THE PRASIOLALES (CHLOROPHYTA) OF ATLANTIC EUROPE: AN ASSESSMENT BASED ON MORPHOLOGICAL, MOLECULAR, AND ECOLOGICAL DATA, INCLUDING THE CHARACTERIZATION OF *ROSENVINGIELLA RADICANS* (KÜTZING) COMB. NOV.[1]

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Despite a simple morphology and intensive studies carried out for more than two centuries, the systematics of the Prasiolales still presents several unsolved problems. The taxonomic relationships of several common species of Prasiolales, mostly from northern Europe, were investigated by a combination of morphological observations, culture experiments, and molecular analyses based on *rbc*L sequences. The results indicate that *Rosenvingiella* and *Prasiola* are separate genera. The capacity for production of tridimensional pluriseriate gametangia and the presence of unicellular rhizoids are the morphological features that discriminate *Rosenvingiella* from filamentous forms of *Prasiola*. The molecular data indicate that uniseriate filaments can be produced in at least three different species of *Prasiola*. The genetic diversity of uniseriate filamentous Prasiolales is higher than their simple morphology would indicate, and the provisional retention of *Schizogonium* Kützing 1843 as independent genus is recommended. The *rbc*L phylogeny confirms that *Prasiola calophylla*, *P. crispa*, and *P. stipitata* are distinct species, whereas *P. stipitata* and *P. meridionalis* are probably conspecific. *Rosenvingiella polyrhiza* is a strictly marine alga, and most records of *Rosenvingiella* in Europe are referable to *Rosenvingiella radicans*, proposed here as a new combination based on *Ulothrix radicans* Kützing 1849. This is a primarily terrestrial alga that can occur from upper intertidal rock to locations situated hundreds of kilometers inland. The great confusion that has arisen in Europe between these two species in the last century is mostly due to misidentifications of marine populations of *R. radicans*.

Key index words: Chlorophyta; distribution; Europe; phylogeny; *Prasiola*; Prasiolales; *rbc*L gene; *Rosenvingiella*; *Schizogonium*; subaerial algae

Abbreviations: BP, bootstrap proportions; ML, maximum likelihood; MP, maximum parsimony; NJ, neighbor joining; *rbc*L, gene of the large subunit of RUBISCO

The Prasiolales is an order of marine, freshwater, and terrestrial green algae widespread in polar and cold temperate regions (Burrows 1991, Sherwood et al. 2000), characterized by a stellate axial chloroplast with a central pyrenoid, flagellate cells with four microtubular roots in a cruciate arrangement and anticlockwise rotation of basal bodies, closed mitosis with a persistent telophase spindle, and cytokinesis by transverse wall deposition (O'Kelly et al. 1989, van den Hoek et al. 1995, Sherwood et al. 2000). Its phylogenetic affinities are not yet completely clear, but recent molecular evidence indicates that the Trebouxiophyceae is the class to which the Prasiolales is most closely related (Sherwood et al. 2000, Friedl and O'Kelly 2002).

Although relatively simple, the morphology of the Prasiolales is very diverse and includes uniseriate filaments, narrow ribbons, expanded blades, and sarcinoid colonies (van den Hoek et al. 1995). As presently circumscribed, the order contains four widely accepted genera: *Prasiococcus* Vischer, *Prasiola* Meneghini, *Prasiolopsis* Vischer, and *Rosenvingiella* P. C. Silva. The validity of a fifth genus, *Schizogonium* Kützing, is disputed, and despite recommendations to retain it as an independent genus (Silva 1980), in more recent literature it is usually not considered, having been apparently regarded as a developmental stage of *Prasiola* and *Rosenvingiella* (Burrows 1991).

The circumscription of prasiolalean taxa at both species and genus levels remains one of the most problematic areas in green algal taxonomy, as these algae show considerable morphological plasticity (Burrows 1991, Hamilton and Edlund 1994, Kováčik and Batista Pereira 2001). Over the last 150 years, the taxonomic relationships of filamentous and blade-like forms of Prasiolales have been examined in detail, and several monographic studies on this group have been published (Jessen 1848, Imhäuser 1889, Knebel 1935). Despite this, many problems, at both the genus and species levels, still remain to be resolved.

The genus *Prasiola* was established by Meneghini (1938, p. 36) for a group of leafy green algae formerly considered a tribe of *Ulva* (Agardh 1823, p. 416). Although the original concept of the genus was limited to blade-like forms, it was realized that the leafy morphology may show a transition to different morphotypes as early as in the first half of the 19th century (Agardh 1828, p. 44, Kützing 1843, p. 246). *Prasiola*

crispa in particular, the lectotype species of *Prasiola* (Silva 1980), occurs as both uniseriate filaments and expanded blades, with a complete range of intermediate forms (West 1916, Knebel 1935, Kobayasi 1967, Burrows 1991).

Rosenvinge (1893) erected the genus *Gayella* for filamentous prasiolalean algae able to produce pseudo-parenchymatous gametangia by transversal divisions of uniseriate filaments. Because *Gayella* Rosenvinge is a later homonym of *Gayella* Pierre (Sapotaceae, Magnoliophyta), the genus was subsequently renamed *Rosenvingiella* by Silva (1957). Whether *Rosenvingiella* constitutes a separate genus from *Prasiola* remains controversial. In Europe, the notion that *Rosenvingiella polyrhiza* (Rosenvinge) P. C. Silva, the generitype, may represent a developmental stage of *Prasiola* has arisen because it occurs frequently mixed with species of *Prasiola* and intermediates seem to occur between them (Børgesen 1902, Edwards 1975, Burrows 1991, John 2002). Wille (1901), apparently in ignorance of Rosenvinge's work, described *Prasiola crispa* f. *submarina* for an alga clearly corresponding to *Rosenvingiella*. Børgesen (1902) created the new name *Prasiola crispa* subsp. *marina* for the same alga and placed *Gayella polyrhiza* in synonymy. He was followed in this by Cotton (1912) and Newton (1931). More recently, Edwards (1975) supported the hypothesis of a relationship between *Rosenvingiella* and *Prasiola*, although not specifying it in detail, and Ettl and Gärtner (1995) also listed *Gayella polyrhiza* as a synonym of *Prasiola crispa*. Other authors, however (Collins 1909, Setchell and Gardner 1920a, Knebel 1935, Waern 1952, Kornmann and Sahling 1974, Womersley 1984, Burrows 1991), regarded *Rosenvingiella* and *Prasiola* as separate genera, and all recent lists of northern European seaweeds include *Rosenvingiella polyrhiza* as a species independent of *P. crispa* (Nielsen et al. 1995, Bartsch and Kuhlenkamp 2000, Gunnarsson and Jonsson 2002, Hardy and Guiry 2003, Rindi and Guiry 2004a). Similar problems are found on the Pacific coast of North America with regard to the relationship between *Rosenvingiella constricta* (Setchell & Gardner) P. C. Silva and *Prasiola meridionalis* Setchell & Gardner (Bravo 1962, 1965, Hanic 1979).

The taxonomic relationships of a filamentous terrestrial prasiolalean alga common in northern Europe are particularly problematic. This entity is widespread in the cool humid parts of the continent, and in the past it has been given several different names. *Lyngbya muralis* (Dillwyn) C. Agardh (Agardh 1824), *Ulothrix radicans* Kützing (Kützing 1849), and *Prasiola crispa* f. *radicans* (Kützing) Brand (Brand 1914) are among those most frequently applied. From morphological and ecological reports available in the literature (Boye Petersen 1928, Knebel 1935, Barkman 1958, Gilbert 1991), it is evident that in the last century this entity has been unanimously treated as a filamentous form of *P. crispa*. In previous studies, we noted that this alga is extremely common in urban habitats in western Ireland (Rindi et al. 1999, Rindi and Guiry 2003) and other urban areas of Atlantic Europe (Rindi and Guiry

2004b). Because this species shows the characteristic features of *Rosenvingiella* (transversal division of the axial cells and production of pseudo-parenchymatous gametangia), we concluded that it was a terrestrial form of *Rosenvingiella polyrhiza* (Rindi et al. 1999, Rindi and Guiry 2003). It was recognized, however, that this conclusion was not completely satisfactory, because its morphology is not in perfect agreement with the features of the material originally described by Rosenvinge (1893) from Greenland.

Taxonomic problems also exist in *Prasiola* sensu stricto. The relationships at species level between some European species of *Prasiola* are not yet completely clear. It has been suggested by some authors that *Prasiola calophylla* (Carmichael ex Greville) Kützing and *P. stipitata* Suhr ex Jessen might be conspecific (Waern 1952, Edwards 1975), with *P. stipitata* being considered as a marine form of *P. calophylla*. It has also been proposed that *P. stipitata* may possibly be conspecific with some extra-European species, such as *P. meridionalis* (Bravo 1965).

It is therefore evident that despite two centuries of intensive work, many problems concerning the taxonomy of European Prasiolales remain to be resolved. The application of molecular techniques to algal systematics has now proven fundamental for investigating algal phylogenetics and has shed light on many taxonomic problems in different algal groups. The DNA sequences of the plastid encoded RUBISCO (*rbc*L) gene, in particular, have proven useful for elucidation of relationships at the species and genus level and have been successfully used for several groups of green algae (McCourt et al. 2000, Hayden and Waaland 2002, Hoham et al. 2002, Sernepont Domis et al. 2003, Zechman 2003, Sakayama et al. 2004). In the present study, we combine the use of *rbc*L sequences with morphological and ecological data for the investigation of the relationships between genera and species currently placed in the Prasiolales. The results presented here provide a definitive answer to several problems that have plagued green algal taxonomists for more than two centuries.

MATERIALS AND METHODS

Collections, morphological observations, and culture studies. From 1997 to 2003 a large number of samples of Prasiolales were collected from several localities in Atlantic Europe, mainly in Ireland. A general assessment of morphology and distribution was made, and preliminary culture trials were carried out. The most detailed studies of isolates were from Galway City, and the information available has been published (Rindi et al. 1999, Rindi and Guiry 2003). On the basis of this general assessment, 30 selected populations referable to the most common forms of Prasiolales occurring in Europe were considered. Details of collections are reported in Table 1; voucher specimens for each population were deposited in the Phycological Herbarium, National University of Ireland, Galway (GALW). These populations were selected with the intention of examining all the common morphological forms of Prasiolales and to cover a large geographical range, encompassing many different habitats and locations at different distances from the sea. The collections were made

TABLE 1. Details of the populations of Prasiolales examined and used for the study.

