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DIVERSITY, LIFE HISTORY, AND ECOLOGY OF *TRENTEPOHLIA* AND *PRINTZINA* (TRENTEPOHLIALES, CHLOROPHYTA) IN URBAN HABITATS IN WESTERN IRELAND[1]

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On the basis of field and culture investigations, five species of the genera *Trentepohlia* and *Printzina* were found to occur in urban habitats in western Ireland: *Trentepohlia abietina* (Flotow) Hansgirg, *T. aurea* (Linnaeus) Martius, *T. iolithus* (Linnaeus) Wallroth, *T.* cf. *umbrina* (Kützing) Bornet, and *Printzina lagenifera* (Hildebrandt) Thompson et Wujek. These species formed perennial populations on a variety of substrata. *T. abietina* occurred on bark of trees; *T.* cf. *umbrina* occurred on stone walls; and *P. lagenifera* grew on several substrata, mainly cement and asbestos sheeting. *T. aurea* and *T. iolithus* were found on old concrete and cement walls; in particular, the latter species formed characteristic, extensive, deep-red patches on many buildings. In culture, best growth and reproduction of these species were observed at 10 and 15° C, 16:8 h light:dark. Both in culture and in the field, reproduction took place by release of biflagellate swarmers behaving as asexual spores, germinating to produce new plants without any evidence of sexual fusion; release of biflagellate swarmers in the field was generally observed in all seasons throughout a whole annual cycle. Confirmation of the occurrence of sexual reproduction in *Trentepohlia* was not obtained.

Key index words: **building surfaces; diversity; ecology; Ireland; life history; phenology; *Printzina*; subaerial algae; *Trentepohlia*; urban habitats**

Abbreviations: **ANOVA, analysis of variance; MS, mean square**

Trentepohlia Martius 1817 *nom. cons.* is a common genus of subaerial branched filamentous green algae, found on wood, bark, leaves, and rock (Fritsch 1935, Ho et al. 1983, Chapman 1984). As currently circumscribed, the genus includes about 40 species (Hoek et al. 1995) that are typical members of the subaerial vegetation of many tropical and subtropical areas (Fritsch 1907), but some are also commonly distributed in temperate regions (Printz 1964).

Organisms belonging to this genus were originally described by Linnaeus as *Byssus aureus* (= *Trentepohlia aurea* (Linnaeus) Martius, the type species; Linnaeus 1759) and *Byssus iolithus* (= *Trentepohlia iolithus* (Linnaeus) Wallroth; Linnaeus 1753). In the last two centuries, a plethora of studies has been carried out on all aspects of the biology of *Trentepohlia*. Many of these investigated morphology, distribution, and reproduction (Printz 1939, and references therein); others concerned physiology (Howland 1929, Tan et al. 1993, Abe et al. 1999), photosynthesis (Ho et al. 1983, Ong et al. 1992), and ultrastructure (Graham and McBride 1975, 1978, Chapman and Good 1978, Roberts 1984, Chapman et al. 2001). However, several features render species of *Trentepohlia* very difficult organisms with which to work, and consequently some aspects of their biology have not been completely elucidated. The morphology of many species is very variable, and some have long been regarded as highly polymorphic organisms (Hariot 1889, Printz 1939, 1964); this has created considerable taxonomic and determination difficulties. Taxonomic criteria at the species level are mainly based on shape and size (length and width) of vegetative cells, presence of hair-like cells (setae), branching pattern, position, and morphology of reproductive structures (De Wildeman 1888, Hariot 1889, Printz 1921, 1939, 1964). However, some of these features are unstable and can vary in relation to ecological conditions (Thompson and Wujek 1997). To elucidate the range of variation, morphometric characters (such as cell length and width) should be measured carefully and analyzed using a suitable statistical treatment, which has been done only very rarely (e.g. Akiyama 1971). Unfortunately, cultural observations have not been particularly helpful in solving this kind of problem, perhaps because most studies have used a very limited range of culture conditions. Additionally, *Trentepohlia* species grow very slowly, and in culture they rarely produce structures, such as zoosporangia, useful for species-level determination (e. g. Uyenco 1965).

Another aspect of the biology of *Trentepohlia* poorly understood is the life history. The most widespread hypothesis is that the life history probably consists of an isomorphic alternance of diploid sporophytes producing quadriflagellate meiospores and haploid gametophytes producing biflagellate gametes, which fuse isogamously (Wille 1878, Oltmanns 1922, Printz 1964, Thompson and Wujek 1997). Evidence for this, however, is inconclusive (Hoek et al. 1995); fusion of gametes has been directly observed rarely (Wille 1878, Lagerheim 1883). Conversely, it has been repeatedly reported that putative biflagellate gametes are able to germinate and produce new algae without fusing sexually (Fritsch 1935, Meyer 1936, Printz 1964, Graham and McBride 1975, Bourrelly 1990). Furthermore, with a few exceptions (e.g. Howland 1929), the details available in the literature about the reproductive phenology (such as temporal patterns in the production

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of reproductive structures and release of gametes and zoospores) are generally scanty.

Western Ireland is a cold-temperate area characterized by high levels of rainfall and humidity. Unpublished observations suggest that many terrestrial habitats in this region support a rich and diversified algal flora; however, with the exception of a short paper by Schlichting (1975) and a monographic treatment of the Prasiolales of Galway city (Rindi et al. 1999), virtually nothing is known about the subaerial algae of Ireland. Ó Séaghdha (1966), in an unpublished masters thesis in Irish, found that species of *Trentepohlia* were among the most common members of the subaerial flora of the city of Galway and other areas of western Ireland. His observations were confirmed by repeated observations made by one of us (M. D. G.) over the last 25 years. Some forms attributable to this genus grow on an extraordinary variety of artificial structures, frequently becoming an aesthetic and practical nuisance, with considerable economic repercussions. These algae produce extensive red or pink growths that can obscure posts and inscriptions and form ugly patches on buildings, making it necessary to repaint such surfaces more regularly than normal; expensive and highly toxic ingredients must also be frequently included in the paints to achieve prevention (Ryan 1983). This kind of problem is well known for some tropical localities, such as Singapore (Wee and Lee 1980, Ho et al. 1983), but it has not been reported for temperate zones. In general, however, information on the distribution and ecology of algae in urban environments is very rudimentary. For this reason, a field and culture study of the species of *Trentepohlia* occurring in Galway was initiated in October 1999. The closely related genus *Printzina*, erected by Thompson and Wujek (1992) for nine species previously attributed to *Trentepohlia*, was also considered. Our aim was to elucidate the diversity and some basic aspects of the ecology of these genera, with particular emphasis on how the species of *Trentepohlia* and *Printzina* reproduce and complete their life history in the field.

MATERIALS AND METHODS

Field studies. In October 1999, the city center of Galway and peripheral areas nearby were surveyed for the presence of *Trentepohlia* and *Printzina*; these algae form orange, red, or pink patches that can be easily recognized with the unaided eye. Collections were made at every site at which such algae were observed. Samples for laboratory examination were collected by scraping a surface of a few cm^2 with a scalpel or a sharp knife. Twenty-eight sites were found to support trentepohliacean algae; for each site a number of environmental factors were noted (orientation of the colonized surface, width of the intervening space, substratum type, kind of habitat, height from the ground, and distance from sea), and the species present was determined by microscopic examination in the laboratory.

Seasonality and reproductive phenology of the species of *Trentepohlia* and *Printzina* were followed for a period of 15 months (January 2000 to March 2001) at 13 selected permanent sites (Fig. 1); the main features of these sites are reported in detail in Table 1. Different sampling procedures were designed to assess (1) variation in the temporal and spatial distribution of the two species most commonly recorded on artificial substrata (*Trentepohlia iolithus* and *Trentepohlia* cf. *umbrina*); (2) variation in the width of cells, a feature of critical taxonomic importance in *Trentepohlia* (Printz 1939, 1964), at several different spatial and temporal scales; and (3) reproductive phenology. The criteria used in choosing sampling dates (Table 2) are explained in detail below. Because of the large number of collections examined in this study, the samples were usually collected and examined in the 3–4 days around each date rather than all on the same day.

Spatial and temporal distribution of T. iolithus *and* T. *cf.* umbrina. Three sampling dates were randomly selected for each season; to achieve a good spread of the sampling dates within the season, two conventions were followed: (1) for each season the first and the last week were excluded from sampling and (2) an interval of at least 15 days elapsed between one sampling date and the next. For each sampling date, two selected sites, located at least 200 m apart, were sampled (Dyke Road and Claddagh Hall for *T. iolithus*, Quadrangle and St. Nicholas Church for *T.* cf. *umbrina*). The sites corresponded to stretches of walls with an extent of several meters square on which there was a well-developed population of the species in question. For each sampling date, at each site 10 measurements of percentage cover (36-cm^2 quadrats) were taken; care was taken not to resample quadrats previously sampled. The null hypothesis that

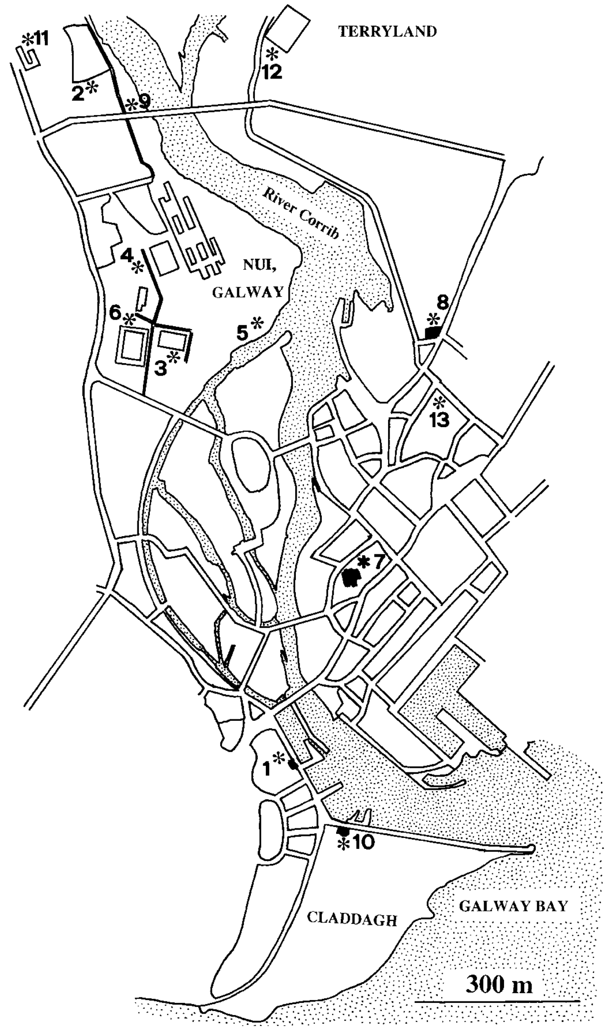

FIG. 1. Study area in Galway city. Asterisks indicate permanent observation sites during the period from January 2000 to March 2001.

