* gene sequences

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Published online: 20 Feb 2014.

To cite this article: Ruth Nielsen, Karl Gunnarsson, Niels Daugbjerg & Gitte Petersen (2014) Description of *Ulvella elegans* sp. nov. and *U. islandica* sp. nov. (Ulvellaceae, Ulvophyceae) from Iceland - a study based on morphology of species in culture and *tufA* gene sequences, *European Journal of Phycology*, 49:1, 60-67

To link to this article: <http://dx.doi.org/10.1080/09670262.2014.888483>

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Description of *Ulvella elegans* sp. nov. and *U. islandica* sp. nov. (Ulvellaceae, Ulvophyceae) from Iceland – a study based on morphology of species in culture and *tufA* gene sequences

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(Received 11 April 2013; revised 4 September 2013; accepted 29 September 2013)

Two new *Ulvella* species, *U. elegans* R. Nielsen & K. Gunnarsson and *U. islandica* R. Nielsen & K. Gunnarsson are described. These microfilamentous marine green algae were found in the sublittoral zone in northern Iceland, epiphytic on *Euthora cristata* and associated with a calcareous polychaete tube, respectively. Unialgal cultures were established from field-collected material for morphological observations. In culture, *Ulvella elegans* was characterized by rosettes of monostromatic pseudoparenchyma consisting of radiating filaments with a margin of mutually free filaments. Each cell had one pyrenoid. Hairs were not observed. *Ulvella islandica* had a heterotrichous morphology, consisting of dense tufts of upright broad branches and much narrower, rhizoid-like branches. *Acrochaete*-type hairs occurred; these are hyaline non-septate merocytic extensions from a more or less bulbous base, which may be separated from the vegetative cell below. Most cells had one pyrenoid except for a few broad cells which had two or three. In a phylogenetic reconstruction based on the chloroplast-encoded *tufA* gene, the sequences for the two species were clearly distinct from any other *Ulvella* sequence available for this gene. *Ulvella islandica* was placed in a clade together with *U. lens*, *U. wittrockii*, *U. reticulata* and *U. pseudorepens*. *Ulvella elegans* occupied a branch deep in the phylogeny but the position was poorly supported.

Key words: *Acrochaete*, Chlorophyta, culture studies, Iceland, marine microfilamentous green algae, North Atlantic, phylogeny, Ulvophyceae, *Ulvella*, *tufA*

Introduction

Modern taxonomy of microfilamentous green algae is based on observations in nature and in culture, complemented with molecular investigations (Nielsen *et al.*, 2013). Several species often grow mixed together on the same substratum in nature, making it difficult to distinguish individual plants. Species-specific characters such as hairs and the size and morphology of sporangia are often missing at the time of collection, making identification impossible. Culture studies enable separation of species and permit additional characters to develop (Kornmann, 1959, 1960; Nielsen & Pedersen, 1977). Molecular methods have supplied data to reveal phylogenetic relationship between genera and species (e.g. O'Kelly *et al.*, 2004). In addition, molecular data supply important information for assessing species boundaries and for testing morphology-based species circumscriptions (Rinkel *et al.*, 2012; Nielsen *et al.*, 2013).

A recent revision of *Ulvella* based on culture studies, supported by molecular analyses of the chloroplast-encoded *tufA* gene, documented that species previously referred to *Acrochaete*, *Endophyton*, *Entocladia*, *Pringsheimiella*, *Pseudodictyon* and *Ulvella* belong to a single genus for which *Ulvella* has priority (Nielsen *et al.*, 2013). The concept of *Ulvella* was thus extended and several new species described. Species were distinguished by their growth form in culture, cell shape and size, the number of pyrenoids per cell, the positions of *Acrochaete*-type hairs (hyaline non-septate merocytic extensions from a more or less bulbous base, which may be separated from the vegetative cell below: Nielsen, 1979) and sporangia, the shape and size of sporangia, and whether germlings are with or without an empty spore-wall. The phylogenetic relationships of species were investigated by analyses of chloroplast *tufA* sequences (Nielsen *et al.*, 2013).

In a detailed study of the microfilamentous marine green algae of Iceland, based on morphological structures of naturally occurring plants and of numerous

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unialgal isolates (Nielsen & Gunnarsson, in prep), two new *Ulvella* species were detected. The aim of the work reported here was to describe these new species using a combination of phenotypic and genotypic characters.

