

GENETIC AND MORPHOLOGICAL IDENTIFICATION OF *FUCUS RADICANS* SP. NOV. (FUCALES, PHAEOPHYCEAE) IN THE BRACKISH BALTIC SEA¹

Lena Bergström²

Department of Ecology and Environmental Science, Umeå Marine Science Center, Umeå University, SE-910 20 Hörnefors, Sweden

Andrey Tatarenkov, Kerstin Johannesson

Department of Marine Ecology, Tjärnö Marine Biological Laboratory, Göteborg University, SE-452 96 Strömstad, Sweden

Rita B Jönsson

Department of Biology and Environmental Sciences, Kalmar University, SE-391 82 Kalmar, Sweden

and

Lena Kautsky

Department of Botany, Stockholm University, SE-106 91, Stockholm, Sweden

Brown seaweeds of the genus *Fucus* occupy a wide variety of temperate coastal habitats. The genus is evolutionary dynamic with recent radiations to form morphologically distinct taxa. In the brackish Baltic Sea, fucoids are the only perennial canopy-forming macroalgae. The most northern populations of *Fucus* occur permanently submerged in extremely low salinity (3–5 psu). These are currently referred to as *Fucus vesiculosus* L. but are morphologically distinct with a narrow frond without bladders. We report here that a population of this unique morphotype is reproductively isolated from a truly sympatric population of common *F. vesiculosus* and conclude that the northern morphotype represents a previously undescribed species. We describe *Fucus radicans* sp. nov., which is attached and dioecious with broadly elliptic receptacles, characterized by a richly branched narrow flat frond (2–5 mm), short thallus (<26 cm), and a high capacity for vegetative recruitment of attached plants. Analysis of five highly polymorphic microsatellite DNA loci showed genetic differentiation between sympatric populations of *F. radicans* and *F. vesiculosus*, whereas allopatric populations of the same species revealed a coherent pattern of genetic variation. Sequences of the RUBISCO region in *F. radicans* were identical to or differing at only one to two dinucleotide positions from those of *F. vesiculosus*, indicating a recent common origin of the two species.

Key index words: asexual reproduction; brackish water; clonality; Fucaceae; marginal environment;

microsatellite DNA; morphology; RUBISCO sequence; species description

Population diversity is recognized as a crucial aspect of biodiversity, not only for the ecological function of today's species, but also to ensure their continued evolutionary potential facing environmental change (Luck et al. 2003). Marginal populations deserve special interest in this respect, because they typically represent small or isolated populations subjected to strong or atypical forces of natural selection. This may favor population diversity through the establishment of unique gene combinations and new genetic traits.

The Baltic Sea is a unique marginal ecosystem because it is the largest brackish sea on Earth, with a surface salinity ranging from 1 to 2 psu in its inner parts up to 25 psu at its entrance to the Skagerrak, and has predominantly atidal conditions (HELCOM 1996). It has a well-documented postglacial history, driven by the suites of a globally increased sea level and regional isostatic rebound after the retreat of the last continental ice sheet 11,000 years ago (Ignatius et al. 1981). After a freshwater stage, the main part of its current marine biota is thought to have colonized the Baltic Sea around 8–3 ka BP, when its entrance to the Atlantic ocean was larger than today and the salinity of the Baltic Sea was higher (i.e. 5–25 psu) (Snøeijrs 1999). After this period, the salinity progressively approached current levels, prompting a rapid evolutionary challenge of the marine fauna and flora, with species incapable of adapting to the new environment becoming extinct (Russell 1985).

