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Running heads: New monotypic family for *Plagiospora* (Rhodophyta)

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**A new monotypic family for the enigmatic crustose red alga**

***Plagiospora gracilis***

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**ABSTRACT**

*Plagiospora gracilis*, a mucilaginous crustose red alga growing on subtidal pebbles on both coasts of the North Atlantic Ocean, forms distinctive tetrasporangia (red algal meiotic structures that release haploid tetraspores) but gametophytes have never been reported. In the absence of gametangia, the taxonomic position of this monotypic genus has always been uncertain; it is currently placed provisionally in the Gloiosiphoniaceae (Gigartinales) by comparison with sporophytes of *Gloiosiphonia* obtained in culture.

Dioecious gametophytic crusts of *P. gracilis* are now reported for the first time, forming gametangia in inconspicuous superficial sori. There is no evidence that fertilization ever occurs in the field although fertile males and female were collected together. In culture, tetraspores grew into tetrasporophytes for three successive generations, by presumed apomictic sporophyte recycling. The life history of *P. gracilis* may represent a late stage in the loss of sexual reproduction leading to tetraspore-to-tetrasporophyte life histories such as that in *Hildenbrandia*. Phylogenetic analysis of sequences of the *rbcl*, LSU (28S) rDNA and *cox1* (COI-5P) genes for *P. gracilis* with other Gigartinales resolved *P. gracilis* as a distinct lineage in a well-supported clade of the families Sphaerococcaceae, Gloiosiphoniaceae, Endocladaceae, Nizymeniaceae and Phacelocarpaceae. We here propose the monotypic Plagiosporaceae fam. nov. to accommodate *P. gracilis*.

**ADDITIONAL KEYWORDS:** apomixis – evolution – Gigartinales – life-history – molecular systematics – phylogeny – Rhodophyta

## INTRODUCTION

The Rhodophyta (red algae) are an ancient eukaryotic lineage of mostly photosynthetic marine organisms, including what is generally believed to be one of the oldest taxonomically resolved eukaryotic fossils, the 1.2 billion year old *Bangiomorpha pubescens* Butterfield (Butterfield, 2000; Yoon *et al.*, 2004). ~~Their~~ Red algal ordinal and familial classification is going through a period of rapid change as morphological and ultrastructural studies are complemented and elucidated by molecular data (e.g. Saunders & Hommersand, 2004; Yoon *et al.*, 2006; Verbruggen *et al.*, 2010; Yang *et al.*, 2015; Saunders *et al.*, 2016).

The monotypic red algal genus *Plagiospora* is based on *P. gracilis* Kuckuck (1897), collected once (in the winter of 1895/6) at Helgoland in the North Sea. Kuckuck described the tetrasporangia of this small mucilaginous crustose alga but found no male or female reproductive organs. He characterized *Plagiospora* by its obliquely divided tetrasporangia, which he had previously observed only in the crustose red algal genus *Hildenbrandia* Nardo, placing it provisionally in the Squamariaceae J.Agardh, nom. illeg. (Denizot, 1968), a heterogeneous group that included the Peyssonneliaceae Denizot (1968). Batters (1902) transferred *P. gracilis* to *Cruoriopsis* L.Dufour (1864) on the basis of its tetrasporangial morphology. As *C. gracilis* (Kuckuck) Batters, this species was reported occasionally from European and North American coasts (Rosenvinge, 1917; Taylor, 1957) and was moved to the Cruoriaceae Kylin by Kylin (1956).

*Plagiospora* was reinstated by Denizot (1968) mainly on account of its very small vegetative cells but left *incertae sedis* because it was very rare and poorly known. South & Hooper (1980) suggested that *P. gracilis* might be involved in the life history of *Gloiosiphonia capillaris* (Hudson) Carmichael because its tetrasporangia resembled

those of *G. capillaris* obtained in culture (Edelstein, 1970). For the same reason, Parke & Dixon (1976) placed *P. gracilis* provisionally in the [gigartinean order](#) Gloiosiphoniaceae Schmitz (Wynne & Kraft, 1981; Irvine, 1983), where it remains ([Schneider & Wynne, 2007](#); Guiry & Guiry, 2016). ~~The Gloiosiphoniaceae are members of the large red algal order Gigartinales (Saunders et al., 2004).~~

*Plagiospora gracilis* has a wide distribution in the temperate North Atlantic Ocean from Sweden to southern England, and from Newfoundland to Massachusetts, but it is confined to the subtidal and has rarely been reported (Table 1). The aim of the present study was to determine the systematic and evolutionary position of this enigmatic genus by integrating morphological and life history observations in the field and in culture with molecular phylogenetic analyses. For this purpose, *P. gracilis* was collected subtidally around the British Isles and eastern Canada, including year-round sampling of a population in Northern Ireland where gametangia were observed for the first time.

## MATERIALS AND METHODS

### FIELD COLLECTIONS AND SAMPLE PROCESSING

Phenological sampling was carried out at Cloghy Rocks, Strangford Narrows, Northern Ireland (54°21'38.16"N, [05°32'47.84"W](#)). Strangford Narrows is a channel 8 km long, on average 30 m deep and 0.5 km wide at the narrowest part, linking the main body of Strangford Lough to the Irish Sea. Although currents in the central Narrows reach 3.5 m s<sup>-1</sup>, Cloghy Rocks is relatively sheltered, experiencing maximum currents of less than 0.8 m s<sup>-1</sup> (Kregting & Elsässer, 2014). Substrata at the sampling depths of 5-12 m at Cloghy

Rocks ranged in size from 2 cm cobbles to large boulders. Cobbles were sampled on 4 August 1983, monthly from October 1983 to February 1985, and on 15 May and 16 December 1985 (Table S2). On each date about 20 cobbles 2-15 cm in length, with visible crustose algae, were collected by divers. Measurements of bottom temperature were taken on each dive using a hand-held thermometer and monthly means of surface sea temperature measurements from 1983-1985 for Port Erin, Isle of Man, in the Irish Sea about 60 km from Strangford Narrows, were obtained from CEFAS (<https://www.cefas.co.uk/cefas-data-hub/sea-temperature-and-salinity-trends/>). Daylength was determined from tables as described by Maggs & Guiry (1987).

Cobbles were returned to the laboratory and carefully examined under a dissecting microscope; all species of crustose red algae, including *Plagiospora gracilis*, were sampled and identified with a compound microscope (Leitz Dialux). The reproductive status of all crustose species was recorded for each collection of cobbles.

*Plagiospora gracilis* was also collected ~~by during~~ diving surveys around the British Isles and Canada (Table 1). Collections of other Gigartinales for phylogenetic analysis were made intertidally or in the subtidal ~~by diving~~ with a subsample of each specimen dried in silica gel for DNA extraction (Table S1). The remainder of each specimen was stored in silica or pressed to serve as a voucher deposited in UNB (Saunders & McDevit, 2012).

#### MORPHOLOGICAL STUDIES

Fresh material and herbarium samples were prepared as squashes, either unstained or stained with aqueous aniline blue, post-fixed in 1% HCl, and mounted in 80% Karo corn syrup (Bestfoods Inc, NJ, USA) as permanent slide mounts. Voucher material was deposited in BM (BM568808, BM568872, BM569403), GALW and NRCC (herbarium

abbreviations ~~as in~~ after Thiers, 2016). Fresh gametophytic crusts were stained with Hoechst 33258 fluorescein ( $10 \mu\text{g ml}^{-1}$ ) to visualize nuclei with an epifluorescence microscope (Leitz Dialux). Preparations briefly fixed in 4% formalin-seawater before staining with aceto-iron-haematoxylin-chloral hydrate (Wittmann, 1965) or aniline blue also gave good definition of nuclei. Photographs were taken using Kodak Technical Pan film developed in Kodak HC110 liquid developer.

#### CULTURE STUDIES

*Plagiospora gracilis* cultures were initiated from tetraspores released by a thallus collected at Flannery Bridge, Kilkieran Bay, Co. Galway ( $53^{\circ}21'25.13''\text{N}$ ;  $9^{\circ}42'33.77''\text{W}$ ), Ireland on 6 December 1981, and grown in half-strength modified von Stosch enriched seawater (Guiry & Cunningham, 1984). Cultures were at first maintained at  $15^{\circ}\text{C}$  16 h light: 8 h dark, at a photon irradiance of  $5\text{--}7 \mu\text{mol m}^{-2} \text{s}^{-1}$ , and later subjected to a series of transfers between conditions as detailed in the results. For molecular studies, an isolate was established from material collected at Strangford Lough on 17 January 1994.

#### PCR AMPLIFICATION, SEQUENCING AND PHYLOGENETIC ANALYSES

Total genomic DNA was extracted as in Saunders & McDevit (2012). The COI-5P was amplified and sequenced as outlined in Saunders & McDevit (2012), while LSU and *rbcl* followed the protocols of Saunders & Moore (2013). Sequencing was performed at Genome Quebec and all molecular data were uploaded to BOLD (<http://www.barcodinglife.org>) and GenBank (<http://www.ncbi.nlm.nih.gov/>). The new data generated here were added to data for representative Atractophorales, Peyssonneliales and Gigartinales (Table S1; Saunders *et al.*, 2016). Preliminary single-gene analyses for

LSU and *rbcL* both positioned *Plagiospora gracilis* within the Gigartinales among the families Endocladaceae Kylin, Gloiosiphoniaceae F. Schmitz, Nizymeniaceae Womersley, Phacelocarpaceae Searles and Sphaerococcaceae Hauck (data not shown). To reduce distant outgroups, the representative Atractophorales and Peyssonneliales were removed from the alignment, which was reanalysed including only Gigartinales. As a result, a multigene alignment (LSU + *rbcL* + COI-5P) was established, partitioned by gene and then by codon for the two protein-coding genes, and analysed using maximum likelihood (ML) methods in RAxML employing a GTR+I+G model in Geneious Pro 9.0.4 (Kearse *et al.*, 2012). Branch support was estimated using nonparametric bootstrapping (1000 replicates).