TABLE 1. Location, habitat, and species of *Trentepohlia* and *Printzina* occurring in permanent sites monitored in Galway city.

Site	Location	Habitat	Species
1	Claddagh Quay	Old cement wall, facing N	*Trentepohlia aurea*
2	NUI, Galway, Football Ground	Bark of trees	*Trentepohlia abietina*
3	NUI, Galway, Tennis Grounds	Bark of trees	*Trentepohlia abietina*
4	NUI, Galway, Library	Bark of trees	*Trentepohlia abietina*
5	Boat Club	Old asbestos building	*Printzina lagenifera*
6	NUI, Galway, Quadrangle Building	Limestone wall, facing N	*Trentepohlia* prox. *umbrina*
7	St. Nicholas Church	Limestone wall, facing N	*Trentepohlia* prox. *umbrina*
8	Dyke Road	Concrete wall, facing N	*Trentepohlia jolithus*
9	Quincentennial Bridge	Cement parapet of the Bridge, facing N	*Trentepohlia jolithus*
10	Claddagh Hall	Concrete wall, facing S	*Trentepohlia jolithus*
11	NUI, Galway, St. Anthony's Building	Concrete wall, facing NE	*Trentepohlia jolithus*
12	Terryland Park	Concrete wall, facing SW	*Trentepohlia jolithus*
13	Bóthar na mBan	Cement wall, facing NE	*Trentepohlia jolithus*

the abundance of *T. iolithus* and *T.* cf. *umbrina* did not vary significantly with season, sampling date, or site was tested by analyzing the percentage cover data using a multifactorial analysis of variance (ANOVA) (Underwood 1997). Season was treated as fixed orthogonal factor, sampling date as random factor nested in season, and site as a random orthogonal factor. The assumption of homogeneity of variances was assessed using Cochran's C test (Underwood 1997), and appropriate data transformations were applied if necessary (this was also done for all other statistical analyses; see below). This kind of experimental design does not provide an appropriate denominator to check the effect of the fixed factor. Indications of the effect of season were obtained by calculating the statistic F as a ratio between the mean square for season and a denominator, which was subjectively selected as the most appropriate among the other mean squares depending on the results obtained. The statistical package GMAV5 for Windows (A.J. Underwood and M.G. Chapman, Institute of Marine Ecology, University of Sydney) was used for these analyses.

Spatial and temporal variation of the width of cells. For *Trentepohlia abietina*, *T. iolithus*, and *T.* cf. *umbrina*, the three most common and widespread species, three sampling dates and three sites (for *T. abietina*: National University of Ireland [NUI] Galway football ground, NUI Galway tennis grounds, NUI Galway Library) or two sites (for *T. iolithus*: Dyke Road and Quincentennial Bridge; for *T.* cf. *umbrina*: NUI Galway Quadrangle and St. Nicholas's Collegiate Church) were selected using the criteria mentioned above. For each sampling date at each site, three samples (4- to 9-cm^2 plots) were removed from areas in which patches of *Trentepohlia* were apparent. Every sample was collected at a distance of 0.2–10 m from all other samples. Each sample was examined microscopically in the laboratory, and the widths of 15 randomly chosen cells were measured. The null hypothesis that the width of cells of *T. abietina*, *T. iolithus*, and *T.* cf. *umbrina* did not vary significantly with season, sampling date, site, or plot was tested by analyzing the cell width data using a model of ANOVA similar to the previous one, but including also the plot as random factor nested in the interaction season × date × site. The statistical treatment was the same and indications of the effect of season were obtained using the same criteria as above.

TABLE 2. Sampling dates.

Season	Dates
Winter 2000	21 January 2000
	20 February 2000
	10 March 2000
Spring 2000	21 April 2000
	23 May 2000
	14 June 2000
Summer 2000	12 July 2000
	17 August 2000
	11 September 2000
Autumn 2000	15 October 2000
	20 November 2000
	8 December 2000
Winter 2001	15 January 2001
	19 February 2001
	8 March 2001

Trentepohlia aurea and *Printzina lagenifera* were not as abundant as the other three species. Abundant populations occurred only at a single site for both species, and this prevented an evaluation of the variation in cell width on the spatial scale of different sites. For these species, seasonal collections were made, and the null hypothesis that the width of cells did not vary significantly in different seasons was tested by a one-way ANOVA (45 replicated measurements for each season). For pairs of morphologically similar species of uncertain taxonomic relationship (*T. aurea–T. abietina* and *P. lagenifera–T.* cf. *umbrina*, respectively), Student's *t*-tests for independent samples (Zar 1999) were performed for each sampling date to assess if width of cells was significantly different between similar species; at least 15 replicates were used for each test. The STATISTICA package (StatSoft 1994) was used for one-way ANOVAs and *t*-tests. For all analyses, $\alpha = 0.05$ was regarded as significant.

Reproductive phenology. This was studied by microscopic examination of the same samples used for the study of the variation of the width of cells; all samples were examined no later than a few hours after collection. For each sample the following observations were made: (1) presence of reproductive structures, (2) kind of reproductive structure, (3) release of swarmers (zoospores or gametes), and (4) number of flagella of the swarmers. Additionally, during the period October 1999 to December 2000, occasional collections were made at several sites in other localities of western Ireland where growths of *Trentepohlia* and *Printzina* were observable (mainly the western counties of Clare and Mayo and the southwestern county of Cork), and the determinations were made by microscopic examination.

For all collections, voucher specimens have been deposited in the Phycological Herbarium, National University of Ireland, Galway.

Culture study. Cultures were initiated from vegetative tips (five to six apical cells) excised from erect branches of *Trentepohlia* and *Printzina* collected at several sites in Galway and placed at 15 and 20° C, 16:8-h light:dark (L:D), and 20–40 μmol photons·m^{-2}·s^{-1}. However, for all species (with the sole exception of *T. iolithus*) new cultures were subsequently obtained by release of biflagellate swarmers that settled and germinated on the bottom of the culture dishes. Morphology and reproduction were examined by growing the algae in a variety of temperature and light regimes (Table 3), with photon irradiances of 10–90 μmol photons·m^{-2}·s^{-1}. Throughout the text we use the terms "low" and "high irradiances" for conditions corresponding to 10–30 μmol photons·m^{-2}·s^{-1} and 70–90 μmol pho-

TABLE 3. Temperature and day length conditions in culture.

Temperature (° C)	Day length (light:dark, h)
10	16:8, 8:16
15	16:8, 8:16
20	16:8
25	16:8

tons·m^{-2}·s^{-1}, respectively. For each combination of factors at least four specimens were used. Jaworski's medium and soil water medium (Tompkins et al. 1995) were used, both as liquid and agar media. For observations on reproduction, only Jaworski's liquid medium was used (specimens grown in liquid medium generally had a more open branching pattern, which allowed the reproductive structures to be seen more clearly). Plastic dishes containing approximately 30 mL of medium (for liquid cultures) or 60 mL (for agar cultures) were used; in the liquid cultures the medium was replaced every 7–10 days.

Permanent cultures of the species of *Trentepohlia* and *Printzina* recorded are currently maintained in the culture collection of the Department of Botany, Martin Ryan Institute, National University of Ireland, Galway.

RESULTS

Five members of the Trentepohliaceae were found in the urban area of Galway: *Trentepohlia iolithus* (Linnaeus) Wallroth, *T. abietina* (Flotow) Hansgirg, *T. aurea* (Linnaeus) Martius, *T.* cf. *umbrina* (Kützing) Bornet, and *Printzina lagenifera* (Hildebrandt) Thompson et Wujek.

Distribution and ecology. Trentepohlia iolithus: This is the most common representative of the genus ins the urban area of Galway and probably in all of Ireland. In Galway it was found at 14 sites; only 2 of these were in the city center, with the others in suburban areas, mainly NUI, Galway, Terryland, and the Claddagh. *T. iolithus* was much more abundant at some sites than at others. This seems to be a very slow-growing organism in nature, and during the period of the study its abundance at individual sites did not show any apparent temporal variation, either between dates within a season or between seasons. Furthermore, differences of abundance between sites seemed to be constant. These patterns of distribution are shown by the results of ANOVA on the percentage cover data (Table 4). The effect of site was highly significant, whereas the effect of sampling date was not significant. Even with the limitations reported in Materials and Methods, there is also a strong indication that the effect of season

TABLE 4. Results of the ANOVA performed on the percentage cover of *Trentepohlia iolithus.*

Source of variation	df	MS	F	F tested on
Season = Se	3	143.61		No test
Date (Se) = Da	8	405.64	1.77	Si × Da (Se)
Site = Si	1	189506.4	*826.14**	Si × Da (Se)
Se × Si	3	627.97	2.74	Si × Da (Se)
Si × Da (Se)	8	229.39	0.65	Residual
Residual	216	350.98		

Italic indicates significance: *$P < 0.001$. Cochran's test: C = 0.0875, $P > 0.05$.

was not significant. Furthermore, differences between sites were consistent in time, as the interactions season × site and site × date (season) were not significant.