Materials and methods

Cultures were established from field-collected material. Isolate IS06013-1 was initiated from plants on calcareous polychaete tubes sampled at 30 m depth at Laugakambur, Hrísey, Iceland (66° 01.95' N, 18° 24.47' W) on 13 June 2006. Isolate IS06015 was initiated from epiphytes on *Euthora cristata* sampled between 1–5 m depth at Laugakambur, Hrísey (66° 01.75' N, 18° 24.47' W), also on 13 June 2006. Crude cultures were established from fragments of host material placed in test tubes with growth medium. Unialgal cultures were obtained for each species by isolating and serial washing single plants of the target species into clean plastic Petri dishes with 15 ml of growth medium, using a fine glass pipette. The strains are available from the Scandinavian Culture Collection of Algae & Protozoa, Copenhagen (SCCAP). The growth medium was a modified Provasoli medium (MV30: Christensen, 1982) with GeO₂ added to prevent diatom growth (Lewin, 1966). The cultures were maintained at 4 °C with a 12 : 12 h light : dark cycle at an irradiance of *c.* 16 μmol photons m⁻² s⁻¹ provided by fluorescence lamps (Philips TL 40W, Philips, Amsterdam, the Netherlands). Observations were made once or twice per week of plants that were kept growing actively by transfer to fresh medium every other week or monthly. Photos were taken with an Olympus digital camera DP-70 mounted on an Olympus AX70 microscope (Olympus, Tokyo, Japan). A saltwater immersion 20× objective was used to study the morphology of prostrate plants growing in plastic Petri dishes.

Following the procedure of Doyle & Doyle (1987), total genomic DNA was extracted from two cultures kept frozen at -18 °C. PCR amplifications and sequencing were performed using primers *tufAF* and *tufAR* (Famà *et al.*, 2002) following the procedure described in Nielsen *et al.* (2013). Sequence editing was done using Sequencher version 4.10 (Gene Codes Corporation, Ann Arbor, Michigan, USA). Sequences were deposited in GenBank under accession numbers KF444923 for isolate IS06013-1 (*U. elegans*) and KF444924 for IS06015 (*U. islandica*). The two new *tufA* sequences were manually inserted into the matrix previously used by Nielsen *et al.* (2013). A list of specimens included in the matrix is provided in Table 1.

Phylogenetic analyses were based on Bayesian inference, maximum likelihood and parsimony. MrBayes (v. 3.1.2, Ronquist & Huelsenbeck, 2003) was used to perform Bayesian analysis with a general time reversible (GTR) substitution model. The choice of model was based on jmodeltest ver. 2.1.3 (Darriba *et al.*, 2012) which selected the best fit for our data matrix of *tufA* sequences from 56 different models of DNA evolution. We used partitioning of the codon positions for the protein-encoded chloroplast gene and ran two independent Markov Chain Monte Carlo (MCMC) (each comprising one cold and three heated chains) with 4 × 10⁶ generations. Parameter values and trees were sampled and saved every 1000th generation.

The numbers of substitution types allowed were two for first and second codon positions (lset = 2) and six types for third codon positions (lset = 6). We assumed that all the model parameters were unlinked and rate multipliers were variable across partitions. We plotted the log likelihood values as a function of generations, which showed that the lnL values converged at *c.* 5530 after 20 000 generations leaving 3981 trees. These were imported into PAUP* vers. 4.0b8 (Swofford, 2001) to produce a 50% majority rule consensus tree. Posterior probabilities were also obtained from the 3981 trees.

Maximum likelihood analysis was performed using PhyML (Guindon *et al.*, 2010) via the online version available on the Montpellier bioinformatics platform at <http://www.atgc-montpellier.fr/phyml>. For this we used a GTR model with 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.

Parsimony analyses were performed using PAUP* and the following options: heuristic search, 100 random addition sequences, five trees held each step and TBR swapping. PAUP* v. 4.0b8 was preferred to the most recent version 10 (Swofford, 2002), as 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 non-additive. 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.

Results

The *tufA* matrix included 50 sequences (43 *Ulvella* spp. and seven outgroup) and 894 characters. The 50% majority rule consensus tree derived from Bayesian inference is shown in Fig. 1, which shows the posterior probabilities and also bootstrap support values from the maximum likelihood and parsimony analyses. The parsimony analysis included 227 informative characters and resulted in 1154 equally parsimonious trees of length 756 with a consistency index of 0.491, and a retention index of 0.692.

Isolate IS06015 (KF444924) was placed in a clade together with *U. lens*, *U. wittrockii*, *U. reticulata* and *U. pseudorepens*. This clade had a very low posterior probability and no bootstrap support from either maximum likelihood or parsimony (Fig. 1). The position of isolate IS06015 within the clade was also unresolved. However, based on the level of *tufA* sequence divergence between IS06015 and the other species of the clade we recognize it as a distinct species, described below as *Ulvella islandica*.

Isolate IS06013-1 (KF444923) was placed at a relatively basal position within *Ulvella*, but its position was unresolved with respect to a poorly supported clade including *U. glabra* and the unnamed *Ulvella* sp. C., and the large clade including all other *Ulvella* species except *U. gigas* and *U. endozoica*. Based on the pronounced level of sequence divergence from all

Table 1. Species included in the phylogenetic analysis with associated data.

