European Journal of Phycology

ISSN: 0967-0262 (Print) 1469-4433 (Online) Journal homepage: https://www.tandfonline.com/loi/tejp20

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Stefan Kraan , Jan Rueness & Michael Guiry

To cite this article: Stefan Kraan , Jan Rueness & Michael Guiry (2001) Are North Atlantic *Alaria esculenta* and *A. grandifolia* (Alariaceae, Phaeophyceae) conspecific?, European Journal of Phycology, 36:1, 35-42, DOI: 10.1080/0967026011000173517 8

To link to this article: https://doi.org/10.1080/09670260110001735178

Published online: 03 Jun 2010.

Are North Atlantic *Alaria esculenta* and *A. grandifolia* (Alariaceae, Phaeophyceae) conspecific?

STEFAN KRAAN[1], JAN RUENESS[2] AND MICHAEL D. GUIRY[1]

[1] *Department of Botany, Martin Ryan Institute, National University of Ireland, Galway, Republic of Ireland*
[2] *Department of Biology, Section for Marine Botany, PO Box 1069, Blindern, 0316 Oslo, Norway*

(Received 15 December 1999; accepted 19 October 2000)

Alaria (Alariaceae, Phaeophyceae) is a common genus of kelps in the northern hemisphere. Fourteen species are currently recognized, of which three, *Alaria esculenta* (L.) Greville, *A. pylaii* (Bory de Saint-Vincent) Greville and *A. grandifolia* J. Agardh, are reported for the cold-temperate North Atlantic Ocean. *Alaria esculenta*, the type species described originally from the North Atlantic, exhibits a range of biogeographically correlated morphotypes suggesting the possibility of multiple species, subspecies or hybrids. In Ireland we discovered an *A. esculenta* population with unusually long stipes resembling the type specimen of *A. grandifolia* described from Spitsbergen by J. Agardh in 1872. These and other plants of *A. esculenta* from Ireland were compared with plants from Spitsbergen fitting the description of *A. grandifolia*, using sexual hybridization, relative growth rate measurements and DNA sequence comparisons. Complete interfertility was observed between the different isolates. Three nucleotide substitutions (0·37%) were found in the *rbc*L and RuBisCo spacer of *A. grandifolia*, and two in the partial 18S rRNA gene and ITS1 sequences. The relative growth rate at 10 °C of an Irish self-cross was significantly lower than those of all the other crosses. Comparison of RuBisCo spacer sequences of the Spitsbergen *A. grandifolia* and six *A. esculenta* isolates showed that *A. grandifolia* was identical to *A. esculenta* from Halifax, Canada. The partial 18S rRNA gene and ITS1 sequence of *A. grandifolia* was identical to that of *A. praelonga* from Japan and differed by a single substitution from *A. esculenta* from Scotland and by two nucleotide substitutions from the isolate from Ireland. The intraspecific differences in *A. esculenta*, together with the hybridization and morphometric results, suggest that *A. grandifolia* is to be considered conspecific with *A. esculenta*, and that *A. grandifolia* is most probably a large deep-water morphological variant, subspecies or ecotype of *A. esculenta*.

Key words: *Alaria esculenta*, *Alaria grandifolia*, Atlantic, crossing experiments, hybridization, relative growth rates, RGR, RuBisCo spacer

Introduction

Alaria Greville (Alariaceae, Phaeophyceae) is a common genus of kelps generally found intertidally along rocky shores subjected to strong wave exposure. The genus is widely distributed in the Northern Hemisphere with 11 species reported from the North Pacific and three species listed for the North Atlantic (Widdowson, 1971*a*). The three North Atlantic species, *Alaria esculenta* (L.) Greville, *A. pylaii* (Bory de Saint-Vincent) Greville and *A. grandifolia* J. Agardh, are currently distinguished on the basis of morphological criteria only. With the known extreme morphological plasticity in *Alaria* this has led to considerable taxonomic confusion (South, 1970*a*; Widdowson, 1971*b*; Feller-Demalsy & Demalsy, 1974). J. Agardh (1872: 26) suggested that *A. grandifolia* might represent older individuals of *A. esculenta*, because of the structure of the plants, or a form of *A. membranacea* J. Agardh in view of the shape of the blade. Yendo (1919) subsequently proposed merging *A. membranacea* with *A. pylaii*. Kjellman (1883) considered that *A. grandifolia* in the Agardh herbarium at Lund (L) were young *A. membranacea* plants, but that *A. membranacea* strongly resembled *A. pylaii*. Rosenvinge (1893) and Jónsson (1904) both concluded that *A. grandifolia* was a variety of *A. pylaii*. However, Yendo (1919) did not fully agree with this conclusion and suggested that the limits of the varieties of the forms of *A. pylaii* were not very clear.

Edelstein *et al.* (1967) reported a deep-water *Alaria* population at 12–19 m depths in Nova Scotia, Canada. They concluded that these plants were *A. grandifolia* after comparing several morphological features with *A. esculenta*. Separation of the two entities was primarily based on the differences in stipe and sporophyll length together with the overall length and width of the putative *A. grandifolia* plants. They further concluded that *A. grandifolia* is a deep-water species.

In several marine algal checklists *A. esculenta*, *A. pylaii* and *A. grandifolia* are reported as separate species (South, 1970*b*, 1983; Vinogradova, 1995), although South (1983) concluded that *A. grandifolia* was a large subtidal ecotype of *A. esculenta*, and Vinogradova (1995) stated that both *A. pylaii* and *A. grandifolia* are most probably conspecific with *A. esculenta*. Lüning (1990) considered *A. pylaii* and *A. grandifolia* to be northern forms of *A. esculenta*.

Sexual hybridization has been used in kelps to examine species relationships (Lüning *et al.*, 1978; Bolton *et al.*,

Correspondence to: S. Kraan. e-mail: stefan.kraan@seaweed.ie.