This species grows most commonly on old concrete or cement walls (either painted or not, although the most abundant populations are usually observed on unpainted concrete), with a very wide intervening space (many tens of meters); almost all populations found in Galway occurred in such situations. No well-developed populations of *T. iolithus* were observed on other kinds of substrata. No correlations were evident in relation to height from the ground or to orientation of the colonized surface: *T. iolithus* was found growing both a few centimeters from the ground or many meters from the ground, and it was also found on walls facing in all compass directions. In the early phases of colonization, *T. iolithus* forms deep red patches with the appearance of irregular vertical stripes (Fig. 2A) and, in some years, these populations can grow to cover, more or less completely, many m^2 of surface (Fig. 2, B–D). They have a characteristic appearance and to the trained eye they are easy to recognize, even from a considerable distance. Visual inspections indicate that *T. iolithus* is widely distributed in the west of Ireland; large growths are observable in urban areas and rural districts of the counties of Galway, Clare, Mayo, Kerry, Cork, Roscommon, and Westmeath, where they invariably occupy the same kind of habitat observed in Galway city.

Trentepohlia abietina: In Galway, *T. abietina* was found only in the area of the campus of the NUI, Galway, where it formed brownish orange patches on the bark of several species of deciduous ornamental trees (Fig. 2E), such as cherries (*Prunus* sp.), beeches (*Fagus sylvatica* Linnaeus), alders (*Alnus* sp.), sycamores (*Acer pseudoplatanus* Linnaeus), and ashes (*Fraxinus excelsior* Linnaeus). Populations were found with different orientations and at different heights from the ground on different trees. The most conspicuous populations were observed in more or less weather-exposed habitats and generally on cherries, but their distribution seemed to be associated with certain areas rather than with one or more species of tree. This species colonized only bark of trees in Galway; plants of *T. abietina* were also found on this kind of substratum in the campus of University College, Cork and in Baltimore, west Cork. However, specimens collected at Glendalough (Co. Wicklow) and Lough Hyne (Co. Cork), on the east and southwest coasts of Ireland, occurred on large limestone rocks in well-shaded habitats in deciduous forests.

Trentepohlia aurea: This species was rare in Galway, being present only at a single site (Claddagh Quay; see Fig. 1). The site was an old cement wall facing north, with a large intervening space (many tens of meters). Many small isolated patches were present, brownish orange in color, growing at a height of 1.5–2 m from the ground. The species was mixed with lichens and other subaerial algae, mainly blue green (provi-

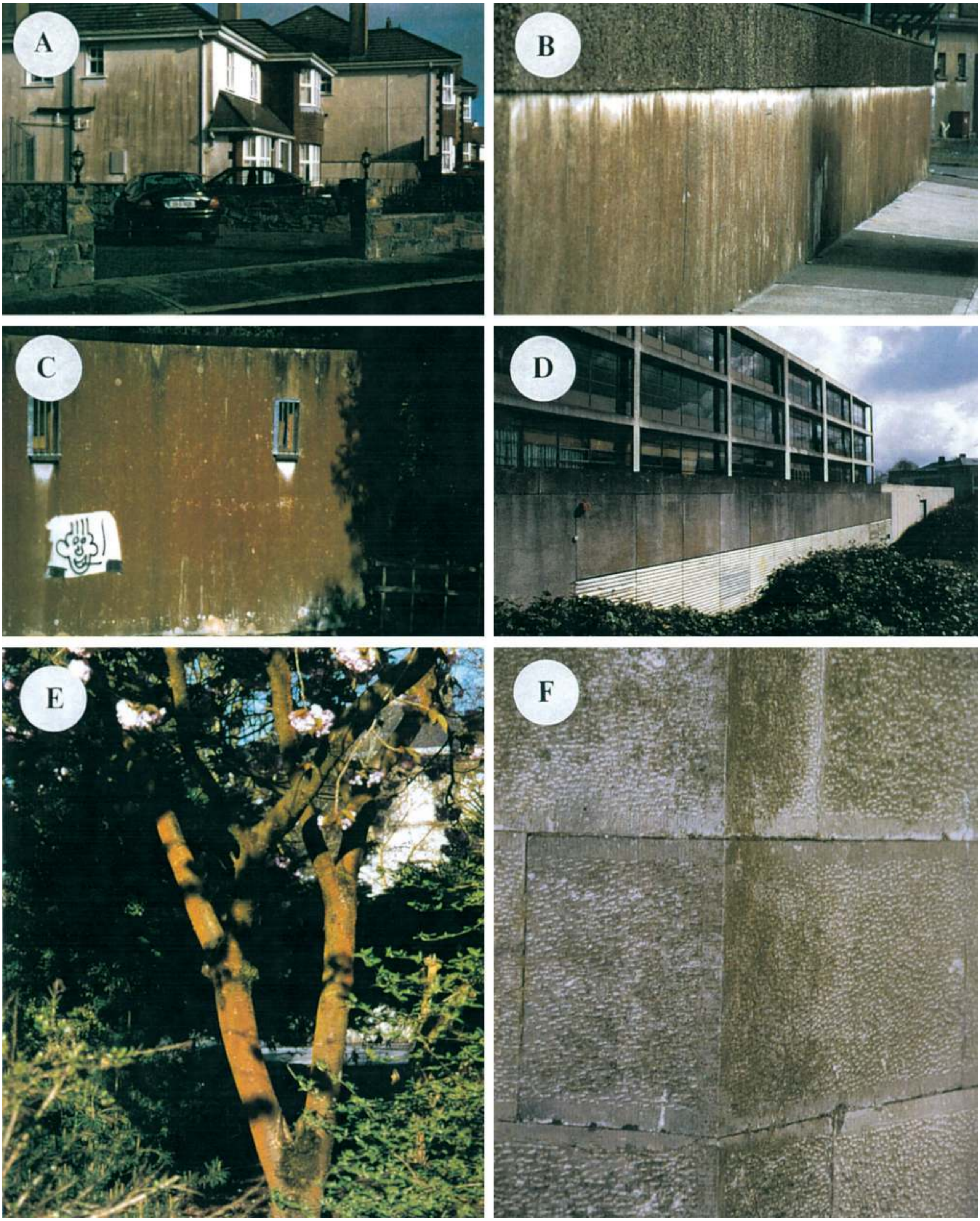

FIG. 2. Habitats occupied by species of *Trentepohlia* in Galway. (A) *T. iolithus* on the painted surfaces of suburban houses. Extensive streaking is evident on west-facing surfaces but is absent on south-facing walls. (B and C) *T. iolithus* on concrete unpainted walls. (D) *T. iolithus* on the NUI, Galway Library in 1994. Note the contrast between older surfaces (left) and the newer surface of extension (right). (E) *T. abietina* on the bark of a flowering cherry tree. (F) *T.* prox. *umbrina* on the carved limestone blocks of the Quadrangle Building, NUI, Galway.

sionally identified as *Gloeocapsa* spp. and *Chroococcus* spp.). *T. aurea* was not collected at other Irish localities during this study.

Trentepohlia cf. *umbrina*: This species was found at seven sites, most of which were concentrated in the area of the campus of NUI, Galway. In all cases it occurred on old limestone walls, usually facing north. At most sites, small isolated red-brown patches were apparent, and only two hosted a well-developed population: a stretch of the wall of the Quadrangle Building of NUI, Galway, which dates from 1845, facing north (Fig. 2F) and the northern side of the Collegiate Church of St. Nicholas (dating from the 12th century). The patterns of distribution of this species were similar to those observed for *T. iolithus*. *T.* cf. *umbrina* was more abundant at some sites than at others, and its abundance did not show any apparent temporal variations. This is highlighted by the results of the ANOVA on the percentage cover data; as for *T. iolithus*, a strongly significant effect of site and no significant effect of sampling date (and, very probably, of season) were detected (Table 5). Even for this species, differences between sites were consistent in time, as the interactions season × site and site × date (season) were not significant.

Specimens of *T.* cf. *umbrina* were also collected in April 2000 on the northern wall of the Quadrangle Building of University College Cork (where it occurred in a habitat virtually identical to the one of the Quadrangle in Galway and which was built at the same time in the 19th century) and on the Powerscourt House Building in Wicklow (forming a red powdery crust on granite columns).

Printzina lagenifera: In Galway, specimens referable to this species were collected at 10 sites. As with *T.* cf. *umbrina*, this species was most common in the area of NUI, Galway (only a single site supporting a recognizable population was found in the city center). Of the species recorded, it seemed to be the most versatile from an ecological point of view, occurring on a variety of substrata (bark of trees, asbestos sheeting, cement, and limestone) and habitats. However, a well-developed population was only found at a single site, a building of asbestos sheeting dating from the 1940s at the rear of the campus, with a very extensive intervening space. On this sheeting *P. lagenifera* formed a powdery layer, reddish brown to pink in color, extending for many meters along the walls of the building, more or less uniformly from the base to the roof. Other sites where *P. lagenifera* was collected were quite different, varying from shaded places to more exposed habitat, at different heights from the ground. No collections attributable with certainty to *P. lagenifera* from other Irish locations were made.

TABLE 5. Results of the ANOVA performed on the percentage cover of *Trentepohlia* cf. *umbrina*.

