*Cryptogamie, Algol., 2006, 27 (1): 17-30*


# A genetic investigation of two morphotypes of *Palmaria palmata* (Palmariales, Rhodophyceae) using Rubisco spacer and ITS 1 and ITS 2 sequences

*Stefan KRAAN*[*] *& Michael D. GUIRY*

*Irish Seaweed Centre, Martin Ryan Institute, National University of Ireland, Galway, Republic of Ireland.*

*(Received 4 February 2005, Accepted 23 September 2005)*

**Abstract** — Numerous varieties and formae have been described of *Palmaria palmata* (Linnaeus) O. Kuntze (Palmariales, Rhodophyceae), which is essentially a morphologically plastic entity. The most striking of these is the *sarniensis-sobolifera* varietal complex. Four isolates of this complex and nine of the type variety were genetically compared using plastid-encoded Rubisco spacer sequences and the Internal Transcribed Spacer (ITS 1 and 2) sequences of the nuclear ribosomal cistron. Analysis of the Rubisco spacer sequences resulted in one phylogenetically informative site (excluding the out-group). Phylogenies inferred from the ITS 1 and 2 showed the European isolates separating from a single east Canadian isolate, but did not resolve monophyly of any of the two morphotypes. The average of the Jukes-Cantor distances fell within the range found for conspecific samples of other palmarialean algae and, therefore, we conclude that the type and entities from the *sarniensis-sobolifera* complex are phylogenetically indistinguishable with respect to the DNA examined. We therefore propose that the use of a varietal designation for the *sarniensis-sobolifera* and *palmata* complex be discontinued.

**Hybrids / ITS 1 and 2 / morphotypes / *Palmaria palmata* / phylogenetics / Rubisco spacer / *sarniensis-sobolifera***

**Résumé** — **Etude génétique de deux morphotypes de *Palmaria palmata* (Palmariales, Rhodophyceae) utilisant les séquences de l'espaceur de la Rubisco et des ITS1 et 2.** De nombreuses variétés et formes ont été décrites pour *Palmaria palmata* (Linnaeus) O. Kuntze (Palmariales, Rhdodophyceae) qui est une entité morphologiquement plastique. La plus frappante de ces formes est le complexe variétal *sarniensis-sobolifera*. Quatre isolats de ce complexe et neuf de la variété type ont été comparés génétiquement en utilisant les séquences plastidiales de l'espaceur de la Rubisco et celles des espaceurs internes transcrits (ITS 1 et 2) du cistron ribosomique nucléaire. L'analyse des séquences de l'espaceur de la Rubisco indique un site phylogénétiquement informatif (l'outgroup étant exclu). Les phylogénies construites à partir des séquences des ITS 1 & 2 montrent les isolats européens se séparant d'un seul isolat de l'est du Canada, mais n'indiquent la monophylie d'aucun des deux morphotypes. La moyenne des distances Jukes-Cantor entre morphotypes s'inscrit dans la variation trouvée pour les échantillons conspécifiques des

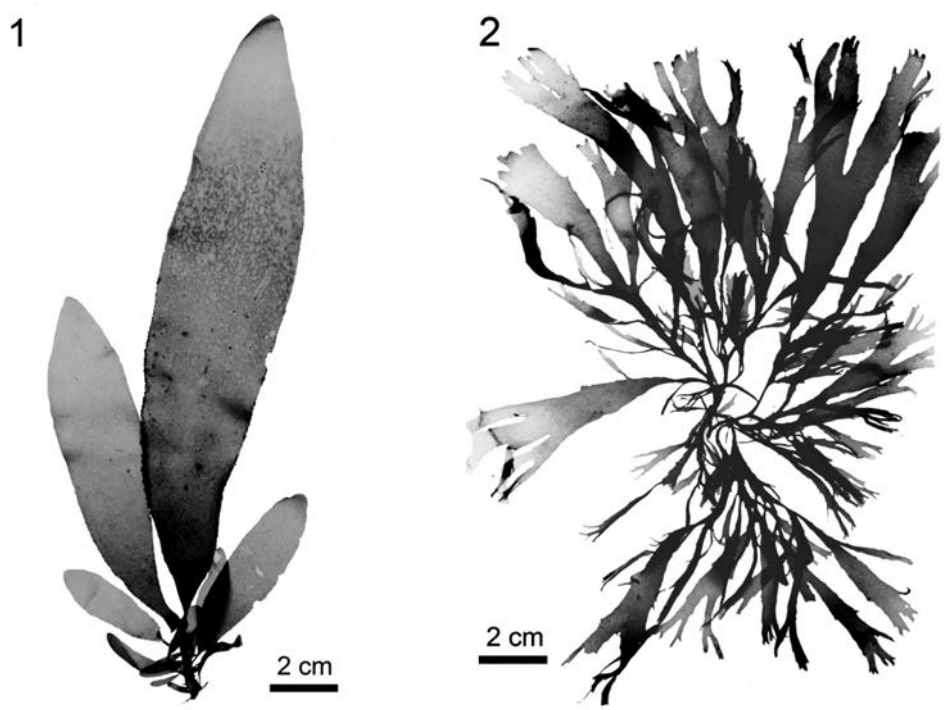
[*] Correspondence and reprints: Stefan.kraan@nuigalway.ie
Communicating editor: Fabio Rindi

autres Palmariales et nous concluons donc que la variété type et les entités du complexe *sarniensis-sobolifera* sont phylogénétiquement indistincts au regard de l'ADN examiné. Nous proposons par conséquent d'arrêter l'usage d'une désignation variétale pour le complexe *sarniensis-sobolifera* et *palmata*.