1983; tom Dieck, 1992). Care should be exercised in using hybridization results alone. For example, tom Dieck & de Oliveira (1993) found interfertility between different geographically separated species, which is inconsistent with normally expected reproductive isolation (Manhart & McCourt, 1992). Hybridization experiments in combination with molecular analysis can provide a more powerful tool for species delineation (Kraan & Guiry, 2000).

The plastid-encoded RuBisCo spacer separates the large and the small subunits of the ribulose-1,5-bisphosphate carboxylase/oxygenase genes. Molecular phylogenetic analysis of the RuBisCo spacer has been used to examine relationships among populations, isolates and species in red algae (Destombe & Douglas, 1991; Maggs *et al*., 1992; Goff *et al*., 1994), and in brown algae (Stache-Crain *et al*., 1997; Siemer *et al*., 1998). However, its utility has been questioned by Müller *et al*. (1998) for analysis of bangiophyte red algae. The relatively fast evolving internal transcribed spacers (ITS1 and ITS2) of the ribosomal cistron separate the 18S, 5·8S and 26S rDNA genes (Saunders & Druehl, 1993) and have been used to examine relationships among species in green algae (Blomster *et al*., 1998), red algae (Patwary *et al*., 1998) and brown algae (Stache-Crain *et al*., 1997).

At Corbet Head, Co. Down, Northern Ireland in April 1997, at a depth of 8 m we collected *Alaria* specimens with unusually long stipes resembling the type specimen of *A. grandifolia* from Spitsbergen as described by J. Agardh (1872: 26). To establish whether this population and *A. grandifolia* from its type locality represent an entity separate from *A. esculenta* we combined classical methods with molecular tools. We therefore compared plants of *A. esculenta* from Ireland with plants of *A. grandifolia* from Spitsbergen using morphometrics, hybridization of gametes, relative growth measurements and molecular analysis of the RuBisCo spacer and internal transcribed spacers (ITS1 and ITS2).

Materials and methods

Species, morphometrics and gametophyte cultures

Plants of *A. esculenta* were sampled in the low intertidal at Trácht Each on Inis Oírr, Aran Islands, Co. Galway (53° 4·1′ N; 09° 31·5′ W), Ireland, 22 April 1996. Specimens of *A. esculenta* resembling *A. grandifolia* were sampled by SCUBA diving at a depth of 8 m off Corbet Head, Co. Down (54° 14·0′ N; 5° 38·9′ W), Northern Ireland, 30 April 1998. Specimens of *A. grandifolia* were collected by SCUBA diving at 5–10 m depth at Sagaskjær, Isfjorden (78° 21·6′ N; 15° 62·5′ E), Spitsbergen, 12 May 1997 by J. Rueness. These specimens are preserved in the phycological herbarium, Martin Ryan Institute, Galway (GALW).

Morphometric characters (stipe length and width, lamina length and width and sporophyll length and width) were measured using a video camera with image analysis software, or with a clear ruler, and compared with the results of the study of Edelstein *et al*. (1967).

Male and female gametophyte cultures, isolated from zoospores, were obtained from Irish and Spitsbergen populations in accordance with South (1970*a*) and Nakahara & Nakamura (1973). The *A. grandifolia* gametophyte cultures from Spitsbergen were maintained in a vegetative state at 3 °C, 10:14 h L:D, 15 μmol m^{-2} s^{-1}, in plastic 20 ml dishes containing sterile enriched seawater; the *A. esculenta* gametophyte cultures were maintained in a vegetative state at 10 °C, 10:14 h L:D, 15 μmol m^{-2} s^{-1} in glass dishes containing sterile enriched seawater, as formulated by Guiry & Cunningham (1984), which was changed monthly.

Hybridization studies

Hybridization experiments were carried out in accordance with methods described for *Alaria* by Kraan & Guiry (2000). Hybrids were incubated at 10 °C, 16:8 h L:D, 20 μmol m^{-2} s^{-1}. The length and width of 20 successful hybrid sporophytes were measured with a dissecting microscope, and blade length, hapteron condition, general appearance and cultivation time were recorded. In addition, controls of 20 self-crosses and 20 isolated male and female gametophytes were incubated in parallel with each crossing experiment to identify possible parthenogenesis or apogamy (see Nakahara & Nakamura, 1973). After 2 months, viable healthy sporophytes with a length of 2 cm were transferred to 20 ml Universal containers on a table-shaker. Daylength conditions were changed to 14:10 h L:D, 30 μmol m^{-2} s^{-1} to mimic spring or autumn conditions and initiate fast growth of the developing sporophytes. The medium was changed weekly or fortnightly.

Growth experiments

Relative growth rates (RGR) were determined at 10 °C for length and width of the 20 self-crosses and hybrid sporophytes of the crosses between Spitsbergen *A. grandifolia* and *A. esculenta* from Inis Oírr only. Lengths were measured weekly using a clear ruler. RGR (mm day^{-1}) was calculated for each plant during the exponential growth phase using the following equation: $\mathrm{RGR} = \ln(l_2) - \ln(l_1)/t_2 - t_1$, where l_2 and l_1 are the lengths of the plant (mm) at days t_2 and t_1, respectively. The resulting data set was statistically examined by a one-way ANOVA to test the null hypothesis that the means of the relative growth rates in all crosses were not significantly different. Multiple comparisons were made *a posteriori* using the Tukey HSD test if the null hypothesis was rejected (Zar, 1996).