Species	Locality	Distance from sea	Habitat	Herbarium (collector)/ GenBank accession number
Prasiola calophylla	Amsterdam, the Netherlands	25 km	Ground at the base of lamp post	GALW011518 (MDG)
Prasiola calophylla	Copenhagen, Denmark	1 m	Concrete, base of step in landing stage for boats	GALW015263 (FR)
*Prasiola calophylla**	Galway City, Ireland	1 km	Concrete, ground at the base of lamp post	GALW014331 (FR) AY694194
Prasiola cfr. *calophylla**	Athlone, Co. Westmeath, Ireland	80 km	Concrete, base of old wall	GALW015249 (FR) AY694193
Prasiola crispa (leafy)*	Galway City, Ireland	700 m	Bare ground at the base of old wall	GALW010001 (FR) AY694196
Prasiola crispa (filamentous)*	Cork City, Ireland	10 km	Concrete, base of old wall	GALW010332 (FR) AY694196
Prasiola crispa (filamentous)	Durham, England	16 km	On bark, base of birch tree in public park	GALW014233 (FR)
Prasiola crispa (filamentous)	Manchester, England	60 km	Concrete, ground at the base of old wall	GALW014368 (FR)
*Prasiola meridionalis**	Newport, Oregon, U.S.A.	0	Upper intertidal rock	GALW014422 (FR) AY694191
*Prasiola stipitata**	Claddagh, Galway, Ireland	0	Upper intertidal rock	GALW015248 (FR) AY694192
Prasiola sp.*	Manchester, England	60 km	Concrete, ground at the base of old wall	GALW014367 (FR) AY694197
*Rosenvingiella polyrhiza**	Juneau, Alaska, U.S.A.	0	Upper intertidal, granite outcrop	GALW014418 (SL) AY694205
*Rosenvingiella polyrhiza**	Millport, Great Cumbrae, U.K.	0	Upper intertidal rock, mixed with *Prasiola stipitata*	GALW010139 (MDG) AY694206
Rosenvingiella polyrhiza	Kinnacorra, Clare Island, Co. Mayo, Ireland	0	Large boulder in upper intertidal	GALW014397 (FR)
*Rosenvingiella radicans**	Amsterdam, the Netherlands	25 km	On bark, base of plane tree	GALW011557 (MDG) AY694198
*Rosenvingiella radicans**	Copenhagen, Denmark	1 m	Concrete, base of step in landing stage for boats	GALW014366 (FR) AY694201
Rosenvingiella radicans	Robin Hood's Bay, Yorkshire, England	50 m	Concrete, step at the entrance of public toilets	GALW014240 (FR)
*Rosenvingiella radicans**	Old City of Oviedo, Spain	25 km	Limestone, base of old wall	GALW015255 (JLMV) AY694199
Rosenvingiella radicans	Old City of León, Spain	110 km	Limestone, base of old wall	GALW011550 (JLMV)
Rosenvingiella radicans	Galway City (Mill Street), Ireland	700 m	Limestone, base of old wall	GALW015259 (FR)
Rosenvingiella radicans	Galway City (NUIG football ground), Ireland	2 km	On bark, base of cedar tree	GALW014370 (FR)
*Rosenvingiella radicans**	Cork City, Ireland	10 km	Base of old painted wall	GALW010332 (FR) AY694203
Rosenvingiella radicans	Dublin, Ireland	5 km	On bark, base of plane tree	GALW010333 (MDG)
*Rosenvingiella radicans**	Athlone, Co. Westmeath, Ireland	80 km	Concrete, base of old wall	GALW014223 (FR) AY694200
*Rosenvingiella radicans**	Claddagh, Galway, Ireland	0	Upper intertidal rock	GALW015236 (FR) AY694204
*Rosenvingiella radicans**	Blackrock, Co. Cork, Ireland	0	Estuary; rock at high water mark	GALW014341 (FR) AY694202
Rosenvingiella radicans	Clare Island Harbour, Co. Mayo, Ireland	0	Concrete wall facing the sea, supralittoral zone	GALW010089 (FR)
Rosenvingiella radicans	Roundstone, Co. Galway, Ireland	1 m	Top of cement wall facing the sea	GALW013366 (FR)
Rosenvingiella radicans	Kinvara, Co. Galway, Ireland	0	On concrete jetty, high water mark	GALW014398 (FR)
Rosenvingiella radicans	Cobh, Co. Cork, Ireland	1 m	Concrete, artificial jetty	GALW010331 (FR)

Collectors: FR, Fabio Rindi; MDG, Michael Guiry; JLMV, Juan Luis Menéndez Valderrey; SL, Sandra Lindstrom. Populations used for phylogenitic analysis are marked by an asterisk.

from sites where light to dark green patches, referable to prasiolalean algae, were observable with the unaided eye. Several characteristics of the site were noted (type of habitat, substratum, orientation of the colonized surface, width of intervening space, distance from sea); the base of old urban walls was the type of habitat where most collections were made.

Each sample was examined microscopically a few days after collection, and a detailed assessment of the morphology was made. For filamentous forms, a number of characters were noted: 1) width of filaments (measured in 25 randomly chosen filaments); 2) width-to-length ratio of cells (measured in eight randomly chosen cells); 3) presence of unicellular rhizoids; 4) presence of pluriseriate blade-like parts; 5) presence of pluriseriate pseudo-parenchymatous gametangia; 6) if gametangia were present, approximate percentage of filaments producing gametangia (checking the presence of gametangia in 10 randomly chosen filaments); and 7) if gametangia were present, width of gametangia (measured in 10 gametangia randomly chosen). For comparative purposes, herbarium specimens of relevant entities conserved in several European herbaria were examined (Appendix 1), and similar observations were made. Fragments of these specimens were rehydrated by soaking the material in a detergent solution, and permanent slides in 80% Karo corn syrup were prepared for microscopic observation. Photomicrographs of Prasiolales were taken with a DXM1200 digital camera (Nikon UK., Ltd., Surrey, UK) and mounted in plates using Adobe Photoshop 4.0® (Adobe Systems Software Ireland, Ltd., Saggart, Ireland).

For each population of the filamentous forms, stock unialgal cultures were isolated using either a marine (VS5, Von Stosch enriched seawater modified following Guiry and Cunningham 1984) or a freshwater culture medium (JM, Jaworski's Medium; Tompkins et al. 1995). Plastic dishes containing approximately 30 mL of medium were used, and the medium was replaced weekly. A general indication of the tolerance to salinity variation was obtained by culturing the algae in both media. Growth in culture was checked at 10 and 15° C, 16:8-h light:dark cycle, 30–35 µmol photons $\cdot$ m^{-2} $\cdot$ s^{-1}; glass dishes containing approximately 400 mL were used, in which the medium was replaced every 15 days. If good growth occurred, cultures were maintained for at least 6 months, and all the characters considered for the field specimens were also assessed in cultures at least 3 months old.

Molecular studies. DNA was extracted from 16 selected populations (marked by an asterisk in Table 1) either by a CTAB method, modified after Doyle and Doyle (1990), or by using the Qiagen DNeasy Plant Mini Kit (Qiagen GmbH, Hilden, Germany), according to the manufacturer's instructions. With the exception of leafy *Prasiola crispa*, *P. meridionalis*, and *P. stipitata*, DNA extractions were made from material grown in unialgal cultures.

For PCR amplification, a PTC-200 DNA Engine (MJ Research Inc., Waltham, MA, USA) was used. All PCR amplifications were carried out using primers PF2 as the forward primer and PR2 as the reverse primer (Table 2), designed using previously published *Prasiola* sequences (Sherwood et al. 2000). Each reaction contained 200 µM each of dATP, dCTP, dGTP, and dTTP; 0.3 µM of each primer; 2.5 mM MgCl₂; and 1.6

TABLE 2. Oligonucleotide primer sequences used for PCR and sequencing.

Primer name	Direction	Sequence
PF2	Forward	5′ TTCGTATGACTCCTCAATCAG 3′
PR2	Reverse	5′ TTACATGCTGCACGAATA 3′

units of Taq polymerase (Bioline, London, UK). The PCR cycle consisted of an initial denaturing phase of 10 min at 94° C, followed by 35 cycles of 94° C for 1 min, 55° C for 1 min, and 72° C for 2 min, with a final extension phase of 5 min at 72° C.

Approximately 1160 base pairs (bp) of the *rbc*L gene were amplified using PF2 and PR2. The success of the PCR reactions was confirmed by running products on a 1% Tris-acetic acid EDTA agarose gel, stained with ethidium bromide, and visualized under UV light. The PCR fragments for sequencing were then purified using the High Pure PCR Product Purification Kit (Roche Diagnostics Ltd., Lewes, UK), according to the manufacturer's instructions. The PCR products were then directly sequenced commercially by MWG-Biotech (Ebersberg, Germany).

Sequence alignment and phylogenetic analysis. The DNA sequence alignments were constructed by eye using MacClade (Maddison and Maddison 2000). No insertions or deletions were present, making the 1155-bp alignment unambiguous. The sequence data were analyzed using maximum parsimony (MP), neighbor joining (NJ), and maximum likelihood (ML), using PAUP 4*b10 (Swofford 1998). The parameters for the ML analysis were determined using ModelTest version 3.06 (Posada and Crandall 1998). All phylogenetic trees were rooted using *Kornmannia leptoderma* (Kjellman) Bliding (Ulvophyceae, GenBank accession number AF499677), *Trebouxia anticipata* Archibald (Trebouxiophyceae, AF189069), *Myrmecia biatorellae* Vinatzer (Trebouxiophyceae, AF499685), and *Pleurastrum erumpens* Deason and H. C. Bold (Trebouxiophyceae, AF189068) as outgroups.

The most parsimonious tree was determined using a heuristic search, and the input order was randomized 20 times. For the ML analysis, the model selected was TIM + I + G, which specified base frequencies (freq A = 0.2844, freq C = 0.1610, freq G = 0.2194, freq T = 0.3352), a rate matrix (R(a) [A–C] = 1.0000, R(b) [A–G] = 2.5332, R(c) [A–T] = 1.5716, R(d) [C–G] = 1.5716, R(e) [C–T] = 6.1626, R(f) [G–T] = 1.0000), with the proportion of invariable sites (I) = 0.4864, and a gamma distribution shape parameter = 0.7262. For all NJ analyses, an ML distance matrix was used as input, again using the TIM + I + G model. The robustness of the data was determined by bootstrapping the data set (Felsenstein 1985) 1000 times for MP and NJ. The program MrBayes (v. 3.04, Huelsenbeck and Ronquist 2001) was used to complete Bayesian inference of phylogeny under the parameters specified by ModelTest. Ten Markov chains were used, specifying a burn-in period of 1000 generations, and 10⁶ generations were run, with sampling every 100 generations.

RESULTS

Morphology, ecology, and culture responses. *Prasiola calophylla* (Charmichael ex Greville) Kützing is a terrestrial alga in northern Europe, although populations of this species can occasionally occur at sites situated very close to the sea and freshwater bodies. This species commonly occurs on the ground at the base of lamp posts, and two of the populations examined (Amsterdam and Galway) occurred in this type of habitat. The population from Copenhagen consisted of a few specimens mixed in a mat of *R. radicans* occurring on a step of a landing stage for boats in Copenhagen Harbour.

Field specimens of *P. calophylla* consist of a short stipe, 15–20 µm wide, expanding into a monostromatic ribbon-like blade several millimeters long and up to 1 mm wide (Fig. 1A). In surface view, cells are 4–5 × 5–12 µm, arranged in longitudinal and transversal rows. In the
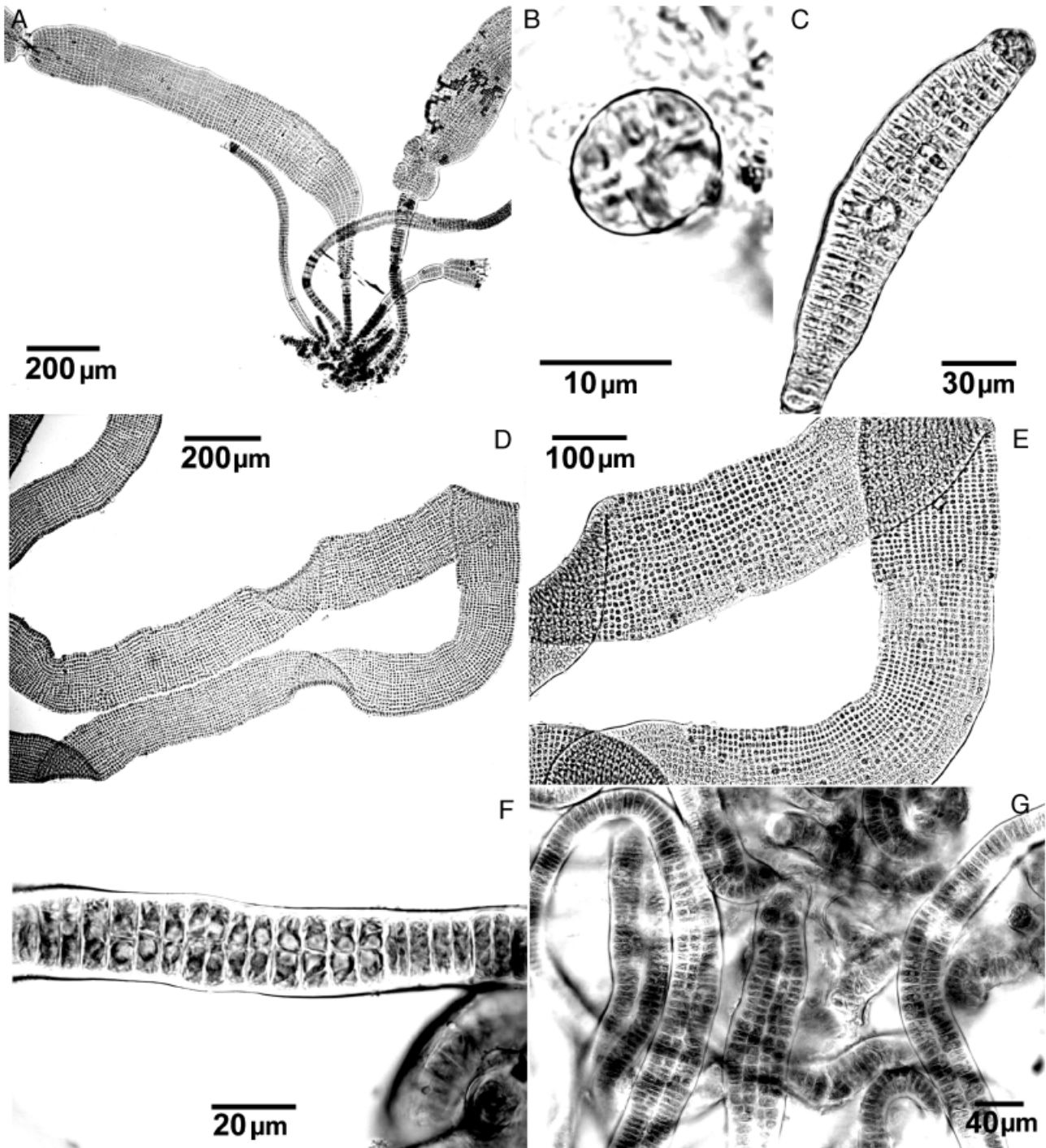
FIG. 1. *Prasiola calophylla* and *Prasiola* cfr. *calophylla*. (A–E) *Prasiola calophylla*. (A) Habit of field material. (B) An aplanosporangium released by a cultured specimen. (C) A young specimen produced in cultured by germination of an aplanospore. (D) Habit of a cultured specimen. (E) Detail of the same specimen at stronger magnification. (F–G) *Prasiola* cfr. *calophylla*. (F) Detail of a uniseriate filament with some cells dividing transversally. (G) Habit of cultured material, consisting mostly of narrow pluriseriate blades.

three populations examined, reproduction was not observed in field specimens but took place in culture.