Today, the Baltic Sea is a species-poor ecosystem where marine species coexist with freshwater species, especially in its inner areas. Approximately 840 species of marine macrofauna (Kautsky and Kautsky 2000) and 220 marine macroalgae (Snøeijrs 1999) are recorded at the Baltic Sea entrance (salinity 25 psu), decreas-

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²Author for correspondence and present address: Department of Botany, Stockholm University, SE-106 91, Stockholm, Sweden.
E-mail lena.bergstrom@botan.su.se.

ing down to approximately 80 and 60 species, respectively, in the Baltic Proper (salinity 6–7 psu). Many of the species subjected to more detailed study show at least some degree of physiological adaptation to the brackish water conditions (Rietema 1991, 1993, 1995, Kristiansen et al. 1994, Düwel 2001). In addition, a number of species show marked genetic differences between Baltic and North Sea populations (Väinölä and Hvilson 1991, Luttikhuisen et al. 2003, Olsen et al. 2004), although much of these differences might be introduced through the invasion into the Baltic of separate evolutionary lineages rather than locally evolved (Väinölä 2003).

The preconditions for phenotypic and genetic radiation are presumably strong within the genus *Fucus*, as evident from recent evolutionary events (Leclerc et al. 1998, Serrão et al. 1999b). Furthermore, *Fucus* species are widely distributed in cold and temperate coastal areas (White 2004) and typically show strong morphological and physiological variation (Knight and Parke 1950, Burrows and Lodge 1951, Pérez-Ruzafa et al. 1993, Malm et al. 2001, Ruuskanen and Bäck 2002). A strong potential for evolution is also indicated by substantial genetic structuring of populations on small geographic scales (<2 km) (Coyer et al. 2003, unpublished data). This is probably supported by a restricted dispersal of gametes, in the range of a few meters only (Serrão et al. 1997), although long-distance dispersal by drifting reproductive plants may occur.

Bladderwrack *Fucus vesiculosus* L. is among the most widely distributed species in the Baltic Sea and the only perennial canopy-forming seaweed over vast areas (Kautsky and Kautsky 2000). It has long been considered a typical example of a species with profound intraspecific variation (Powell 1963), this being a prerequisite for local adaptation and adaptation to marginal environments such as the Baltic Sea (Chapman 1995). Populations of *F. vesiculosus* from the Baltic Sea differ from marine populations in being better adapted to low salinity (Andersson et al. 1992, Bäck et al. 1992), less tolerant of emersion stress as they are permanently submerged (Pearson et al. 2000), and may also show morphological differentiation by a gradual size reduction with decreasing salinity (Ruuskanen and Bäck 1999a). However, it is uncertain whether the level of differentiation of Baltic *F. vesiculosus* should warrant any taxonomic recognition, and the situation is further complicated by the presence of noticeable phenotypic variation in *F. vesiculosus* even within the Baltic Sea (Waern 1952, Powell 1963).

Observations of morphological variation within Baltic *F. vesiculosus* resulted in detailed descriptions of an intraspecific taxonomy by early botanists (Kjellman 1890, Svedelius 1910) that was, however, later abandoned as being too imprecise (Waern 1952). Clearly, one obvious example of morphological variation in *F. vesiculosus* is a gradual, mainly environmentally induced, size reduction and loss of bladders with decreasing salinity and increasing wave exposure (Lüning 1990, Ruuskanen and Bäck 1999a). In marine *F. ves-*

iculosus, the morphological response to wave exposure may be recognized as a series of gradual forms, ranging from *F. vesiculosus* f. *vadorum*, which is heavily bladdered and characteristic of sheltered sites; *F. vesiculosus* f. *vesiculosus*, the typical form; to *F. vesiculosus* f. *linearis*, which lacks bladders and is characteristic of exposed sites (Powell 1963). These forms may also broadly be applied to Baltic *F. vesiculosus*. Phenotypic variation that cannot be explained by a direct environmental induction is, however, also present. One example is the occurrence of what is considered a dwarf morphotype of *F. vesiculosus*, which grows attached in mixed populations with common *F. vesiculosus* in the southern Gulf of Bothnia, northern Baltic Sea (Waern 1952, Kautsky H. et al. 1992) (Fig. 1). This dwarf morphotype is dominant further north, where it is not further reduced in size. It is the only morphotype present in the central Gulf of Bothnia, reaching a distributional limit with respect to low salinity at 3–4 psu (Serrão et al. 1996, Bergström and Bergström 1999).