DNA extraction, sequencing and analysis

DNA was extracted from fresh laboratory-grown sporophytic material of *A. grandifolia*. DNA extraction, double-

Table 1. Source of RuBisCo spacer sequences analysed in this study

Species and origin	GenBank accession no.	Reference
Alaria esculenta Ireland	AF 109795	Kraan & Guiry (2000)
Alaria esculenta Scotland	AF 109796	Kraan & Guiry (2000)
Alaria esculenta Norway	AF 109797	Kraan & Guiry (2000)
Alaria esculenta Iceland	AF 109798	Kraan & Guiry (2000)
Alaria esculenta Canada	AF 109799	Kraan & Guiry (2000)
Alaria esculenta France	AF 109800	Kraan & Guiry (2000)
Alaria grandifolia Spitsbergen	AF 177175	This study
Alaria praelonga Japan	AF 109801	Kraan & Guiry (2000)

stranded PCR, primer sequences and annealing positions for the RuBisCo spacer were as previously described (Kraan & Guiry, 2000). Primer pair P1 and G4 was used to amplify the region in the ribosomal cistron from position 1542 in the 18S rRNA gene across the ITS1, the 5·8S gene, ITS2 and to position 42 in the 26S gene (Saunders & Druehl, 1993). Double-stranded amplifications were performed in a Hybaid Omn-E thermal cycler with an initial denaturation step of 95 °C for 3 min followed by 30 cycles with the following temperature profile: 1 min 95 °C, 2 min 54 °C and 2 min 72 °C and ending with one extension step of 72 °C for 5 min. Reaction volume, primer concentrations, dNTPs, reaction buffer, $MgCl_2$ and units of *Taq* were as previously described (Kraan & Guiry, 2000).

Double-stranded PCR products were sequenced using a LI-COR 4200 system (MWG Biotech UK Limited, Milton Keynes, UK), using a nested amplification technique. The new sequence for *A. grandifolia* was submitted to GenBank (Table 1). Sequences were initially aligned together with other sequences of *A. esculenta* isolates already available in GenBank (Table 1) and edited using GENEDOC (Nicholas & Nicholas, 1997). Final adjustments were made by eye. Another representative of the genus *Alaria*, *A. praelonga* Kjellman, collected by M. Masuda at Katsurakoi, Kushiro, Hokkaido, Japan, 17 May 1996, was chosen as an outgroup because of the close relationship of *A. praelonga* to the *Alaria* species in this study (Kraan & Guiry, 2000).

Results

Morphometric characters

The measured morphometric characters (stipe, lamina and sporophyll length and width) of *A. grandifolia* plants from Spitsbergen, *A. esculenta* from Corbet Head, Co. Down, Ireland, and *A. esculenta* from Inis Oírr, Aran Islands, Co. Galway, Ireland were compared with details provided by Edelstein *et al.* (1967) and are shown in Table 2. It is clear from Table 2 that none of the morphometric characters distinguishes between *A. esculenta* and *A. grandifolia* except for sporophyll length, which is longer for *A. grandifolia* in all cases. Besides, Table 2 shows that the supposedly *A. grandifolia* is only found in deeper water (> 3·5 m), contrary to *A. esculenta* which is also found just below the tidal range.

Hybridization experiments

The results of the hybridization experiments between Irish *A. esculenta* and Spitsbergen *A. grandifolia* are shown in Table 3. All plants crossed successfully only at 10 °C. The Spitsbergen *A. grandifolia* self-cross also produced progeny at 3 °C whereas the other crosses did not,

Table 2. Measurements of stipe, lamina and sporophyll length and width (in cm), of *Alaria grandifolia* from Spitsbergen, *A. esculenta* from Corbet Head, Co. Down, Ireland and *A. esculenta* from Inis Oírr, Aran Islands, Co. Galway, Ireland compared with other studies

	This study			Kjellman[a]		Taylor[a]		Edelstein *et al.*[a]	
	Alaria grandifolia Spitsbergen	*Alaria esculenta* Corbet Head	*Alaria esculenta* Inis Oírr	*Alaria grandifolia* Spitsbergen	*Alaria esculenta* Spitsbergen	*Alaria grandifolia* East coast North America	*Alaria esculenta* East coast North America	*Alaria grandifolia* Nova Scotia	*Alaria esculenta* Nova Scotia
Stipe									
Length	> 120[b]	> 100	30–62	125[b]	15[b]	60–150	10–30	50–70	10–30
Width	4·0[b]	0·5–1·0	0·5–1·0	2·0[b]	1·0[b]	> 1·5	0·5–1·2	1·5–2·0	0·6–1·0
Lamina									
Length	270[b]	200–250	353[b]	> 100[b]	150[b]	100–200	–	300	100–250
Width	45[b]	25[b]	17[b]	30[b]	12[b]	20–45	4–25	50	To 25
Sporophyll									
Length	42[b]	5–15	4–14	60[b]	12[b]	20–40	7–25	30–40	To 30
Width	5[b]	2–4	2·0–4·5	7[b]	1·0[b]	< 3·5	1·2–2·5	2·0–4·0	2·0–4·0
Habitat	5–10 m	2–8 m	Low water mark	4–27 m	Below tidal range	3·5–18 m	Below tidal range	12–19 m	To 8 m

[a]Data (including Kjellman's and Taylor's) taken from Edelstein *et al.* (1967).
[b]The largest specimen measured.

Table 3. Hybridization results between *A. esculenta* from Ireland and *A. grandifolia* from Spitsbergen over a 4-month cultivation period ($n = 20$)

	Male gametophyte		
Female gametophyte	*A. esculenta* Corbet Head	*A. esculenta* Inis Oírr	*A. grandifolia* Spitsbergen
A. esculenta Corbet Head	100% Slender NM	100% Slender L = 72·6 ± 37·9 W = 5·16 ± 1·94	100% Broad NM
A. esculenta Inis Oírr	100% Slender L = 61·7 ± 16·4 W = 6·09 ± 1·46	100% Slender L = 145 ± 72·7 W = 4·9 ± 3·3	100% Broad L = 35·9 ± 7·9 W = 3·83 ± 1·35
A. grandifolia Spitsbergen	100% Broad NM	100% Broad L = 30·3 ± 1·34 W = 3·76 ± 0·9	100% Broad L = 23·8 ± 1·76 W = 4·05 ± 0·92

Morphology of the progeny of the crosses is shown as percentage of individuals that formed broad or slender blades. Sizes of progeny are given as mean length and width (in mm) with standard deviation.
Broad, sporophytes grew fast in width and slower in length producing triangular-shaped sporophytes, broader at the base and thinner towards the top. Slender, sporophytes grew fast in length and slower in width producing ribbon-like sporophytes. NM, not measured.