## RESULTS

### PHENOLOGY

At the Strangford Narrows study site, *Plagiospora gracilis* was one of the most frequently observed crustose red algae on cobbles collected at 5-12 m depth, although because of small individual size the total coverage was small. A marked reproductive periodicity was apparent (Fig. 1; Table S2). Tetrasporangia first developed in late November and were produced abundantly from December to April. A few sporangia were still present in late May and early June. Non-pigmented necrotic sporangia were sometimes observed in crusts collected in July and August. Water temperatures measured *in situ* at Strangford Narrows were generally 0.5-1.0°C below the mean monthly temperatures for Port Erin (Fig. 1); the daily temperature range in Strangford Narrows is up to reaches 2.0°C (Kregting & Elsässer, 2014: fig. 2). Tetrasporangia of *P. gracilis* were initiated when the

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water temperature dropped below about 10°C, at a daylength of ca. 8.5 h, and tetrasporangia were present until the temperature increased to 8-9°C in May/June, when the daylength was 15 h. Gametangia were observed on only four occasions (Fig. 1; Table S2), between late November and late February.

#### MORPHOLOGY

Crusts were usually up to 15 mm (rarely to 45 mm) in diameter, and 70-135 µm thick, closely adherent to the substratum, with a smooth glossy surface that was bright red under good illumination. They were mucilaginous and squashed easily under slight pressure, composed of a single basal layer of parallel filaments of cells measuring 6-9 x 2.5-4.0 µm, between which abundant cell fusions (Fig. 2A) were formed. Each basal layer cell gave rise to 1-2 sparsely branched erect filaments of isodiametric cells 5-6 µm wide basally, tapering to 3-4 µm near the apices (Fig. 2 B-D), and up to 22 cells in length. Apical cells in surface view were rounded, separated by mucilaginous walls. Cell fusions occurred between neighbouring erect filament cells with varying frequency. All cells were uninucleate unless fused, when pairs of fused cells showed two nuclei near their original positions (Fig. 2K). Pig plugs were narrow and not always visible, even in stained material.

Tetrasporocytes developed laterally on erect filaments by division of intercalary cells, cut off by anticlinal divisions of the enlarged supporting cell, pyriform or ovoid, up to 9 x 6 µm before division (Fig. 2B). In some thick crusts they formed terminally on 1-3-celled lateral branches, and occasionally a single filament bore two sporangia at different levels. Mature tetrasporangia were 10-17(-22) x 9-11 µm excluding walls, obliquely divided, irregularly cruciate (Fig. 2C,D) in a pattern that is very similar to irregularly zonate

division (Guiry, 1978: 117). In surface view of fertile crusts, mature sporangia were visible over all but the thallus margins.

The gametangial crusts were dioecious, forming male or female gametangia in superficial slightly pigmented mucilaginous sori up to 1.5 mm in diameter. Tetrasporangia were sometimes closely associated with gametangial sori, although they were never borne on filaments that terminated in gametangial branches. It was not clear whether the same crusts can form both sporangia and gametangia, or whether these observations result from coalescence of separate individual thalli.

Male sori (Fig. 2F-H) developed by apical extension of erect filaments above the level of the surrounding vegetative thallus, other filaments remaining vegetative. Spermatangial filaments were slightly pigmented and deeply staining, except for the basal cell which was intermediate in morphology with the vegetative cells. They elongated initially by apical division, forming unbranched filaments of 8-9 cells 3-4  $\mu\text{m}$  wide (Fig. 2E-F) that subsequently cut off irregularly pinnate series of lateral branches of 1-3 cells about 3  $\mu\text{m}$  in diameter (Fig. 2F-H). The distal cells of each filament tended not to form lateral cells, but instead cut off obliquely up to 4 ovoid spermatangia, 3.0-4.5 x 1.5-2.0  $\mu\text{m}$ , in a paired or whorled arrangement (Fig. 2F-H). All but the lowermost lateral branch cells also functioned as spermatangial mother cells, budding off obliquely 1-3 spermatangia (Fig. 2H). Mature spermatangial filaments were 35-40  $\mu\text{m}$  long, and covered by a thick mucilaginous layer. Released spermatia were ovoid, about 5  $\mu\text{m}$  long, highly vacuolate and possibly necrotic.

Female sori (Figs 2I-M, 3A-F) likewise developed superficially from vegetative apical cells. They were composed of thick-walled, semi-slightly pigmented cells 3.5-6.0  $\mu\text{m}$  wide (Fig. 2J-M), with larger nuclei and pit plugs than vegetative cells, usually forming filaments but occasionally remaining as single large cells. The filaments were either unbranched and sterile, up to 7 cells long (Fig. 2J), or up to 13 cells in length, with a

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8 pinnate, alternate, or secund arrangement of 1-3 lateral branches 5-8 (-11) cells long,  
9 that were themselves sometimes branched once (Fig. 2I,K-M). The unbranched  
10 filaments are not interpreted as a developmental stage of the branched ones, because  
11 they were also present in mature sori. Many of the lateral branches terminated in hairs  
12 1.5  $\mu\text{m}$  wide, connected by pit plugs to elongate conical cells. Two different types of  
13 reproductive branch systems were observed in female sori, one bearing carpogonial  
14 branches, the other auxiliary cells.  
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Carpogonial branches (Figs 3A-C, 4A-K) developed in a lateral position on the branch systems, usually initiated as a single elongate cylindrical cell (Figs 3A, 4A-B) that developed an apical protuberance before dividing by a cross-wall to form a 2-celled carpogonial branch (Figs 3B, 4C-F). In some cases a small wedge-shaped cell was present below the carpogonial initial (Fig. 4E); division of this initial could give rise to a 3-celled carpogonial branch (Fig. 4G). In mature carpogonial branches that were 3-4 cells long (Figs 2L, 4G-H), only the carpogonium and hypogynous cell were differentiated from sterile branch cells by their dense cytoplasmic contents. In one example (Fig. 3C), a single sterile lateral cell was formed by one of the carpogonial branch cells. Developing carpogonia (Fig. 3A-C, 4A-G) were ovate-conical, c.  $9 \times 5 \mu\text{m}$ , with a single nucleus in an apical position,  $3 \mu\text{m}$  wide, containing a nucleolus of  $1 \mu\text{m}$  diameter. Mature carpogonia (Figs 2L, 3B-C, 4I-J) were usually conical,  $5 \times 5 \mu\text{m}$ , bearing trichogynes  $2.5 \mu\text{m}$  in diameter and up to  $225 \mu\text{m}$  long. Older carpogonia decreased to  $3.5 \times 3.5 \mu\text{m}$ , and the nucleus was no longer visible. Hypogynous cells (Fig. 4F-J) were usually binucleate, containing equal-sized nuclei  $1.5 \mu\text{m}$  in diameter; the other carpogonial branch cells, where present, were uninucleate. Supporting cells of carpogonial branches were sometimes binucleate (Fig. 4J), and not otherwise distinguished from other cells of reproductive branch systems. Spermata were never seen on trichogynes, and there was no evidence that fertilization had occurred, although in some cases the hypogynous cell

had formed a lateral protuberance (Fig. 4J) or cut off a small cell. Old necrotic carpogonia on binucleate hypogynous cells (Fig. 4K) were frequently observed, sometimes in the same reproductive branch system as mature carpogonial branches, but never on the same supporting cell. More than one mature carpogonium was never present in a single branch system.

Auxiliary cell branch systems (Figs 3D-F, 4L-N) were similar to those bearing carpogonial branches. Auxiliary cells were intercalary, usually the basal or suprabasal cell of a lateral branch of 4-6 (-9) cells, but were rarely in an unbranched filament. The auxiliary cells were initially slightly larger than other cells (Fig. 3D), enlarging to 8  $\mu\text{m}$  diameter, spherical or ovoid in shape, with much enlarged pit plugs (Figs 3D-F, 4L-N). Their nuclei (Figs 3D-F, 4L-N) were about 4  $\mu\text{m}$  diameter, with a nucleolus 2.5  $\mu\text{m}$  in diameter, compared to the 1.5  $\mu\text{m}$  diameter nuclei of the sterile cells.

Neither carpogonial branches nor auxiliary cells ever showed evidence of post-fertilization development, and cystocarps were not observed, despite the presence of mature male and female gametangia over a two-month period.