The perception that a taxonomically distinct brackish water-adapted entity of *Fucus* exists in the inner parts of the Baltic Sea has persisted despite a general tendency of lumping furoid taxa rather than splitting them (Waern 1952, Powell 1963). Luther (1981), for example, concluded that “the evesiculate, narrow-winged Gulf of Bothnia *Fucus*” probably was a separate taxonomic entity from common *F. vesiculosus* but remarked that a genetic assessment was needed. “The Gulf of Bothnia *Fucus*,” also referred to as the “dwarf morphotype,” is dioecious with a flat even frond and a distinct midrib. Thus, it resembles *F. vesiculosus*, but it is more richly branched with narrower fronds and does not have vesicles. It grows attached to hard substrates, typically at 2–8 m depth in salinities of 3–5 psu at exposed to semiexposed sites.

The present study aimed to resolve the taxonomic status of the dwarf morphotype of *F. vesiculosus* present in the Baltic Sea. We used morphometric analysis of phenotypic traits and genetic analysis of five highly polymorphic microsatellite DNA loci (Engel et al. 2003) as well as sequencing of one chloroplast DNA region to assess the phenotypic and genetic relationship between the Gulf of Bothnian populations of the dwarf morphotype and populations of the common *F. vesiculosus* from the same area and from separate areas. Our results show that unique morphological characteristics of the dwarf morphotype are preserved in populations sympatric with common *F. vesiculosus*. We also show that the dwarf and common morphotypes are strongly genetically differentiated when present in mixed populations. The results indicate that these two morphotypes are reproductively isolated and that the evesiculate narrow-winged Gulf of Bothnian *Fucus* should be considered a separate species.

MATERIALS AND METHODS

Sample material. Populations of the dwarf morph of *Fucus* were sampled in the northern Gulf of Bothnia (D1, site