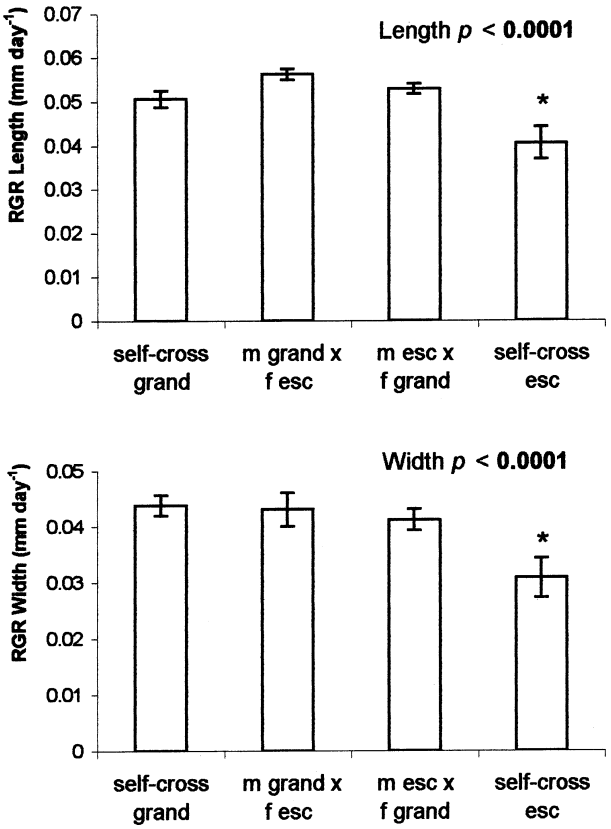

Fig. 1. Mean relative growth rates (mm day^{-1}) for length and width (with 95% confidence limits, $n = 20$) of the progeny of crosses between *A. grandifolia* from Spitsbergen and *A. esculenta* from Inis Oírr, Aran Islands, Co. Galway, Ireland, grown at 10 °C. f = female gametophyte, m = male gametophyte, esc = *A. esculenta*, grand = *A. grandifolia*, * = significant difference.

although there was no noticeable growth after fertilization in the Spitsbergen *A. grandifolia* self-cross at 3 °C. The progeny of all the crosses that involved Spitsbergen *A. grandifolia* gametophytes had a broad morphology, whereas all the crosses between *A. esculenta* from Ireland formed slender progeny. Sporophytes derived parthenogenetically in the female parallel cultures were easily identified because of their small, round, clump-like appearance and the lack of a hapteron.

Relative growth rates

The relative growth rates (RGR) for length and width of the hybrid crosses between *A. esculenta* from Inis Oírr and Spitsbergen *A. grandifolia* are shown in Fig. 1. The null hypothesis for similarity of RGR for length and width amongst the crosses was rejected (length: $p < 0{\cdot}0001$; width: $p < 0{\cdot}0001$). The *A. esculenta* self-cross showed a significantly slower RGR for length and width compared with the other crosses, which did not differ significantly.

Alignment of the RuBisCo spacer and ITS regions

The RuBisCo spacer of *A. grandifolia* from Spitsbergen was sequenced entirely on both strands. The sequence was 815 bases, of which 362 represented the 3′ end of the *rbc*L gene, 283 the spacer and 170 the 5′ end of the *rbc*S gene. A pairwise sequence comparison between Spitsbergen *A. grandifolia* and Irish *A. esculenta* revealed three nucleotide substitutions (Table 4). Table 4 shows that the sequence of *A. grandifolia* is identical to that of *A. esculenta* from Canada. Alignment of the sequences with five other *A. esculenta* isolates and *A. praelonga* resulted in a data set 815 nucleotides in length. There were no insertions or deletions. *A. praelonga* is clearly separated from *A. esculenta* and *A. grandifolia* by eight apomorphic positions. *A. esculenta* from Norway and from France both have a single autapomorphic substitution. *A. esculenta* from Iceland has two autapomorphies, both transitions. *A. esculenta* from Canada and *A. grandifolia* from Spitsbergen both share three synapomorphies, all transversions, at positions 150

Table 4. Positions at which the sequences varied among the six isolates of *Alaria esculenta, A. grandifolia* and *A. praelonga*

	Sequence position														
	*rbc*L							RuBisCo spacer						*rbc*S	
	76	78	104	126	150	359	360	398	420	437	475	501	509	733	755
A. esculenta Ireland	C	T	T	T	C	A	A	C	A	A	C	C	T	A	A
A. esculenta Scotland	C	T	T	T	C	A	A	C	A	A	C	C	T	A	A
A. esculenta Norway	C	T	T	**C**	C	A	A	C	A	A	C	C	T	A	A
A. esculenta Iceland	C	T	T	T	C	A	A	C	A	A	C	C	T	**G**	**G**
A. esculenta Canada	C	T	T	T	**G**	A	**C**	C	A	A	C	**G**	T	A	A
A. esculenta France	C	T	**A**	T	C	A	A	C	A	A	C	C	T	A	A
A. grandifolia Spitsbergen	C	T	T	T	**G**	A	**C**	C	A	A	C	**G**	T	A	A
A. praelonga Japan	**A**	**C**	T	T	C	**C**	A	**A**	**G**	**C**	**T**	C	**A**	A	A

Apomorphic positions are printed in **bold**. *rbc*L, large subunit; *rbc*S, small subunit.

and 360, in *rbc*L, and at position 501, in the RuBisCo spacer (Table 4). Of the aligned data set 15 sites are variable (1·84%), of which three are phylogenetically informative (0·37%).