Species	GenBank accession number	Strain number	Collecting site, habitat, collector	Coordinates	Collection date
<i>Ochlochaete hystrix</i>	AY454406	MA1 8d1	USA, Quissett Estuary, Woods Hole, MA, dead <i>Zostera marina</i>	No information	No date
<i>Percursaria percursora</i>	AY454403	UTEX 1423	No information	No information	No date
<i>Ruthnielsenia tenuis</i>	JQ302995	RNtenuis72	Denmark, Northern Kattegat, Læsø, on mollusc shell. Coll.: R. Nielsen	57°15'N, 10°53'E	20 Jun. 1971
<i>Ruthnielsenia tenuis</i>	AY454404	WA2 2263	USA, False Bay, San Juan Island, WA, mollusc shell	No information	No date
<i>Ulva californica</i>	AY454401	FH 3.2	USA, False Bay, San Juan Island, WA	No information	No date
<i>Ulva intestinalis</i>	AY454399	4.2KEK	USA, False Bay, San Juan Island, WA	No information	No date
<i>Ulvaria obscura</i>	AY454402	1.4MW	USA, False Bay, San Juan Island, WA	No information	No date
<i>Ulvella aequicrassa</i>	JQ302998	RN281083-12-4-1	Chile, Puerto Aldea, on mollusc shell. Coll.: R. Nielsen	30°12'S 71°26'W	28 Oct. 1983
<i>Ulvella dasykala</i>	JQ302969	RN310186-1-1	Spain, Arrieta, North of town, Lanzarote, Canary Islands, on <i>Champia parvula</i> . Coll.: R. Nielsen	29°08'N, 13°28'W	31 Jan. 1986
<i>Ulvella elegans</i>	KF444923	IS06013 01	Iceland, Hrisey at Laugakambur, 30 m depth on a polychaete tube. Coll.: K. Gunnarsson	66°02'N, 18°24'W	13 Jun. 2006
<i>Ulvella endostraca</i>	JQ303002	RN050979c/K0220	New Zealand, Kaikoura, on mollusc shell. Coll.: Ø. Moestrup	42°24'S, 173°41'E	5 Sep. 1979
<i>Ulvella endozoica</i>	JQ302993	UTEX B 2352	USA, Bache Shoals, N. Florida Keys, 3 m depth, on <i>Pseudoplexaura</i> sp. Coll.: Goldberg et al.	25°29'N, 80°10'W	No date
<i>Ulvella gigas</i>	JQ302982	RN00019 01-1	Japan, Ohra, Nabeta Bay, Shimoda, Shizuoka Prefecture, on <i>Sargassum ringgoldianum</i> . Coll.: R. Nielsen, M. Ima	32°40'N, 138°56'E	24 Apr. 2000
<i>Ulvella glabra</i>	JQ302999	RN161183-1-1	Chile, Coquimbo, Bahía Herradura de Guayacan, littoral, on pebble. Coll.: R. Nielsen	29°59'S 71°21'W	16 Nov. 1983
<i>Ulvella globocaeapitosa</i>	JQ302985	RN00019 03-2	Japan, Ohra, Nabeta Bay, Shimoda, Shizuoka Prefecture, on <i>Padina arborescens</i> . Coll.: R. Nielsen, M. Ima	32°40'N, 138°56'E	24 Apr. 2000
<i>Ulvella heteroclada</i>	JQ303004	PC-2V/K0219	Canada, Nova Scotia, Peggy's Cove, on <i>Chondrus crispus</i> . Coll.: J. Correa	44°30'N 63°55'W	11 Jun. 1985
<i>Ulvella inopinata</i>	JQ302981	RN00015 01-2	Japan, Yura, Awaji Island, on <i>Chondrus ocellatus</i> . Coll.: R. Nielsen, M. Ima	34°16'N, 134°56'E	20 Apr. 2000
<i>Ulvella islandica</i>	KF444924	IS06015	Iceland, Hrisey at Laugakambur, 5–1 m depth on <i>Euthora cristata</i> . Coll.: K. Gunnarsson	66°02'N, 18°24'W	13 Jun. 2006
<i>Ulvella lens</i>	JQ302996	RN191083-8-3	Chile, Coquimbo, Bahía Herradura de Guayacan, Polvorin, 5 m depth, on pebble. Coll.: H. Black	29°59'S 71°21'W	18 Oct. 1983
<i>Ulvella leptochaete</i>	JQ302971	HO01001 03	Japan, Ryukyus, Ishigaki-jima Island, Kabira, Okinawa Prefecture, on mollusc shell. Coll.: H. Ohba	24°27'N, 124°08'E	30 Jan. 2001
<i>Ulvella leptochaete</i>	JQ302986	RN00007 12-1	Japan, Katsuma, Shikanojima Island, Fukuoka City, Northern Kyushu, on <i>Acrosorium flabellatum</i> . Coll.: R. Nielsen, M. Ima	33°41'N, 130°17'E	21 Mar. 2000
<i>Ulvella leptochaete</i>	JQ302994	RN05035-1	Germany, Sylt, on <i>Chaetomorpha linum</i> . Coll.: E. Herre	54°54'N, 08°18'E	24 Jun. 2005
<i>Ulvella leptochaete</i>	AY454409	NY 1b	USA, Ocean/Orient Point, Long Island, NY, endophytic in <i>Champia</i> sp.	No information	No date
<i>Ulvella marchantiae</i>	JQ302991	RNC 17-1	St. Lucia, Cas-en-bas, on <i>Laurencia</i> sp. Coll.: J. McLachlan	14°05'N, 60°56'W	21 Oct. 1982
<i>Ulvella operculata</i>	JQ303005	Pf 1a/K0008	Canada, Nova Scotia, Pubnico Point, on <i>Chondrus crispus</i> . Coll.: J. Correa	43°37'N 65°47'W	14 Oct. 1985
<i>Ulvella pachypes</i>	JQ302997	RN230888-2-2	Denmark, Endelave, 11 m depth, on pebble. Coll.: M. B. Rasmussen	55°46'N, 10°20'E	23 Aug. 1988