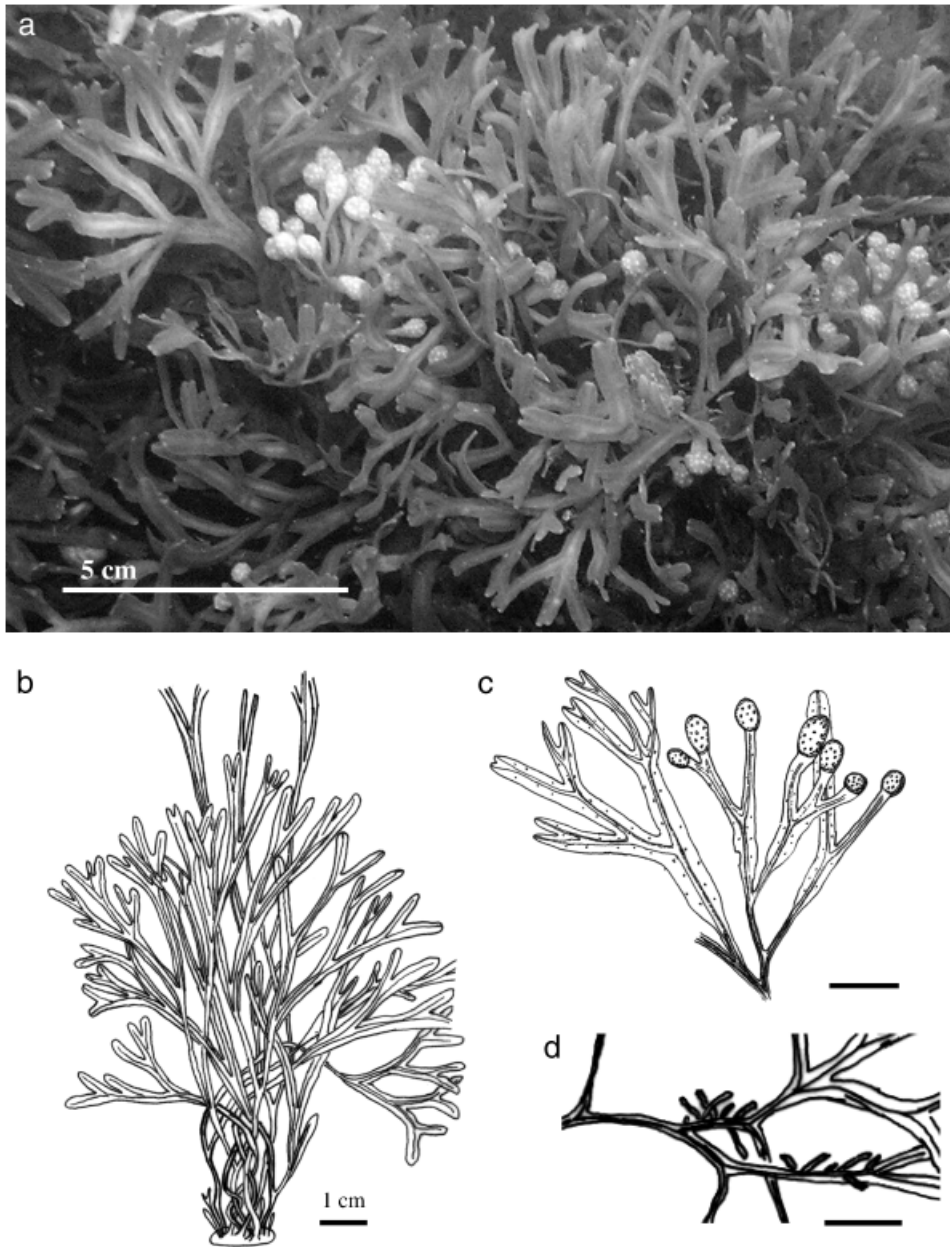


FIG. 1. *Fucus radicans*. (a) Pictured at 3 m depth south of site D1 in the Gulf of Bothnia (63°26' N, 19°25'E). (b) Lower part of frond showing multiple generations protruding from the same holdfast. (c) Upper part of frond with vegetative and reproductive tips. (d) Adventitious branchings from the stem. Scale bars, 1 cm.

Järnäs, salinity 4 psu) and in the southern Gulf of Bothnia (DM, site Öregrund, 5 psu). At Öregrund, the dwarf morphotype occurs side by side with common *F. vesiculosus*, which was also sampled (CM). Reference data of common *F. vesiculosus* were obtained from the Swedish west coast (C1, site Tjärnö, and C2, site Lysekil, 20 psu) and the Baltic Proper (C3-4, site Öland, 7 psu). Also, previously unpublished data from the Baltic proper was included in the analyses of morphology (C5, site Askö, salinity 6 psu). The reference material was chosen to represent a wide range of geographic distributions and conditions of wave exposure in common *F. vesiculosus* (Fig. 2).

Populations from Järnäs (only dwarf) and Öregrund (dwarf and common mixed) were sampled in August 2003 from 3 to 3.5 m depth, which corresponds to the depth of maximum abundance of *Fucus* at these sites. Sampling was performed by collecting all individuals growing within a quadrat of 0.25 m²

in the field and subsequently within another quadrat placed adjacent to the previous one, until at least 50 individuals representing each population were obtained (Fig. 2). The total area sampled was 1.75 m² at Järnäs and 2 m² at Öregrund. This sampling procedure was used to minimize the risk of morphological differentiation among individuals due to local environmental effects and to obtain an unbiased sample of the full range of morphotypes present side by side in the field. Minimizing the sample area was also conceived to maximize the probability of gene flow through sexual reproduction between individuals of different morphotypes at the sympatric site (Öregrund). The total depth distribution of *Fucus* at Öregrund was between 1 and 5 m depth for both the dwarf and the common morphotypes. Both morphotypes were maximally developed at 2–4 m depth, where the dwarf morphotype was estimated to cover 50%–100% of the total available substrate and the common morphotype, 25%–50%. The subst-