We were not able to succesfully sequence the complete rDNA cistron. Due to a long mononucleotide C repeat in the ITS2 template slippage occurs during PCR. This leads to unreadable sequence ladders at the beginning and a high error rate. However, we were able to sequence the first 411 base pairs (bp) of *A. esculenta* from Ireland and Scotland, *A. praelonga* and *A. grandifolia*, of which 285 bp represented the 3′ end of the 18S gene and 126 bp the ITS1 (data not shown). Pairwise comparisons revealed one synapomorphic substitution (transition, T into C) in *A. grandifolia* and *A. praelonga* at position 132 in the 3′ end of the 18S gene, and one autapomorphic substitution (transition, A into G) in *A. esculenta* from Ireland at position 406, in ITS1.

Discussion

Comparison of morphological characters (Table 2) showed that there is substantial variation in length and width in the plants sampled and in the literature reports. These characters would not therefore appear to be particularly suitable for the separation of *A. grandifolia* from *A. esculenta*. Similar observations have been made for other kelps by Mathieson *et al.* (1981), who concluded that due to extreme phenotypic plasticity many entities that formerly seemed quite distinct are now known to overlap in taxonomically critical features. The largest plants examined were those of *A. esculenta*, not *A. grandifolia* as would have been expected from literature descriptions. Table 2 shows that only sporophyll length seems to be a valid character in the separation of the two species. In terms of habitat *A. grandifolia* plants are generally found in deeper water, in contrast to *A. esculenta*, which is usually present just below low water. This is in agreement with Edelstein *et al.* (1967) who indicated that *A. grandifolia* is a deep-water species; however, we do not agree that stipe length and thickness, a much wider lamina and larger sporophylls are sufficient characters to distinguish *A. grandifolia* from *A. esculenta* (Table 2). Judging from the morphological characters we agree with South (1983) who considered *A. grandifolia* to be a large, subtidal ecotype of *A. esculenta*.

Further evidence to support this latter conclusion is given by our hybridization results (Table 3). We observed complete interfertility among all combinations of crosses between *A. grandifolia* and *A. esculenta*, indicating that the two entities are conspecific. A recent cultivation project with several strains of *A. esculenta* confirmed these results, showing that hybrids of *A. esculenta* and *A. grandifolia* became fertile when cultured in the sea (Kraan & Guiry, unpublished observations). However, many kelp species are known to hybridize interspecifically (tom Dieck & de Oliveira, 1993) or even intergenerically (Lewis & Neushul, 1995). Liptack & Druehl (2000) showed an interfamilial cross between *Alaria marginata* Postels & Ruprecht and *Lessoniopsis littoralis* (Tilden) Reinke, supported by molecular evidence. Fertilization in *Alaria* species is facilitated by the sex pheromone lamoxirene, which can be found in all species of the Alariaceae, Laminariaceae and Lessionaceae (Müller *et al.*, 1985). Yarish *et al.* (1990) postulated that the sharing of a common sex pheromone is sufficient reason to expect interfertility but incompatibility has been shown between several species of the genus *Alaria* (Kraan & Guiry, 2000).

Remarkably, all cross combinations involving *A. grandifolia* produced broad sporophytes. This finding is in contrast with the *A. esculenta* crosses, which were all slender. Differences in morphology of the young sporophytes may be due to an ecotypic adaptation of *A. grandifolia* sporophytes to deep water and hence less wave action. Differences in morphology due to environmental conditions, like currents and wave action, have often been observed in other kelps but cannot be attributed to genotypic or phenotypic differences at present (Widdowson, 1971*a*; Chapman, 1974; Mathieson *et al.*, 1981).

The RGR of the progeny of the various crosses grown under laboratory conditions do not differ significantly

except for the *A. esculenta* self-cross, which grew significantly slower at 10 °C. Normally, *Alaria grandifolia* from Spitsbergen is subjected to temperatures of −2 °C (winter temperature) to 6 °C (summer temperature) and a polar light-regime (U.S. Navy, 1981). Placed under favourable conditions in the laboratory, the young *A. grandifolia* sporophytes might jump-start and grow at a faster rate at 10 °C than under normal conditions in nature, compared with *A. esculenta* sporophytes for which 10 °C represents winter. Makarov *et al*. (1999) concluded that temperature seems not to be a factor in determining seasonal changes in growth. They showed in *A. esculenta* from the Barents Sea, with a negative RGR during the polar night, that transplantation to a 12:12 h L:D light regime in the laboratory resulted in a faster RGR similar to that observed in spring, suggesting that *A. esculenta* is in a forced rest during the polar night. This scenario might apply to the progeny of the crosses involving *A. grandifolia* gametophytes from Spitsbergen, which are adapted to low temperatures and a polar light regime. It is possible that, after exposure to favourable conditions in the laboratory, progeny of the crosses involving *A. grandifolia* gametophytes showed a faster RGR than normally expected, compared with the *A. esculenta* self-cross.