(continued)

Table 1. Continued.

Species	GenBank accession number	Strain number	Collecting site, habitat, collector	Coordinates	Collection date
<i>Ulvella parasitica</i>	JQ302972	RN060972	Denmark, Læsø, Nordre Rønner, on <i>Fucus serratus</i> . Coll.: R. Nielsen	57°22'N, 10°55'E	6 Sep. 1972
<i>Ulvella porphyrae</i>	JQ302976	RN014578	Italy, Sorrento, on <i>Porphyra</i> sp. Coll.: R. Nielsen	40°38'N, 14°23'E	1 May 1978
<i>Ulvella pseudorepens</i>	JQ302975	RN071076a	Denmark, Lyngså Strand, on drift <i>Chorda filum</i> . Coll.: R. Nielsen	57°15'N, 10°33'E	7 Oct. 1976
<i>Ulvella ramosa</i>	JQ302988	RN00007 35	Japan, Katsuma, Shikanoshima Island, Fukuoka City, Northern Kyushu, on <i>Gloiopeltis tenax</i> . Coll.: R. Nielsen, M. Ima	33°41'N, 130°17'E	21 Mar. 2000
<i>Ulvella ramosa</i>	JQ302980	RN000008 01-3	Japan, Oshoro, Otaru, Hokkaido, on <i>Chondrus yendoi</i> . Coll.: R. Nielsen, M. Ima	33°12'N, 140°51'E	05 Apr. 2000
<i>Ulvella ramosa</i>	JQ303001	RN021185	Canada, Pacific Ocean, Western Canada, on <i>Iridaea cordata</i> . Coll.: J. Correa	No information	1985
<i>Ulvella ramosa</i>	AY454410	WA1 12A1	USA, Mar Vista, San Juan Island, WA, endophytic in <i>Mazzaella oregona</i>	No information	No date
<i>Ulvella ramulosa</i>	JQ302973	RN070778	Denmark, Northern Kattegat, Hirsholm, on <i>Chaetomorpha linum</i> . Coll.: R. Nielsen	57°29'N, 10°38'E	7 Jul. 1978
<i>Ulvella repens</i>	JQ302984	RN090704-1	Germany, Helgoland, Vorhafen, near Trockendock, on <i>Chorda filum</i> . Coll.: I. Bartsch	54°11'N, 07°53'E	5 Jul. 2004
<i>Ulvella repens</i>	JQ302974	RN260275	Denmark, Northern Kattegat, Hirsholm, on <i>Fucus vesiculosus</i> . Coll.: R. Nielsen	57°29'N, 10°38'E	26 Feb. 1975
<i>Ulvella reticulata</i>	JQ302970	RNN97085	Norway, Næroy, 10-15 m depth, on <i>Saccharina latissima</i> . Coll.: K. Gunnarsson	61°38'N, 04°59'E	04 Sep. 1997
<i>Ulvella scutata</i>	JQ302990	RN020273/K0289	Denmark, Northern Kattegat, Hirsholm, on <i>Polysiphonia stricta</i> . Coll.: R. Nielsen	57°29'N, 10°38'E	25 Aug. 1972
<i>Ulvella scutata</i>	JQ302989	RN00009 02-6	Japan, Charatsunai, Muroan, Hokkaido, on <i>Chondrus yendoi</i> . Coll.: R. Nielsen, M. Ima	42°18'N, 140°59'E	7 Apr. 2000
<i>Ulvella scutata</i>	JQ303006	WAz3/CCMP 1675	USA, Washington, San Juan Island, Friday Harbor. Epiphyte on <i>Osmudea spectabilis</i> . Coll.: C.J. O'Kelly	48°54'N, 123°01'W	June 1977
<i>Ulvella</i> sp.	AY454407	MA1 2a1	USA, Brant Rock, Marshfield, MA, on <i>Phycodryis rubens</i>	No information	No date
<i>Ulvella</i> sp. A	JQ302977	RN00002 38-2	Japan, Saikai-bashi, Nagasaki Prefecture, on balanid or mollusc shell. Coll.: R. Nielsen, M. Ima	33°03'N, 129°45'E	10 Mar. 2000
<i>Ulvella</i> sp. B	JQ302979	RN00008 20-1	Japan, Oshoro, Otaru, Hokkaido, on <i>Hyalosiphonia caespitosa</i> . Coll.: R. Nielsen, M. Ima	33°12'N, 140°51'E	5 Apr. 2000
<i>Ulvella</i> sp. C	JQ302978	RN00009 05-1a	Japan, Charatsunai, Muroan, Hokkaido, on <i>Ahnfeltiopsis flabelliformis</i> . Coll.: R. Nielsen, M. Ima	42°18'N, 140°59'E	7 Apr. 2000
<i>Ulvella testarum</i>	JQ303000	RNKæ	Sweden, Kristineberg, on <i>Mya arenaria</i> . Coll.: R. Nielsen	58°16'N, 11°27'E	22 Jun. 1971
<i>Ulvella vacuospora</i>	JQ302987	RN00015 02-3	Japan, Yura, Awaji Island, on <i>Laurencia</i> sp. Coll.: R. Nielsen, M. Ima	34°16'N, 134°56'E	20 Apr. 2000
<i>Ulvella vacuospora</i>	JQ302983	RN00019 08-1	Japan, Ohra, Nabeta Bay, Shimoda, Shizuoka Prefecture, on <i>Ecklonia cava</i> . Coll.: R. Nielsen, M. Ima	32°40'N, 138°56'E	24 Apr. 2000
<i>Ulvella viridis</i>	JQ302968	RN275478/K0338	Italy, Naples, Posillipo at Villa Volpicelli, on <i>Derbesia balbisiana</i> (or <i>Bryopsis disticha</i>). Coll.: R. Nielsen	40°48'N, 14°12'E	28 Apr. 1978
<i>Ulvella waernii</i>	JQ302992	RN182981	Finland, Tvärminne, Shore at Zoological Station, on <i>Phragmites australis</i> . Coll.: R. Nielsen	59°51'N 23°15'E	18 Sep. 1980
<i>Ulvella wittrockii</i>	JQ303003	RN260979	Denmark, Stevns Klimt, S of Mandehoved, on <i>Pylaiella littoralis</i> . Coll.: T. Christensen	55°15'N, 12°24'E	26 Sep. 1979