#### LIFE HISTORY IN CULTURE

Tetraspores grown at 15°C 16:8 h (long days; LD) often developed into filaments (Fig. 5A) before forming crustose sporelings (Fig. 5B) with a multiaxial marginal meristem. After 4 months the crusts were 3 mm in diameter and 50  $\mu\text{m}$  thick. Examined from below (Fig. 5C), a radial arrangement of basal filaments with numerous cell fusions was apparent. In surface view (Fig. 5D), apical cells of erect filaments were rounded and embedded loosely in mucilage. Erect filaments branched occasionally and consisted of up to 9 more or less isodiametric cells 3-6  $\mu\text{m}$  diameter (Fig. 5E) that formed a few cell fusions. The crusts were then transferred to 15°C, 8:16 h (short days; SD) and after a

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7 further 3 months, to 10°C SD. Within 2 months, intercalary erect filament cells had  
8 differentiated into tetrasporangial supporting cells that cut off tetrasporocytes (Fig. 5E).  
9 When mature, obliquely cruciately divided tetrasporangia 11-20 x 7-11 µm (Fig. 5F)  
10 released spores.  
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14 Germination of released tetraspores occurred by division of the spore into several  
15 cells, one of which often grew out as a filament when spores were poorly attached.  
16 Sporelings grown at 15°C LD and 10°C LD formed crusts identical to those of the parent  
17 culture. While maintained under LD for 11 months, no reproduction occurred. Replicate  
18 cultures transferred to 10°C SD had formed abundant tetrasporangia 1.5 months later,  
19 representing the third successive sporangial generation of a tetraspore-to-  
20 tetrasporophyte life history.  
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#### 28 29 PHYLOGENETIC ANALYSES 30 31

32 Phylogenetic analysis of LSU, *rbcL* and COI-5P sequences placed *Plagiospora gracilis* in  
33 a strongly supported clade within the Gigartinales consisting of representatives of the  
34 families Sphaerococcaceae, Gloiosiphoniaceae, Endocladiaceae, Nizymeniaceae and  
35 Phacelocarpaceae (Fig. 6). *Sphaerococcus* and *Plagiospora* constituted separate  
36 lineages sister to a robust grouping of the representatives of the remaining families,  
37 including the Gloiosiphoniaceae. There was a close relationship between the  
38 Nizymeniaceae and Phacelocarpaceae and a robust but more distant relationship  
39 between the Gloiosiphoniaceae and the Endocladiaceae.  
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#### 52 53 DISCUSSION 54 55 56 57 58 59 60

We report here the first discovery of gametangia in *Plagiospora gracilis*. Field collections and culture studies indicate that a short-day response is involved in the induction of tetrasporangia, and gametangia likewise appear to be formed under short days.

Development of tetrasporangial plants from tetraspores in culture, the extreme rarity of gametangial plants in the field, and the lack of post-fertilization development strongly suggest that the principal life history is recycling of tetrasporophytes.

      Mixed development of tetraspores into both gametophytes and tetrasporophytes (facultative apomixis) has been observed in *Gloiosiphonia capillaris* (Maggs, 1988). The (uncompleted) isomorphic life history distances *P. gracilis*, however, from *G. capillaris* which has an erect, ephemeral much-branched gametophytic phase as well as the crustose phase. The situation in *P. gracilis* might represent an extension of a trend towards an increased frequency of tetrasporophyte recycling, to the point where fertilization never occurs and gametangia may be non-functional. Obligate apomeiosis is thought to be rare in the red algae, a phenomenon observed mostly in crustose species (Hawkes, 1990).

Our molecular phylogenetic analyses (Fig. 6) place *Plagiospora* in a distinct lineage in a well-supported clade among the five gigartinalean families Sphaerococcaceae, Gloiosiphoniaceae, Endocladaceae, Nizymeniaceae and Phacelocarpaceae. Searles (1968: 77) was probably the first author to draw attention to similarities between the Phacelocarpaceae (including the Nizymeniaceae, later segregated by Womersley, 1971) and the Endocladaceae and Gloiosiphoniaceae, despite their position in two different orders at the time. The resemblance included the uniaxial vegetative construction and similar polycarpogonial procarps. Molecular analyses showed that the Nizymeniaceae and Phacelocarpaceae are closely related and might be better placed in the same family (Saunders & Kraft, 1994; Saunders *et al.*, 2004). In

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7 previous multi-gene analyses without *Plagiospora*, this five-family clade including  
8 Sphaerococcaceae received only poor support although [the](#) Gloiosiphoniaceae,  
9 Endocladaceae, Nizymeniaceae and Phacelocarpaceae were grouped robustly  
10 (Verbruggen *et al.*, 2010). As *Plagiospora* represents a lineage distinct from the other  
11 families in this clade, its position could be treated taxonomically either by merging all five  
12 families, with Sphaerococcaceae Hauck (1885: 17) having priority, or by recognizing a  
13 new family.  
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20 The most important morphological characters for systematics of the red algae are  
21 features of the female reproductive system and post-fertilization development (Kylin,  
22 1956), but since post-fertilization development is unknown in *Plagiospora*, only  
23 vegetative, gametangial and tetrasporangial characters can be used to inform our  
24 judgement regarding familial assignment. The crustose thallus of *Plagiospora* is relatively  
25 simple and its morphology can only be compared directly with other species with crustose  
26 phases. Of the five families in this clade, only the Gloiosiphoniaceae and  
27 Sphaerococcaceae form a crustose phase as part of their heteromorphic life histories.  
28 The crusts of *Gloiosiphonia capillaris* (Maggs, 1988; Maggs, unpublished observations)  
29 resemble *Plagiospora* in being mucilaginous and forming abundant cell fusions and  
30 obliquely divided cruciate tetrasporangia, [these](#) but differ in the presence of a compact  
31 pseudoparenchymatous layer that grows downwards. *Sphaerococcus* crusts are tough,  
32 not mucilaginous, and also differ greatly from *Plagiospora* in the complex morphology,  
33 with rows of large cells equivalent to axial filaments (Maggs & Guiry, 1982). Life histories  
34 in [the](#) Nizymeniaceae, Phacelocarpaceae and Endocladaceae, where known, exhibit  
35 isomorphic erect gametophytes and sporophytes.  
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49 The female reproductive structures of *Plagiospora*, consisting of 2-4-celled  
50 carpogonial branches borne singly in sparsely branched, quite variable, reproductive  
51 branch systems, have some similarities to those of the five families in the clade.  
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8 However, *Plagiospora* differs from the others in the relative position of carpogonial and  
9 auxiliary cell branches. Whereas all five families are procarpic (i.e. the auxiliary cell  
10 branches and carpogonial branches are in proximity, usually in the same branch system;  
11 Searles, 1968), in *Plagiospora* carpogonial and auxiliary cell branches are always  
12 separate (non-procarpic). *Sphaerococcus* has a simple procarpic system, with only one  
13 carpogonial branch per auxiliary cell (Kylin, 1930), but the four other families are  
14 polycarpogonial – each fertile branch system has several carpogonial branches in  
15 association with one auxiliary cell. An analysis (Norris, 1957) of the reproductive branch  
16 systems of gigartinalean families including the Gloiosiphoniaceae and Endocladaceae  
17 concluded that procarpic families probably developed from non-procarpic families,  
18 possibly more than once. Because *Gloiosiphonia* is not always functionally procarpic (the  
19 post-fertilization connecting filament may contact a distant auxiliary cell; Kylin, 1930), it  
20 may be an early stage in the development of typical procarps (Kylin, 1956).

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22 The plane of division of spermatangial mother cells is an important character in  
23 red algae which can be diagnostic at the ordinal level (e.g. Ahnfeltiales Maggs &  
24 Pueschel, 1989). Spermatangia are cut off obliquely in the Gloiosiphoniaceae (Kylin,  
25 1928; Lee & Yoo, 1979), and in the Nizymeniaceae and Phacelocarpaceae, which are  
26 mostly Southern Hemisphere endemics (Womersley, 1994). Although vegetative thalli in  
27 the Nizymeniaceae and Phacelocarpaceae are large, erect and morphologically complex,  
28 with a pseudoparenchymatous construction, male gametangia are formed in distinctive  
29 superficial filamentous nemathecium (Searles, 1968; Womersley, 1994). The male  
30 filaments of *Nizymania conferta* (Harvey) Chiovitti, G.W.Saunders & Kraft [as *Stenocladia*  
31 *australis* (Sonder) P.C.Silva (Searles, 1968, fig. 16; Womersley, 1994, fig. 138)] are  
32 uniseriate and form whorls of spermatangial mother cells ~~that~~ each of which cuts off  
33 obliquely one or two spermatangia, in the same manner as *Plagiospora*. The obliquely  
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budded spermatangia in *Plagiospora* differ markedly from those of *Endocladia*, in which spermatangia are formed in short rows by transverse division (Kylin, 1928).

Tetrasporangia in *Sphaerococcus* and *Phacelocarpus* are terminal and regularly zonate (Searles, 1968; Maggs & Guiry, 1982; Chiovitti *et al.*, 1995; Womersley, 1994), in contrast to *Plagiospora*. However, tetrasporangia in *Nizymania australis* Sonder (Womersley, 1994, fig. 137) are formed laterally in superficial tufts of filaments and divide irregularly cruciately, as in *Plagiospora*.

Despite the stark contrast between the general morphology of *Plagiospora* and the other five families in the clade, some potentially significant similarities can be seen among *Plagiospora* and the other taxa. These are presumably linked to the shared evolutionary history demonstrated by our molecular analyses, but do not justify mergers among the families. We therefore propose the monotypic Plagiosporaceae fam. nov. to accommodate the genus *Plagiospora*.

Plagiosporaceae Maggs & G.W. Saunders, fam. nov.

**Description:** Thalli crustose, non-calcified, mucilaginous, with marginal meristems forming prostrate filaments that bear erect filaments of cuboid cells 6–9  $\mu\text{m}$  in diameter; vegetative cell fusions common. Tetrasporangia borne laterally on erect filaments, dividing obliquely cruciately. Gametophytes crustose; gametangial sori formed in inconspicuous superficial sori developing by apical extension and differentiation of erect vegetative filaments. Carpogonial branches lateral, 2–4-celled; auxiliary cells distant, intercalary in lateral branches; spermatangial filaments bearing short lateral branches that function as spermatangial mother cells, budding off spermatangia obliquely.

Postfertilization development unknown.

Type genus: *Plagiospora* Kuckuck, 1897: 393.

#### ACKNOWLEDGEMENTS

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### Figure legends

**Figure 1.** Phenology of *Plagiospora gracilis*, collected at Strangford Lough Narrows, 1983-1985, showing water temperature measured *in situ* during collections (dashed line) and monthly means of seawater temperature at Port Erin, Isle of Man (continuous line). Tetrasporangia are indicated by the quartered circle. nd, no data.