**Espaceur de la Rubisco / hybrides / ITS 1 and 2 / morphotypes / *Palmaria palmata* / phylogénétique / *sarniensis-sobolifera***

## INTRODUCTION

*Palmaria palmata* (Linnaeus) O. Kuntze (= *Rhodymenia palmata* (L.) Greville, see Guiry, 1974a, 1974b) is an edible red alga known in the north Atlantic as Dillisk or Dulse, where it is sold dried in several countries as a sea-vegetable (Morgan *et al.,* 1980; Galland-Irmouli *et al.,* 1999). The species is widely distributed in the cold and temperate waters of the north Atlantic, occurring from Arctic Russia (Zinova, 1953) to Portugal (Ardré, 1970) and the Azores (Neto, 1994), and from Arctic Canada to New Jersey (South & Tittley, 1986). Populations occur mainly in the mid-intertidal and shallow subtidal zones (rarely to 20 m) growing on rock and epiphytically on several algae, notably on the stipes of the kelp *Laminaria hyperborea* (Gunnerus) Foslie. Thalli are perennial and very variable in shape, resulting in an extraordinary range of morphotypes, which have been described as varieties and formae and, occasionally, as separate species (e.g., Greville, 1830). In the north Atlantic, the most extreme of these varieties or formae (Guiry, 1975; Irvine & Guiry, 1983) are known as the varieties *sobolifera* (when the blade divisions are wedge-shaped and finely dissected above) and *sarniensis* (when the blade has numerous linear divisions throughout), see Figs 1 and 2. Greville (1830: 96), as *Rhodymenia palmata*, stated "No species that I am acquainted with is so apt to vary in the width of the frond and its segments. In some of the specimens, the whole has a narrow linear character, while in others there is a considerable expansion". He described *Rhodymenia palmata* var. *sarniensis* as brighter in colour, more fugitive and with fronds evidently marked with fairly large though obscure reticulations, which are sufficient to distinguish it from the typical form of *Rhodymenia palmata*. The plants of the *sarniensis-sobolifera* complex have been reported from the Orkney Islands (Greville, 1830), Galway Bay, Ireland (van der Meer, 1987), Atlantic France (Feldmann, 1954), Norway (Lyngbye, 1819), and Alaska (Lindstrom, 1977). With respect to the latter location, a narrow-fronded plant named as *Rhodymenia palmata* forma *sarniensis* has been reported from Orca, Alaska (Botanical Expedition to Alaska, 25-27. viii 1899, no. 5166 British Museum; Guiry, 1975). *P. palmata* var. *sarniensis* is found within the distribution range of *P. palmata*, but apparently only at wave-sheltered sites, often with silty conditions (Irvine & Guiry, 1983; Kraan, pers. obs.). *Palmaria palmata* from the north west Pacific is thought to be *P. mollis* (Yabu & Yasui, 1984; Deshmukhe & Tatewaki, 1990) or, perhaps, an undescribed species, and no records of the finely dissected *P. palmata* are known from the north-western Pacific. The varieties are so different from the type variety that they have frequently been misidentified as different algae (Irvine & Guiry, 1983). It is difficult to refer to these entities formally, as nomenclatural combinations for these taxa do not exist. In this account they will be collectively referred to as "the *sarniensis-sobolifera* complex". See Guiry *et al.* (2005) for the taxonomic status of the varieties and formae.




Figs 1-2. Two morphotypes of *Palmaria palmata* collected at Finavarra Co. Galway, Ireland in May 1999. **1.** Typical plant. **2.** Plant referred to as the *sarniensis-sobolifera* complex.

Van der Meer (1987) found that the plants of the type variety and those of the *sobolifera-sarniensis* complex "hybridised" freely in culture, suggesting that they are referable to a single species, although this criterion is no longer considered adequate to prove conspecificity among algae (Kraan *et al*., 2001). Hybridization experiments between Canadian and Irish male and female gametophytes resulted in differences in morphology between crossings and reciprocal crossings, suggesting genetic differences between the Irish and Canadian varieties (van der Meer & Bird, 1985; van der Meer, 1987). A molecular study on phylogenetic radiation within the Palmariaceae using ribosomal DNA internal transcribed spacers ITS1 and ITS2 revealed 1.2% divergence between plants referred to as the variety *sarniensis* from Ballyhenry Bay, northern Ireland and *P. palmata* from Halifax, Nova Scotia, Canada (Lindstrom *et al.*, 1996). A similar level of divergence was found between conspecific samples of another species of the genus *Palmaria* Stackhouse, *P. callophylloides* Hawkes & Scagel. The authors therefore concluded that *P. palmata* var. *sarniensis* from Ballyhenry Bay, northern Ireland and *P. palmata* from Halifax, Nova Scotia, Canada are likely to be conspecific (Lindstrom *et al.*, 1996). However, observations on chromosome pairing and on abnormality of growth and viability of the surviving sporelings of transatlantic hybrids show that there is a considerable amount of karyotypic divergence between Irish and Canadian populations (van der Meer, 1987). The observations that the varieties occur under different environmental conditions

(Irvine & Guiry, 1983; van der Meer, 1987) suggest phenotypic plasticity under influence of the environment. In order to clarify the taxonomic status of the *sarniensis-sobolifera* complex, we obtained sequences of the internal transcribed spacers ITS1 and ITS2 of the nuclear ribosomal DNA and the Rubisco spacer of the chloroplast DNA for twelve isolates of *P. palmata*, including four isolates referable to the *sarniensis-sobolifera* complex, from several locations in the eastern north Atlantic. We tested the hypothesis that no genetic differentiation occurred between different morphotypes. Confirmation of this possibility would suggest that different morphotypes are simply produced by morphological plasticity linked to the influence of environmental factors, as reported for other species of seaweeds (e.g. *Halimeda opuntia* from wave affected and sheltered, shaded sites: Kooistra & Verbruggen, 2005). Conversely, detection of genetic differentiation would indicate the existence of cryptic species diversity.