A sequence divergence of 0·37% was found in the RuBisCo spacer between *A. esculenta* from Ireland and *A. grandifolia* from Spitsbergen. Similar amounts were found among sequences of all other *A. esculenta* isolates. Analysis showed that the Spitsbergen *A. grandifolia* RuBisCo sequence was identical to the sequence of the *A. esculenta* isolate from Halifax, Canada, both sharing three synapomorphies compared with the sequence of *A. esculenta* from Ireland. Due to the low information content of the alignment we cannot conclude with certainty a phylogenetic relationship between the *Alaria* species under investigation; however, it was clear that *A. praelonga* was separated from all other *A. esculenta* isolates and *A. grandifolia* by eight apomorphic substitutions. The three synapomorphies encountered in the sequences of *A. grandifolia* and *A. esculenta* of Canada compared with the other isolates seems small; however, due to the highly conserved RuBisCo spacer and the recent radiation of the Laminariales (Yoon & Boo, 1999) they appear to be phylogenetically important. Similar numbers of synapomorphies in RuBisCo spacer sequences within populations have been found in the supposedly primitive brown alga *Ectocarpus siliculosus* (Dillwyn) Lyngbye (Stache-Crain *et al*., 1997). However, these authors concluded that the RuBisCo spacer is too conservative to resolve differences between and within populations, which is in agreement with Yoon & Boo (1999) and Kraan & Guiry (2000).

In the red algal genus *Porphyra* C. Agardh, the separation of four different *Porphyra* species is based, besides gross morphology, on one and three nucleotide substitutions only in the spacer region (Brodie *et al*., 1998). Yoon & Boo (1999) showed a difference of two nucleotide substitutions between *Undaria pinnatifida* (Harvey) Suringar, *U. undarioides* (Yendo) Okamura and *U. peterseniana* (Kjellmann) Okamura, which are closely related species to the genus *Alaria*. They stated that the latter two are difficult to distinguish and that *U. undarioides* might be an ancient hybrid of *U. pinnatifida* and *U. peterseniana* judging from the ability to hybridize and their biogeographical overlap. The *A. grandifolia* isolate in this study differs by three to five nucleotide substitutions from the other *A. esculenta* isolates, which suggests that they are different species. However, the morphological differences and hybridization results indicate conspecificity. Besides, a similar amount of sequence divergence was found amongst other *A. esculenta* isolates (Kraan & Guiry, 2000). Furthermore, the *A. grandifolia* sequence is identical to that of *A. esculenta* from Halifax, Canada. This might suggest that *A. esculenta* from Canada is wrongly identified and is in fact *A. grandifolia*.

Our *A. esculenta* sequence from Sandy Cove, Halifax, Nova Scotia, Canada differs by three substitutions from *A. esculenta* from Ketch Harbour, Nova Scotia, Canada (Yoon & Boo, 1999) at positions 261 and 262 in the *rbc*L, and position 408 in the RuBisCo spacer. This again suggests that our *A. esculenta* from Canada might in fact be *A. grandifolia*. However, the Canadian *A. esculenta* sequence in the study by Yoon & Boo (1999) differs by seven substitutions from our sequence of *A. esculenta* from Ireland, perhaps suggesting that they are different species. In conclusion, it seems that there is a lot of variation in *A. esculenta* RuBisCo spacer sequences. Similarly, in *A. praelonga*, there are 16 nucleotide substitutions and a 10 base pair insertion at position 394 of the RuBisCo spacer that differ between accessions from Akkeshi, Hokkaido, Japan (Yoon & Boo, 1999) and *A. praelonga* from Katsurakoi, Kushiro, Hokkaido, Japan (present study).

Siemer *et al*. (1998) recommended using the RuBisCo operon in parallel with the nuclear ribosomal cistron for further phylogenetic studies in brown algae. Unfortunately we only managed to sequence a part of the rDNA cistron. However, sequence comparison between *A. grandifolia*, *A. esculenta* and *A. praelonga* showed a single nucleotide substitution at position 406 in the ITS1 of *A. esculenta* from Ireland. *A. grandifolia* and *A. praelonga* shared one synapomorphy at position 132 in the 18S gene. Similar single nucleotide substitutions in the 18S genes have been found in non-digitate *Laminaria* species (Yotsukura *et al*., 1999).

In the genus *Alaria* intraspecific differences are larger than interspecific differences, which is in agreement with Kraan & Guiry (2000). This discrepancy shows that phylogenetic studies based on only one specimen might have a completely different outcome depending on where the specimen is sampled from. Besides, despite the very recent radiation in the Laminariales (Stam *et al*., 1988), it shows that sufficient differences have accumulated within certain species of *Alaria* for the RuBisCo spacer to be adequate for delineation at the population level in some *Alaria* species.

The intraspecific differences in *A. esculenta*, together with the hybridization and morphometric results, suggest

that *A. grandifolia* is to be considered conspecific with *A. esculenta*, and that *A. grandifolia* is most probably a large deep-water ecotype of *A. esculenta* as suggested by South (1983).

The taxonomic position of *A. pylaii* remains unclear. It has been reported from the east coast of Canada (Widdowson, 1971*b*), east Greenland (Lund, 1959; Pedersen, 1976), Iceland (Caram & Jónsson, 1972), Norway (Rueness, 1997) and the Faroes (Irvine, 1982, with doubt). The geographical distribution of *A. pylaii* in the North Atlantic falls within the distribution area of *A. esculenta* (Widdowson, 1971*b*). Conspecificity of *A. pylaii* with *A. esculenta* as well as *A. grandifolia* has been suggested (J. Agardh, 1872; Kjellman, 1883; Rosenvinge, 1893; Jónsson, 1904; Yendo, 1919). Unfortunately, we have not been able to obtain specimens of *A. pylaii*; however, we strongly suspect that *A. pylaii* is another form, variety or ecotype of *A. esculenta* as proposed by Lüning (1990) and Vinogradova (1995).

Acknowledgements

We are grateful to Dr F. Rindi for translating J. Agardh's paper and to Professor M. Masuda for providing the *Alaria praelonga* plants. This study was supported under contract IR.95.MR.011 of the Marine Research Measure (Operational Programme for Fisheries 1994–1999), in co-operation with the Irish Seaweed Industry Organisation (ISIO) and the National University of Ireland, Galway.