**Figure 2.** Vegetative and reproductive morphology of *Plagiospora gracilis*, collected at Strangford Lough Narrows, 23 Jan 1985. A, Basal layer, with numerous cell fusions (arrows), giving rise to erect filaments. B, Thin crust with tetrasporocyte (tmc) borne on intercalary supporting cell. C, Mature obliquely cruciate tetrasporangia (t). D, Thick crust with tetrasporangia. E, Young spermatangial sorus with unbranched filaments. F, Mature spermatangial sorus. G, H, Spermatangial filaments showing spermatangial mother cells obliquely budding off up to 3 spermatangia. I, Female sorus formed above vegetative thallus (arrow indicates original surface of crust). J, Sorus consisting of single enlarged apical cells with thick membranes around them. K, Female sorus showing cell fusions between vegetative filaments; reproductive filaments have enlarged nuclei. L, Four-celled carpogonial branch (ca) includes a binucleate hypogynous cell. M, Binucleate cell (hy, of unknown function) formed laterally on female gametangial filaments.

**Figure 3.** Female reproduction in *Plagiospora gracilis*, collection data as preceding figure, stained with Wittmann's haematoxylin or aniline blue. A, Large carpogonial branch initial (ci) before trichogyne formation. B, Young 2-celled and developing 3-celled carpogonial branches, carpogonium with large nucleus in an apical position. First branch

cell of the 3-celled branch is small and wedge-shaped. C, Four-celled carpogonial branch; first cell has cut off a sterile lateral cell (arrow). D, Female sorus with branched reproductive filaments; one cell (arrow) may be a developing auxiliary cell. E, F, Intercalary auxiliary cells (a); note large nucleus (arrow).

**Figure 4.** Female reproductive structures of *Plagiospora gracilis*, collection data as before. Figs A-K stained with Wittmann's haematoxylin; Figs L-N with aniline blue. Upper scale bar applies to Figs A-K; lower scale bar to Figs L-N. A, Carpogonial branch initial (ci) prior to formation of trichogyne. B, Carpogonial branch initial showing trichogyne initiation. C, D, Carpogonial branch initial has apparently cut off hypogynous cell (h) to form 2-celled carpogonial branches; carpogonia (c) contain single large nucleus. E, Carpogonial branch initial borne on small wedge-shaped cell (b), possibly a stage in the formation of 3-celled carpogonial branches. F, Carpogonial branch consisting of carpogonium with elongating trichogyne borne on binucleate hypogynous cell. G, H, Developing 3- and 4-celled carpogonial branches. I, Old carpogonial branch, with small conical carpogonium. J, Mature carpogonial branch, with conical carpogonium bearing long trichogyne; hypogynous cell has formed a protuberance. K, Necrotic carpogonium (nc) on 3-celled carpogonial branch. L-N, Auxiliary cells (a) intercalary in lateral auxiliary cell branches. Auxiliary cells contain single large nuclei and have enlarged pit connections.

**Figure 5.** *Plagiospora gracilis* grown in culture. A, Germinating tetraspores. B, Crust grown from tetraspores released in culture. C, Crust from below, showing abundant cell fusions in basal layer. D, Crust from above, showing rows of rounded cells embedded in mucilage. E, Erect filaments forming tetrasporocytes (tmc) on intercalary supporting cells, following transfer to short-day conditions. F, Mature obliquely cruciate tetrasporangia.

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**Figure 6.** Maximum likelihood phylogeny for the multigene alignment restricted to the included Gigartinales (Table S1). Bootstrap values (1000 replicates) indicated along branches with >50 % support. Scale bar indicates substitutions per site.

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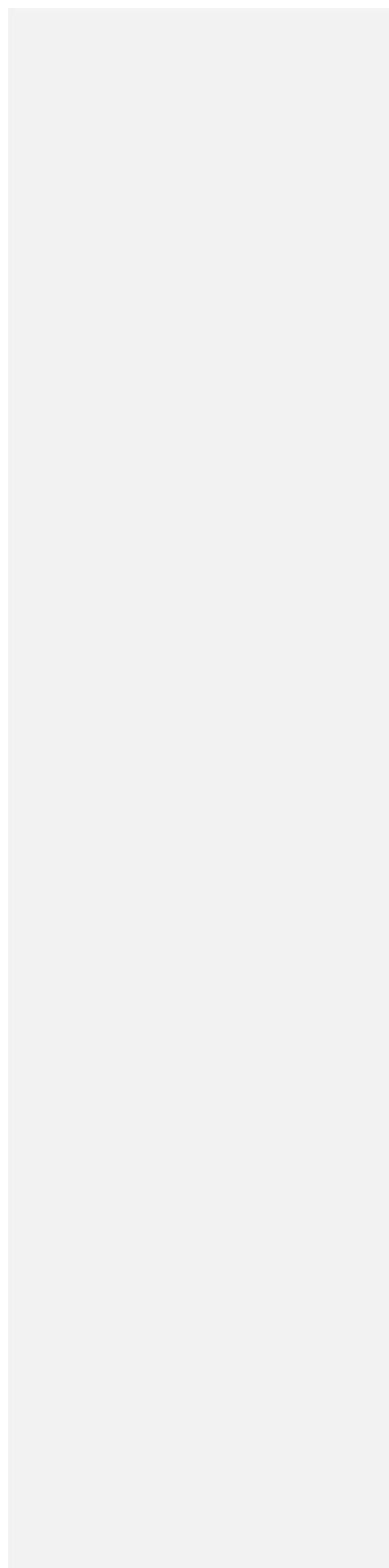


Table 1. Distribution and reproduction of *Plagiospora gracilis* Kuckuck. All published records are as *Cruoriopsis gracilis* (Kuckuck) Batters, except Kuckuck (1897) and Batters (1896; as *Cruoriopsis crucialis* L.Dufour).

Locality (North to South by country)	Habitat	Tetrasporangia present?	Reference/collector voucher
<b>NE Atlantic</b>			
Sweden: Kristineberg	5-10 m, stones	Yes, Oct to Dec	Kylin (1944)
Denmark: Middelfart	15 m, stones	Yes, July	Rosenvinge (1917)
Germany: Helgoland, North Sea	10 m, stones	Yes, Winter 1895/96	Kuckuck (1897)
Scotland: Islay, Argyll	Subtidal stones	Few, June 1982	CAM
N. Ireland: Rathlin Is, Co. Antrim	15 m, stones	None, 29 Aug 1985	CAM
N. Ireland: Strangford Lough, Co. Down	5-12 m, stones	See Table S1	CAM
Ireland: Flannery Bridge, Kilkieran Bay, Co. Galway	5 m, stone	6 Dec 1981	CAM
Wales: Bardsey, Caernavon	12-17 m, stones	None, 22 Aug 1983	CAM <b>BM568872</b>
Wales: Bardsey, Caernavon	9 m, stones	None, 25 Aug 1983	CAM

			<b>BM569403</b>
Wales: Skomer, Pembrokeshire	5-10 m, stones	None, Aug 1982	S. Hiscock
Wales: Martinshaven, Pembs	5-10 m, stones	None, Aug 1982	S. Hiscock
England: Starehole Bay, Salcombe, Devon	3 m, stones	None, 8 Sept 1985	CAM <b>BM568808</b>
England: Yealm Estuary, Plymouth, Devon	"Deep" shells	Yes; no date given	Batters (1896)
<b>NW Atlantic</b>			
Newfoundland: Bonne Bay & Fortune Bay	10-20 m	Yes, July	South & Hooper (1980)
Prince Edward Is: Rustico	18 m, stones	Undivided sporocytes, 22 Aug 1986	CAM
Prince Edward Is: Gala Pt.	8 m, stones	Yes, very rare; also undivided sporocytes and abortive bisporangial stages, 29 July 2008	GWS
Nova Scotia: Peggys Cove	15 m, stones	None, 19 July 1986	GWS

Nova Scotia: Diligent River	2-3 m, stones	Yes, 12 Dec 1986	CAM
Maine: Casco Bay	4-8 m, stones	Few, July	Phycotheca Boreali- Americani, no. 1650 (Collins 1911).
Massachusetts: S. Cape Cod	8-22 m, stones	Apr to Nov	Sears & Wilce (1975)

CAM, Christine Maggs; GWS, Gary Saunders

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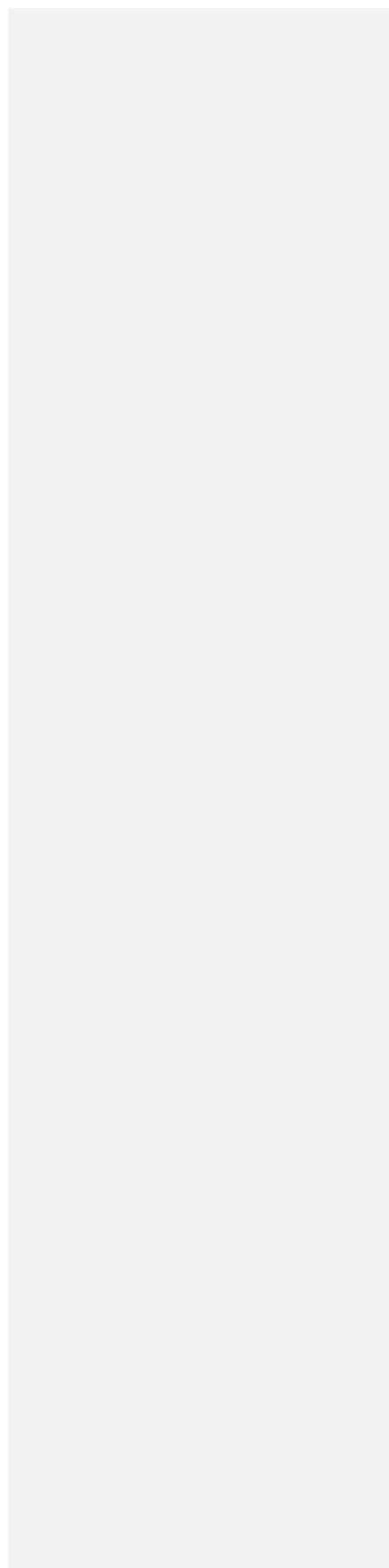
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**Supplementary files**

Table S1

Table S2

PDF Proof



**Table S1.** A list of the taxa used in this study with the corresponding GenBank accessions for the three genes used in phylogenetic analyses.