In culture, *P. calophylla* did not survive in VS5 but grew well and reproduced in JM. Cultured thalli consisted of blades similar to field material but with the tendency to become much more twisted (more at 15° C than at 10° C). Old cultured specimens showed a very irregular habit, with production of uniseriate filaments and new blades from the margins of the blades. Reproduction took place by production and release of
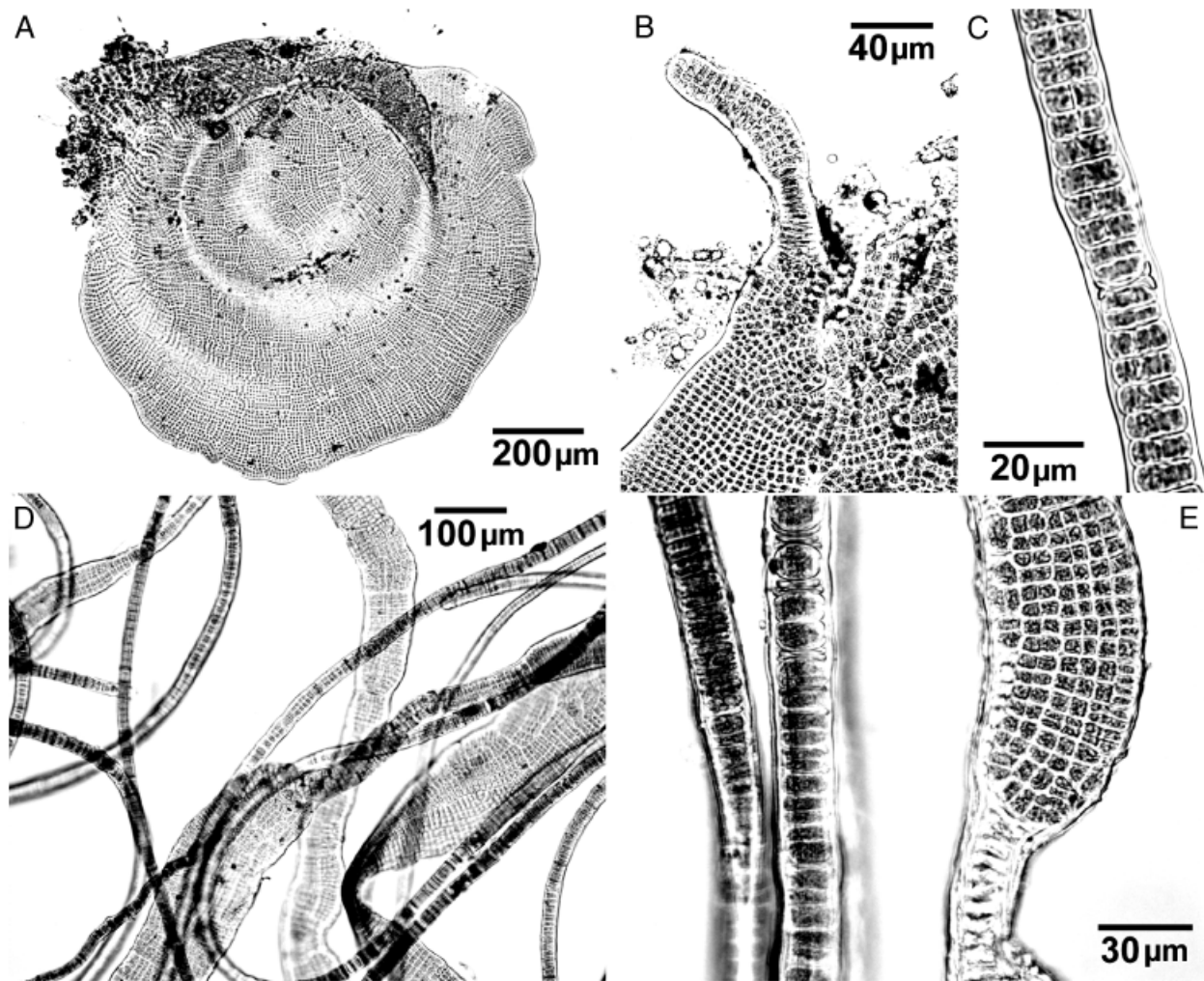
FIG. 2. *Prasiola crispa*. (A) Habit of a young blade of the population from Galway. (B) Detail of the edge of a blade, showing tendency to produce uniseriate parts and narrow blades. (C) Detail of a filament with some cells dividing transversally. (D) A mixture of uniseriate filaments and narrow pluriseriate blades. (E) Detail of two uniseriate filaments and a blade; note superficial corrugations in the uniseriate filaments.

globular aplanosporangia, 10–12 μm in diameter, from the apical parts of the blades (Fig. 1B). Each aplanosporangium released a variable number of aplanospores, globular to elliptical in shape and 2–4 μm in diameter, which produced new thalli (Fig. 1, C–E). This was observed in all three populations examined. It was the only form of reproduction found in *P. calophylla*, and several generations were completed in this way. No filamentous forms similar to *Rosenvingiella* were observed at any developmental stage or in any subsequent generation.

A filamentous *Prasiola* collected in Athlone, Ireland was found at the base of an old concrete wall. Field-collected material consisted mainly of uniseriate filaments, 15–22 μm wide, which tended to produce narrow pluriseriate blades (Appendix 2). When placed in culture, the alga grew well in JM but not in VS5 (Appendix 3). Cultured material showed an irregular morphology, consisting of uniseriate filaments (Fig. 1F)

alternating with narrow pluriseriate blades (Fig. 1G). In old cultures, the uniseriate parts were liable to become irregularly branched. Neither aplanospores nor any other reproductive bodies were observed, and the cultured material reproduced only vegetatively.

Prasiola crispa (Lightfoot) Meneghini was examined for four populations. This alga occurred mostly at the bases of old walls, either on concrete or bare ground. The sample from Durham formed a bright green mat at the base of a birch tree in a public park.

The population from Galway consisted of typical crisped blades, with irregular shape and growing to several centimeters in width (Fig. 2A). The tendency to produce filamentous stages from the edge of the blades was observed in some specimens (Fig. 2B). In surface view, cells were 2–4 × 5–7 μm. The other populations consisted primarily of uniseriate filaments, with the tendency to divide transversally and to produce narrow pluriseriate blades (Fig. 2, C–E).
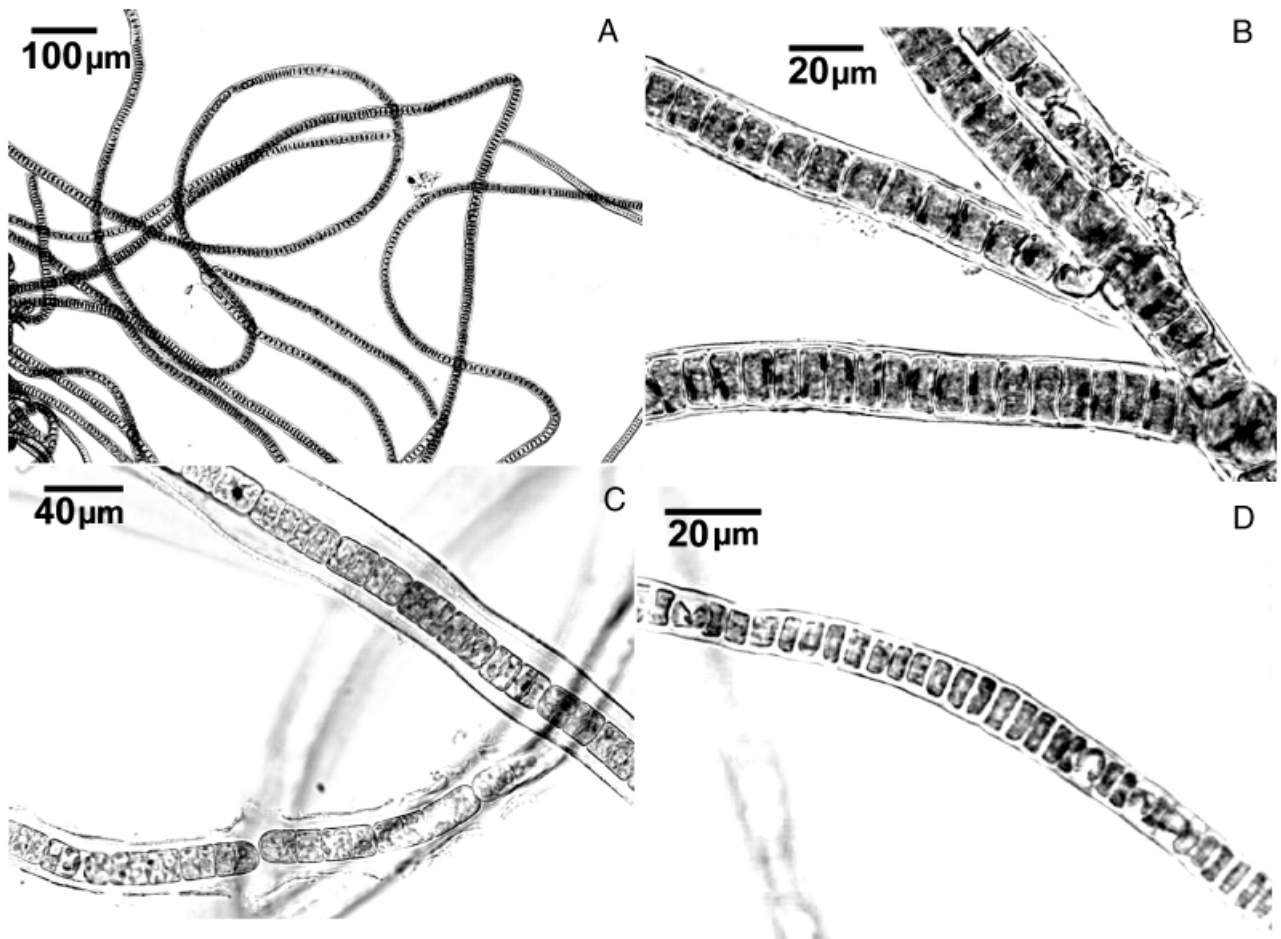
FIG. 3. *Prasiola* sp. from Manchester and *Schizogonium murale*. (A–C) *Prasiola* sp. (A) Habit of field material. (B) Detail of some filaments. (C) Cultured filaments showing enlargement and fragmentation of the original wall. (D) *Schizogonium murale*. Specimen L 0489817, designated as lectotype. Detail of a filament.

Superficial corrugations of the wall were observed in many uniseriate filaments (Fig. 2E). The width of the uniseriate filaments varied between 12 and 25 μm, showing some variability in different populations (Appendix 2); the material from Cork was generally thinner (mostly 12–15 μm) than the algae from Manchester (15–18 μm) and Durham (16–20 μm). Individual cells were 2–6 times wide as long, although this character was also variable in different populations (mostly 2.5–3.5 for Cork, 4–6 for Manchester and Durham).