Source of variation	df	MS	F	F tested on
Season = Se	3	354.39		No test
Date (Se) = Da	8	312.57	1.56	Si × Da (Se)
Site = Si	1	114800.00	*571.50**	Si × Da (Se)
Se × Si	3	101.91	0.51	Si × Da (Se)
Si × Da (Se)	8	200.87	0.62	Residual
Residual	216	323.82		

Italic indicates significance: $^{*}P < 0.001$. Cochran's test: C = 0.0774, $P > 0.05$.

Morphology, growth, and reproduction. Trentepohlia iolithus: Individual plants form small cushions that grow in culture with a regular hemispherical shape (Fig. 3A); creeping axes form abundantly branched erect axes, with unilateral or alternate arrangement (Fig. 3, B–D). In the field, however, growth is much more irregular; the "erect" parts tend to assume a decumbent habit, with a very irregular branching, and are not easily recognizable from the creeping axes. The result is that well-developed field specimens generally produce a sort of crust, with an almost pseudo-parenchymatous growth form. The shape of the cells varies from perfectly cylindrical to globular; erect axes have mainly cylindrical cells, whereas in the prostrate parts the shape is more commonly swollen or globular. In both cases, however, a complete range of variation may be found. In culture, growing parts of the thallus usually produce regularly cylindrical cells. Cell length was 15–40 μm. Cell width was 10–30 μm; as shown by the results of the ANOVA (Table 6), the range of variation of this character was more or less constant in space and time. No significant differences between sampling dates, sites, and plots were found. No significant interactions were found. For this analysis, however, a significant effect of season (not directly testable) cannot be dismissed.

In culture, growth of *T. iolithus* was observed at 10, 15, and 20° C, both in long and short days and at low and high irradiances. In general, growth at 15 and 20° C was distinctly better than at 10° C. Algae grown at 16:8-h L:D at high irradiances were more densely branched than specimens grown in low irradiances or short days. At 25° C, the growth was very poor at low irradiances and virtually absent at high irradiances. In culture, vegetative growth and fragmentation were the only forms of reproduction observed for *T. iolithus*; no specialized reproductive structures were produced in any of the conditions tested, even in cultures up to 14 months old. However, other forms of reproduction seemed to occur very rarely in the field. Release of biflagellate swarmers was observed only in five samples, collected in November and December 2000 (Table 7). They were ovoid or pear shaped, 10–15 μm wide and 15–20 μm long, bigger than in other species of *Trentepohlia*; no evidence of sexual fusion was found. Unfortunately, due to the irregular and compact branching of the field specimens, it was not possible to observe the reproductive structures that released these swarmers.

Trentepohlia abietina: Field specimens consist of tufted plants with a limited prostrate system of rounded or swollen cells, 10–15 μm in diameter, from which erect axes, 400–750 μm tall, arise (Fig. 4A); the erect axes

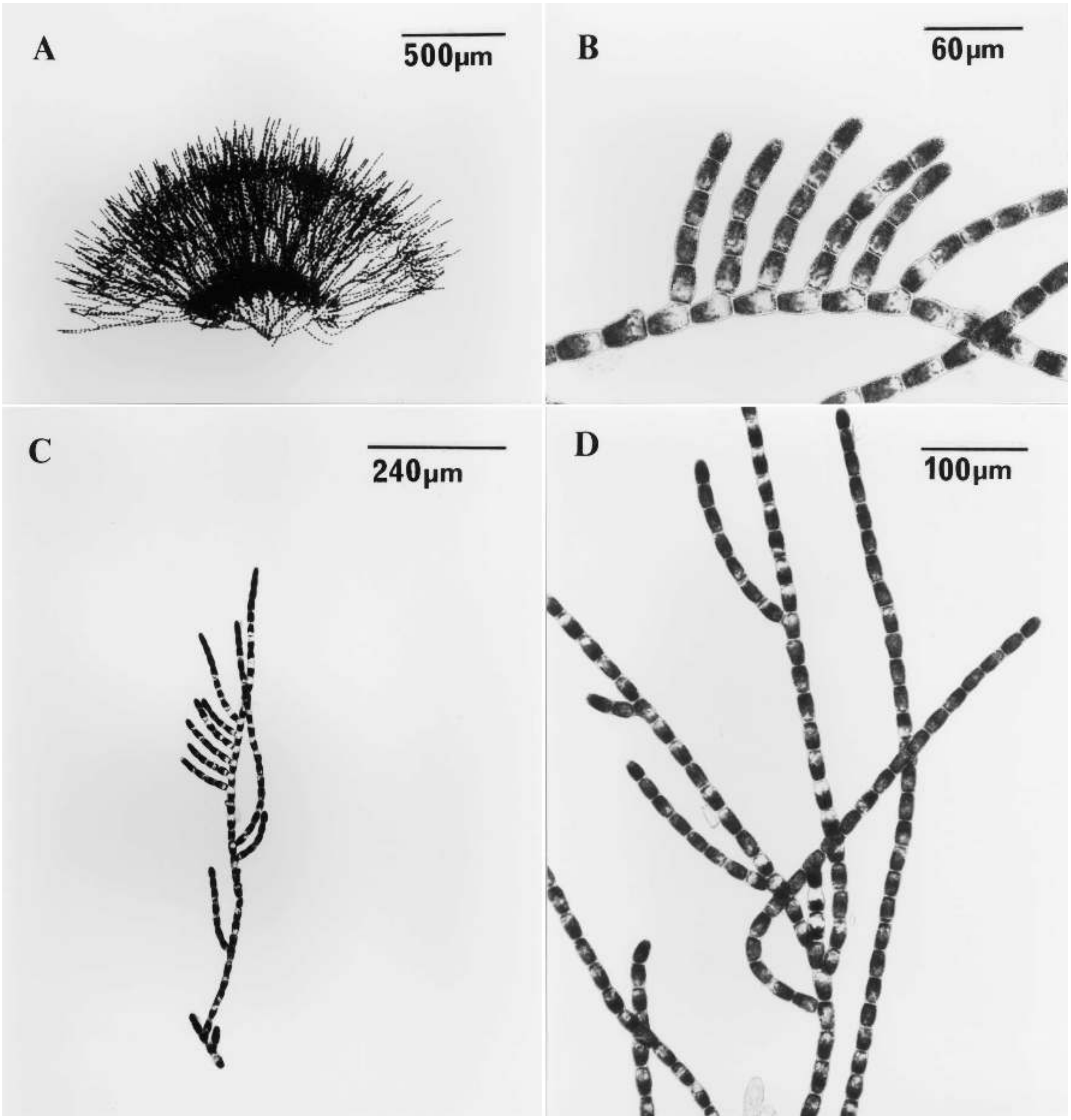

FIG. 3. *Trentepohlia iolithus.* (A) Habit of a cultured specimen. (B) Detail of an erect axis showing unilateral branching. (C) Habit of an erect axis. (D) Detail of branching of erect axes.

are poorly branched, with a few branches unilaterally or irregularly arranged (Fig. 4B). The cells of the erect parts of the thallus are cylindrical, 5–9 μm wide and 10–30 μm long. The ANOVA performed on the cell width data showed that this character varied significantly between different plots (i.e. on a spatial scale of few centimeters to some tens of meters), as shown by the significant effect of plot (Table 8); the effects of date and site were not significant. Although the effect of season was not directly testable, the F statistic calculated as ratio between the mean square (MS) of season and MS of date suggested that a significant effect of season probably occurred as well. No significant interactions were found.

Presumptive gametangia were the only reproductive structures recorded for *T. abietina*; they were globular or ovoid, 10–20 μm in diameter, formed laterally from cells of both erect and prostrate axes, or were produced at the apices of the erect axes (Fig. 4C). Such structures occurred in all samples examined throughout the period of the study; in late spring and early summer they appeared larger than in winter and were replete with mature swarmers (Fig. 4D). Release of biflagellate swarmers took place in the summer and autumn of 2000 (Table 7). When placed on a microscope slide, specimens collected in summer released within a few minutes numerous biflagellate swarmers, which actively swam for 15–20 min before settling on

TABLE 6. Results of the ANOVA performed on the width of cells of *Trentepohlia iolithus*. No significant differences at $P < 0.05$.

Source of variation	df	MS	F	F tested on
Season = Se	3	109.06		No test
Date (Se) = Da	8	3.84	0.63	Si × Da (Se)
Site = Si	1	0.49	0.08	Si × Da (Se)
Plot (Se × Da × Si) = Pl	48	7.07	0.95	Residual
Se × Si	3	22.78	3.72	Si × Da (Se)
Si × Da (Se)	8	6.13	0.87	Pl (Se × Da × Si)
Residual	1008	7.46		

Cochran's test: C = 0.0307, $P > 0.05$.

the slide. The swarmers were initially oval from squeezing through the narrow ostiole of the gametangium, were 4–5 μm × 6–8 μm, but later assumed a more globular shape. Release of swarmers became gradually more infrequent in autumn, and in the samples examined in November 2000 most gametangia were completely empty. In all samples collected in Galway, the swarmers behaved as asexual spores, settling without sexual fusion. However, in a collection from eastern Ireland at Glendalough, Co. Wicklow, fusion of some pairs of biflagellate swarmers was observed; unfortunately, in few minutes the zygotes disintegrated and it was therefore not possible to observe their subsequent development.

For *T. abietina* culture observations were more limited than for the other species. Vegetative portions were placed at 20° C, 16:8 h L:D, 25–40 μmol photons·$m^{-2}·s^{-1}$; after 15–20 days they started to show some signs of growth, issuing new lateral branches. The growth continued for several weeks, producing small densely tufted thalli with the same morphology as the field specimens. Although not as abundant as in field material, presumptive gametangia were produced after 14–21 days of growth. On two occasions, during changes of the medium, release of swarmers was observed. As for the field specimens, no sexual fusion took place; the swarmers settled on the bottom of the dishes and started to germinate after 3–4 weeks, producing short prostrate filaments. However, the viability of such specimens was low and their growth very slow, ceasing entirely when they consisted of 10–15 cells. No further monitoring of their growth was thus possible.