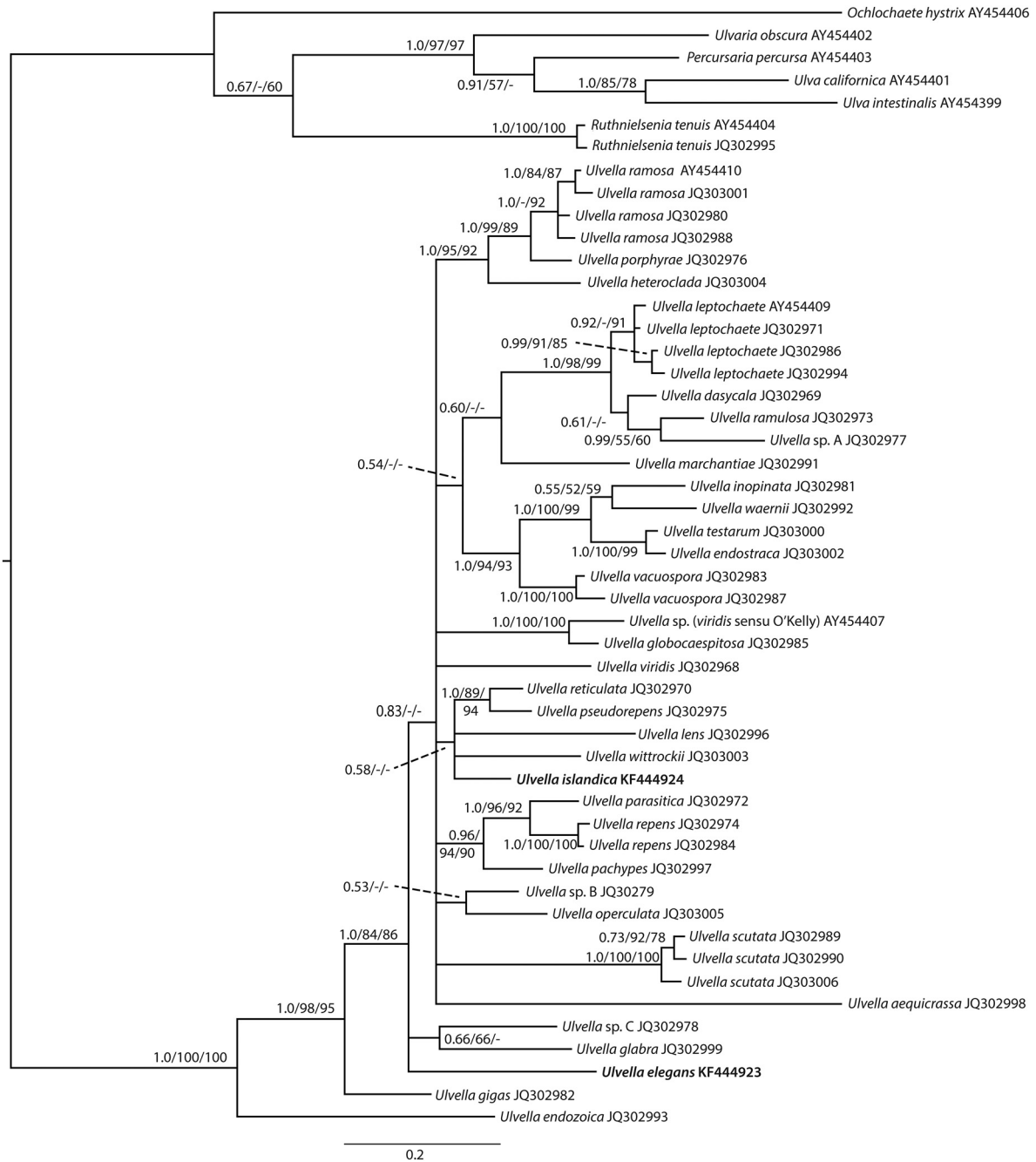


Fig. 1. 50% majority rule consensus tree derived from Bayesian inference. Support values on branches are posterior probabilities, bootstrap support from maximum likelihood, and bootstrap values from parsimony. Bootstrap values below 50 are indicated with a dash. The two new species are shown in bold. For details of other taxa included in the analyses see Table 1.

other species, we recognize IS06013-1 as a distinct species, which we name *U. elegans*.