The filamentous forms of *P. crispa* were unable to grow in VS5, but good growth was observed in JM, where these algae produced filamentous thalli similar to field material (Appendix 3). In culture, however, uniseriate filaments were the main growth form, and the tendency to produce blades was not as marked as in the field material. No growth in culture was obtained for the leafy form, either in JM or in VS5. No reproduction other than vegetative growth and fragmentation was observed in culture.

Prasiola sp., an alga for which identification at species level is presently not possible, was collected from the base of an old urban wall in the city center of Man-chester, where it formed a bright green mat on concrete. The field material consisted of uniseriate filaments 10–18 μm wide (mostly 12–15 μm), provided with a corrugated wall (Fig. 3A). No rhizoids or pluriseriate parts were observed. Individual cells were 2–4 times wide as long, mostly 2–2.5 times (Fig. 3B).

In culture, the alga grew vigorously in JM and weakly in VS5 (Appendix 3). Filaments grown in JM were usually wider than in the field (mostly 16–20 μm) and produced occasionally biseriate parts, but no extensive blades were observed. In old filaments, the alga tended to fragment with a characteristic pattern: The wall became very thick and eventually disintegrated, releasing fragments of variable length, which produced new filaments (Fig. 3C). No other forms of reproduction were observed.

Rosenvingiella polyrhiza (Rosenvinge) P. C. Silva appears to be a strictly marine alga and was confirmed at three sites: Juneau (Alaska), Clare Island (Ireland), and Great Cumbrae Island (United Kingdom). At these locations, *R. polyrhiza* occurred on rocky outcrops and large boulders in the upper intertidal zone. In field specimens, most of the thallus consisted
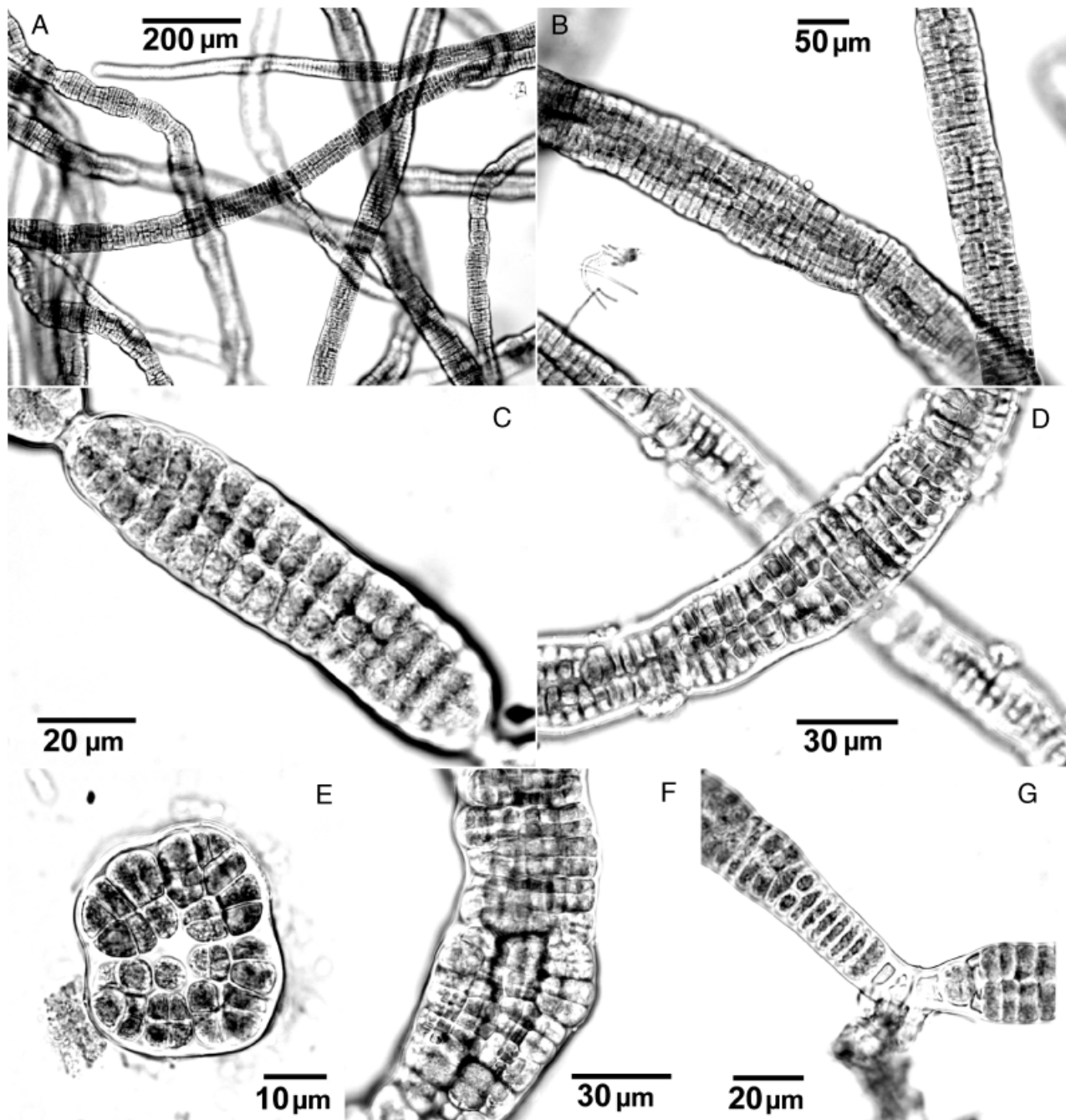
Fig. 4. *Rosenvingiella polyrhiza*, field material. (A–D) Habit of field specimens, consisting mostly of pluriseriate pseudo-parenchymatous gametangia. (E) Transversal section of a gametangium. (F) Detail of a gametangium, showing small male gametangial cells (left down) and large female gametangial cells (the other parts). (G) Rhizoidal part of the thallus, showing uniseriate parts attached to the substratum by a couple of rhizoids.

of pseudo-parenchymatous gametangia, irregularly rounded or polygonal in section (Fig. 4, A–E), produced by anticlinal division of the axial cells. Fully developed gametangia were up to 80 μm in diameter, sometimes constricted at intervals (Appendix 4). In surface view, the gametangial cells were subquadrate and grouped in packets of two different sizes. The female cells (which were most abundant) were 5–10 μm in side, whereas the male cells were 2–3 μm in side (Fig. 4F). The uniseriate parts of the thallus consisted of filaments 9–20 μm wide (11–15 μm in the holotype), formed by cells two to six times (mostly three to five times) wide as long (Appendix 2). These were attached to the substratum by unicellular rhizoids produced as prolongations of vegetative cells (Fig. 4G). Rhizoids occurred either singly, in pairs, or in series of up to six cells.
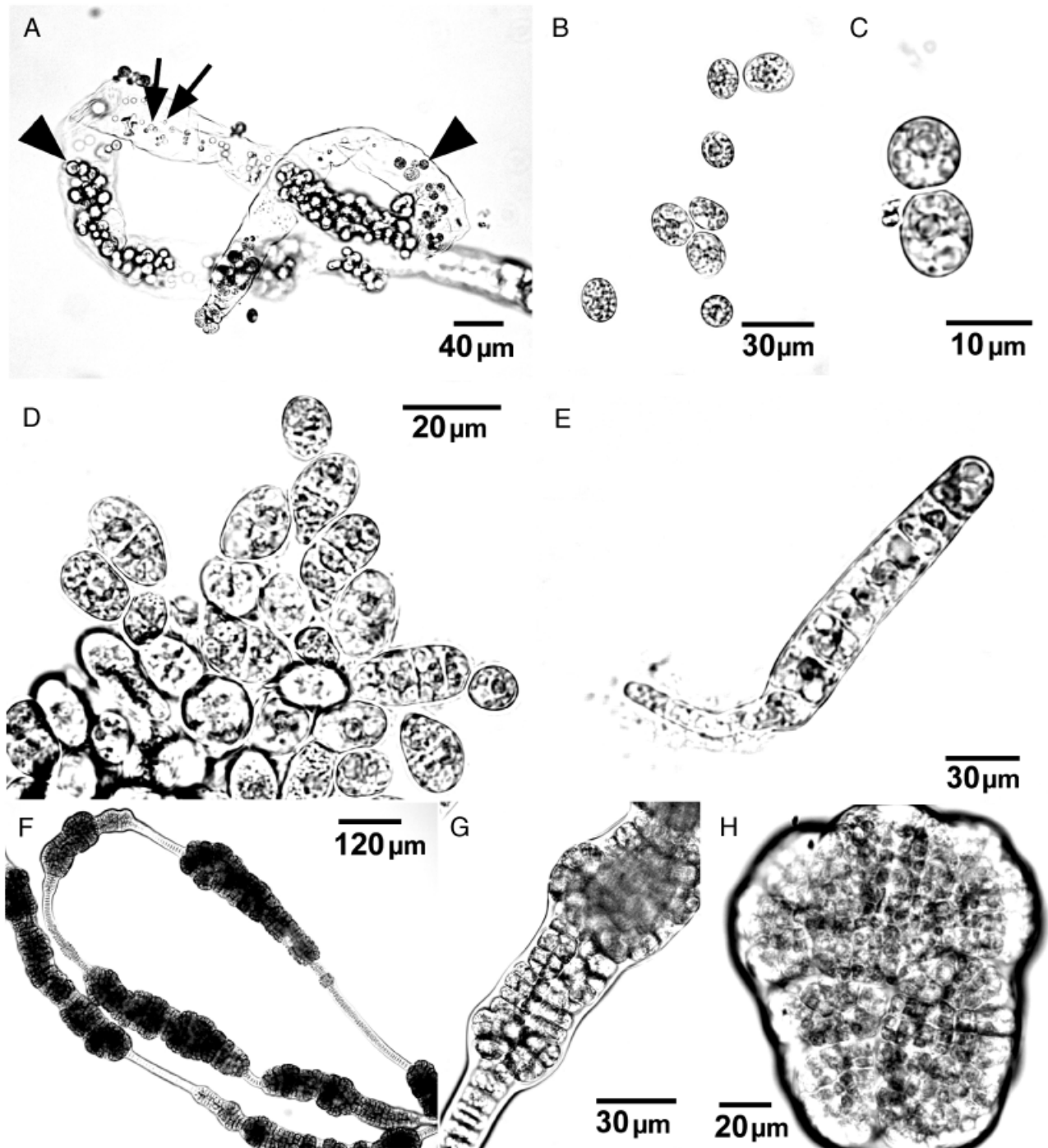
FIG. 5. *Rosenvingiella polyrhiza*, cultured material. (A) Habit of a gametangium disintegrating and releasing large female gametes (arrowheads) and small male gametes (arrows). (B and C) Detail of some female gametes after release. (D) Germination of female gametes, showing germlings at several stages of growth. (E) A young cultured specimens, consisting of a short uniseriate specimen attached by a unicellular rhizoid. (F) A mature cultured specimen, showing development of gametangia. (G) Detail of a gametangium. (H) Habit of a gametangium with irregular shape.

In culture, *R. polyrhiza* grew in both VS5 and JM, but growth in VS5 was faster and more vigorous (Appendix 3). The culture from Alaska grew better at 10 °C than at 15 °C. The morphology of the uniseriate filaments was identical to the field specimens. Gametangia were produced in all the three populations, but their morphology differed from field material in two features. Cultured thalli consisted mostly of uniseriate parts, and the gametangia represented a relatively limited portion of the alga. Furthermore, many

gametangia had a different morphology, consisting of masses of cells with very irregular shape, generally wider than long, and much larger than gametangia observed in field specimens. Some regular cylindrical gametangia were observed only in the material from Alaska (in which the two morphologies coexisted). The most detailed observations were possible for the culture from Alaska, in which release of female gametes took place in culture. About 2 weeks after the field material was placed in culture, the contents of the gametangia started disintegrating (Fig. 5A). Most cells appeared to be female gametes, globular or slightly elliptical and 8–9 μm in diameter (Fig. 5, B and C). Some male gametes were observed mixed with them. They were also globular, 4–5 μm in diameter, with two very long flagella, and were immobile at the time of examination. In subsequent days, the walls of the original alga ruptured and the female gametes were released in large amounts on the bottom of the dishes, where they germinated (Fig. 5D). No sexual fusion was observed, and presumably parthenogenesis took place. The germlings attached to the dishes by a unicellular rhizoid and grew, producing uniseriate filaments (Fig. 5E), which grew profusely, showing the morphology of uniseriate field specimens. After 3 months of growth, production of gametangia became observable (Fig. 5, F–H). After about 5 months, release of female gametes took place as previously observed, giving rise to similar germlings.