Trentepohlia aurea: Specimens collected in the field were formed by poorly branched erect axes, arising from a reduced system of prostrate axes (Fig. 5A). The cells were regularly cylindrical in the erect parts, swollen in the prostrate parts, 10–18 μm wide, and up to four times long as wide. The width of the erect filaments did not vary significantly seasonally (one-way ANOVA, $P > 0.05$). The habit of this species is basically similar to *T. abietina*, but the Student's *t*-tests showed that, for each sampling date, width of the cells was significantly larger in *T. aurea* than in *T. abietina* (always $P < 0.00001$). Furthermore, the erect axes were distinctly longer in *T. aurea* (800–1200 μm) than in *T. abietina* (400–750 μm). By contrast with field specimens, cultures of *T. aurea* showed a more tufted bushy habit and were more abundantly branched, with a spiral or irregular arrangement of the branches (Fig. 5B).

Even for *T. aurea*, reproduction took place by production of presumptive gametangia releasing biflagellate swarmers. These gametangia occurred laterally or apically both on the erect and prostrate axes; they were usually globular in field specimens, ovoid or flask-shaped in culture, and 25–30 μm wide (Fig. 5, C and D). In cultured specimens, the ostiole of mature gametangia often occurred at the end of a very long beak (10–15 μm), rarely observable in wild plants. In the field, presumptive gametangia were observed on several occasions, but they were never as abundant as in *T. abietina*. Release of swarmers took place only three times, in April, August, and September 2000 (Table 7). The swarmers were 8–10 μm long and 5–8 μm wide and were never observed to fuse sexually.

TABLE 7. Phenology of *Trentepohlia iolithus*, *T. abietina*, *T. aurea*, *T.* cf. *umbrina*, and *Printzina lagenifera*.

	Dec '99	Jan '00	Feb '00	Mar '00	Apr '00	May '00	Jun '00	Jul '00	Aug '00	Sep '00	Oct '00	Nov '00	Dec '00	Jan '01	Feb '01	Mar '01
Trentepohlia iolithus																
Dyke Road	--	---	---	---	---	---	---	---	---	---	---	---	+--	---	---	---
Millennium Bridge	nd	nd	nd	nd	---	---	---	---	---	---	---	++-	++-	---	---	---
Bothar na mBan	-	---	---	---	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Grattan Road	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Terryland			-	-	-	-	-	-	-	-	-	-	-	-	nd	nd
St. Anthony's, NUI			-	-	-	-	-	-	-	-	-	-	-	-	nd	nd
Trentepohlia abietina																
NUI, Football Ground	-	---	---	---	---	---	--+	+++	+++	+++	+++	+++	---	nd	nd	nd
NUI, Library	-	---	---	---	---	---	--+	+++	+++	++-	--+	+++	---	nd	nd	nd
NUI, Tennis Grounds	-	---	---	---	---	--+	---	--+	+++	+++	--+	--+	---	nd	nd	nd
Trentepohlia aurea																
Claddagh Quay	-	-	-	-	+	-	-	-	+	+	-	-	-	nd	nd	nd
Trentepohlia cf. *umbrina*																
NUI,Quadrangle	+	---	+--	+--	+--	---	+--	---	---	---	+--	---	+--	nd	nd	nd
St. Nicholas Church	+-	---	---	---	+--	---	+--	---	---	---	---	++-	+++	nd	nd	nd
Printzina lagenifera																
Boat Club	nd	nd	+	+	-	-	-	+	-	-	---	++-	+--	---	---	---

-, No release of biflagellate swarmers was observed; +, release of biflagellate swarmers was observed; nd, no data available. The number of symbols indicates the number of samples examined for each site and sampling date; see Table 1 for the exact dates.

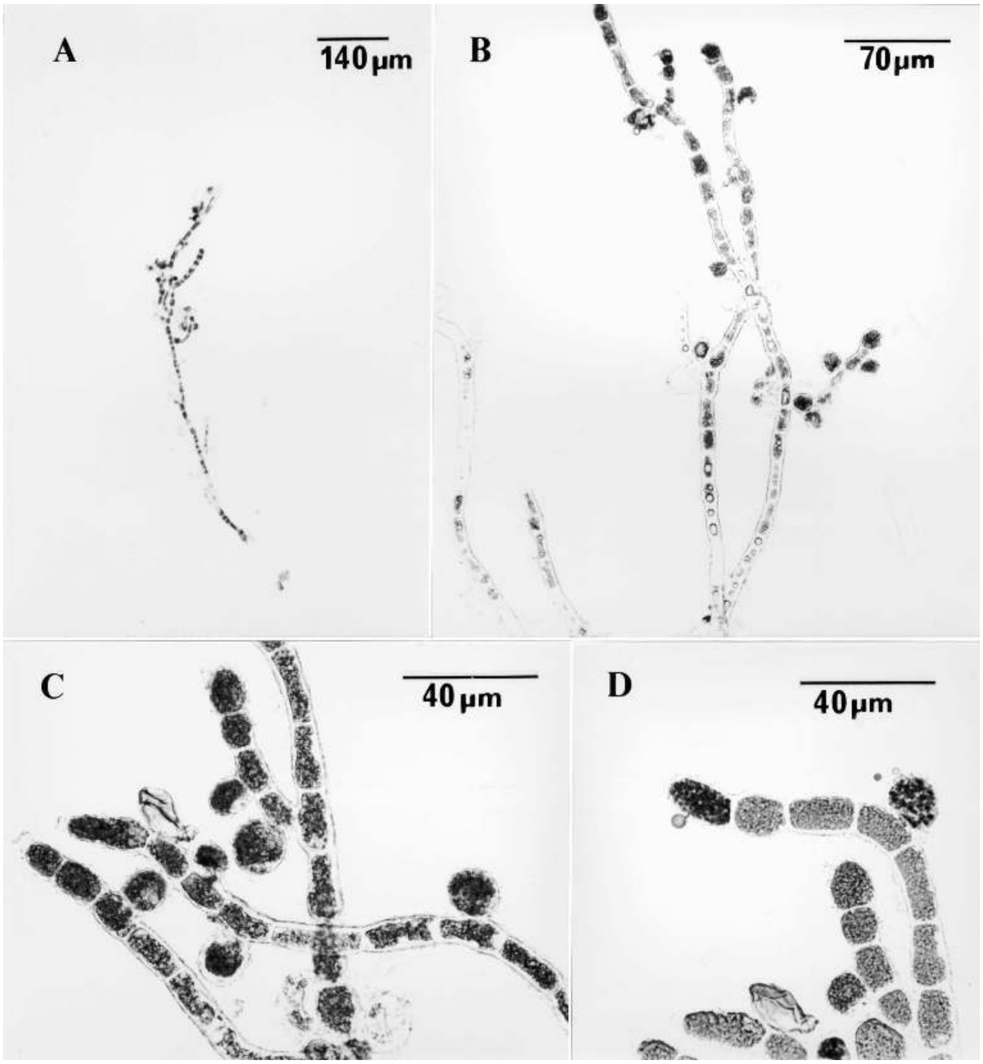

FIG. 4. *Trentepohlia abietina.* (A) Habit of an erect axis. (B) Detail of branching of an erect axis. (C) Erect axes with putative gametangia. (D) A putative gametangium close to maturation.

Cultures of *T. aurea* were initially started from excised tips of erect axes placed at 15 and 20° C, 16:8-h L:D, and low irradiances. After 7–10 days under these conditions new branches were produced, and after a further 14–21 days the algae grew into well-developed tufts, with an open and prolific branching. After 5 weeks (at 20° C) and 8 weeks (at 15° C), respectively, the algae began to produce presumptive gametangia. In the following weeks this phenomenon gradually extended to the whole thallus, and eventually every vegetative cell produced two to three gametangia-like structures. When mature, these released biflagellate swarmers that settled and germinated. After all the supposed gametangia were emptied, vigorous vegetative growth resumed, and in 3–4 more weeks the algae resumed the tufted habit shown previously.

TABLE 8. Results of the ANOVA performed on the width of cells of *Trentepohlia abietina.*

Source of variation	df	MS	F	F tested on
Season = Se	3	13.28		No test
Date (Se) = Da	8	1.77	2.12	Si × Da (Se)
Site = Si	2	0.72	0.87	Si × Da (Se)
Plot (Se × Da × Si) = Pl	72	1.07	*1.38**	Residual
Se × Si	6	0.91	1.09	Si × Da (Se)
Si × Da (Se)	16	0.84	0.78	Pl (Se × Da × Si)
Residual	1512	0.77		

Italic indicates significance: $^*P < 0.05$. Cochran's test: C = 0.0226, $P > 0.05$.

Sporelings from these cultures were moved to the culture regimes listed in Table 1. They grew at all temperatures tested (although better at 15 and 20° C than at 10 and 25° C), both in long and short days and in high and low irradiances. However, production of supposed gametangia and release of swarmers did not take place under any culture regime. Even in conditions that were clearly favorable to growth, no reproduction was observed in cultures up to 8 months old.