Taxon	Voucher	LSU	<i>rbcL</i>	COI-5P
<b>Atractophorales</b>				
<b>Atractophoraceae</b>				
<i>Atractophora hypnoides</i> P.Crouan & H.Crouan	GWS005200	GQ497323	KU382063	GQ497303
<b>Gigartinales</b>				
<b>Aerotylaceae</b>				
<i>Aerotylus australis</i> J.Agardh	G0023 GWS034942	GQ406346 ND	KC130223 ND	ND XXXXX
<b>Areschougiaceae</b>				
<i>Areschougia congesta</i> (Turner) J.Agardh	GWS002474 GWS014882	GQ406347 ND	KC130213 ND	ND HM917458
<i>Austroclonium charoides</i> (Harvey) Min- Thein & Womersley	G0360	KF026501	KF026485	HM915819

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Chondrymeniaceae				
Chondrymeniaceae sp.	GWS005891	KF026513	KF026497	HM918785
<i>Crebradomus gongylocarpus</i> Kraft & G.W.Saunders	GWS002030	KF026500	KF026482	HM915973
<i>Dissimularia withallii</i> Kraft & G.W.Saunders	JAR-1201	KF026509	KF026492	KF026473
Cruoriaceae				
<i>Cruoria cruoriiformis</i> (P.Crouan & H.Crouan) Denizot	SA21654	KC130241	KC130214	HM916098
<i>Cruoria pellita</i> (Lyngbye) Fries	GWS000563	GQ406348	KT310698	ND
<i>Pseudopolyides furcellatus</i> Barbara, Gallardo, Cremades, Barreiro, Maneiro & G.W.Saunders	SA19548	KC130239	ND	KC130189
	SA19559	ND	KC130212	KC130194
Cystoeloniaceae				
<i>Calliblepharis jubata</i> (Goodenough & Woodward) Kützing	GWS000323	KF026511	ND	ND

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	GWS014653	ND	KF026494	KF026474
<i>Cystoclonium purpureum</i> (Hudson)				
Batters	G0421	KC130242	KC130217	ND
	GWS002294	ND	ND	HM918475
<i>Hypnea charoides</i> Lamouroux	G0358	GQ406354	KC130220	HM915818
Dicranemataceae				
<i>Dicranema revolutum</i> (C.Agardh)				
J.Agardh	G0036	GQ406349	ND	ND
	GWS014819	ND	KC130231	HM917423
Dumontiaceae				
<i>Dasyphloea insignis</i> Montagne	G0034	DQ343688	ND	ND
	GWS014869	ND	XXXXX	ND
	GWS015838	ND	ND	XXXXX
<i>Dilsea carnosa</i> (Schmidel) Kuntze	GWS000746	EF033609	JN403065	AY971151
<i>Dudresnaya hawaiiensis</i> R.K.S.Lee	GWS001024	KC130243	KC130219	KC130201
<i>Dumontia contorta</i> (Gmelin) Ruprecht	CSM005B	ND	JN403062	AY971155
	GWS001815	JX296139	ND	AY970583

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<i>Gibsmithia hawaiiensis</i> Doty	GWS001343	GU176297	KT310689	ND
Endocladiaceae				
<i>Endocladia muricata</i> (Postels & Ruprecht) J. Agardh	G0155	KF026504	ND	ND
	GWS020093	ND	KF026496	KF026475
<i>Gloiopeltis furcata</i> (Postels & Ruprecht) J. G. Agardh				
	GWS002264	EF033612	JX969801	ND
	GWS013649	ND	ND	HM916317
Furcellariaceae				
<i>Furcellaria lumbricalis</i> (Hudson) Lamouroux				
	GWS001772	GQ406350	ND	ND
	GWS003509	ND	KC130215	ND
<i>Turnerella mertensiana</i> (Postels & Ruprecht) F. Schmitz				
	GWS003012	XXXXX	XXXXX	XXXXX
Gigartinaceae				
<i>Chondracanthus exasperatus</i> (Harvey & Bailey) Hughey				
	GWS002829	GQ338089	JN403073	GQ398091

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<del><i>Rhodoglossum gigartinoides</i> (Sonder)</del>				
Edyvane & Womersley	G0098	GQ338091	JN403074	ND
	GWS029663	KF026505	ND	KF026468
<b>Gloiosiphoniaceae</b>				
<del><i>Gloiosiphonia capillaris</i> (Hudson)</del>				
Carmichael in Berkeley	GWS000374	GQ406352	ND	ND
	GWS013313	ND	KU382056	HM915532
<b>Haemeschariaceae</b>				
<i>Haemescharia polygyna</i> Kjellman	GWS001849	GQ406353	KC130218	ND
<b>Kallymeniaceae</b>				
<i>Callophyllis edentata</i> Kylin	GWS001145	AY171604	KC130228	JX034247
<i>Glaphyrymenia pustulosa</i> J.Agardh	GWS015923	KF280962	KF280988	HM917946
<i>Kallymenia cribrosa</i> Harvey	GWS000466	KF280953	KF280978	KF280930
<del><i>Meredithia crenata</i> C.W. Schneider,</del>				
C.E.Lane & G.W.Saunders	GWS001247	AY171612	KC157632	KC157617
<i>Polycoelia laciniata</i> J.Agardh	GWS001906	JX296144	KF280983	KT307606
<i>Psaromenia</i> sp._1LH	GWS002058	JX296141	KC157627	HM915984

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<i>Mychodeaceae</i>				
<i>Mychodea terminalis</i> Harvey	DV023	XXXXXX	XXXXXX	XXXXXX
<i>Mychodeophyllaceae</i>				
<i>Mychodeophyllum papillitectum</i> Kraft	G0385	GQ406355	KF026493	HM915826
<i>Nizyeniaceae</i>				
<i>Nizyenia australis</i> Sonder	GWS001581	GQ406356	KC130227	ND
	GWS014843	ND	ND	HM917440
<i>Phaeocarpaceae</i>				
<i>Phaeocarpus peperocarpus</i> (Poiret) Wynne, Ardré & Silva	GWS000417	GQ406357	KC130222	HM915869
<i>Phyllophoraceae</i>				
<i>Archestenogramma profundum</i> C.W.Schneider, T.Chengsupanimit & G.W.Saunders	BDA0368	JN403059	JN403075	HQ933374
<i>Mastocarpus californianus</i> S.C.Lindstrom, J.R.Hughey & P.T.Martone	GWS000073	GQ338094	GQ338144	GQ380171

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<del><i>Stenogramma phyllophoroides</i></del>				
(J.Agardh) Millar	GWS001506	JN403060	GQ338123	GQ380377
<b>Plagiosporaceae</b>				
<del><i>Plagiospora gracilis</i></del>	GWS000293	XXXXX	XXXXX	ND
<b>Polyidaceae</b>				
<i>Polyides rotundus</i> (Hudson) Greville	G0344	FJ848972	KC130221	ND
	GWS002676	ND	ND	HM918499
<b>Rhizophyllidaceae</b>				
<del><i>Contarinia</i> sp.</del>	6091	KC130244	KC130224	ND
	6168	ND	ND	XXXXX
<i>Portieria hornemannii</i> (Lyngbye) Silva	G0232	FJ848973	JX996090	ND
<b>Solieriaceae</b>				
<i>Solieria robusta</i> (Greville) Kylin	GWS001590	GQ406360	KC130210	HM915934
<b>Sphaeroceceaceae</b>				
<del><i>Sphaeroceceus coronopifolius</i></del>				
Staekhouse	GWS001833	FJ848974	KC130216	ND
<b>Peyssonneliales</b>	CM04	KU501313	JX969782	JX969703

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<i>Peyssonneliaceae</i>				
<i>Peyssonnelia atropurpurea</i> P.L.Crouan & H.M.Crouan				
<i>Peyssonnelia dubyi</i> P.L.Crouan & H.M.Crouan	CM03	KU501316	JX969785	JX969715
<i>Ramierusta textilis</i> C.M.Pueschel & G.W.Saunders	GWS001755	FJ848970	KC130226	JX969749
<i>Sonderophycus</i> sp.	G0418	AF419125	KC130225	KC130203

**Table S2.** Reproductive status of *Plagiospora gracilis* at Cloghy Rocks, Strangford Lough, N. Ireland, collected on pebbles at depths of 5 to 12 m.