In the culture from Great Cumbrae, release of male gametes was observed, but female gametes were never released. No release of gametes was observed in the culture from Ireland, either at 10 or 15 °C. In these cultures the gametangia showed only the irregular morphology, and no further observations on the life history were possible.

Rosenvingiella radicans (Kützing) Rindi, McIvor & Guiry, comb. nov. is a primarily terrestrial alga, but it may also occur in littoral and supralittoral habitats; most of the prasiolalean populations examined in the course of this study were attributed to this species. The populations of Blackrock, Claddagh, and Kinvara (Table 1) occurred on rock and concrete at high water mark, where they are submerged only infrequently and for short times. Populations at Clare Island and Roundstone occurred on surfaces very close to the sea, exposed to large amounts of marine spray. Most populations, however, occurred in completely terrestrial habitats, in some cases in locations situated tens of kilometers from the sea (Amsterdam, Athlone, León, and Oviedo; Table 1). Damp sites at the bases of old urban walls facing North seem to be the favored habitat of this species. The alga is also common in other urban habitats (corners, bases of poles, protruding parts of buildings) and on bark at the bases of trees.

Field material consisted of uniseriate filaments 7–20 μm wide, mostly 8–14 μm (Fig. 6, A and B). These were attached to the substratum by unicellular rhizoids, produced singly or in pairs but not in multiple series (Fig. 6, C–E). Rhizoids were mostly produced in thinner filaments (8–10 μm wide); in most populations they were frequent, but in some (Athlone, Dublin, Galway football ground) they were observed only rarely. Individual cells were 1–5 times wide as long, mostly 2–2.5 times (Appendix 2). Gametangia were rare in field specimens, showing a characteristic temporal pattern: They are produced only in late winter to early spring. Among the populations examined in this study, only the material from Kinvara showed well-developed gametangia at the time of collection (they were also observed in the population at Mill Street in Galway but not at the time in which the material was collected and isolated in culture). The gametangia were usually observable only in a limited number of filaments, rarely more than 10% in a sample (Appendix 4). When fully developed, the gametangia were 12–35 μm wide (Appendix 4), and in surface view the cells were small, subquadrate, and 2 to 7 μm in side (Fig. 6, F–H).

In culture, *R. radicans* showed a rapid and vigorous growth in both VS5 and JM (Appendix 3). When placed in culture, the alga attached itself to the bottom of the dishes by the production of numerous rhizoids, even in populations in which rhizoids were rare in the field material. In young cultures, the algae consisted of uniseriate filaments morphologically similar to the field material, and no differences between material grown in VS5 and JM were evident. In older cultures, however, the morphology showed some variation. The widths of the filaments often exceeded those of field specimens (Appendix 2), and in some filaments the cell wall became very thick (up to 3 μm). The abundance of rhizoids showed considerable variation. Although several populations continued to produce abundant rhizoids even after years in culture, in other populations (Claddagh, Clare Island, Copenhagen) rhizoids were not observable in old cultures (Appendix 2). In culture *R. radicans* reproduced only by growth, and vegetative fragmentation and gametangia were never observed at any stage, either in VS5 or JM. When filaments bearing gametangia were placed in culture, they fragmented and decayed, and the uniseriate parts of the filaments restarted vegetative growth. No release of reproductive bodies was observed.

Molecular analyses. Of the 1155 characters included in the *rbc*L sequence analyses, 722 were constant and 341 were parsimony informative. Within the Prasiolales, sequence divergence between genera ranged from 6.32% (*R. polyrhiza* vs. *P. linearis*/*P. furfuracea*/*P. meridionalis*/*P. stipitata*) to 8.25% (*Prasiola* sp. New Zealand vs. *R. radicans* Copenhagen). Intrageneric sequence divergences ranged from 0.71% (*P. stipitata* vs. *P. linearis*, *P. furfuracea*, and *P. meridionalis*) to 5.7% (*P. crispa* vs. *P. fluviatilis*). The *rbc*L sequence obtained from *P. meridionalis* from Oregon was identical to the sequences *of P. meridionalis*, *P. linearis*, and *P. furfuracea* from GenBank and differed from the sequence of *P. stipitata* from Galway obtained for this study by only 0.7%. Intraspecific sequence divergence ranged from 0% (*R. radicans* from Cork vs. *R. radicans* from Oviedo) to 0.86% (*R. radicans* from Copenhagen vs. *R. radicans* from Athlone).
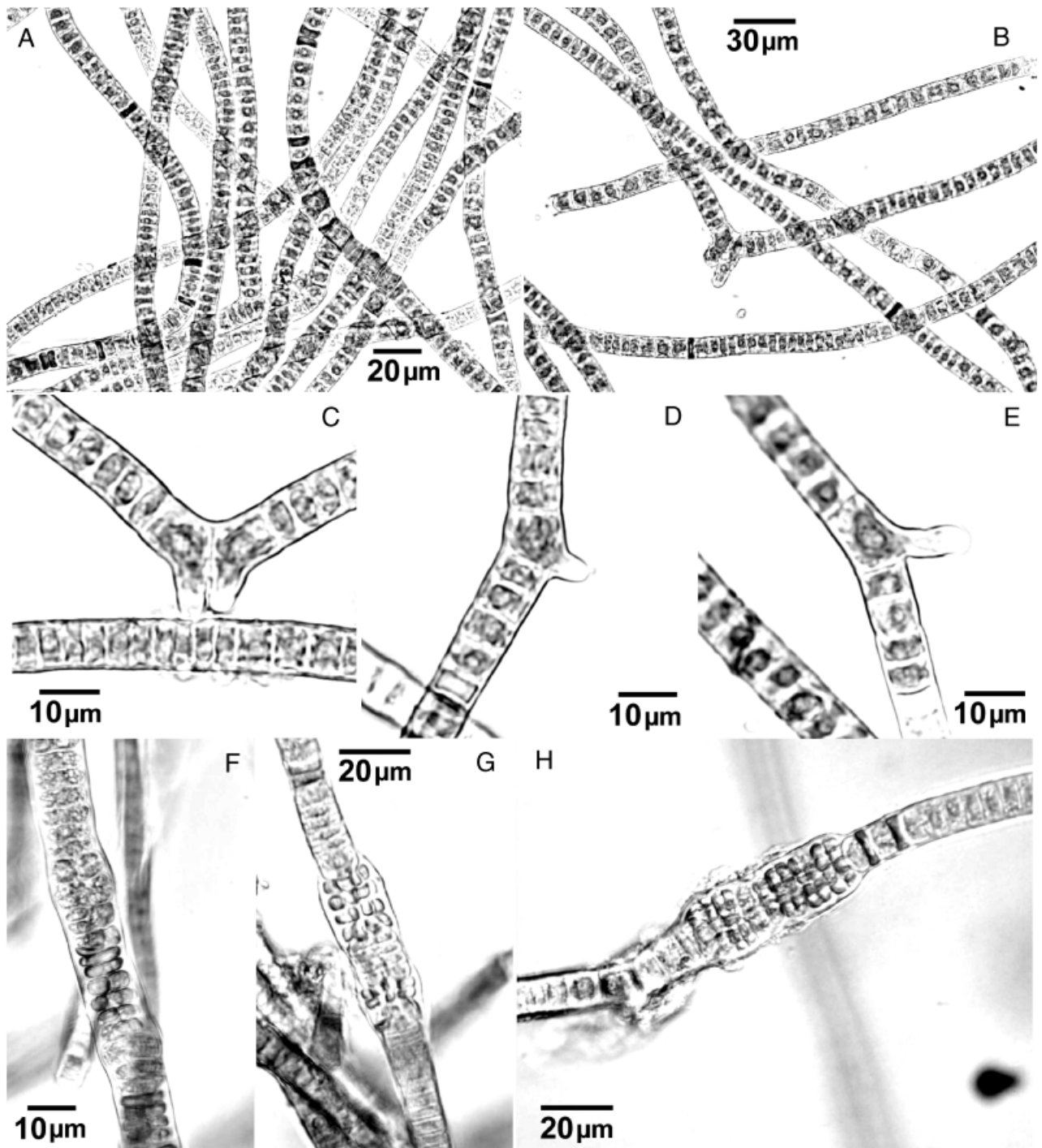
FIG. 6. *Rosenvingiella radicans*. (A and B) Habit of uniseriate filaments. (C–E) Detail of rhizoids, produced in couple and singly. (F) Detail of a filament starting to develop gametangia. (G and H) Detail of gametangia.

Parsimony analysis (not shown) resulted in six most parsimonious trees of length 815 steps (consistency index = 0.629, retention index = 0.772, homoplasy index = 0.371) and differed only in the placement of the *R. radicans* samples and *P. stipitata*, *P. linearis*, *P. furfuracea*, and *P. meridionalis* relative to each other. Parsimony analysis was congruent with the ML analysis,

shown in Fig. 7, with bootstrap proportions (BP) from MP and NJ analyses and posterior probabilities from the Bayesian analysis shown at the nodes. Posterior probabilities were 1.00 unless stated, and those less than 0.95 are not indicated on the phylogenetic tree.

In all analyses, the Prasiolales formed a monophyletic group with high bootstrap support (BP = 100%).
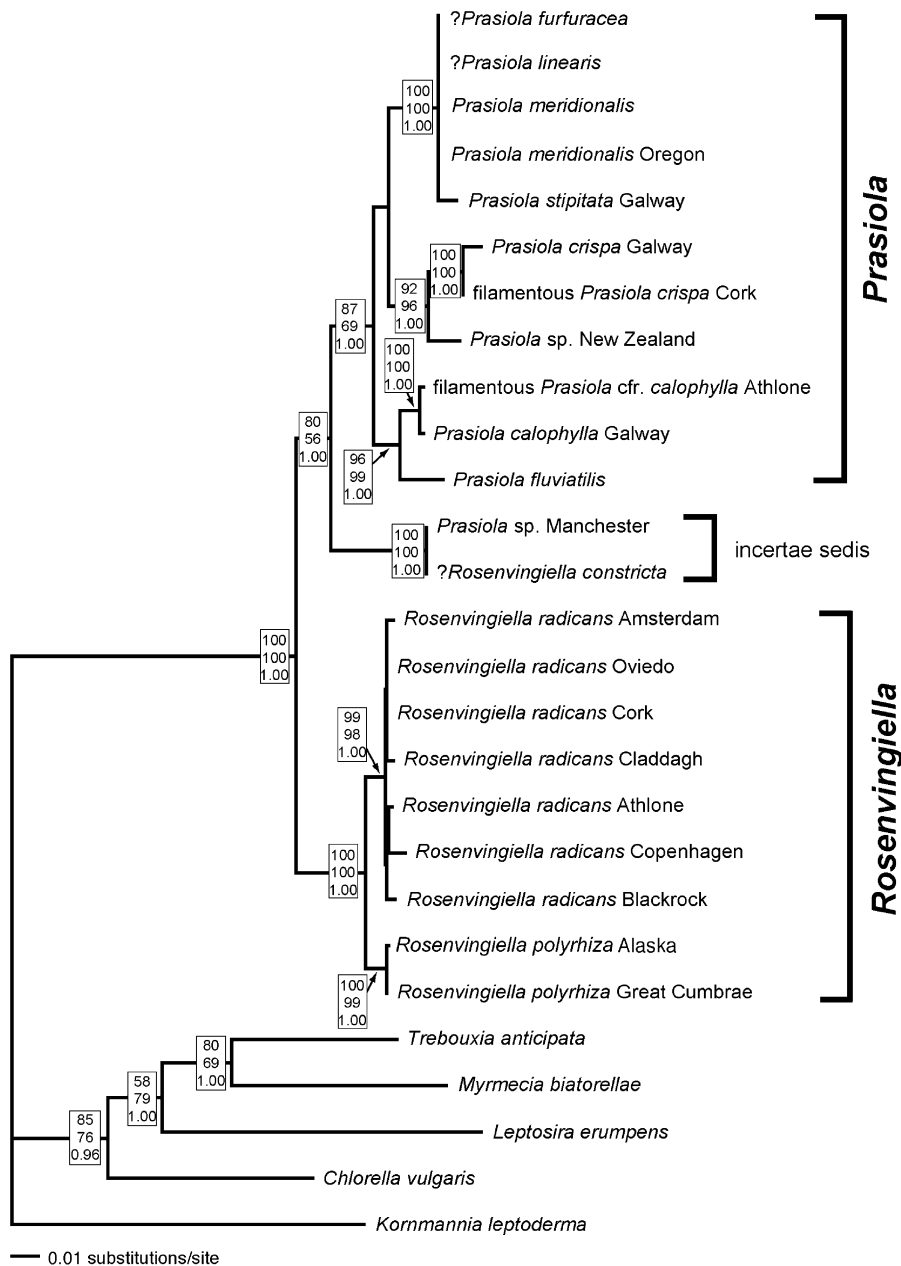
FIG. 7. Phylogenetic tree produced from maximum likelihood analysis of *rbc*L sequences of *Prasiola*, *Rosenvingiella*, and outgroup sequences obtained from GenBank, with bootstrap proportions from maximum parsimony (top, 1000 replications), neighbor joining (middle, 1000 replications with maximum likelihood distance algorithm as input), and posterior probabilities from Bayesian analysis (bottom) given at nodes. Bootstrap support less than 50% is not indicated, nor are posterior probabilities less than 0.95, and all branch lengths are drawn proportional to the amount of sequence change.