## MATERIAL AND METHODS

The isolates of *P. palmata* used in this study are listed in Table 1. Specimens were collected from rocks, mussels, or from other algae. Voucher specimens from each collection are preserved in the phycological herbarium, Martin Ryan Institute, National University of Ireland, Galway (GALW).

### DNA extraction and purification

Plants from the sites listed in Table 1 were transported to the laboratory in a cool box, or were sent by courier alive, or dried in silica gel. Plants were cleaned with sterile seawater on arrival and clean, epiphyte-free fragments were processed for DNA extraction. All DNA was extracted from tetrasporophytes. DNA was extracted using the LiCl extraction protocol as described by Hong *et al.* (1992) and modified by van Oppen *et al.* (1994). RNA was removed by incubation (30 min, 37ºC) with 10 µL RNase according to the manufacturer's protocol (Boehringer Mannheim, Germany).

### Double-stranded PCR

Double-stranded ITS regions of the nuclear rRNA cistron sequences were amplified using the primer pair TW5 and Z28B (Table 2) in an Omni-E thermal cycler (Hybaid Ltd., Midllesex, England) 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 50° C and 2 min 74°C and ended with one extension step of 72°C for 5 min. The reaction mix was as described by Kraan & Guiry (2000). Rubisco sequences were amplified using the primer pair RBS1 and RBS2 (Table 2) at a similar temperature profile except for the annealing temperature, which was 55°C. Amplifications were checked for correct length, purity and yield on 1.5% agarose TAE gels stained with Ethidium Bromide in accordance with the methods of Sambrook *et al.* (1989).

Table 1. Source of *Palmaria* specimens used for sequencing ITS regions and Rubisco Spacer.

| *Species* | *Origin* | *Collection date* | *Specifications* | *GenBank accession no.* | |
|---|---|---|---|---|---|
| | | | | *ITS* | *Rubisco spacer* |
| *P. palmata* | Spiddal, Co. Galway, Ireland | May 1999 | On rock | AY029384 | AY029373 |
| *P. palmata* | Carraroe, Co. Galway, Ireland | May 1999 | Creatnach, small type growing on mussel | AY029385 | AY029374 |
| *P. palmata* | Black Head, Co. Clare, Ireland | May 1999 | On rock | AY029386 | — |
| *P. palmata* | Finavarra, Co. Clare, Ireland | May 1999 | On *Fucus serratus* Linnaeus | AY029387 | AY029375 |
| *P. palmata sarniensis-sobolifera* complex | Finavarra, Co. Clare, Ireland | May 1999 | On rock | — | AY029376 |
| *P. palmata* | Strangford Lough, Co. Down, Northern Ireland. | September 1998 | On rock | AY029388 | AY029378 |
| *P. palmata sarniensis-sobolifera* complex | Strangford Lough, Co. Down, Northern Ireland | September 1998 | On rock | AY029393 | AY029379 |
| *P. palmata sarniensis-sobolifera* complex | Ballyhenry Bay, Co. Down, Northern Ireland | September 1994 | ?[a] | Lindstrom *et al.*, 1996 | — |
| *P. palmata* | Paimpol, Bretagne, France | April 1999 | On *Laminaria hyperborea* (Gunnerus) Foslie | AY029391 | AY029381 |
| *P. palmata sarniensis-sobolifera* complex | Paimpol, Bretagne, France | April 1999 | On rock | AY029390 | AY029380 |
| *P. palmata* | Trondheim, Norway | April 1999 | On rock | AY029389 | AY029382 |
| *P. palmata* | Cudillero, Spain | March 1999 | On rock | AY029392 | AY029383 |
| *P. palmata* | Nova Scotia, Canada | March 1994 | ?[a] | Lindstrom *et al.,* 1996 | — |
| *P. palmata* | Nova Scotia, Canada | June 1995 | ? | — | U28421 |
| *P. mollis* | Shaman Island, Alaska | April 1994 | ?[a] | Lindstrom *et al.,* 1996 | — |
| *P. hecatensis* | Shaman Island, Alaska | April 1994 | ?[a] | Lindstrom *et al.*, 1996 | — |

[a] Probably from rocks, however not mentioned in Lindstrom *et al.* (1996)

Table 2. Primer sequences used for polymerase chain reaction amplification and sequencing of the ITS 1 & 2, and Rubisco spacer region.

| *Code* | *Seq.*[a] | *Sequence* | *Anneals to* |
|---|---|---|---|
| RBS1 [b] | F | 5'-TGTGGACCTCTACAAACAGC-3' | End of large subunit |
| RBS2 [b] | R | 5'-CCCCATAGTTCCCAAT-3' | Begin of small subunit |
| TW5 [c] | F | 5'-AACTTAAAGGAATTGACGGGA-3' | End of 18S |
| Z28B [c] | R | 5'-GGTCCGTGTTTCAAGACGGG-3' | Begin of 28S |
| AP1 [d] | F | 5'-TGAGATGCCCTTAGATG-3' | End of 18S |
| AP2 [d] | R | 5'-GCATTCCCAAGCTACCCG-3' | Begin of 28S |
| AP3 [d] | F | 5'-GAGAAGTCGTAACAAGG-3' | End of 18S |
| AP4 [d] | R | 5'-GGTCCGTGTTTCAAGACGGG-3' | End of ITS2 |

[a] Sequencing direction: F = forward, R = reverse
[b] Maggs *et al.* (1992)
[c] Lindstrom *et al.* (1996)
[d] Designed in the Department of Botany, NUI, Galway