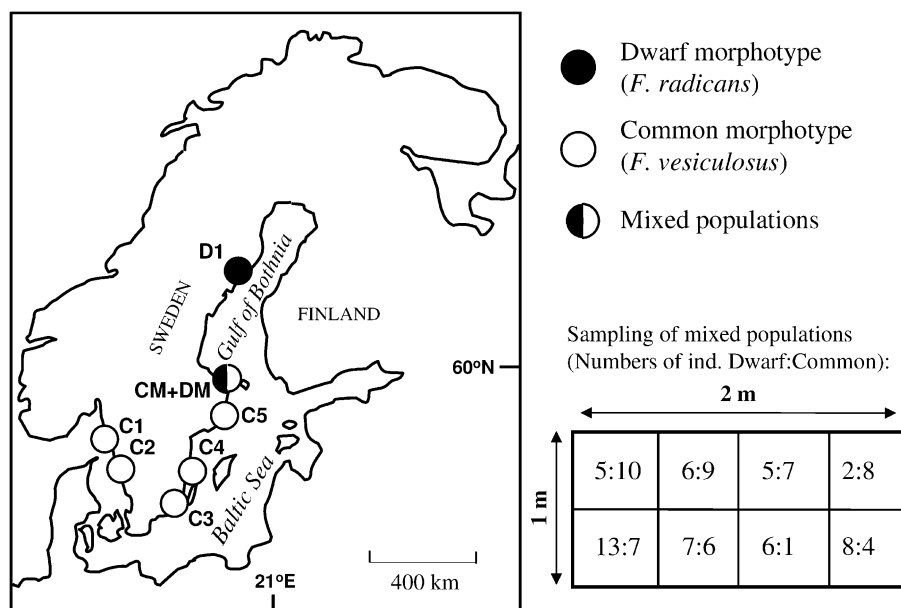


FIG. 2. Sampling sites of the dwarf morphotype (*Fucus radicans*, populations D1 and DM) and of common *F. vesiculosus* at the Swedish west coast (populations C1–C2) and in the Baltic Sea (populations C3–C5 and CM). Populations CM and DM occurred completely mixed and were sampled as illustrated in the sampling scheme to the right, at 3 m depth.

rate in this depth interval consisted of gently sloping granite rock. Below 5 m depth, *Fucus* was excluded due to lack of suitable substrate, as this consisted predominately of sand. Reference populations of *F. vesiculosus* in the southern Baltic Sea and at the Swedish west coast were sampled in June 2002 at 0.5–1 m depth, which corresponds to their maximum depth of abundance. Fifty reproductive individuals were collected within 2 m² at each site.

All sampled algae grew attached on rock. One individual was defined as the sum of all fronds emerging from the same holdfast, clearly separated from other holdfasts, and individuals were kept separate throughout the sampling. One vegetative apical tip from each individual was silica dried and stored for genetic analysis, and the remaining part was deep frozen and stored for morphological characterization. In the reference material, a random subset of 20 individuals in each population was analyzed morphologically.

Additionally, the sex ratio of each morphotype was determined for reproductive individuals collected at the Öregrund site and its vicinity (200 m along the shore) on four occasions in June and July 2004. The season of sampling corresponds to the main reproductive period for both morphotypes. Receptacles from 10 to 44 individuals of each morphotype and occasion were analyzed to determine the presence of either oogonia (female) or antheridia (male) in the conceptacles. On two occasions, receptacle morphology was characterized on 12 fertile individuals of the dwarf and common morphotypes, respectively. Egg size was calculated for individuals collected on one of the occasions (June 30), after egg release artificially induced by dehydration and rehydration of the ripe female receptacles.

Genetic analysis. The DNA was extracted from 10 mg of dried algal tissue using DNeasy Plant MiniKit (Qiagen, Valencia, CA, USA). Eluates of the first and second elutions were kept separately. Usually the second eluate (diluted 1:10 with water) was used in the PCR reaction. When the first eluate was used, it was diluted 1:200. We genotyped the samples using five microsatellite loci (L20, L38, L58, L85, L94) developed by Engel et al. (2003). The PCR reactions were performed in 12 µL of solution containing 2 µL of template DNA, 0.6 units of Taq polymerase (MBI Fermentas), 1 × PCR buffer (MBI Fermentas, St. Leon-Rot, Germany: 10 mM Tris-