Date ——— Reproductive state

4 Aug 1983 ——— Vegetative

24 Oct 1983 ——— Vegetative

3 Nov 1983 ——— Tetrasporocytes

15 Dec 1983 ——— Tetrasporangia

23 Jan 1984 ——— Tetrasporangia

1 Mar 1984 ——— Tetrasporangia

26 Mar 1984 ——— Tetrasporangia

18 Apr 1984 ——— Tetrasporangia

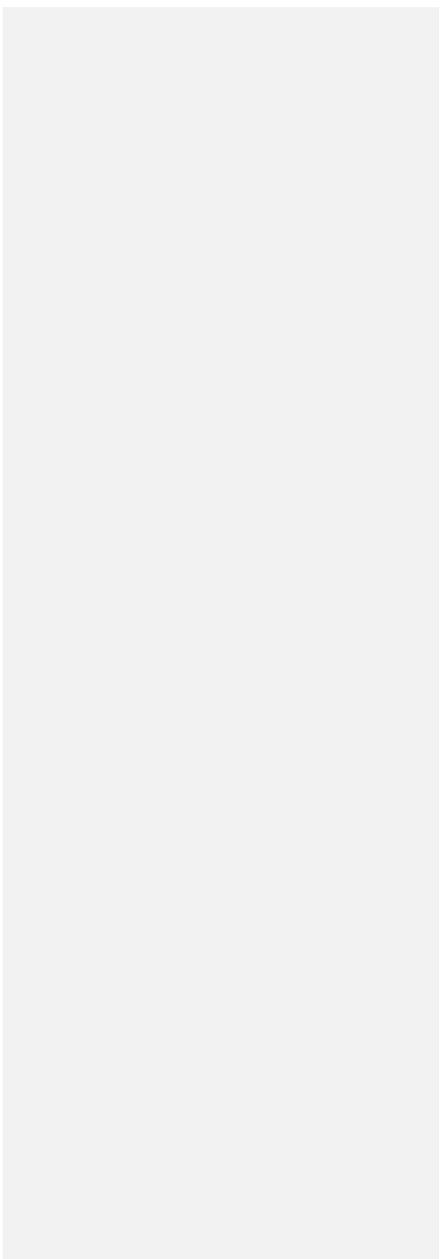
29 May 1984 ——— Tetrasporangia (few)

27 Jun 1984 ——— Vegetative

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- 27 Jul 1984 — Vegetative
- 29 Aug 1984 — Vegetative
- 26 Sep 1984 — Vegetative
- 2 Nov 1984 — Vegetative
- 28 Nov 1984 — Tetrasporangia; spermatangia
- 19 Dec 1984 — Tetrasporangia
- 23 Jan 1985 — Tetrasporangia; spermatangia; carpogonia
- 30 Jan 1985 — Tetrasporangia
- 26 Feb 1985 — Tetrasporangia; carpogonia
- 15 May 1985 — Tetrasporangia
- 16 Dec 1985 — Spermatangia; carpogonia

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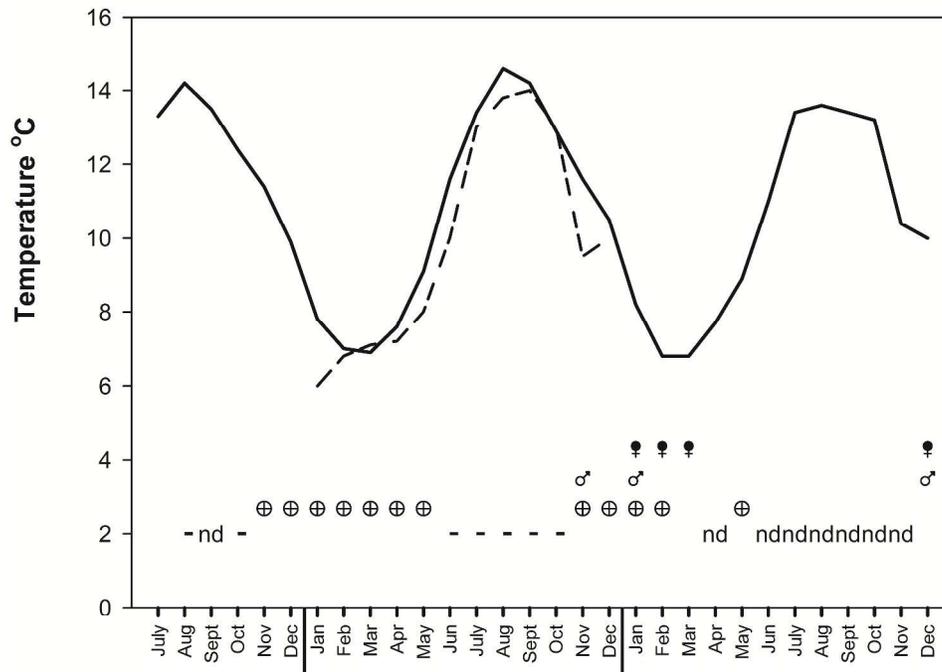


Figure 1. Phenology of *Plagiospora gracilis*, collected at Strangford Lough Narrows, 1983-1985, showing water temperature measured in situ during collections (dashed line) and monthly means of seawater temperature at Port Erin, Isle of Man (continuous line). Tetrasporangia are indicated by the quartered circle. nd, no data.

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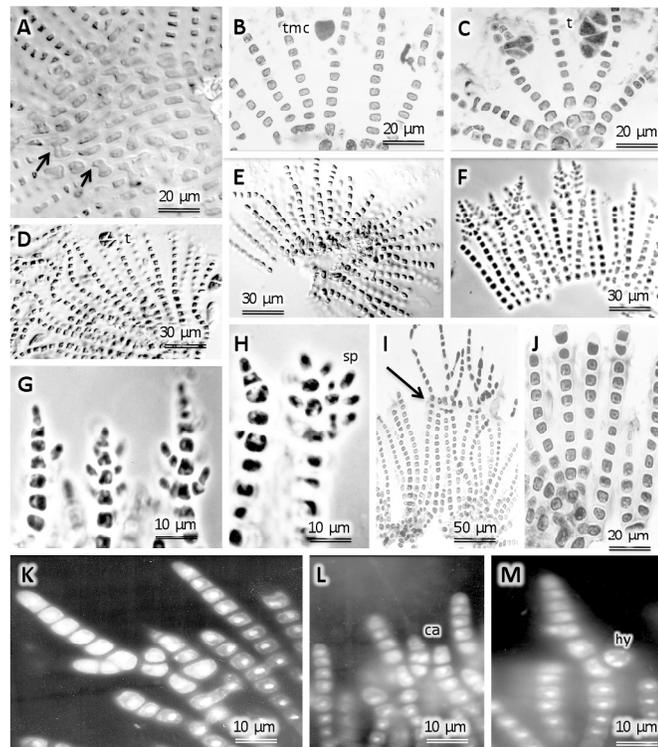


Figure 2. Vegetative and reproductive morphology of *Plagiospora gracilis*, collected at Strangford Lough Narrows, 23 Jan 1985. A, Basal layer, with numerous cell fusions (arrows), giving rise to erect filaments. B, Thin crust with tetrasporocyte (tmc) borne on intercalary supporting cell. C, Mature obliquely cruciate tetrasporangia (t). D, Thick crust with tetrasporangia. E, Young spermatangial sorus with unbranched filaments. F, Mature spermatangial sorus. G, H, Spermatangial filaments showing spermatangial mother cells obliquely budding off up to 3 spermatangia. I, Female sorus formed above vegetative thallus (arrow indicates original surface of crust). J, Sorus consisting of single enlarged apical cells with thick membranes around them. K, Female sorus showing cell fusions between vegetative filaments; reproductive filaments have enlarged nuclei. L, Four-celled carpogonial branch (ca) includes a binucleate hypogynous cell. M, Binucleate cell (hy, of unknown function) formed laterally on female gametangial filaments.

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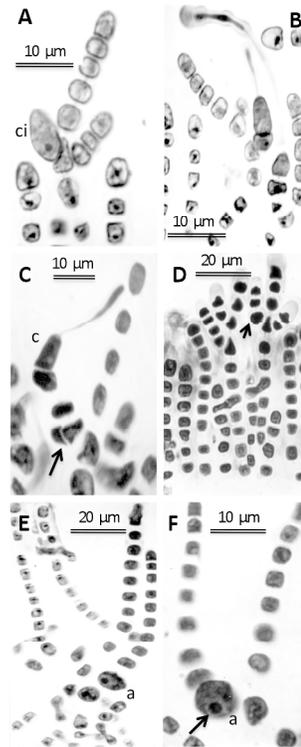


Figure 3. Female reproduction in *Plagiospora gracilis*, collection data as preceding figure, stained with Wittmann's haematoxylin or aniline blue. A, Large carpogonial branch initial (ci) before trichogyne formation. B, Young 2-celled and developing 3-celled carpogonial branches, carpogonium with large nucleus in an apical position. First branch cell of the 3-celled branch is small and wedge-shaped. C, Four-celled carpogonial branch; first cell has cut off a sterile lateral cell (arrow). D, Female sorus with branched reproductive filaments; one cell (arrow) may be a developing auxiliary cell. E, F, Intercalary auxiliary cells (a); note large nucleus (arrow).  
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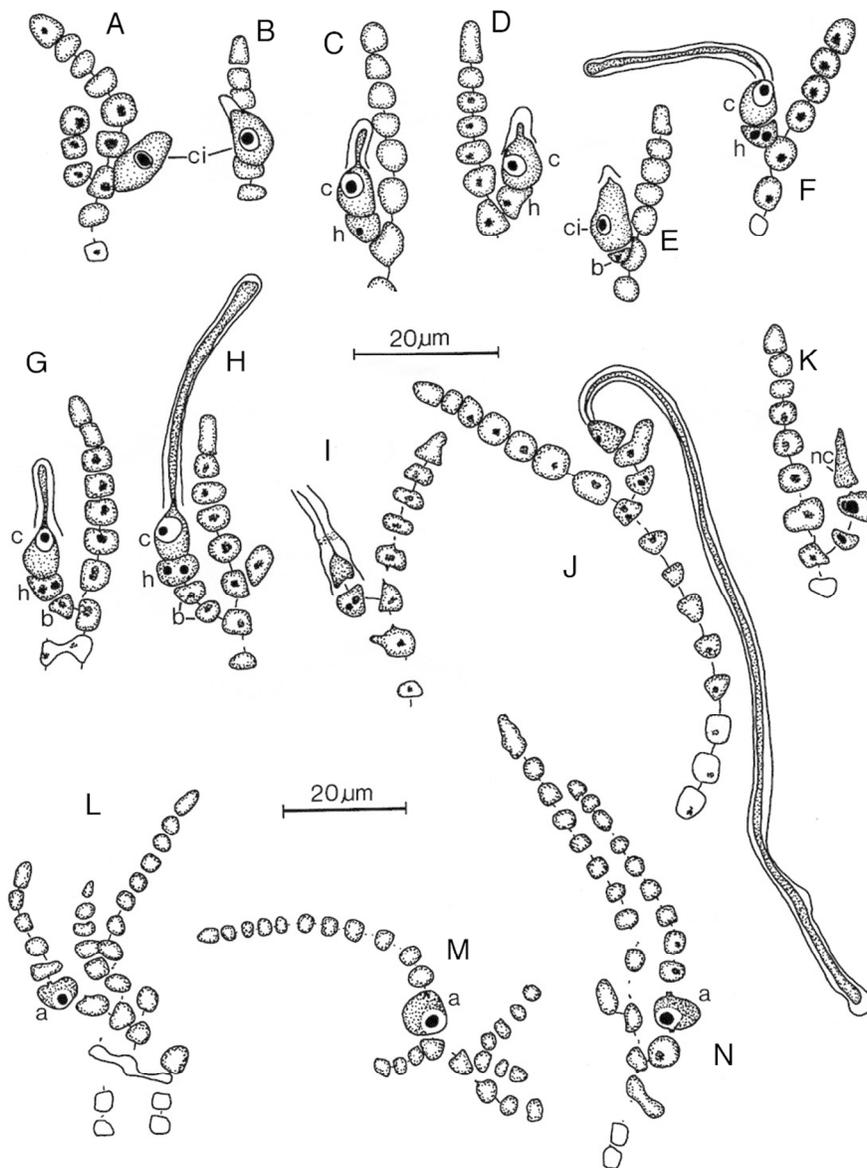