## Sequencing and phylogenetic analysis

Double-stranded PCR products were custom-sequenced on an ABI-PRISM 373 system using a nested amplification technique (Lark technologies Inc., Saffron Walden, Essex, UK). Primer pair RBS1 and RBS2 was used for the Rubisco spacer and several internal primers were used for the ITS regions (Table 2). The Rubisco spacer and ITS regions of *P. palmata* of at least two individuals from each population at all collecting sites were sequenced entirely on both strands. Sequences were initially aligned and edited using GENEDOC (Nicholas & Nicholas, 1997) and final adjustments were made by eye; the alignments used for the analyses are available from the authors on request. Other representatives of the genus *Palmaria*, i.e., *P. mollis* (Setchell & Gardner) van der Meer & Bird and *P. hecatensis* Hawkes, were chosen as outgroup taxa. All sequences were submitted to GenBank; accession numbers are reported in Table 1. Only ITS1 and ITS 2 (for reasons explained below) data were analysed with maximum parsimony (MP) using TBR and branch-swapping, and Maximum likelihood (ML) methods with PAUP* version Beta 8 for MAC (Swofford, 1999). All sites were weighted equally and alignment gaps were excluded. Modeltest (Posada & Crandall, 1998) was used to determine the correct parameters for the ML analysis. The robustness of each analysis was tested by bootstrapping the dataset 1000 times for MP and 100 times for ML. Genetic distances were calculated in order to make direct comparisons with genetic distances calculated for other Palmariaceae by Lindstrom *et al.* (1996). A distance matrix was generated using the computer program MEGA (Kumar *et al.*, 1993). Pair-wise distances were calculated using the Jukes-Cantor option (Jukes & Cantor, 1969), which is applied when the four nucleotides do not substantially deviate from 25%, the nucleotide substitutions per site between different sequences are between 0.05 and 0.3 and the transition/transversion (ts/tv) ratio is < 2 (Kumar *et al.*, 1993). The between-sequences ts/tv ratios were calculated with the computer program MEGA (Kumar *et al.*, 1993).

## RESULTS

### Alignment of the Rubisco spacer

The Rubisco spacer sequence was 350 basepairs, of which 106 represented the 3´-end of the *rbc*L gene, 87 the spacer and 157 the 5'-end of the *rbc*S gene. Attempts to sequence the Rubisco spacer of *P. palmata* from Black Head were unsuccessful. In the aligned data set, 3 sites were variable (0.86%), of which one was phylogenetically informative (0.29 %). Due to the low information content it was not possible to perform phylogenetic analysis or to use distance methods.

### Alignment of ITS 1 and 2

The ITS alignment contained 2102 bases, of which 629 represented the 3'-end of the 18S gene, 367 the ITS1, 132 the 5.8S, 413 the ITS2 and 561 the 5' end of the 28S gene. Of the aligned data set (excluding the two outgroup taxa), 105 sites were variable (4.97%), of which 35 were found in the ITS 1, 5.8S and ITS 2 region and of which twenty were phylogenetically informative (0.95%). Only ITS 1 and 2 were used for the distance analysis. Attempts to obtain ITS 1 and 2 sequences failed for an isolate from Finavarra, Co. Clare, Ireland belonging to the *sarniensis-sobolifera* complex. The isolate was therefore omitted from the analysis. The nucleotide composition within sequences showed a moderate T bias (A=26.2%, T=32%, C=21%, G=21%). Ts/tv ratios (data not shown) between sequences were in general larger than 1, indicating no substitutional saturation (Bakker, 1995). Fourteen single-gaps had to be inserted in the alignment; only the two outgroup species, *P. hecatensis* and *P. mollis*, had an eight-nucleotide-insert at the beginning of ITS 2, which was absent from the *P. palmata* isolates.

### Distance analysis

The average Jukes-Cantor distance of the ITS region of *P. palmata* isolates was 1.27% (Table 3). The average distance of a representative of the *sarniensis-sobolifera* complex from Paimpol (France) compared to other *P. palmata* isolates was 4.58%, while another representative of the *sarniensis-sobolifera* complex from Strangford (northern Ireland) had a lower distance of 1.92%. The average genetic distance among different species of *Palmaria* was 11.22%. The maximum distance was found between the *sarniensis-sobolifera* complex representative from Paimpol (France) and *P. hecatensis* (Alaska): 15.20%.

### Phylogenetic analysis on ITS 1 and 2 data

Parsimony analysis (exhaustive search and all gaps removed) showed a single most parsimonious tree of 186 steps (CI=0.919, RC=0.749; tree not shown). Results of Modeltest specified a Tamura-Nei plus Gamma model (TrN+G). The rate matrix was specified as [A-C]=1.0000, [A-G]=1.0670, [A-T]=1.0000,

Table 3. Genetic distances calculated from ITS 1 and 2 sequences using Jukes-Cantor method with pair wise deletions between *P. palmata* and *P. palmata sarniensis-sobolifera* complex samples and other *Palmaria* species. Jukes-Cantor distances are given in percentages.