References

Agardh, J.G. (1872). Bidrag till kännedomen af Grönlands Laminarieer och Fucaceer. *Bihang K. Svensk. Vet.-Akad. Handl.*, **10**: 1–31.

Blomster, J., Maggs, C.A. & Stanhope, M.J. (1998). Molecular and morphological analysis of *Enteromorpha intestinalis* and *E. compressa* (Chlorophyta) in the British Isles. *J. Phycol.*, **34**: 319–340.

Bolton, J.J., Germann, I. & Lüning, K. (1983). Hybridization between Atlantic and Pacific representatives of the Simplices section of *Laminaria* (Phaeophyta). *Phycologia*, **22**: 133–140.

Brodie, J., Hayes, P. K., Barker, G.L., Irvine, L.M. & Bartsch, I. (1998). A reappraisal of *Porphyra* and *Bangia* (Bangiophycidae, Rhodophyta) in the northeast Atlantic based on the *rbc*L-*rbc*S intergenic spacer. *J. Phycol.*, **34**: 1069–1074.

Caram, B. & Jønsson, S. (1972). Nouvel inventaire des algues marines de l'Islande. *Acta Bot. Island.*, **1**: 5–31.

Chapman, A.R.O. (1974). The genetic basis of morphological differentiation in some *Laminaria* populations. *Mar. Biol., Berl.*, **24**: 85–91.

Destombe, C. & Douglas, S.E. (1991). RuBisCo spacer sequence divergence in the Rhodophyta alga *Gracilaria verrucosa* and closely related species. *Curr. Genet.*, **19**: 395–398.

Edelstein, T., Craigie, J.S. & McLachlan, J. (1967). *Alaria grandifolia* J. Agardh from Nova Scotia. *J. Phycol.*, **3**: 3–6.

Feller-Demalsy, M.-J. & Demalsy, P. (1974). Recherches sur les *Alaria* (Phaeophyceae) de l'est Canadian. I. Nombres chromosomiques et identité des *Alaria* de l'estuaire du St-Laurent. *Can. J. Bot.*, **52**: 691–694.

Goff, L.J., Moon, D.A. & Coleman, A.W. (1994). Molecular delineation of species and species relationships in the red algal agrophytes *Gracilariopsis* and *Gracilaria* (Gracilariales). *J. Phycol.*, **30**: 521–537.

Guiry, M.D. & Cunningham, E.M. (1984). Photoperiodic responses in the reproduction of north-eastern Atlantic *Gigartina acicularis* (Rhodophyta: Gigartinales). *Phycologia*, **23**: 357–367.

Irvine, D.E.G. (1982). Seaweeds of the Faroes. 1: The flora. *Bull. Br. Mus. Nat. Hist. (Bot.)*, **10**: 109–131.

Jónsson, H. (1904). The marine algae of East Greenland. *Meddel. Grønl.*, **30**(1): 1–73.

Kjellman, F.K. (1883). The algae of the Arctic sea. *K. Svensk. Vet.-Akad. Handl.*, **20**: 1–350.

Kraan, S. & Guiry, M.D. (2000). Sexual hybridization experiments and phylogenetic relationships as inferred from RuBisCo spacer sequences in the genus *Alaria* (Alariaceae, Phaeophyceae). *J. Phycol.*, **35**: 190–198.

Lewis, R.J. & Neushul, M. (1995). Intergeneric hybridization among five genera of the family Lessoniaceae (Phaeophyceae) and evidence for polyploidy in a fertile *Pelagophycus* × *Macrocystis* hybrid. *J. Phycol.*, **31**: 1012–1017.

Liptack, M.K. & Druehl, L.D. (2000). Molecular evidence for an interfamilial Laminarialean cross. *Eur. J. Phycol.*, **35**: 135–142.

Lund, S. (1959). The marine algae of East Greenland. I. Taxonomic part. *Meddel. Grønl.*, **156**(1): 1–247.

Lüning, K. (1990). *Seaweeds: Their Environment, Biogeography and Ecophysiology*. (Yarish, C. & Kirkman, H., editors). Wiley, New York.

Lüning, K., Chapman, A.R.O. & Mann, K.H. (1978). Crossing experiments in the non-digitate complex of *Laminaria* from both sides of the Atlantic. *Phycologia*, **17**: 293–298.

Maggs, C.A., Douglas, S.E., Fenety, J. & Bird, C.J. (1992). A molecular and morphological analysis of the *Gymnogrongus devoniensis* (Rhodophyta) complex in the North Atlantic. *J. Phycol.*, **28**: 214–232.

Makarov, V.N., Makarov, M.V. & Schoschina, E.V. (1999). Seasonal dynamics of growth in the Barents Sea seaweeds: endogenous and exogenous regulation. *Bot. Mar.*, **42**: 43–49.

Manhart, J.R. & McCourt, R.M. (1992). Molecular data and species concepts in algae. *J. Phycol.*, **28**: 730–737.

Mathieson, A.C., Norton, T.A. & Neushul, M. (1981). The taxonomic implications of genetic and environmentally induced variation in seaweed morphology. *Bot. Rev.*, **47**: 313–347.

Müller, D.G., Maier, I. & Gassmann, G. (1985). Survey on sexual pheromone specificity in Laminariales (Phaeophyceae). *Phycologia*, **24**: 475–477.

Müller, K.M., Sheath, G., Vis, M.L., Crease, T.J. & Cole, K.M. (1998). Biogeography and systematics of *Bangia* (Bangiales, Rhodophyta) based on the Rubisco spacer, *rbc*L gene and 18S rRNA gene sequences and morphometric analyses. 1. North America. *Phycologia*, **37**: 195–207.