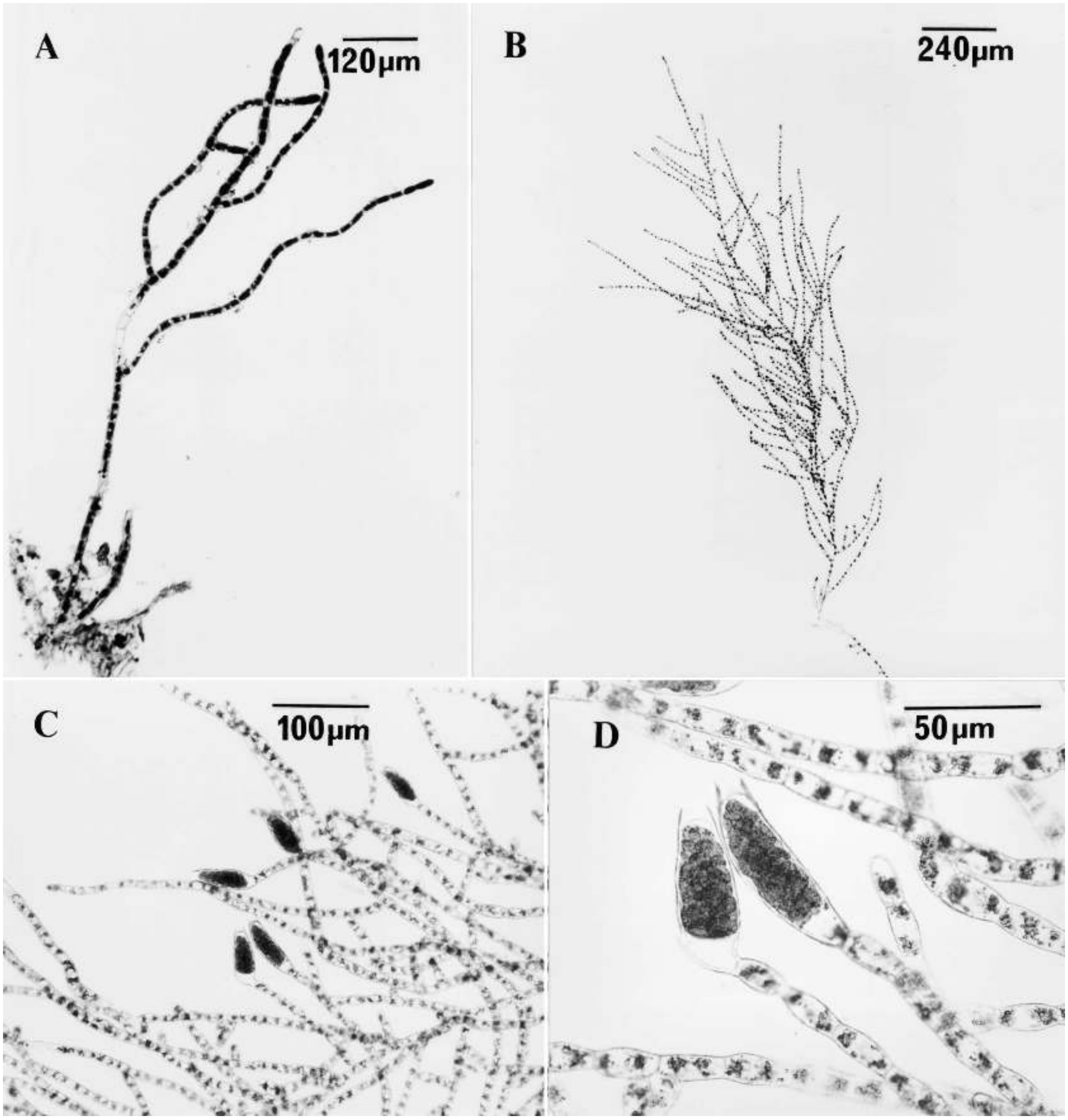

FIG. 5. *Trentepohlia aurea.* (A) Habit of an erect axis (field specimen). (B) Habit of an erect axis (cultured specimen). (C) Branches of a cultured specimen, bearing putative gametangia. (D) Detail of two putative gametangia.

Seemingly, the only way in which reproduction could be stimulated was to change the culture regime. Some specimens, initially cultured at 15° C, 16:8-h L:D, and high irradiances, started to produce presumptive gametangia (and subsequently released swarmers) 2–3 weeks after being moved to 8:16-h L:D or being transferred from liquid to agar culture.

Trentepohlia cf. *umbrina*: Individual specimens of this entity form small compact masses of short irregularly branched filaments, consisting of globular or swollen cells, 8–20 μm wide and one to two times as long as wide (Fig. 6A). In general, there was not a clear distinction between erect and prostrate axes. As shown by the results of the ANOVA, the width of the cells in wild specimens showed some variation on small spatial and temporal scales (Table 9). Significant differences between plots occurred; furthermore, width of cells varied significantly with sampling date at a site, as evidenced by the significant interaction site × date (Table 9). Date and site considered separately had no significant effects, and there is strong indication that the same was true for season.

In both the field and in culture, the only form of reproduction observed for this species was the production of presumptive gametangia that released biflagellate swarmers; however, the swarmers were never observed to fuse sexually. In the field, release of swarmers was observed in all seasons, but without a clear pattern. Frequently, release of swarmers took place in some samples but not in others collected at the same site on the same day (Table 7). In culture, the swarmers settled on the bottom of the dishes and after 3–4 weeks germination took place; the new algae produced the same kind of reproductive structures

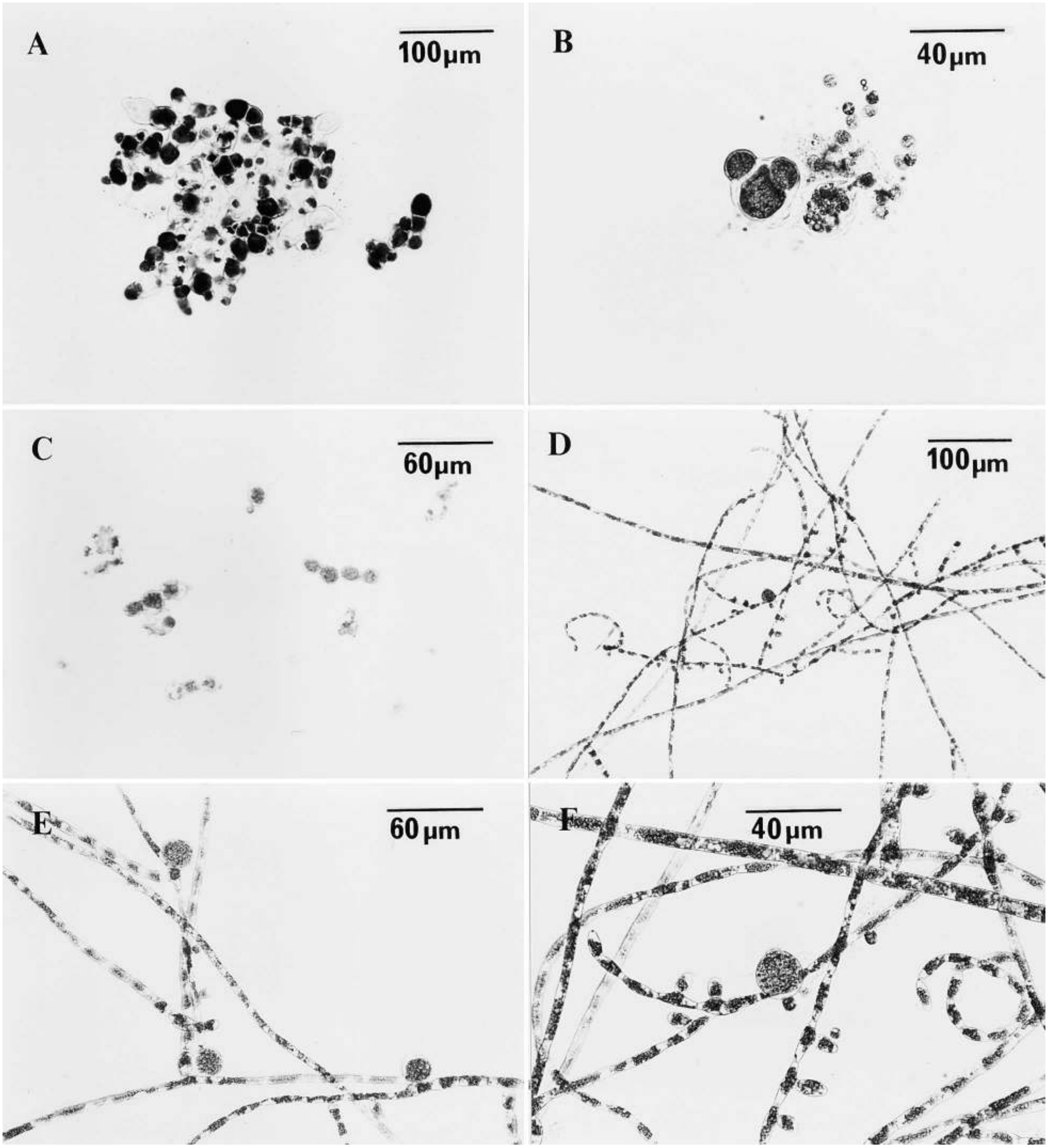

FIG. 6. *Trentepohlia* prox. *umbrina* and *Printzina lagenifera.* (A) *T.* prox. *umbrina*: habit of a cultured specimen. (B) *T.* prox. *umbrina*: a presumptive gametangium releasing gametes. (C) *P. lagenifera*: habit of some cells from field specimens. (D) *P. lagenifera*: habit of a cultured specimen. (E) *P. lagenifera*: three putative gametangia. (F) *P. lagenifera*: detail of a putative gametangium.

and released biflagellate swarmers. All cells of the thallus seemed to have the potential to develop into mature presumptive gametangia that were globular or flask shaped and 15–20 µm in diameter (Fig. 6B). The resulting swarmers were initially oval, 4-6 µm × 8–10 µm, later becoming swollen or globular.