| | *2* | *3* | *4* | *5* | *6* | *7* | *8* | *9* | *10* | *11* | *12* | *13* | *14* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 *P. palmata*-Spiddal, Co. Galway, Ireland | 0.26 | 0.52 | 1.56 | 0.65 | 1.43 | 0.78 | 1.30 | 4.26 | 0.13 | 0.39 | 1.82 | 10.24 | 11.37 |
| 2 *P. palmata*-Carraroe, Co. Galway, Ireland | — | 0.78 | 1.83 | 0.65 | 1.43 | 0.78 | 1.30 | 4.26 | 0.13 | 0.39 | 1.82 | 10.11 | 11.23 |
| 3 *P. palmata*-Black Head, Co. Clare, Ireland | | — | 1.69 | 1.17 | 1.96 | 1.30 | 1.56 | 4.68 | 0.65 | 0.91 | 2.35 | 10.84 | 11.67 |
| 4 *P. palmata*-Finavarra, Co. Clare, Ireland | | | — | 2.22 | 3.02 | 2.36 | 2.76 | 5.38 | 1.69 | 1.69 | 3.43 | 11.15 | 12.45 |
| 5 *P. palmata*-Strangford Lough, Co. Down, northern Ireland | | | | — | 1.04 | 1.17 | 1.69 | 4.26 | 0.52 | 0.78 | 2.22 | 10.39 | 11.67 |
| 6 *P. palmata sarniensis-sobolifera* complex - Strangford Lough, Co. Down, northern Ireland | | | | | — | 1.96 | 2.49 | 4.13 | 1.30 | 1.56 | 3.02 | 11.30 | 12.45 |
| 7 *P. palmata sarniensis-sobolifera* complex - Ballyhenry Bay, Co. Down, northern Ireland | | | | | | — | 1.56 | 4.27 | 0.65 | 0.65 | 1.17 | 9.65 | 10.61 |
| 8 *P. palmata*-Paimpol, Bretagne, France | | | | | | | — | 4.82 | 1.17 | 1.17 | 2.62 | 11.15 | 11.99 |
| 9 *P. palmata sarniensis-sobolifera* complex - Paimpol, Bretagne, France | | | | | | | | — | 4.82 | 4.13 | 5.38 | 14.46 | 15.20 |
| 10 *P. palmata*-Trondheim, Norway | | | | | | | | | — | 0.26 | 1.69 | 10.09 | 11.21 |
| 11 *P. palmata*-Cudillero, Spain | | | | | | | | | | — | 1.69 | 10.09 | 11.21 |
| 12 *P. palmata*-Nova Scotia, Canada | | | | | | | | | | | — | 10.52 | 10.46 |
| 13 *P. mollis*-Shaman Island, Alaska | | | | | | | | | | | | — | 7.75 |
| 14 *P. hecatensis*-Shaman Island, Alaska | | | | | | | | | | | | | — |

[C-G]=1.0000, [C-T]=1.6836, and [G-T]=1.0000, with base frequencies at A=0.2609, C=0.2121, G=0.2104 and T=0.3166. The gamma distribution was set at 0.7534. A maximum likelihood tree (Fig. 3) showed an identical tree topology except for the *P. palmata* isolate from Finavarra, which was clustered with the Black Head (Ireland) isolate in the MP method. The *sarniensis-sobolifera* type of Paimpol (France) and Strangford (northern Ireland) formed one clade supported by a bootstrap value of 60-66 %. However, the *sarniensis-sobolifera* isolate from Ballyhenry Bay (northern Ireland) was placed basally in a separate clade close to the Canadian isolates and as a sister clade to all other European (east Atlantic) *P. palmata* isolates, and was supported by a bootstrap value of 59-91%. The ML and MP analyses did resolve monophyly of the European taxa, but not monophyly of the morphotypes. The *P. palmata* isolate from Strangford (northern Ireland) was grouped with the representatives of the *sarniensis-sobolifera* complex of the present study with 59-73 % bootstrap support.