Rosenvingiella formed a monophyletic group with 100% bootstrap support in all analyses, with the samples attributed to *R. polyrhiza* and those attributed to *R. radicans* forming two well-supported sister groups to each other (BP = 98%–100%). The published sequence of *R. constricta* was shown to be identical in sequence to the *Prasiola* sp. collected from Manchester and did not group with any *Rosenvingiella* sequence included in this study. Both *Prasiola* sp. from Manchester and *R. constricta* formed a sister group to the remaining *Prasiola* species used in this study, although the bootstrap support was moderate to weak (BP = 56%–80%). The remaining *Prasiola* species were grouped together with moderate to good support (BP = 69%–87%) and were further divided into three well-supported groups

(BP = 92%–100%), although the relationships between these groups remained equivocal and could not be resolved with certainty. The filamentous form of *P. crispa* from Cork differed from the leafy *P. crispa* from Galway by only 6 bp, and *Prasiola* sp. from New Zealand (recovered from GenBank) was placed basal to these. The *rbc*L sequence obtained from *P.* cfr. *calophylla* from Athlone differed from *P. calophylla* by only 2 bp, and both taxa formed an extremely well-supported group, with *P. fluviatilis* from North America placed basally in this group (BP = 96%–100%) and the remaining *Prasiola* species placed as a sister group in both MP and ML analyses, although this relationship was not supported in any of the bootstrapped analyses. The sequences of *P. meridionalis*, *P. linearis*, and *P. furfuracea* were

identical; these grouped together with *P. stipitata* (BP = 100%), with *P. stipitata* differing by 6 bp.

DISCUSSION

When combined together, *rbc*L sequences, morphological data, and culture observations provide a clear and unambiguous picture of the species and genus relationships of the most commonly occurring Prasiolales in northern Europe.

Relationships at genus level. At the genus level, our results clearly show that *Prasiola* and *Rosenvingiella* should be regarded as separate genera. The *rbc*L phylogeny shows that the populations of *Rosenvingiella* sequenced in this study form a well-supported monophyletic group, separated from all species of *Prasiola*. Sequences of specimens referable with certainty to the generitypes of both genera (*P. crispa* and *R. polyrhiza*) are included in our results, which renders the relationships between *Prasiola* and *Rosenvingiella* unambiguous. Noticeably, the only *rbc*L sequence of *Rosenvingiella* previously available in GenBank (referred to *R. constricta*) occurs in a separate group, which is not in agreement with our results, and we believe this to be the result of a misidentification. The study in which this sequence was produced (Sherwood et al. 2000) focused on phylogeny at the order level and did not provide morphological details on the material sequenced.

The separation of *Rosenvingiella* from *Prasiola* is also well supported on morphological and physiological grounds. The production of pluriseriate pseudo-parenchymatous gametangia by anticlinal divisions of the axial cells, used by Rosenvinge (1893) to erect *Rosenvingiella* (as *Gayella*), is clearly shown to be a good character at the generic level. Pluriseriate gametangia have never been reported for *Prasiola* (except for studies in which *Rosenvingiella* was placed in synonymy with *Prasiola*) and are constantly observable in *R. polyrhiza*. In *R. radicans* the production of gametangia shows a clear seasonal pattern: In Galway City, it takes place only in late winter and early spring (Rindi et al. 1999, Rindi and Guiry 2003), and the few reports available for other north European regions indicate that this is probably a general pattern (Kornmann and Sahling 1974, Edwards 1975, Menéndez-Valderrey, personal communication). The tendency to produce narrow pluriseriate blades, typical of filamentous *Prasiola*, was never observed in *Rosenvingiella*. We also agree with Waern (1952, p. 44) that the capacity to produce unicellular rhizoids is a valid character in the separation of the two genera. Rhizoids are typically produced in *R. radicans* and *R. polyrhiza* but were never observed in filamentous forms attributable with certainty to *Prasiola*. This character, however, should not be regarded as an absolute criterion for the identification of individual specimens, because its abundance (both in the field and in culture) is quite variable. Rhizoids are profuse in most collections of *Rosenvingiella*, but in some populations they are either very rare or com-

pletely absent. The correct generic attribution of field-collected filamentous specimens 10–5 µm wide, devoid of both rhizoids and gametangia, as either *Rosenvingiella* or *Prasiola*, is usually impossible. However, when placed in culture, even filaments of *Rosenvingiella* devoid of rhizoids in the field will produce abundant rhizoids, thereby allowing generic discrimination. Other characters, such as width of uniseriate filaments (usually thicker in *Prasiola*), width-to-length ratio of cells (usually higher in *Prasiola*), thickness of the wall (thicker in old filaments in both genera), and presence of corrugations on the wall (occasionally observable in *Prasiola*), in our opinion show too wide a range of variation to be used in the distinction between *Rosenvingiella* and uniseriate *Prasiola*. In both genera, these characters may be considerably influenced by environmental conditions. Conditions favorable to growth generally produce thicker filaments, and the cells will divide more quickly, producing a higher width-to-length ratio; the cell wall is generally thicker in old slow-growing filaments in both genera.

Growth in culture also supports the separation of *Rosenvingiella* from *Prasiola*. *Rosenvingiella radicans* shows a wide tolerance to variations in salinity, growing well in both marine and freshwater media. Although growth in freshwater media is not as vigorous, *R. polyrhiza* is also able to grow in both types of media. Conversely, *P. crispa* and *Prasiola* sp. from Manchester grow well in freshwater media, but their growth in marine media is weak or absent. *Prasiola calophylla* is totally unable to survive in marine media and, although sometimes found very close to the sea, appears completely segregated from the marine environment proper. The fast growth and reproduction by aplanosporangia observed in the freshwater medium for this species are in agreement with the observations of Kornmann and Sahling (1974). For none of the cultured populations of *R. radicans* and *R. polyrhiza* was any evidence found that these populations may be part of a life history involving also forms of *Prasiola*. It is noteworthy that despite the controversial circumscription of the two genera, most of the culture studies that have concerned species of *Rosenvingiella* provided the same results (Setchell and Gardner 1920a, Kornmann and Sahling 1974, Hooper and South 1977). Only the study of Bravo (1965) on *Prasiola meridionalis* came to different conclusions, but Hanic (1979) pointed out that this was likely to have been caused by contaminations in Bravo's cultures.

Prasiola crispa is the only terrestrial species of *Prasiola* for which production of uniseriate stages is reported (Knebel 1935, Kobayasi 1967, Rindi et al. 1999, Kováčik and Batista Pereira 2001). However, our phylogenetic analyses show that filamentous uniseriate forms of terrestrial *Prasiola* are attributable to at least three different *Prasiola* species. Whereas the filamentous alga from Cork is clearly *P. crispa*, the alga from Athlone is conspecific with *P. calophylla* and the alga collected in Manchester appears to represent a taxon distinct from both, for which identification at species level is not

possible at present. These filamentous algae are so morphologically similar that it is currently impossible to establish boundaries at species level between them. Further collections and observations are necessary for a better characterization of these forms, in particular for the alga from Manchester. In our analyses, this species is placed as a sister group to all other species of *Prasiola*. It is unclear whether this group represents a separate genus; however, it is clear that this represents a distinct evolutionary lineage, and the inclusion of further species is likely to resolve this further. If discrimination at the genus level proves justified for this taxon, the use of the name *Schizogonium*, rather than the creation of a new genus, seems to us the appropriate solution. *Schizogonium* was erected by Kützing (1843, p. 246) for prasiolalean algae consisting of uniseriate filaments with the capacity to divide transversally, producing narrow blades. Silva (1980) designated *Schizogonium murale* Kützing as the lectotype species. As defined and illustrated by Kützing (1843, 1845, 1849, 1850), most species of the genus are probably developmental stages of *P. crispa*, and with very few exceptions (Collins 1909, Thompson 1938, Massuk and Kostikov 1984), in the last century this genus seems to have generally been placed in synonymy with *Prasiola*. Authentic specimens of *Schizogonium murale* from the Herbarium Kützing (conserved in L) were examined during this study, and the specimen L 0489817 is designated here as lectotype. The specimen consists of uniseriate filaments 8–13 μm wide, which occasionally divide to produce biseriate parts (Fig. 3D); no rhizoids are observable. The material corresponds well with Kützing's illustrations (Kützing 1843, fig. 7 in pl. 3; Kützing 1850, fig. 1 in pl. 98) and, except for the smaller width, resembles closely our alga from Manchester. Further observations and *rbc*L sequences are clearly necessary to assess in detail the relationships between uniseriate forms of *Prasiola* and clarify the position of *Schizogonium*. In any case, our results suggest for these filamentous forms a much higher level of genetic diversity than is indicated by their simple and uniform morphology. We agree with Silva (1980) that, pending further investigations, *Schizogonium* should be retained as independent genus. *rbc*L sequences for *Prasiola velutina* (Lyngbye) Wille, a freshwater species morphologically similar to narrow forms of *P. crispa* and *Schizogonium* (Knebel 1935, Printz 1964, Lokhorst and Star 1988), are also desirable.

Relationships at species level in Prasiola. Our *rbc*L phylogeny indicates that *P. crispa*, *P. calophylla*, and *P. stipitata* are different species. They occur in three separate groups, which roughly reflect a division in marine species (*P. stipitata* group), freshwater-terrestrial species (*P. calophylla* group), and completely terrestrial species (*P. crispa* group). The variation of the *rbc*L sequences in the marine group is unexpectedly low, considering the clearly different morphology of the species included. *Prasiola stipitata* and *P. meridionalis* are the only two species for which *rbc*L sequenc-

es were obtained in this study, and the possible conspecificity suggested by our analyses is not particularly surprising, considering the close similarity in habit (Setchell and Gardner 1920b, Knebel 1935, Bravo 1962, Burrows 1991), the very similar life history (Friedmann 1959, 1969, Cole and Akintobi 1963), and the identical habitat. More difficult to explain are the identical *rbc*L sequences of *P. linearis* and *P. furfuracea*, which were obtained from GenBank. These species have a distinctive morphology (Knebel 1935, Jao 1937, Printz 1964, Burrows 1991), and the culture study of Kornmann and Sahling (1974) did not indicate any possible relationship between *P. furfuracea* and *P. stipitata*. The incongruence between the 18S results and the *rbc*L data from the same samples in the study in which these sequences were published (Sherwood et al. 2000) complicates matters, and we suggest that further studies need to be carried out to clarify the situation. Because *P. furfuracea* and *P. stipitata* are well-distinct morphologically (Knebel 1935, Kornmann and Sahling 1974, Burrows 1991), we believe that the results obtained using the 18S sequence data are more likely to be correct. Further studies will also be necessary to resolve the relationship between *P. calophylla* and the freshwater species *P. fluviatilis* (Sommerfelt) Areschoug.