In culture, growth and reproduction of *T.* cf. *umbrina* did not show a clear and predictable pattern. On agar, most algae grew regularly in all conditions tested, producing in some months conspicuous cushions several millimeters wide that usually bore presumptive sporangia. In liquid cultures, some thalli continued to grow for several months. However, most specimens gradually stopped growing, and at some stage, generally when 10–50 cells in extent, they produced presumptive gametangia that released biflagel-

TABLE 9. Results of the ANOVA performed on the width of cells of *Trentepohlia* cf. *umbrina*.

Source of variation	df	MS	F	F tested on
Season = Se	3	10.16		No test
Date (Se) = Da	8	14.22	1.17	Si × Da (Se)
Site = Si	1	3.39	0.28	Si × Da (Se)
Plot (Se × Da × Si) = Pl	48	0.04	*1.55**	Residual
Se × Si	3	11.02	0.90	Si × Da (Se)
Si × Da (Se)	8	12.20	*273.67***	Pl (Se × Da × Si)
Residual	1008	0.03		

Italic indicates significance: *$P < 0.05$, **$P < 0.01$. Transformation: Ln (X+1); Cochran's test: C = 0.0395, $P > 0.05$.

late swarmers. Thereafter, they gradually lost pigmentation and died. This situation was clearly not dependent on particular culture conditions, because at some stage it was observed for all the combinations of temperature, day length, and irradiance tested in culture.

Printzina lagenifera: Field specimens consist of small cushion-like masses, formed by irregularly branched deeply entangled filaments without a clear distinction between erect and prostrate parts. The cells were globular to barrel-shaped, rarely cylindrical, 6–12 μm wide, and one to three times as long as wide (Fig. 6C); width did not show significant seasonal variation (one-way ANOVA, $P > 0.05$). Field material of *P. lagenifera* is very similar to *T.* cf. *umbrina*, and for some specimens a specific attribution to either can be problematical. However, width of the cells was significantly larger in *T.* cf. *umbrina* (*t*-tests, $P < 0.05$ for each sampling date), and in culture the two species showed a completely different morphology. Young specimens of *P. lagenifera* arose as widely spreading creeping axes, that in subsequent stages produced elongated erect axes with a unilateral, alternate, or, less commonly, opposite branching. The cells were cylindrical or slightly swollen, usually thinner (3–8 μm wide), and comparatively much longer (up to 10 times as long as wide) than in field specimens (Fig. 6D).

Presumptive gametangia were frequently observed both in field and cultured specimens. They were terminal, lateral, or intercalary in position and occurred both on prostrate and erect parts of the thallus (Fig. 6E). Shape was variable from globular to flask-shaped, 10–25 μm in diameter, with a neck that was usually more pronounced in field specimens (Fig. 6F). Biflagellate swarmers were released; these were initially oval, 4–6 μm × 8–10 μm, and subsequently becoming globular. In the field, release of swarmers seemed to occur more frequently at the coldest times of the year but was also observed in summer (Table 7). Fusion of biflagellate swarmers was never observed; these cells settled on the bottom of the dishes, and in favorable conditions they germinated after few days. As in *T.* cf. *umbrina*, the new algae produced the same kind of reproductive structures and released biflagellate swarmers. At 15° C, 16:8-h L:D, and low and high irradiances, we could follow up to five consecutive generations produced in this way. Zoosporangia or other reproductive structures were never observed.

In culture, growth and reproduction of *P. lagenifera* were much more predictable than for *T.* cf. *umbrina.* Best growth was observed at 10 and 15° C at 16:8-h L:D; at 20° C growth was usually slower and the algae showed a denser branching and a more tufted bushy habit. At 25° C, this entity appeared unable to grow and survive in the long term; after a few weeks the algae were completely colorless. Reproduction by biflagellate swarmers was commonly observed in most conditions tested in culture. At 15° C, 16:8-h L:D, and high irradiances, specimens started from settled spores bore mature gametangia and released biflagellate swarmers in 30–40 days; however, sooner or later all specimens cultured at 10 and 15° C, either in 16-h or 8-h days and in high and low irradiances, produced gametangia and released swarmers (which germinated). The same was also observed at 20° C and 16:8-h L:D for cultures grown both in low irradiances and high irradiances; however, at this temperature, high irradiances seemed unfavorable to growth and reproduction, and production of supposed gametangia took much longer time than in other culture regimes.

DISCUSSION

Distribution and ecology. This is the first study to report in detail the diversity, distribution, and ecology of *Trentepohlia* and *Printzina* in temperate urban habitats. At least 12 species of *Trentepohlia* and one of *Printzina* have long been known to occur in Europe (Printz 1964), and some of them have been described as occurring on artificial substrata. Most records, however, refer to rural environments (Schlichting 1975, John 1988, Noguerol-Seoane and Rifón-Lastra 1997) and only a few report on urban occurrences (e.g., Rishbeth 1948). The present study is the first report of the general and widespread presence of *T. iolithus* in western Ireland. There, the distribution of this species is directly correlated with human presence; well-developed populations are observable only on old concrete and cement walls and basically any vertical surface built with these materials can be expected to be colonized by *T. iolithus* in a relatively short time. However, the impression of one of us (M. D. G.) is that in the center of Galway and other western Irish cities, *T. iolithus* is not as abundant as it was 10–15 years ago. The recent boom in the Irish economy and the improvement of the financial situation of many families, which has led to more frequent repainting or cleaning of houses, may explain this. This is also perhaps the main reason why *T. iolithus* and the other species of *Trentepohlia* are actually more common on public buildings (such as old walls or parapets of bridges) than private houses and in peripheral areas (such as NUI Galway and Terryland) than in the city center of Galway.

Ecologically, the species of *Trentepohlia* occurring in western Ireland differ in their preference for particular substrata, usually forming well-developed populations only on a surface type (cement for *T. aurea* and *T. iolithus*; tree bark for *T. abietina*; limestone for *T.* cf. *umbrina*). By contrast, *P. lagenifera* occupies a wider range of substrata. At the same time, however, the five species recorded in the present study seem to share several common ecological and physiological traits. Both in the field and in culture, Irish species of *Trentepohlia* and *Printzina* seem in general slow-growing algae, in agreement with previous reports (Howland 1929, Ho et al. 1983). By the use of ANOVA, we demonstrated that the abundance of wild populations of *T. iolithus* and *T.* cf. *umbrina* does not vary significantly on a temporal scale of seasons or months, and this pattern is consistent on a spatial scale of hundreds of meters or kilometers. These algae must therefore grow very slowly in nature, and the temporal scale on which the abundance of their populations varies significantly is years; this is in good agreement with casual observations by one of us (M. D. G.) in the previous 25 years. It is also interesting that for all five species the best conditions for growth and reproduction in culture are a combination of temperatures between 10 and 20° C and day lengths of 16 h rather than 8 h. Thermally, therefore, these algae seem perfectly adapted to the environmental conditions of western Ireland. This area is characterized by a very mild climate in comparison with other areas of northern Europe, with temperatures ranging between 0 and 20° C and relatively limited seasonal variation. Snow and ice are generally absent, and rainfall and humidity are relatively high throughout the year.

Reproductive patterns differ in some degree between the species. For populations of *T. abietina* occurring in Galway, release of swarmers is clearly associated with summer and early autumn. Our results suggest that high temperatures may be an important requirement for the maturation of gametangia, but further observations are necessary to confirm this. Conversely, for the other species, considerable variation in the maturation of gametangia and release of swarmers is apparent both temporally and spatially. For *T. aurea*, *T.* cf. *umbrina*, and *P. lagenifera*, release of swarmers occurred in several seasons and sampling dates, and it was frequently observed in some samples but not in others collected at the same site on the same day. This unpredictability is in remarkable contrast to the relatively regular reproductive behavior observed in culture. *Trentepohlia* cf. *umbrina* and *P. lagenifera* regularly released swarmers in almost all combinations of factors tested. The situation was different for *T. aurea*: algae grown in culture reproduced only after a drastic change of the culture conditions. However, constant and optimal conditions used for culture experiments do not of course reflect the variability of chemical and physical factors in subaerial environments. Moisture, in particular, which is not a limiting factor in culture, is of critical importance in the field and periods of dry weather can be limiting for reproduction even if other conditions are favorable. Brand (1902) and Howland (1929) suggest that in many temperate populations of *Trentepohlia*, swarming depends less on the season than on local conditions of temperature and moisture, with which we are in agreement. The unpredictability of reproduction in these algae is probably a reflection of the unpredictable nature of the subaerial environments in which they live. In the case of *T. iolithus*, it is difficult to make generalizations, because the role of flagellated cells in the propagation of the species is not clear; release of swarmers occurs very rarely in the field and was never observed in culture.

Life history. There is no evidence that an isomorphic alternance of diploid sporophytes and haploid gametophytes, regarded as the theoretical life history in this genus (e.g. Wille 1878, Oltmanns 1922, Printz 1964), normally takes place in western Irish populations. Biflagellate swarmers behaving as asexual spores and reproducing the same morphological phase are the only specialized form of reproduction that we observed both in the field and in culture. The lack of stalked zoosporangia in cultures of *Trentepohlia* is a well-known character (Graham and McBride 1975, Chapman 1984, Nakano and Handa 1984, Nakano and Ihda 1996), and these structures have been recorded in cultured strains only rarely (Uyenco 1965). They are, however, relatively common in field collections of most species (Hariot 1889, Printz 1939, 1964, Nakano and Handa 1984). It is therefore very peculiar that they are extremely rare in western Irish populations. So far, in the 385 Irish collections examined, only a single sample was found bearing zoosporangia, a specimen referred with doubt to *T.* cf. *umbrina*, collected from the front wall of the "Louis Renouf" Field Station at Lough Hyne, southwestern Ireland on 28 October 2000. Zoosporangia were never observed in any specimen from Galway. Similarly, sexual reproduction in Irish populations of *Trentepohlia* seems to occur rarely, if at all. Sexual fusion of some biflagellate swarmers was observed in a single collection (*T. abietina* from Glendalough, Co. Wicklow), but we could not follow the subsequent development and we do not feel able to confirm the occurrence of this phenomenon in Irish populations. In this regard, however, our observations are no different from those of other authors. Sexual fusion of gametes has been mentioned in many reports (Hariot 1889, Karsten 1891, Meyer 1909, Oltmanns 1922, Howland 1929, Fritsch 1935, Printz 1939, Smith 1955), but most of them refer to Wille's (1878) and Lagerheim's (1883) studies, which seem to be the only ones in which fusion of gametes was observed personally by the authors.