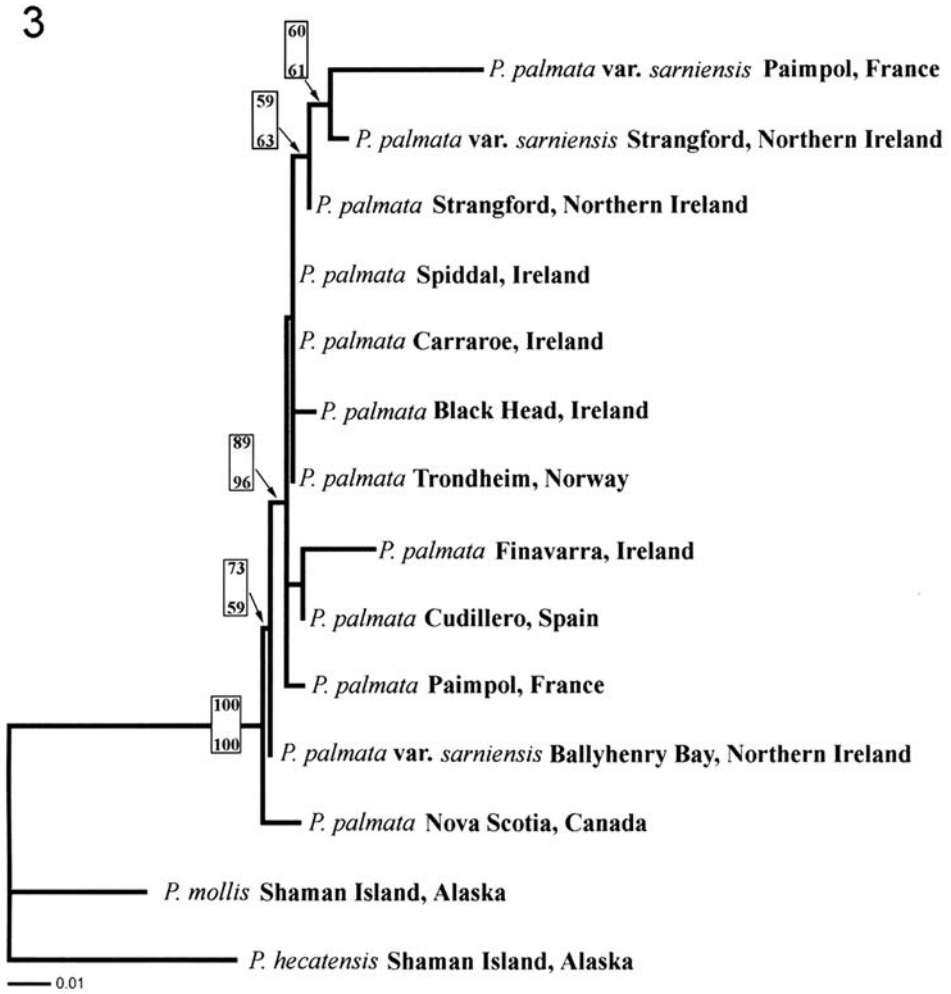



Fig. 3. Maximum Likelihood tree for ITS1 and 2 spacer sequences of *P. palmata* species, rooted with *Palmaria* species from Alaska. Branch lengths are proportional to the amount of sequence change, and bootstrap values for MP (top, 1000 replicates) and ML (bottom, 100 replicates) analyses are indicated at the nodes.

## DISCUSSION

*Palmaria palmata* has long been recognized as a very variable species with an extraordinary range of described varieties and formae, some of which have been treated as species at various times (e.g., see Greville, 1830: 96, as *Rhodymenia palmata*). This diverse range of morphotypes assumes a genetic basis and hence justifies a genetic study of the different morphotypes. A direct comparison of Rubisco spacer sequences showed that *P. palmata* isolates from Norway, Spain,

France, and Spiddal, Carraroe and Finavarra in Ireland, together with a sample of the *sarniensis-sobolifera* complex from Finavarra, Ireland show only one phylogenetically informative position, which is insufficient to perform any analysis to examine relationships among populations. This is in agreement with the conclusions of Müller *et al.* (1998) who questioned the utility of the Rubisco spacer for analysis of bangiophycean red algae. However, Kamiya *et al.* (1998) demonstrated in the florideophycean red alga *Caloglossa leprieurii* (Montagne) J. Agardh that molecular analysis using Rubisco spacer sequences did resolve populations, and these sequences corresponded with the results of morphological and hybridization experiments. Rubisco spacer sequences used in this study are too conserved to resolve populations in the genus *Palmaria* and, most probably, within the family Palmariaceae.

Comparisons of the ITS 1 and ITS 2 sequences were more successful, with 1.8% phylogenetically informative sites (excluding the outgroup). The tree in Fig. 3 shows the European isolates separated from the Canadian sample and the outgroup species with the support of a high bootstrap value. Nevertheless, this separation is based on only one isolate from Canada, for which the sequence was obtained from Genbank. The aim of this study was to examine the relationships between different morphotypes and we were not specifically interested in genetic differences between east and west Atlantic populations; further studies, based on a larger dataset and including a larger number of samples from North America, will be necessary for this. Our ITS analysis, however, did not resolve the European *P. palmata* from the *sarniensis-sobolifera* complex. The average genetic distance of 1.27% among *P. palmata* isolates in this study corresponds well with a distance of 1.2% found by Lindstrom *et al.* (1996). The *sarniensis-sobolifera* isolate from France, compared to other *P. palmata* isolates used in this study, showed most divergence with a genetic distance of 4.58% (Table 3). The genetic distance of the *sarniensis-sobolifera* samples from Strangford Lough (northern Ireland) and Ballyhenry Bay (northern Ireland) falls within the range of divergence for the type. The clustering of the *P. palmata* isolate from Strangford Lough, with the *sarniensis-sobolifera* isolates from Strangford Lough and Paimpol, although supported by a relatively low bootstrap value, could be due to the fact that this isolate was a hybrid of the two types. It is possible that the *sarniensis-sobolifera* type from Strangford Lough, (northern Ireland) and the *palmata* type from the same locality are both hybrids of true *sarniensis* and *palmata* plants, thus explaining why the genetic distance is more similar to that of other *P. palmata* isolates with respect to the DNA examined. Remarkably, the putative "*sarniensis*" sample from Ballyhenry Bay (Lindstrom *et al.*, 1996), about 15 km north in Strangford Lough, appears not to belong in any cluster and is as closely related to *P. palmata* from Nova Scotia, Canada as to the other European isolates (Fig. 3). Perhaps this anomaly results from sample confusion, as it is peculiar to see it in a separate clade. If this is not due to sample confusion, then a cryptic introduction (Ribera & Boudouresque, 1995) might explain this discrepancy. A possible explanation of the molecular comparisons is that the *sarniensis-sobolifera* sample from France could be genuine *sarniensis-sobolifera* whilst the other *sarniensis-sobolifera* samples with a closer genetic distance to the other *P. palmata* isolates were perhaps hybrids. Van der Meer (1987) showed that plants of the *sarniensis-sobolifera* complex and *palmata* plants from Ireland should be considered to be part of a single genetic entity, as they hybridized freely and produced normal tetrasporangial and gametangial plants. A study by Provan *et al.* (2005), using plastid PCR-RFLP haplotypes, revealed the existence of six different haplotypes in *P. palmata* plants, with the occurrence of an endemic haplotype for

the Galway Bay area (Finavarra, Cararoe and Spiddal in this study) and the highest level of five haplotypes in the English channel (Paimpol in this study). Provan *et al.* (2005), however, studied only plants of the type variety and no isolates of the *sarniensis-sobolifera* complex were included in their study. Nevertheless, they demonstrated a large amount of intraspecific variation in plastid DNA within the type variety itself.

At the genus level, *P. mollis* and *P. hecatensis* showed a genetic distance of 10 - 15% compared to other *P. palmata* samples. This is 2 to 3 times higher than values found for the *sarniensis-sobolifera* sample from France compared to other *P. palmata* samples. Lindstrom *et al.* (1996) demonstrated a genetic distance of 5% within conspecific samples of *P. hecatensis* isolates from geographically distant locations. This level of divergence is similar to that found between the *sarniensis-sobolifera* sample from France and other *P. palmata* samples. The other two *sarniensis-sobolifera* samples from Strangford and Ballyhenry Bay (northern Ireland) are within the genetic distance range of other *P. palmata* samples; we therefore believe that they cannot be regarded as separate genetic entities.