Figure 4. Female reproductive structures of *Plagiospora gracilis*, collection data as before. Figs A-K stained with Wittmann's haematoxylin; Figs L-N with aniline blue. Upper scale bar applies to Figs A-K; lower scale bar to Figs L-N. A, Carpogonial branch initial (ci) prior to formation of trichogyne. B, Carpogonial branch initial showing trichogyne initiation. C, D, Carpogonial branch initial has apparently cut off hypogynous cell (h) to form 2-celled carpogonial branches; carpogonia (c) contain single large nucleus. E, Carpogonial branch initial borne on small wedge-shaped cell (b), possibly a stage in the formation of 3-celled carpogonial branches. F, Carpogonial branch consisting of carpogonium with elongating trichogyne borne on binucleate hypogynous cell. G, H, Developing 3- and 4-celled carpogonial branches. I, Old carpogonial branch, with small conical carpogonium. J, Mature carpogonial branch, with conical carpogonium bearing long trichogyne; hypogynous cell has formed a protuberance. K, Necrotic carpogonium (nc) on 3-celled carpogonial branch. L-N, Auxiliary cells (a) intercalary in lateral auxiliary cell branches. Auxiliary cells contain single large large nuclei and have enlarged pit connections.

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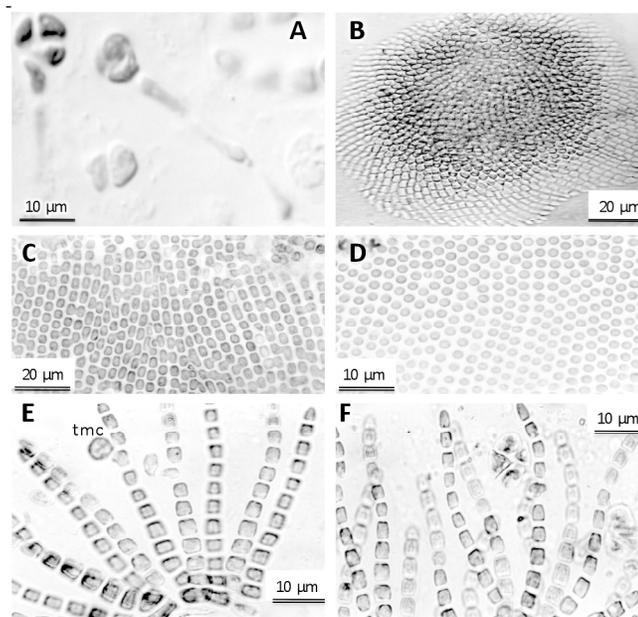


Figure 5. *Plagiospora gracilis* grown in culture. A, Germinating tetraspores. B, Crust grown from tetraspores released in culture. C, Crust from below, showing abundant cell fusions in basal layer. D, Crust from above, showing rows of rounded cells embedded in mucilage. E, Erect filaments forming tetrasporocytes (tmc) on intercalary supporting cells, following transfer to short-day conditions. F, Mature obliquely cruciate tetrasporangia.

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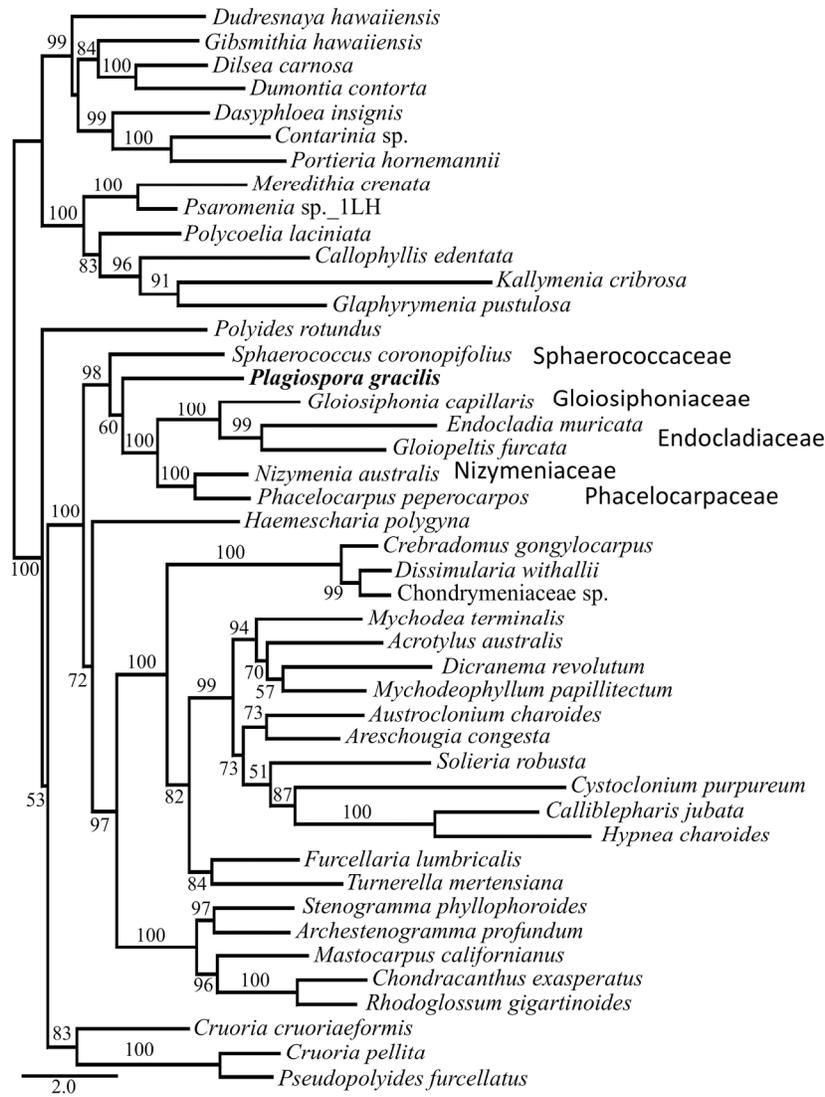


Figure 6. Maximum likelihood phylogeny for the multigene alignment restricted to the included Gigartinales (Table S1). Bootstrap values (1000 replicates) indicated along branches with >50 % support. Scale bar indicates substitutions per site. 250x333mm (300 x 300 DPI)

Table S1. A list of the taxa used in this study with the corresponding GenBank accessions for the three genes used in phylogenetic analyses.

Taxon	Voucher	LSU	<i>rbcL</i>	COI-5P
<b>Atractophorales</b>				
Atractophoraceae				
<i>Atractophora hypnoides</i> P.-Crouan & H. Crouan	GWS005200	GQ497323	KU382063	GQ497303
<b>Gigartinales</b>				
Acrotylaceae				
<i>Acrotylus australis</i> J.-Agardh	G0023 GWS034942	GQ406346 ND	KC130223 ND	ND XXXXX
Areschougiaceae				
<i>Areschougia congesta</i> (Turner) J. Agardh	GWS002474 GWS014882	GQ406347 ND	KC130213 ND	ND HM917458
<i>Austroclonium charoides</i> (Harvey) Min-Thein & Womersley	G0360	KF026501	KF026485	HM915819
Chondrymeniaceae				
Chondrymeniaceae sp.	GWS005891	KF026513	KF026497	HM918785
<i>Crebradomus gongylocarpus</i> Kraft & G.W.-Saunders	GWS002030	KF026500	KF026482	HM915973
<i>Dissimularia withallii</i> Kraft & G.W. Saunders	JAR-1201	KF026509	KF026492	KF026473
Cruoriaceae				
<i>Cruoria cruoriiformis</i> (P.-Crouan & H. Crouan) Denizot	SA21654	KC130241	KC130214	HM916098
<i>Cruoria pellita</i> (Lyngbye) Fries	GWS000563	GQ406348	KT310698	ND
<i>Pseudopolyides furcellatus</i> Barbara, Gallardo, Cremades, Barreiro, Maneiro &	SA19548	KC130239	ND	KC130189