Nakahara, H. & Nakamura, Y. (1973). Parthenogenesis, apogamy and apospory in *Alaria crassifolia* (Laminariales). *Mar. Biol., Berl.*, **18**: 327–332.

Nicholas, K.B. & Nicholas, H.B. Jr. (1997). GeneDoc: a tool for editing and annotating multiple sequence alignments. Distributed by the author: http://www.cris.com/~ketchup/genedoc.shtml.

Patwary, M.U., Sensen, C.W., MacKay, R.M. & van der Meer, J.P. (1998). Nucleotide sequences of small-subunit and internal transcribed spacer regions of nuclear rRNA genes support the autonomy of some genera of the Gelidiales (Rhodophyta). *J. Phycol.*, **34**: 299–305.

Pedersen, P.M. (1976). Marine benthic algae from southernmost Greenland. *Meddel. Grønl.*, **199**(3): 1–80.

Rosenvinge, K.L. (1893). Grønlands havalger. *Meddel. Grønl.*, **3**: 765–981.

Rueness, J. (1997). Algae. In *Distribution of Marine, Benthic Macro-organisms in Norway* (Brattegard, T. & Holthe, T., editors). A tabulated catalogue. Preliminary edition. Research Report no. 1997-1. Direktoratet for Naturforvaltninhg, Trondheim.

Saunders, G.W. & Druehl, L.D. (1993). Nucleotide sequences of the internal transcribed spacers and 5·8S rRNA genes from *Alaria marginata* and *Postelsia palmaeformis* (Phaeophyta: Laminariales). *Mar. Biol., Berl.*, **115**: 347–352.

Siemer, B.L., Stam, W.T., Olsen, J.L. & Pedersen, P.M. (1998). Phylogenetic relationships of the brown algal orders Ectocarpales, Chordariales, Dictyosiphonales and Tilopteridales (Phaeophyceae) based on RuBisCo large subunit and spacer sequences. *J. Phycol.*, **34**: 1038–1048.

South, G.R. (1970*a*). Experimental culture of *Alaria* in a sub-arctic, free-flowing sea water system. *Helgoländer Wiss. Meeresunters.*, **20**: 216–228.

South, G.R. (1970*b*). Checklist of marine algae from Newfoundland, Labrador and the French island of St Pierre and Miquelon. *MSRL Technical Report 2*. Memorial University of Newfoundland, St John's, Newfoundland, Canada.

South, G.R. (1983). A checklist of marine algae of eastern Canada, second revision. *Can. J. Bot.*, **62**: 680–704.

Stache-Crain, B., Müller, D.G. & Goff, L.J. (1997). Molecular systematics of *Ectocarpus* and *Kuckuckia* (Ectocarpales, Phaeophyceae) inferred from

phylogenetic analysis of nuclear- and plastid-encoded DNA sequences. *J. Phycol.*, **33**: 152–168.

Stam, W.T., Bot, P.V.M., Boele-Bos, S.A., van Rooij, J.M. & van den Hoek, C. (1988). Single-copy DNA-DNA hybridization among five species of *Laminaria* (Phaeophyceae): phylogenetic and biogeographic implications. *Helgol. Meeresunters.*, **42**: 251–257.

Swofford, D.L. (1993). PAUP (Phylogenetic Analysis using Parsimony), version 3.1.1. User's manual by D.L. Swofford & D.P. Begle, Laboratory of Molecular Systematics, Smithsonian Institution, Washington, DC.

tom Dieck, I. (1992). North Pacific and North Atlantic digitate *Laminaria* species (Phaeophyta): hybridization experiments and temperature responses. *Phycologia*, **31**: 147–163.

tom Dieck, I. & de Oliveira, E.C. (1993). The section Digitatae of the genus *Laminaria* (Phaeophyta) in the northern and southern Atlantic: crossing experiments and temperature responses. *Mar. Biol., Berl.*, **115**: 151–160.

US Navy. (1981). *Marine Climatic Atlas of the World*, vol. IX, *World-wide Means and Standard Deviations*. US Government Printing Office, Washington, DC.

Vinogradova, K.L. (1995). The checklist of the marine algae from Spitsbergen. *Bot. Zhr. SSSR.*, **80**(6): 50–61.

Widdowson, T.B. (1971a). A statistical analysis of variation in the brown alga *Alaria*. *Syesis*, **4**: 125–143.

Widdowson, T.B. (1971b). A taxonomic revision of the genus *Alaria* Greville. *Syesis*, **4**: 11–49.

Yarish, C., Brinkhuis, B.H., Egan, B. & Garcia-Ezquivel, Z. (1990). Morphological and physiological bases for *Laminaria* selection protocols in Long Island Sound aquaculture. In *Economically Important Marine Plants of the Atlantic: Their Biology and Cultivation* (Yarish, C., Penniman, C.A. & van Patten, P., editors), pp. 53–94. Groton, Connecticut Sea Grant College Program.

Yendo, K. (1919). A monograph of the genus *Alaria*. *J. Coll. Sci. Tokyo Imp. Univ.*, **153**: 1–145.

Yoon, H.S. & Boo, S.M. (1999). Phylogeny of Alariaceae (Phaeophyta) with special reference to *Undaria* based on sequences of the RuBisCo spacer region. *Hydrobiologia*, **398/399**: 47–55.

Yotsukura, N., DenBoh, T., Motomura, T., Horiguchi, T., Coleman, A.W. & Ichimura, T. (1999). Little divergence in ribosomal DNA internal transcribed spacer-1 and -2 sequences among non-digitate species of *Laminaria* (Phaeophyceae) from Hokkaido, Japan. *Phycol. Res.*, **47**: 71–80.

Zar, J.H. (1996). *Biostatistical Analysis*, 3rd edition, Prentice-Hall, London.