It is not clear why this phenomenon has been observed so rarely. Several authors have hypothesized that many species of *Trentepohlia* are heterothallic and sexual fusion can take place only when gametes of opposite sexes are present (Karsten 1891, Printz 1964, Thompson and Wujek 1997). Even so, it seems strange that so few such fusions have been observed in more than two centuries of studies on this genus. A confirmation of the occurrence of sexual reproduction in

Irish populations would indicate that life history patterns of *Trentepohlia* may be more variable than currently understood. Differences might occur not only at species level but also at the population level, possibly even on relatively small (e.g. regional) spatial scales. For such a generalization, however, further observations of sexual fusion, such as the ones made for *T. abietina* from Co. Wicklow, are necessary (especially considering that despite very numerous and frequent observations, no evidence of sexual reproduction is available for populations of the same species from Galway). It is also possible that sexual reproduction (and perhaps the production of stalked zoosporangia) takes place only if stimulated by particular environmental conditions, even though it seems difficult to indicate what combinations of environmental variables might be favorable. In this regard, our culture studies were not helpful.

Nevertheless, our observations show that for at least four of the five species occurring in Ireland (*T. abietina*, *T. aurea*, *T.* cf. *umbrina*, and *P. lagenifera*) biflagellate swarmers have the potential to germinate and reproduce new specimens without sexual fusion. It is likely, even if they are able to reproduce sexually, that the usual life history of western Irish populations of *Trentepohlia* and *Printzina* consists of a repetition of the same morphological phase, reproducing by biflagellate swarmers. Because the biflagellate swarmers seem to be produced by simple mitosis and there is no indication of events that might produce genetic variability, the genetic differentiation of these populations can thus be expected to be low.

Taxonomy. On the basis of descriptions and illustrations available in the literature (Kützing 1854, De Wildeman 1888, 1891, Hariot 1889, Printz 1921, 1939, 1964, Nakano and Handa 1984), the attribution of our specimens to *T. iolithus* and *P. lagenifera* seems to be well founded. The separation of *T. aurea* and *T. abietina* requires some discussion. For *T.* cf. *umbrina* we do not feel sufficiently confident at this time to propose more than a provisional determination.

The problem of the taxonomic relationship between *T. aurea* and *T. abietina* has been discussed by several authors (De Wildeman 1891, Hariot 1889, Printz 1921). The width of cells in the erect filaments, mostly 5–10 μm in *T. abietina* and more than 10 μm in *T. aurea*, is generally regarded as the main feature separating the two species (Hariot 1889, Printz 1921, 1939, 1964); differences in the texture of the filaments (more rigid in *T. aurea*) and in the arrangement of the sporangia (less regular in *T. abietina*) have been also reported (Hariot 1889). However, it has been commonly acknowledged that the distinction between *T. abietina* and reduced forms of *T. aurea* may be tenuous, and in the older literature these entities have sometimes been treated as conspecific (see, in particular, Printz 1921). For the two entities occurring in Galway, no transitional forms were observed, and our observations with both field and cultured specimens lead us to believe they should be regarded as different entities at the species level. Our ANOVA indicate that the width of the filaments of *T. abietina* can vary significantly either on a seasonal scale and a small spatial scale (a few centimeters to some tens of meters); in spite of this, the range of variation of this feature is definitely different in the two species: the Student's *t*-tests consistently showed significant differences for each sampling date, with the filaments of *T. aurea* being significantly larger. It should be pointed out that in western Ireland populations of these species do not produce zoosporangia and for *T. aurea* this does not allow a complete discrimination from similar species, such as *Trentepohlia uncinata* (Gobi) Hansgirg and *T. annulata* Brand (Printz 1939, 1964). However, *T. aurea* is generally reported as the most common species in Europe (Hariot 1889), and in every morphological feature our material is in complete agreement with previous descriptions (e.g. Hariot 1889, Printz 1939, 1964) and illustrations (e.g. Kützing 1854, pl. 93) of this species. Accordingly, it seems to us reasonable to refer our relatively broad form to *T. aurea* and the narrow one, which in Galway grows on bark of trees, to *T. abietina*. We acknowledge, however, that an examination of more Irish specimens of *T. aurea* is necessary, and other types of data, particularly molecular, would be very helpful in elucidating the taxonomic relationships of these entities.

Trentepohlia umbrina (Kützing) Bornet is also widespread in Europe, where it has been reported as one of the most common corticolous algae (Bornet 1873, Hariot 1889). The nomenclatural history of this species is not clear, as Kützing described with the same specific epithet two similar species, *Microcystis umbrina* (Kützing 1833, p. 373), subsequently moved to the genus *Protococcus* (Kützing 1843, p. 169), and *Chroolepus umbrinus* (Kützing 1843, p. 283), without stating clearly the difference between the two. In subsequent treatments, however, Kützing (1845, 1849) maintained the generic distinction for these entities, and Bornet (1873) proposed the transfer of *C. umbrinus* to the genus *Trentepohlia*. Recently, a transfer of this latter entity to the genus *Phycopeltis* was also proposed (Thompson and Wujek 1997, p. 89). After examination of some original specimens of *C. umbrinus* from Kützing's herbarium (Rjiksherbarium, Leiden), we are not able to confirm the attribution of the plants from western Ireland to this entity. Kützing's material is in good agreement with the plants from Galway in terms of general habit and globular or swollen shape of the cells, but it differs from the Irish form in the habitat (bark of trees instead of rock) and the thickness of the cell wall (often definitely thicker); in Kützing's specimens the size of the cells seems also slightly larger, with some cells that can be up to 25 μm in diameter. Unfortunately, we could not observe any reproductive structures in Kützing's material. Without an examination of fresh collections, it is impossible to assess how taxonomically relevant these differences are. In fact, thickness and diameter of the cells show some degree of variation both in Kützing's and Irish specimens, and it is very difficult to state the taxonomic relevance of the different substratum. For this kind of problem, a conclusive answer can probably be obtained only by a combination of morphological observations with molecular data. We believe that availability of molecular data from material collected in the type localities and referable with absolute certainty to the type forms is an essential requirement to elucidate the taxonomic position of many entities.

Some discussion is also needed in regard to the possible occurrence in Ireland of another species of *Trentepohlia* with globular cells, *Trentepohlia monilia* De Wildeman, for which Printz (1921, p. 23) erected a separate genus, *Physolinum*. This species, described by De Wildeman (1891, p.140) for corticolous plants from Chile, has been reported for several tropical (Printz 1921, Nielsen 1954, Flint 1959) and warm-temperate localities (Handa and Nakano 1988, Davis and Rands 1993, Noguerol-Seoane and Rifón-Lastra 1997). Khan (1951) recorded growth of this species in an agar plate inoculated with material scraped from a bark of tree in Wimbledon, England, and subsequently De Valéra and Ó Séaghdha (1968) used the same name for plants from Galway. These records, however, require critical reassessment. Khan (1951) reported that in his specimens the filaments were richly and irregularly branched, with branches arising with alternate or opposite arrangement; in his illustrations, the cells appear cylindrical or slightly swollen, up to five to six times as long as wide, and the putative gametangia are provided with a pronounced beak. In these features, the morphology of Khan's plants corresponds well to cultured specimens of *P. lagenifera* (Cribb 1968, Handa and Nakano 1988, Nakano and Handa 1984, Nakano and Ihda 1996, this study); at the same time, it appears in sharp contrast with other descriptions of *T. monilia*. Following De Wildeman (1888, 1891), *T. monilia* is characterized by poorly branched filaments, cells globular or lemon-shaped, one to two times as long as wide, with narrow connections to the adjoining cells and smooth cell walls with a conspicuous brownish color. For these reasons we believe that records of *T. monilia* for Britain and Ireland should be rejected and that records for other European localities should also be reassessed.

Finally, it seems appropriate to us to conclude our discussion with some methodological remarks. *Trentepohlia* has been long known to include highly variable and polymorphic organisms (Hariot 1889, Printz 1939, 1964), and our observations confirm that the morphology of some species (*T. aurea* and *P. lagenifera*, in particular) may undergo dramatic variations depending on the conditions of growth. However, to date, no studies have attempted to assess rigorously the temporal and spatial scales on which some features can show significant variation. We have done this with one of the morphological characters regarded as most important in the taxonomy of *Trentepohlia*, the width of the cells. The use of a multifactorial ANOVA showed that cell width can vary significantly at different spatial and temporal scales in different species (season and plot for *T. abietina*, possibly season for *T. iolithus*, plot and interaction site × date for *T.* cf. *umbrina*), which is a very important conclusion. When a detailed morphological study on species of *Trentepohlia* is carried out, it is necessary to replicate sampling at the appropriate temporal and spatial scales; limited sampling in space and time will probably lead to a poor understanding of the range of morphological variation, with multiple possibilities of misidentifications. In our opinion, there is little doubt that the examination of a small number of specimens in many studies of this genus is one of the main reasons of the confused taxonomic relationships of several entities.

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