8	G.W.-Saunders				
9		SA19559	ND	KC130212	KC130194
10	Cystocloniaceae				
11	<i>Calliblepharis jubata</i> (Goodenough &				
12	Woodward) <del>Kütz</del> zingKützing	GWS000323	KF026511	ND	ND
13		GWS014653	ND	KF026494	KF026474
14	<i>Cystoclonium purpureum</i> (Hudson)				
15	Batters	G0421	KC130242	KC130217	ND
16		GWS002294	ND	ND	HM918475
17	<i>Hypnea charoides</i> J.V.-Lamouroux	G0358	GQ406354	KC130220	HM915818
18	Dicranemataceae				
19	<i>Dicranema revolutum</i> (C.-Agardh) J.				
20	Agardh	G0036	GQ406349	ND	ND
21		GWS014819	ND	KC130231	HM917423
22	Dumontiaceae				
23	<i>Dasyphloea insignis</i> Montagne	G0034	DQ343688	ND	ND
24		GWS014869	ND	XXXXX	ND
25		GWS015838	ND	ND	XXXXX
26	<i>Dilsea carnosa</i> (Schmidel) Kuntze	GWS000746	EF033609	JN403065	AY971151
27	<i>Dudresnaya hawaiiensis</i> R.K.S.-Lee	GWS001024	KC130243	KC130219	KC130201
28	<i>Dumontia contorta</i> (Gmelin) Ruprecht	CSM005B	ND	JN403062	AY971155
29		GWS001815	JX296139	ND	AY970583
30	<i>Gibsmithia hawaiiensis</i> Doty	GWS001343	GU176297	KT310689	ND
31	Endocladiaceae				
32	<i>Endocladia muricata</i> (Postels &				
33	Ruprecht) J.-Agardh	G0155	KF026504	ND	ND
34		GWS020093	ND	KF026496	KF026475
35	<i>Gloiopeltis furcata</i> (Postels & Ruprecht)				
36	J.G.-Agardh	GWS002264	EF033612	JX969801	ND
37		GWS013649	ND	ND	HM916317

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Furcellariaceae					
	<i>Furcellaria lumbricalis</i> (Hudson) J.V. Lamouroux	GWS001772	GQ406350	ND	ND
		GWS003509	ND	KC130215	ND
	<i>Turnerella mertensiana</i> (Postels & Ruprecht) F.-Schmitz	GWS003012	XXXXXX	XXXXXX	XXXXXX
Gigartinaceae					
	<i>Chondracanthus exasperatus</i> (Harvey & Bailey) J.R.-Hughey	GWS002829	GQ338089	JN403073	GQ398091
	<i>Rhodoglossum gigartinoides</i> (Sonder) Edyvane & Womersley	G0098	GQ338091	JN403074	ND
		GWS029663	KF026505	ND	KF026468
Gloiosiphoniaceae					
	<i>Gloiosiphonia capillaris</i> (Hudson) Carmichael <del>in Berkeley</del>	GWS000374	GQ406352	ND	ND
		GWS013313	ND	KU382056	HM915532
Haemeschariaceae					
	<i>Haemescharia polygyna</i> Kjellman	GWS001849	GQ406353	KC130218	ND
Kallymeniaceae					
	<i>Callophyllis edentata</i> Kylin	GWS001145	AY171604	KC130228	JX034247
	<i>Glaphyrymenia pustulosa</i> J.-Agardh	GWS015923	KF280962	KF280988	HM917946
	<i>Kallymenia cribrosa</i> Harvey	GWS000466	KF280953	KF280978	KF280930
	<i>Meredithia crenata</i> C.W. <del>S</del> -Schneider, C.E.-Lane & G.W.-Saunders	GWS001247	AY171612	KC157632	KC157617
	<i>Polycoelia laciniata</i> J.-Agardh	GWS001906	JX296144	KF280983	KT307606
	<i>Psaromenia</i> sp._1LH	GWS002058	JX296141	KC157627	HM915984
Mychodeaceae					
	<i>Mychodea terminalis</i> Harvey	DV023	XXXXXX	XXXXXX	XXXXXX
Mychodeophyllaceae					
	<i>Mychodeophyllum papillitectum</i> Kraft	G0385	GQ406355	KF026493	HM915826

8	Nizymeniaceae				
9	<i>Nizyenia australis</i> Sonder	GWS001581	GQ406356	KC130227	ND
10		GWS014843	ND	ND	HM917440
11	Phacelocarpaceae				
12	<i>Phacelocarpus peperocarpus</i> (Poiret)				
13	<u>M.J.</u> Wynne, Ardré & <u>P.C.</u> Silva	GWS000417	GQ406357	KC130222	HM915869
14	Phylloporaceae				
15	<i>Archestenogramma profundum</i> C.W.				
16	Schneider, <del>F.</del> Chengsupanimit & G.W.				
17	Saunders	BDA0368	JN403059	JN403075	HQ933374
18	<i>Mastocarpus californianus</i> S.C.				
19	Lindstrom, J.R. Hughey & P.T.-Martone	GWS000073	GQ338094	GQ338144	GQ380171
20	<i>Stenogramma phylloporoides</i> (J.				
21	Agardh) <u>A.</u> Millar	GWS001506	JN403060	GQ338123	GQ380377
22	Plagiosporaceae				
23	<i>Plagiospora gracilis</i> <u>Kuckuck</u>	GWS000293	XXXXX	XXXXX	ND
24	Polyidaceae				
25	<i>Polyides rotundus</i> (Hudson) Greville	G0344	FJ848972	KC130221	ND
26		GWS002676	ND	ND	HM918499
27	Rhizophyllidaceae				
28	<i>Contarinia</i> sp.	6091	KC130244	KC130224	ND
29		6168	ND	ND	XXXXX
30	<i>Portieria hornemannii</i> (Lyngbye)				
31	<u>P.C.</u> Silva	G0232	FJ848973	JX996090	ND
32	Solieriaceae				
33	<i>Solieria robusta</i> (Greville) Kylin	GWS001590	GQ406360	KC130210	HM915934
34	Sphaerococcaceae				
35	<i>Sphaerococcus coronopifolius</i>				
36	Stackhouse	GWS001833	FJ848974	KC130216	ND
37	<b>Peyssonneliales</b>				
38	Peyssonneliaceae	CM04	KU501313	JX969782	JX969703

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<i>Peyssonnelia atropurpurea</i> P. <del>L.</del> -Crouan & H. <del>M.</del> -Crouan				
<i>Peyssonnelia dubyi</i> P. <del>L.</del> -Crouan & H. <del>M.</del> -Crouan	CM03	KU501316	JX969785	JX969715
<i>Ramicrusta textilis</i> C.M.-Pueschel & G.W.-Saunders	GWS001755	FJ848970	KC130226	JX969749
<i>Sonderophycus</i> sp.	G0418	AF419125	KC130225	KC130203

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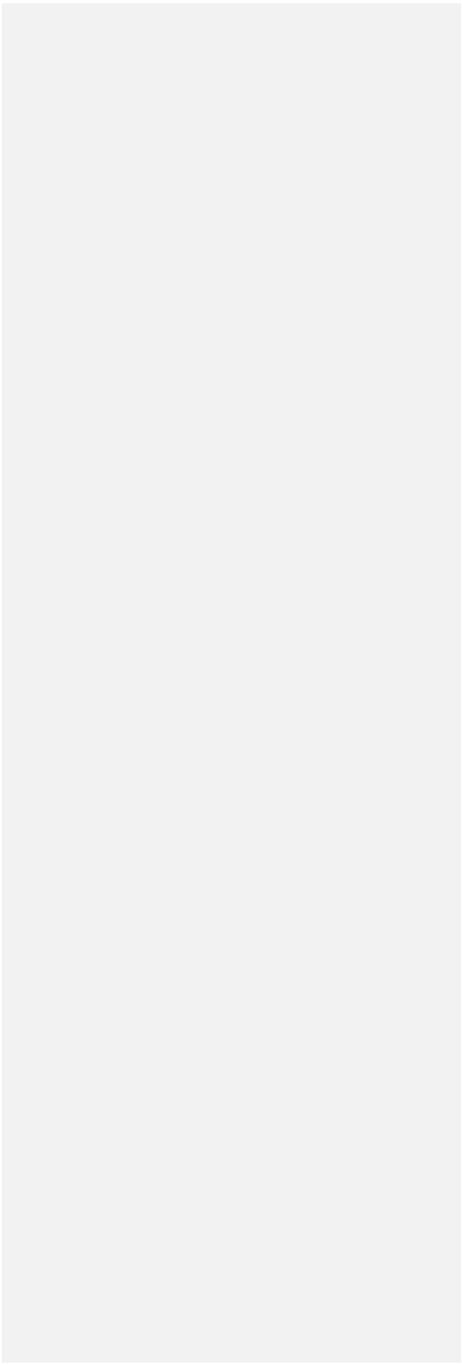


Table S2. Phenology of *Plagiospora gracilis* at Cloghy Rocks, Strangford Lough, N. Ireland, collected on pebbles at depths of 5 to 12 m.

Date	Reproductive state
4 Aug 1983	Vegetative
24 Oct 1983	Vegetative
3 Nov 1983	Tetrasporocytes
15 Dec 1983	Tetrasporangia
23 Jan 1984	Tetrasporangia
1 Mar 1984	Tetrasporangia
26 Mar 1984	Tetrasporangia
18 Apr 1984	Tetrasporangia
29 May 1984	Tetrasporangia (few)
27 Jun 1984	Vegetative
27 Jul 1984	Vegetative
29 Aug 1984	Vegetative
26 Sep 1984	Vegetative
2 Nov 1984	Vegetative
28 Nov 1984	Tetrasporangia; spermatangia
19 Dec 1984	Tetrasporangia
23 Jan 1985	Tetrasporangia; spermatangia; carpogonia
30 Jan 1985	Tetrasporangia
26 Feb 1985	Tetrasporangia; carpogonia
15 May 1985	Tetrasporangia
16 Dec 1985	Spermatangia; carpogonia