***Ulvella elegans* R. Nielsen & K. Gunnarsson, sp. nov.**

Figs 2–6

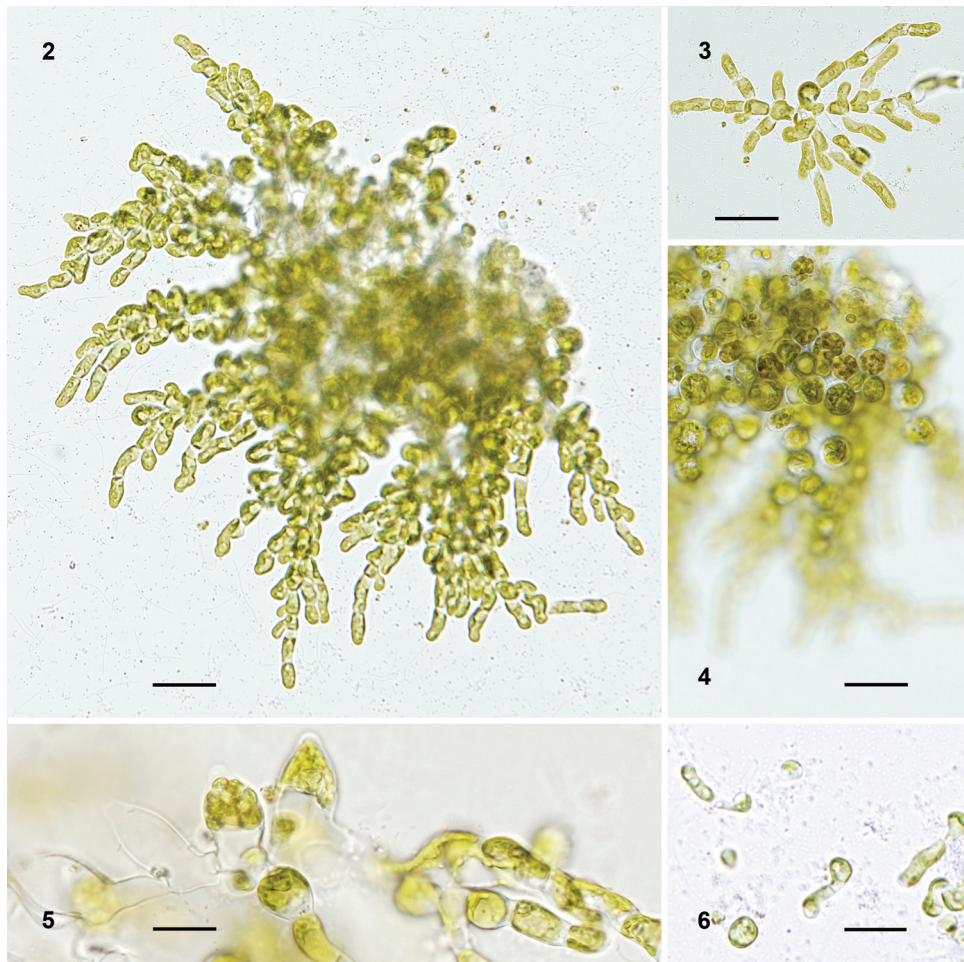
DESCRIPTION: Monostromatic rosettes or mutually free filaments with alternate or opposite branches. The distal, cylindrical cells are 3.5–6.5 μm wide and 2–4 times as long; the rounded middle cells 10.5–12.5 μm across. Vegetative cells contain a parietal chloroplast with one pyrenoid. Hairs have not been observed.