Relationships at species level in Rosenvingiella. For *Rosenvingiella*, our phylogenetic analyses show a clear separation of the marine populations attributable to *R. polyrhiza* and the mostly terrestrial populations that are attributable to a different species, designated here as *R. radicans*. This separation is remarkably well supported by morphological features and culture responses. All specimens of *R. polyrhiza* examined in this study, either directly collected or obtained from herbaria, occurred in marine habitats and consisted mostly of thick pseudo-parenchymatous gametangia, up to 80 μm wide. *Rosenvingiella radicans* occasionally occurs in marine habitats at or near the high water mark, but it is a primarily terrestrial alga. In *R. radicans*, most samples consist of uniseriate filaments and gametangia are rare. As mentioned above, they only occur in late winter and early spring and, if present, are less than 35 μm in width. Our observations also suggest that the morphology of the rhizoidal parts of the thallus is a good character for discrimination at the species level. Whereas in *R. polyrhiza* rhizoids occur commonly in series of four, five, or six adjacent cells, in *R. radicans* rhizoids, if present, are only produced singly or in couples (Rindi et al. 1999, Rindi and Guiry 2003, 2004b). In culture, the growth of *R. polyrhiza* varied in vigor, depending on the population, but production of gametangia took place in all populations at some stage. For *R. radicans*, none of the 16 populations isolated in culture produced gametangia at any stage, and variations in the culture regime were ineffective in stimulating their production (unpublished data). In general, the distinction between these two species is so sharp that it is almost

surprising how much confusion between them occurred in the last century. This confusion probably originated from the fact that populations of *R. radicans* may grow in the marine habitats that are normally typical of *R. polyrhiza*. In the upper intertidal, the two species overlap and may even occur mixed. Judging from the original descriptions, *P. crispa* f. *submarina* (Wille 1901) and *P. crispa* subsp. *marina* (Børgesen 1902) are simply marine populations of *R. radicans*. From descriptions of morphology and ecology, we also believe that the reports of *R. polyrhiza* by Waern (the Öresund population, Waern 1952), Feldmann and Magne (1964), Kristiansen (1972), and Coppejans et al. (1984) should also be referred to *R. radicans*. For Helgoland, Germany, the description of morphology and culture responses and the remark that the alga is widespread in the village of Helgoland clearly indicate that the material examined by Kornmann and Sahling (1974) was also *R. radicans*, whereas the material collected by Edwards (1975) at the mouth of the river Wear, England was most probably a mixture of the two species. Records of terrestrial *R. polyrhiza* for Ireland (Rindi et al. 1999, Rindi and Guiry 2003) and northern Spain (Menéndez Valderrey and Rico 2001) must also be referred to *R. radicans*.

Unfortunately, it has not been possible during this study to obtain fresh material of the other species currently included in *Rosenvingiella*, *R. constricta*. This was originally described (Setchell and Gardner 1920b) and has been widely reported (Scagel et al. 1989, Hansen 1997) from the Pacific coast of North America and is very rare in Europe (Waern 1952, Kornmann and Sahling 1974). The *rbc*L sequence of *R. constricta* obtained from GenBank was found to be identical to our *Prasiola* sp. from Manchester and is incongruent with the morphological evidence. Considering the ambiguity of other results from the same study (Sherwood et al. 2000), we are very unwilling to regard this result as grounds to refine the taxonomy of this species. After examination of the isotype specimen conserved in BM, we consider that the placement of this species in *Rosenvingiella* is beyond doubt. *Rosenvingiella constricta* is very distinct from both *R. polyrhiza* and *R. radicans* with respect to the width of the gametangia and the presence of frequent constrictions, and we believe that it must continue to be regarded as a separate species.

Nomenclature. The choice of a suitable name for the terrestrial species of *Rosenvingiella* is a very difficult nomenclatural problem, and only after a detailed examination of the older literature and many herbarium specimens have we been able to conclude that *R. radicans* is the appropriate combination for it. Our investigations showed that this species was well known to the phycologists of the early 19th century, but its circumscription was very confused. Names that have been used for it with some degree of certainty include *Conferva velutina* Bory de Saint-Vincent (Bory de Saint-Vincent 1796), *Conferva frigida* Roth (Roth 1797), *Oscillatoria parietina* Vaucher (Vaucher 1803), *Conferva muralis* Roth (Roth 1806), *Conferva*

muralis Dillwyn nom. illeg. (Dillwyn 1809), *Oscillatoria muralis* (Dillwyn) C. Agardh (Agardh 1812), *Lyngbya muralis* (Dillwyn) C. Agardh (Agardh 1824), *Rhizoclonium murale* (Dillwyn) Kützing (Kützing 1843), *Hormidium murale* (Dillwyn) Kützing (Kützing 1845), *Ulothrix radicans* Kützing (Kützing 1849), and *Schizogonium julianum* Meneghini in Kützing (Kützing 1849). The search is particularly complicated by the fact that the original descriptions of these species are usually very concise. Most authors just describe these species as filamentous algae forming green mats at the bases of old walls, on damp soil, or woodwork (Bory de Saint-Vincent 1796, Roth 1797, Vaucher 1803, Roth 1806, Dillwyn 1809, Lyngbye 1819, Agardh 1824, Kützing 1843, 1845), and only rarely are some important morphological details provided. We know now that species belonging to at least three genera currently recognized can produce green mats at the base of walls and trees: *Klebsormidium*, *Prasiola*, and *Rosenvingiella* (Rindi and Guiry 2004b). It is clear that the earlier phycologists were not aware of this and their circumscription of species and genera followed completely different concepts. Difficulties in locating authentic specimens further complicate attempts to circumscribe the taxonomic concepts on which these species were based. Because our results have shown that presence of rhizoids and production of pluriseriate gametangia are the morphological markers that unambiguously define *Rosenvingiella*, we believe that the only possibility of resolving this problem unequivocally is to select as basionym the oldest name for which the original description or illustrations indicate the presence of rhizoids or gametangia.

Conferva velutina was reported by Bory de Saint-Vincent (1796, p. 37) as a terrestrial alga forming bright green mats on old wood and bases of walls in cities, but no morphological details useful for attribution to *Prasiola*, *Rosenvingiella*, or other genera were included in the description. The only authentic specimen of this species that we could locate in PC, where Bory's original material is conserved, consists of an alga with uniseriate filaments 10–14 μm wide (Appendix 2). No pluriseriate parts and rhizoids occur. For these reasons, we conclude that this species cannot be referred unambiguously to the terrestrial *Rosenvingiella*.

Roth's *Conferva frigida* (Roth 1797, p. 166) and *C. muralis* (Roth 1806, p. 189) were both used for forms corresponding to the terrestrial *Rosenvingiella* by subsequent authors (Lyngbye 1819, Agardh 1824). Even in this case, however, the original descriptions are too vague to allow discrimination at genus level, and because most of Roth's herbarium was destroyed during World War II (Stafleu and Cowan 1983), there is no possibility to examine authentic specimens.

Oscillatoria parietina was described by Vaucher (1803, p. 196) as forming green patches on rocks, wood, and soil. In this case, however, Vaucher's description and illustration provide enough detail to establish an identity: The original illustration and the observation that

the filaments are able to move clearly indicate that the original material was an oscillatorialean blue-green alga. The attribution of this entity to *Rosenvingiella* (Lyngbye 1819, Agardh 1824) is therefore incorrect.

Conferva muralis nom. illeg. was described by Dillwyn (1809, p. 39, pl. 7) in apparent ignorance of Roth's *C. muralis* and was subsequently used by several authors as basionym for names that were clearly applied to the terrestrial *Rosenvingiella*. C. Agardh proposed for this entity the new combinations *Oscillatoria muralis* (Agardh 1812, p. 108) and *Lyngbya muralis* (Agardh 1824, p. 74). Kützing's *Rhizoclonium murale* (Kützing 1843, p. 261) and *Hormidium murale* (Kützing 1845, p. 193) were based on *Lyngbya muralis* and therefore indirectly on *C. muralis* Dillwyn. From the examination of many herbarium specimens conserved in BM and L, it is evident to us that these names have been mostly applied to the terrestrial *Rosenvingiella*. However, Dillwyn's original description and illustrations provide no evidence that his *C. muralis* is the correct basionym for it. No rhizoids or gametangia are mentioned or illustrated in the original plate. Drouet (1968) designated an original specimen of Dillwyn (conserved in the Agardh herbarium) as lectotype of *C. muralis* and stated that the material was *Schizogonium*. We could not examine the lectotype, but after examination of two authentic specimens of Dillwyn's *C. muralis* conserved in BM, we have no reason to reject Drouet's view. One of these samples is clearly a mixture in which the terrestrial *Rosenvingiella* occurs. However, the most abundant entity is a thicker filamentous alga, 10–16 μm wide and devoid of rhizoids (Appendix 2), which closely resembles Dillwyn's original illustrations and should be considered the authentic *C. muralis*. The other sample consists of a filamentous uniseriate alga, 9–12 μm wide and devoid of pluriseriate parts and rhizoids, for which unambiguous determination is not possible. For the reasons mentioned above, the morphology of these specimens does not seem to correspond to *Rosenvingiella* and is in better agreement with filamentous *Prasiola* or *Schizogonium*, if the genus is to be retained. The use of *C. muralis* Dillwyn as basionym for our terrestrial *Rosenvingiella* would therefore be inappropriate.

Kützing (1849, p. 349) created the new name *Ulothrix radicans* for the species that he had previously called *Rhizoclonium murale* (Dillwyn) Kützing (Kützing 1843) and *Hormidium murale* (Dillwyn) Kützing (Kützing 1845). He did not provide any explanation for the creation of a completely new name, but the fact that in the description (Kützing 1849, p. 349) he listed *C. muralis* Dillwyn as a possible synonym with a question mark suggests that Kützing realized that the name had been misapplied in the previous circumscription of this species. To our knowledge, *U. radicans* is the first name that can be applied without ambiguity to our terrestrial *Rosenvingiella*. The presence of rhizoids is reported both in the original description and in the specific epithet itself (*radicans* = rooting, provided with roots). The figure IIIa of plate 95 in [52]Kützing (1850) provides definitive confirmation. We therefore propose the following new combination as the correct name for the terrestrial *Rosenvingiella*:

Rosenvingiella radicans (Kützing) Rindi, McIvor & Guiry

Basionym: Ulothrix radicans Kützing (*Species algarum*, 1849, p. 349)

In the description, Kützing cited *Schizogonium julianum* Meneghini as a synonym of *U. radicans*. However, this is apparently a *nomen nudum*, which was not published before and has not nomenclatural priority over *U. radicans*.

The sheet L 0055072 (Rijksherbarium, Leiden) was selected as type material of *U. radicans* by Lokhorst, who considered this alga a filamentous form of *Prasiola*. The sheet includes three specimens that were collected (as testified by Kützing's handwriting), respectively, in Zurich (Switzerland), Karlsruhe, and Stuttgart (Germany). On the fragments of sheet supporting the specimens, however, the indication of the locality is specified only for the material from Karlsruhe. Because the type must be a single specimen (Greuter et al. 2000), we select this specimen as lectotype and Karlsruhe as lectotype locality of *Ulothrix radicans*.

Conclusions. Our results clarify well the relationships at genus and species level for the most common forms of Prasiolales of northern Europe. Further research will be necessary to understand better the phylogenetic relationships of several entities difficult to characterize from a morphological point of view (especially the filamentous forms of *Prasiola*) and the relationships between European and extra-European species of *Prasiola*. For *Rosenvingiella*, *rbc*L sequences of authentic *R. constricta* are particularly required, and further studies will need to reassess the relative distribution of *R. polyrhiza* and *R. radicans* in Europe. Whereas *R. radicans* is clearly widespread throughout Europe (from coastal areas to regions several hundreds of kilometers far from the sea), *R. polyrhiza* is probably much more rare than the present records suggest. This alga is probably a northern entity, with a circumpolar distribution and common only in the northernmost parts of Europe. Furthermore, although this study showed once more the usefulness of *rbc*L as a marker at genus and species level, phylogenies based on other more variable molecular markers are necessary for a better characterization of the relationships between populations and species in this group. There is no doubt that despite two centuries of intensive work, there is still a lot to be discovered about the Prasiolales and these algae still offer a great potential for exciting and creative research.

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APPENDIX 1

Authentic herbarium specimens examined.