Although van der Meer (1987) left it to the individual investigator to decide if there is any useful purpose in separating the species into subspecies or varieties, the results provided by Provan *et al.* (2005) and this study strongly support not using subspecies or varieties as independent taxa. We therefore propose that the *sarniensis* and *sobolifera* varietal designation should be discontinued. In a study on population differentiation of *P. mollis*, DNA sequence data for the ITS regions revealed a maximum of 4 base-pair differences, which was not sufficient to provide insight into population differentiation (Lindstrom *et al.*, 1996). In contrast, a study on *P. mollis* using RAPD markers produced results confirming the genetic proximity of individuals from within the same population and suggesting pairs, triplets and larger clusters of populations (Lindstrom *et al.*, 1997). Unfortunately, we were not able to obtain a sequence from the *sarniensis-sobolifera* sample we examined from Finavarra (Ireland), due to contamination of the DNA. A more detailed genetic comparison using other nuclear genes or genetic markers may provide useful insights into the position of the *sarniensis-sobolifera* samples in this study.

The results suggest that the two varieties are conspecific in respect of the DNA examined. Further evidence is provided by the experiments of van der Meer (1987) showing the varieties hybridizing freely in culture. Furthermore, the observations that the varieties occur under different environmental conditions (Irvine & Guiry, 1983; van der Meer, 1987) suggest phenotypic plasticity under influence of the environment. Phenotypic responses to environmental conditions have been shown in other red and green algae (Chapman *et al.*, 1977; Mathieson *et al.*, 1981; Kooistra & Verbruggen, 2005) as well as in brown algae (Widdowson, 1971; Chapman, 1974). Transplantation experiments of young plants of both varieties to detect environmental effects on phenotype would be an interesting subject for further studies.

**Acknowledgements.** Many thanks to Dr. Lynne McIvor for providing help with the phylogenetic analysis. This study was supported under contract CT97-3828 of the EU Fourth Framework FAIR programme.

## REFERENCES

ARDRÉ F., 1970 — Contribution à l'étude des algues marines du Portugal. I. La flore. *Portugalia acta biologica, Série B* 10:137-555.

BAKKER F.T., 1995 — *Time spans and spacers: Molecular phylogenetic exploration in the* Cladophora *complex (Chlorophyta) from the perspective of rDNA gene and spacer sequences.* PhD Thesis, University of Groningen, The Netherlands, pp. 1-119.

CHAPMAN A.R.O., 1974 — The genetic basis of morphological differentiation in some *Laminaria* populations. *Marine biology* 24: 85-91.

CHAPMAN A.R.O., EDELSTEIN T. & POWER P., 1977 — Studies on *Gracilaria*. I. Morphological and anatomical variation in samples from the lower Gulf of St. Lawrence and New England. *Botanica marina* 20: 149-153.

DESHMUKHE G.V. & TATEWAKI M., 1990 — The life history and evidence of the macroscopic male gametophyte in *Palmaria palmata* (Rhodophyta) from Muroran, Hokkaido, Japan. *Japanese journal of phycology (Sôrui)* 38: 215-221.

FELDMANN J., 1954 — Inventaire de la flore marine de Roscoff. Algues, champignons, lichens et spermatophytes. *Travaux de la station biologique de Roscoff, Nouvelle Série* 2, *Supplément* 6: 1-152.

GALLAND-IRMOULI A-V., FLEURENCE J., LAMGHARI R., LUÇON M., ROUXEL C., BARBAROUX O., BRONOWICKI J-P., VILLAUME C. & GUÉANT J-L., 1999 — Nutritional value of proteins from edible seaweed *Palmaria palmata* (Dulse). *Journal of nutritional biochemistry* 10: 353-359.

GREVILLE R.K., 1830 — *Algae britannicae*. Edinburgh & London, 218 p.

GUIRY M.D., 1974a — A reappraisal of the genus *Palmaria* Stackhouse. *British phycological journal* 9: 219

GUIRY M.D., 1974b — A preliminary consideration of the taxonomic position of *Palmaria palmata* (Linnaeus) Stackhouse=*Rhodymenia palmata* (Linnaeus) Greville. *Journal of marine biology association of the United Kingdom* 54: 509-528.

GUIRY M.D., 1975 — An assessment of *Palmaria palmata* forma *mollis* (S. et G.) comb. nov. (=*Rhodymenia palmata* forma *mollis* S. et G.) in the eastern North Pacific. *Syesis* 8: 245-261.

GUIRY M.D., RINDI F. & GUIRY G.M., 2005 — *AlgaeBase version 4.0*. World-wide electronic publication, National University of Ireland, Galway.
http://www.algaebase.org; searched on 27 April 2005.

HONG Y.-K., COURY D.A., POLNE-FULLER M. & GIBBOR. A., 1992 — Lithium chloride extraction of DNA from the seaweed *Porphyra perforata* (Rhodophyta). *Journal of phycology* 28: 717-720.

IRVINE, L.M. & GUIRY M.D., 1983 — Palmariales. *In*: Irvine L.M (Ed.), *Seaweeds of the British Isles*. *Volume 1 Rhodophyta, part 2A Cryptonemiales (sensu stricto), Palmariales, Rhodomeniales*. London, Natural History Museum/HMSO, pp. 65-74.

JUKES T.H. & CANTOR C.R., 1969 — Evolution of protein molecules. *In*: Munro, H.N. (Ed.), *Mammalian Protein Metabolism*. New York, Academic Press, pp. 21-331.