Sporangia are 11–13.8 μm tall, with a conical apex. On release, spores germinate unilaterally and remain a part of the developing plants.

HOLOTYPE: Dried sample of isolate IS06013-1 maintained in the Botanical Museum, Copenhagen (C) as no. CAT 2481.

TYPE LOCALITY: Laugakambur, Hrīsey, Iceland, 30 m depth, on a polychaete tube.

ETYMOLOGY: The name refers to the elegant look of the monostromatic rosettes with free marginal filaments of plants kept in culture.



Figs 2–6. *Ulvella elegans*. 2. Mature plant with a pseudoparenchymatous central area and mutually free distal filaments. 3. Young plant of radiating filaments of cylindrical vegetative cells with a single pyrenoid. 4. Same plant as in Fig. 2 shown in different focus. 5. Barrel-shaped mature and empty sporangia. 6. Germlings. Scale bars = 20 μm (Figs 2–4) and 10 μm (Figs 5, 6).

Individuals formed a rosette in contact with a solid substratum in culture. Cells in the middle part were confluent and formed a monostromatic, pseudoparenchymatous layer, while the margin consisted of mutually free filaments (Figs 2, 3). Plants growing freely formed bushes of free filaments developed around a dense middle part. Cells in the middle were rounded to almost globular and 10.5–12.5 μm in diameter, whereas distal cells were 3.5–6.5 μm wide and 2–4 times as long. Vegetative cells contained a parietal chloroplast with a single pyrenoid. Hairs were not observed. Sporangia developed from the middle cells of substrate-attached plants or from intercalary cells in freely growing plants (Figs 4, 5). They became almost barrel-shaped with a conical apex and were 11–13.8 μm tall. On release, spores germinated unilaterally and remained part of the developing plants (Fig. 6).

***Ulvella islandica* R. Nielsen & K. Gunnarsson, sp. nov.**

Figs 7–12

DESCRIPTION: Tufts of upright branches of cylindrical cells, 10–15 μm wide, with rounded cells at base,

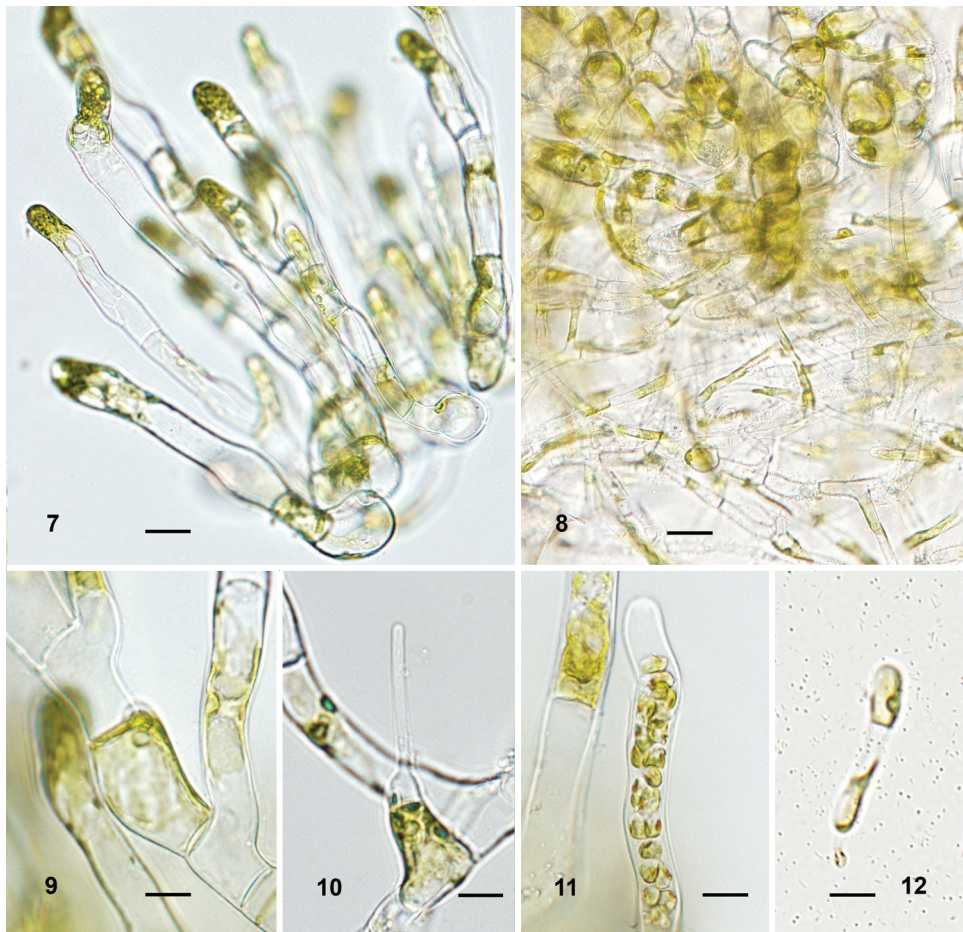
20–32 μm across. Narrow, alternately branched filaments resembling rhizoids develop from both kinds of cells at the base of the tuft; they have cylindrical cells, 4.5–6 μm wide. Vegetative cells contain a parietal, slightly lobed chloroplast with one (to three) pyrenoid(s). *Acrochaete*-type hairs occur apically on cells of broad filaments. Sporangia develop from similar cells and become elongate, linear-cylindrical.