Species	Herbarium	Notes
Conferva muralis Dillwyn	BM	Authentic specimen from Dillwyn's herbarium. No date and locality available.
Conferva muralis Dillwyn	BM	Authentic specimen from Dillwyn's herbarium. No date and locality available.
Conferva velutina Bory	PC (TA 47087)	Authentic specimen from Bory's herbarium. Westphalia, Germany, January 1817.
Gayella constricta Setchell & Gardner	BM	Syntype specimen. Tomales Bay, Marin County, California, USA, August 1916.
Gayella polyrhiza Rosenvinge	C 168	Holotype specimen. Collected by Rosenvinge; Godthåb, Greenland, 2 July 1886.
Schizogonium murale Kützing	L 0489817	Authentic specimen from Kützing's herbarium. Nordhausen, Germany, undated. Designated here as lectotype specimen.
Schizogonium murale Kützing	L 0489815	Authentic specimen from Kützing's herbarium. Gall. = presumably Gallia, France? Undated.
Schizogonium murale Kützing	L 0489818	Authentic specimen from Kützing's herbarium. France (Gallia), undated.
Schizogonium murale Kützing	L 0489816	Authentic specimen from Kützing's herbarium. Switzerland (Schweiz), undated.
Ulothrix radicans Kützing	L 0055072	Herbarium sheet marked as type, including three specimens, respectively from Karlsruhe, Stuttgart, and Zurich. The specimen from Karlsruhe is designated here as lectotype.
Ulothrix radicans f. *schizogonoides* Kützing	L 0374894	Authentic specimen from Kützing's herbarium. Undated and unlocalized.
Ulothrix radicans Kützing	L 0374891	Authentic specimen from Kützing's herbarium. Nordhausen, Germany, undated.

APPENDIX 2

Vegetative morphological characters of herbarium specimens and populations of filamentous Prasiolales examined.

Species and population	Width (field)	Width-to-length ratio (field)	Rhizoids (field)	Width (VS5)	Width-to-length ratio (VS5)	Rhizoids (VS5)	Width (JM)	Width-to-length ratio (JM)	Rhizoids (JM)
Conferva muralis (BM)	13.3 ± 2.5	2.3 ± 0.4	–						
Conferva velutina (PC)	12 ± 1.3	2.9 ± 0.9	–						
Gayella constricta isotype (BM)	21.7 ± 3.2	4.7 ± 1	+						
Gayella polyrhiza holotype (C)	12.4 ± 1.8	4.5 ± 1.1	+						
Schizogonium murale lectotype (L)	11.1 ± 1.2	2.9 ± 1	–						
Ulothrix radicans lectotype (L)	9.5 ± 1.4	2 ± 0.6	+ +						
Prasiola cfr. *calophylla*, Athlone	18 ± 2.1	3.4 ± 0.5	–				21.7 ± 1.1	3.8 ± 0.4	–
Prasiola crispa, Cork	14.3 ± 2	3.4 ± 1	–				14.7 ± 1.8	3.4 ± 0.6	–
Prasiola crispa, Durham	18.4 ± 2.6	4.7 ± 0.7	–				19.2 ± 2.1	5.2 ± 0.7	–
Prasiola crispa, Manchester	16.5 ± 2	4.7 ± 0.9	–				17.4 ± 1.8	5 ± 0.6	–
Prasiola sp., Manchester	13.8 ± 2.6	2.7 ± 0.5	–				15.5 ± 1.6	4.1 ± 1.4	–
Rosenvingiella polyrhiza, Juneau	12.2 ± 1.4	2.7 ± 0.6	+	15.7 ± 1.9	3.5 ± 1	+	16 ± 1.7	2 ± 0.6	+ +
Rosenvingiella polyrhiza, Great Cumbrae	11.8 ± 1.1	2.5 ± 0.7	+	11.1 ± 0.8	3.3 ± 0.8	+	11.5 ± 1.1	3.1 ± 1.1	+ +
Rosenvingiella polyrhiza, Clare Island	10.2 ± 1.1	3.2 ± 0.8	+	11.5 ± 1.2	3.7 ± 0.9	+	11.9 ± 1.4	2.9 ± 0.8	+
Rosenvingiella radicans, Amsterdam	10.1 ± 1.4	2.3 ± 0.5	+ +	12.2 ± 1.9	3.4 ± 0.9	+ +	11.8 ± 1.4	3 ± 0.7	+ +
Rosenvingiella radicans, Copenhagen	10.9 ± 2.1	2.4 ± 0.8	+ +	10.1 ± 1.1	3 ± 0.9	–	10.1 ± 1.5	2.7 ± 0.6	+ +
Rosenvingiella radicans, Robin Hood's Bay	9.2 ± 1.1	2.1 ± 0.5	+ +	8.8 ± 0.9	3.5 ± 0.8	+ +	10.1 ± 1.4	3 ± 0.7	+ +

Appendix 2 (continued)

Species and population	Width (field)	Width-to-length ratio (field)	Rhizoids (field)	Width (VS5)	Width-to-length ratio (VS5)	Rhizoids (VS5)	Width (JM)	Width-to-length ratio (JM)	Rhizoids (JM)
Rosenvingiella radicans, Oviedo	9.7 ± 1.9	2.1 ± 0.8	+ +	10.3 ± 0.7	3.1 ± 0.8	+ +	10.7 ± 0.9	3.2 ± 0.8	+ +
Rosenvingiella radicans, León	9.5 ± 1.4	2.1 ± 0.6	+ +	9.9 ± 1.1	3.4 ± 0.9	+ +	9.8 ± 0.8	3.2 ± 0.7	+ +
Rosenvingiella radicans, Galway (Mill Street)	9.5 ± 1.3	2 ± 0.7	+ +	11.7 ± 1.2	3.1 ± 0.6	+ +	11.5 ± 1.4	3.3 ± 0.8	+ +
Rosenvingiella radicans, Galway (NUIG football ground)	9.1 ± 1.1	2 ± 0.5	+	10.5 ± 0.9	2.9 ± 0.6	+	10.7 ± 0.9	3.1 ± 0.7	+
Rosenvingiella radicans, Cork	7.5 ± 0.4	0.9 ± 0.2	+ +	11.1 ± 1.3	3.3 ± 1	+ +	11.1 ± 1	3.5 ± 0.7	+ +
Rosenvingiella radicans, Dublin	9.9 ± 1.2	2.5 ± 0.6	+ +	10.5 ± 1.2	2.9 ± 0.6	−	11.3 ± 0.9	3.1 ± 0.5	+ +
Rosenvingiella radicans, Athlone	9 ± 1.1	2.4 ± 0.6	+ +	8.6 ± 0.8	3.1 ± 0.6	+ +	8.9 ± 0.6	3.2 ± 0.8	+ +
Rosenvingiella radicans, Claddagh	9.1 ± 1.5	2.5 ± 0.7	+ +	12.1 ± 1.4	2.7 ± 0.7	−	10.1 ± 1.1	2.8 ± 0.7	+
Rosenvingiella radicans, Blackrock	8.3 ± 1.6	2.3 ± 0.9	+ +	10.3 ± 1.1	3.5 ± 0.9	+ +	10.9 ± 0.9	3.4 ± 0.7	+ +
Rosenvingiella radicans, Clare Island	8.9 ± 1.4	2.5 ± 0.6	+ +	10.0 ± 1.2	3.3 ± 0.7	−	10.2 ± 1.1	3.3 ± 0.8	−
Rosenvingiella radicans, Roundstone	8.9 ± 1.5	2.5 ± 0.7	+ +	10.3 ± 0.9	3.6 ± 0.9	+ +	9.7 ± 0.9	3.4 ± 0.8	+ +
Rosenvingiella radicans, Kinvara	9.3 ± 1.6	2.6 ± 0.8	+ +	9.9 ± 0.8	3.8 ± 0.8	+ +	9.8 ± 1	3.6 ± 0.7	+ +
Rosenvingiella radicans, Cobh	8.8 ± 1.2	2.1 ± 0.6	+ +	12.4 ± 0.6	3.3 ± 0.9	+ +	11.1 ± 0.8	3.1 ± 0.9	+ +

Width and width-to-length ratio of cells are referred to uniseriate parts and are expressed in μm as means ± SD ($n + 25$ for width, $n + 8$ for width-to-length ratio). Abundance of rhizoids is expressed following a subjective scale: − , absent; + , rare to infrequent; + + , frequent to abundant.

APPENDIX 3

Culture responses in Von Stosch Medium (VS5) and Jaworski's Medium (JM) and reproductive characters of herbarium specimens and populations of Prasiolales examined.

Species and population	Growth (VS5)	Growth (JM)	Blade-like parts (field)	Blade-like parts (VS5)	Blade-like parts (JM)	Gametangia (field)	Gametangia (VS5)	Gametangia (JM)
Conferva muralis (BM)			−			−		
Conferva velutina (PC)			−			−		
Gayella constricta (BM, isotype)			−			+ +		
Gayella polyrhiza (C, holotype)			−			+ +		
Schizogonium murale (L, lectotype)			+			−		
Ulothrix radicans (L 0055072)			−			+		
Prasiola calophylla, Amsterdam	−	+ + +	+ +		+ +	−		−
Prasiola calophylla, Copenhagen	−	+ + +	+ + + +		+ + +	−		−
Prasiola calophylla, Galway	−	+ + +	+ + + +		+ + +	−		−
Prasiola cfr. *calophylla*, Athlone	−	+ + +	+ + +		+ + +	−		−
Prasiola crispa (leafy), Galway	−	−	−			−		−
Prasiola crispa (filamentous), Cork	−	+ + +	+ + +		+ +	−		−
Prasiola crispa (filamentous), Durham	−	+ + +	+ +		+ +	−		−
Prasiola crispa (filamentous), Manchester	−	+ +	−		+	−		−
Prasiola sp., Manchester	−	+ +	−		−	−	+	−
Rosenvingiella polyrhiza, Juneau	+ +		+ +	−	−	+ +	+	+

Species and population							
Rosenvingiella polyrhiza, Great Cumbrae	+ +	+	+ +	+ +	−	−	+
Rosenvingiella polyrhiza, Clare Island	+ +	+	+ +	+ +	−	−	+
Rosenvingiella radicans, Amsterdam	+ +	+ +	−	−	−	−	−
Rosenvingiella radicans, Copenhagen	+ +	+ +	−	−	−	−	−
Rosenvingiella radicans, Robin Hood's Bay	+ +	+ +	−	−	−	−	−
Rosenvingiella radicans, Oviedo	+ +	+ +	−	−	−	−	−
Rosenvingiella radicans, León	+ +	+ +	+	−	−	−	−
Rosenvingiella radicans, Galway (Mill Street)	+ +	+ +	−	−	−	−	−
Rosenvingiella radicans, Galway (NUIG football ground)	+ +	+ +	−	−	−	−	−
Rosenvingiella radicans, Cork	+ +	+ +	−	−	−	−	−
Rosenvingiella radicans, Dublin	+ +	+ +	−	−	−	−	−
Rosenvingiella radicans, Athlone	+ +	+ +	−	−	−	−	−
Rosenvingiella radicans, Claddagh	+ +	+ +	−	−	−	−	−
Rosenvingiella radicans, Blackrock	+ +	+ +	−	−	−	−	−
Rosenvingiella radicans, Clare Island	+ +	+ +	−	−	−	−	−
Rosenvingiella radicans, Roundstone	+ +	+ +	+	−	−	−	−
Rosenvingiella radicans, Kinvara	+ +	+ +	−	−	−	−	−
Rosenvingiella radicans, Cobh	+ +	+ +	−	−	−	−	−

Growth is quantified following a subjective scale: + +, quick and vigorous growth observed; growth occurring but slow or not particularly vigorous; −, growth absent or very weak. Presence of blade-like parts and pseudo-parenchymatous gametangia is also expressed following a subjective scale: −, absent; +, rare or infrequent; + +, frequent or abundant.

APPENDIX 4

Morphological features of gametangia in herbarium specimens and populations of *Rosenvingiella*.

Species and population	Percentage of filaments with gametangia (field)	Percentage of filaments with gametangia (VS5)	Percentage of filaments with gametangia (JM)	Width of gametangia (field)	Width of gametangia (VS5)	Width of gametangia (JM)
Gayella constricta (BM, isotype)	>90%			112 ± 20.4		
Gayella polyrhiza (C, holotype)	>90%			59 ± 15.5		
Ulothrix radicans (L, 0055072)	<10%			24.5 ± 5.2		
Rosenvingiella polyrhiza, Juneau	>90%	<5%	<5%	55.6 ± 11.6		
Rosenvingiella polyrhiza, Great Cumbrae	>90%	<5%	<5%	29.7 ± 5.4	69.7 ± 21.1	
Rosenvingiella polyrhiza, Clare Island	>90%	<5%	0	30 ± 5.6	105.5 ± 54.9	
Rosenvingiella radicans, Galway (Mill Street)	<10%	0%	0%	21.6 ± 4.5	78 ± 35.9	
Rosenvingiella radicans, Kinvara	<10%	0%	0%	18.4 ± 4.1		

Abundance of gametangia is expressed as percentage of filaments producing gametangia on a total of 10 filaments randomly chosen. Width of gametangia is expressed in μm as means ± SD (n + 10).