KAMIYA M., WEST J.A., KING R.J., ZUCCARELLO G.C., TANAKA J. & HARA Y., 1998 — Evolutionary divergence in the red algae *Caloglossa leprieurii* and *C. apomeiotica*. *Journal of phycology* 34: 361-70.

KOOISTRA W.H.C.F. & VERBRUGGEN H, 2005 — Genetic patterns in the calcified tropical seaweeds *Halimeda opuntia*, *H. distorta*, *H. hederacea*, and *H. minima* (Bryopsidales, Chlorophyta) provide insights in species boundaries and interoceanic dispersal. *Journal of phycology* 41: 177-187.

KRAAN S. & GUIRY M.D., 2000 — Sexual hybridization experiments and phylogenetic relationships as inferred from RuBisCo spacer sequences in the genus *Alaria* (Alariaceae, Phaeophyceae). *Journal of phycology* 35: 190-198.

KRAAN S., RUENESS J. & GUIRY M.D., 2001 — Are North Atlantic *Alaria esculenta* and *A. grandifolia* (Alariaceae, Phaeophyceae) conspecific? *European journal of phycology* 36: 35-42.

KUMAR S., TAMURA K. & NEI M., 1993 — MEGA: Molecular Evolutionary Genetics Analysis, version 1.02. The Pensylvania State University, University Park, PA 16802.

LINDSTROM S.C., 1977 — *An Annotated bibliography of the benthic marine algae of Alaska*. Alaska, Department of Fish and Game 13: 172 p.

LINDSTROM S.C., OLSEN J.L. & STAM W.T., 1996 — Recent radiation of the Palmariaceae (Rhodophyta). *Journal of phycology* 32: 457-468.

LINDSTROM S.C., OLSEN J.L. & STAM W.T., 1997 — Post-glacial recolonization and the biogeography of *Palmaria mollis* (Rhodophyta) along the northeast Pacific coast. *Canadian journal of botany* 75: 1887-1896.

LYNGBYE H.C., 1819 — *Tentamen hydrophytologiae danicae*. Copenhagen, Schultz, t. 32, 248 p.

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. *Journal of phycology* 28: 214-32.

MATHIESON A.C., NORTON T.A. & NEUSHUL, M., 1981 — The taxonomic implications of genetic and environmentally induced variations in seaweed morphology. *Botanical review* 47: 313-347.

MORGAN K.C., WRIGHT J.L.C. & SIMPSON F.J., 1980 — Review of chemical constituents of the red alga *Palmaria palmata* (Dulse). *Economic botany* 34: 27-50.

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.

NETO A.I., 1994 — Checklist of the benthic marine macroalgae of the Azores. *Arguipélago ciências biológicas e marinhas* 12 A: pp. 15-34.

NICHOLAS K.B. & NICHOLAS. H.B. JR., & Deerfield, D.W. II., 1997 — GeneDoc: Analysis and Visualization of Genetic Variation, EMBNEW.NEWS 4:14 http://www.psc.edu/biomed/genedoc

POSADA D. & CRANDALL K. A., 1998 — MODELTEST: testing the model of DNA substitution. *Bioinformatics* 14: 817-818.

PROVAN J., WATTIER R.A. & MAGGS C.A., 2005 — Phylogeographic analysis of the red seaweed *Palmaria palmata* reveals a Pleistocene marine glacial refugium in the English Channel. *Molecular ecology* 14: 793-803.

RIBERA M.A. & BOUDOURESQUE C-F., 1995 — Introduced marine plants, with special reference to macroalgae: mechanisms and impact. *In*: Round F.E. & Chapman D.J. (Eds.), *Progress in phycological research.* Bristol, Biopress Ltd., pp. 187-268.

SAMBROOK J., FRITCH E.F. & MANIATIS T., 1989 — *Molecular cloning: A laboratory manual.* 2nd Edn. New York, Cold Springer Harbor Laboratory Press, 3 vols.

SOUTH G.R. & TITTLEY I., 1986 — *A checklist and distribution index of benthic marine algae of the North Atlantic Ocean*. London and St Andrews, New Brunswick, Canada, British Museum (Natural History) and Huntsman Marine Laboratory, 76 p.

SWOFFORD D.L., 1999 — PAUP* *Phylogenetic Analysis using Parsimony (* and Other Methods)*. Massachusetts, Sinauer Associates, Sunderland.

VAN DER MEER J.P. & BIRD C.J., 1985 — *Palmaria mollis* stat. nov.: a newly recognized species of *Palmaria* (Rhodophyceae) from the northeast Pacific coast. *Canadian journal of botany* 63: 398-403.

VAN DER MEER J.P., 1987 — Experimental hybridization of *Palmaria palmata* (Rhodophyta) from the northeast and northwest Atlantic Ocean. *Canadian journal of botany* 65: 1451-1458.

VAN OPPEN M.J.H., DIEKMANN O.E., WIENCKE C., STAM W.T. & OLSEN J.L., 1994 — Tracking dispersal routes: phylogeography of the Arctic-Antarctic disjunct seaweed *Acrosiphonia arcta*. *Journal of phycology* 30: 67-80.

WIDDOWSON T.B., 1971 — A statistical analysis of variation in the brown alga *Alaria*. *Syesis* 4: 125-43.

YABU H. & YASUI H., 1984 — The male gametophyte of Japanese *Palmaria palmata* (Rhodophyta). *Japanese journal of phycology (Sôrui)* 32: 279-282.

ZINOVA A.D., 1953 — *Opredelitel' burykh vodoroslej severnykh morej SSSR*. Moscow and Leningrad, Akad Nauk SSSR, 226 p.