HOLOTYPE: Dried sample of isolate IS06015 maintained at the Botanical Museum, Copenhagen (C) as no. CAT 2480.

TYPE LOCALITY: Laugakambur, Hrísey, Iceland, 1–5 m depth, on *Euthora cristata*.

ETYMOLOGY: The name refers to the origin of the type material.

Mature plants in culture had a heterotrichous morphology, consisting of a dense tuft of upright broad branches with rounded cells at the base (Fig. 7). The distal cylindrical cells of the broad branches were 10–15 μm in width, whereas the rounded basal cells were 20–32 μm across. Irregularly branched narrow filaments, 4.5–6 μm wide, developed from the



Figs 7–12. *Ulvella islandica*, morphology of heterotrichous plants in culture. **7.** Tuft of broad cylindrical filaments with rounded cells at the base. **8.** Basal part of tuft with rhizoid-like filaments. **9.** Vegetative cell with a parietal chloroplast and one pyrenoid. **10.** *Acrochaete*-type hair. **11.** Cylindrical, apical sporangium. **12.** Germling with evacuated spore-wall attached. Scale bars = 20 μm (Figs 7, 8) and 10 μm (Figs 9–12).

rounded cells and the lower cylindrical cells, and formed a matted cell mass (Fig. 8). Most cells contained a single pyrenoid in a parietal, slightly lobed chloroplast (Fig. 9); however, two or three pyrenoids were observed in a few broad cells. *Acrochaete*-type hairs occurred apically on cells of broad filaments (Fig. 10). Sporangia developed from similar cells and became linear-cylindrical (Fig. 11). Settled spores germinated unilaterally; an evacuated, often brown-pigmented, spore-wall and a germ-tube were visible in young, few-celled plants (Fig. 12).

Discussion

The discovery of two new species of microfilamentous marine green algae from Iceland confirms the prediction of new species of *Ulvella* by Nielsen *et al.* (2013). Species of *Ulvella* are generally separated by growth form, the shape and size of the cells, the number of pyrenoids per cell, the position of *Acrochaete*-type hairs, and the shape and size of sporangia. These characters are usually better developed in plants in culture than in samples collected in nature, where some characters may be missing at the time of

collecting. Therefore, the morphology of the new species reported here was described from actively growing cultured algae. The rosettes of *U. elegans* make this species most similar to *U. marchantiae*, but the two differ in the morphology of the sporangia, which are bottle-shaped with a short neck in *U. marchantiae* and barrel-shaped with a conical apex in *U. elegans*. The presence of *Acrochaete*-type hairs was considered an important diagnostic character by Nielsen (1979) and *Acrochaete*-type hairs were observed only in *U. marchantiae* (Nielsen & McLachlan, 1986, as *Acrochaete marchantiae*), not in *U. elegans*. The lack of *Acrochaete*-type hairs is shared by *U. glabra*, *U. ramosa* and *U. testarum* but none of these species seems to be closely related to *U. elegans* (Fig. 1). Based on the phylogenetic distribution of these characteristic hairs in species of *Ulvella*, they appear to have been repeatedly lost during evolution; their absence is probably not a consequence of culture conditions as isolates lacking *Acrochaete*-type hairs have been subject to the same culture methods as hair-producing *Ulvella* species.

The heterotrichous morphology of *U. islandica* is shared by several *Ulvella* species, among them

U. pseudorepens, which belongs to the same clade as *U. islandica*. The two can be easily distinguished by the number of pyrenoids, with several per cell in *U. pseudorepens* and only one in the majority of the cells in *U. islandica*. *Ulvella repens* is heterotrichous with relatively broad upright filaments (Nielsen *et al.*, 2013) like *U. islandica*. A matted mass of rhizoidal-like branches only occurs in *U. islandica*.

Though phylogenetic analyses have clarified some relationships among the species of *Ulvella*, others remain unresolved including the exact phylogenetic position and sister group of each of the two new species described here. In order to gain a better understanding of their phylogeny, future analyses of additional genes will most likely be helpful.

Acknowledgements

We are grateful to Svanhildur Egilsdóttir, Tryggvi Sveinsson, Erlendur Bogason, Juliet Brodie and Barbara Rinkel for their company and help during the sampling expedition. We thank Charlotte Hansen and Hannah Blossom for assistance with the molecular work. A Carlsberg Foundation grant supported the molecular work and equipment. Financial and logistic support was provided by the EU-program 'Synthesys' during analyses of the culture collections at Botanical Garden and Museum, Natural History Museum of Denmark.

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