HCl pH 8.0, 50 mM KCl, 0.08% Nonidet P40), 0.2 mM dNTP (Sigma, Manchester, UK), 2 mM MgCl₂, and 0.5 µM of each forward and reverse primers. Additionally, BSA was added to the PCR reaction mixture (final concentration, 0.2 µg/µL) when amplifying loci L58, L85, and L94. Amplification was performed as in Engel et al. (2003). Some 6 µL of PCR product was loaded on 8% acrylamide/bisacrylamide gels (ReproGel High Resolution, Amersham Pharmacia, Buckinghamshire, UK) and separated in an ALFexpress II DNA Analyser (combined electrophoresis and laser fluorescent detection system, Amersham Pharmacia). Sizing of the PCR fragments was done with ALFwin Fragment Analyser software (version 1.00, Amersham Pharmacia) based on 50-bp DNA step ladder and internal standards in each well. Additionally, two individuals with known genotypes were included on each gel to ensure repeatability of scoring.

To verify the supposed phylogenetic relationship between the dwarf morphotype and common *F. vesiculosus*, DNA sequences of the plastid-encoded RUBISCO region spanning the large subunit gene (*RbcL*), the small subunit gene (*RbcS*), and the spacer between the subunits were analyzed. The 627-bp region (668 bp with primers) was amplified using forward and reverse primers (forward: 5'-TTGTGGTCAAATGCATCACT-3'; reverse: 5'-AGCCCCCATAATTCCCAATA-3'). We designed primers based on published sequences of *Ascophyllum nodosum* (GenBank accession number AJ287853) and *F. vesiculosus* (AY157695 and AF346700). The amplified DNA was purified using QIAquick purification kit (Qiagen). Amplification primers were used for direct sequencing of the purified DNA on CEQ 8000 capillary sequencer (Beckman Coulter, Fullerton, CA, USA) following manufacturer protocol. Sequences were obtained for two individuals of the common *F. vesiculosus* and four individuals of the dwarf morphotype, all from Öregrund (CM and DM). Because all obtained sequences were identical, they were deposited in GenBank under the single accession number AY878074. Assembling and alignment of the sequences was done in SeqMan and MegAlign modules of the Lasergene software (DNASTAR, Madison, WI, USA).

Morphological characterization. Five morphological variables were measured on each individual: 1) frond length, from the base of the holdfast to the tip of the most distal tip; 2) stipe width, midway between the holdfast and the oldest

dichotomy: 3) frond width, midway between the youngest and next youngest dichotomy, average of three measurements; 4) midrib width, midway between the youngest and next youngest dichotomy, average of three measurements; and 5) distance between dichotomies, measured from the second oldest dichotomy and onward, average of five measurements. These variables have previously proven useful for morphological characterization of *F. vesiculosus* populations (Bäck 1993), and they are all independent of the reproductive stage of an individual. This enables comparisons and identifications using plants sampled in different seasons and reproductive modes. In addition to the five metric variables, the number of fronds per holdfast and the presence or absence of vesicles was noted, and the length, width, and thickness of three receptacles from each of the separately collected fertile individuals were measured.

Statistical analyses. Population differentiation (Raymond and Rousset 1995a) and departures from Hardy-Weinberg equilibrium were assessed using exact tests as implemented in GENEPOP (Raymond and Rousset 1995b). Cavalli-Sforza and Edwards' chord distances (1967) based on allele frequencies were used to construct the neighbor-joining tree with PHYLIP (Felsenstein 1993). This method was chosen because it performed best for reconstructing tree topologies based on microsatellite data in a simulation study by Takezaki and Nei (1996). Support for the tree nodes was assessed by bootstrapping the allele frequency matrix (1000 iterations). The relationship among individuals was assessed by factorial correspondence analysis (FCA) as implemented in GENETIX 4.04 (Belkhir et al. 2002).

The expected frequency of a multilocus genotype, P_{gen} , and the probability of observing n individuals of each multilocus genotype by chance in a sample of N individuals, P_{sex} , were calculated according to Ivey and Richards (2001). P_{gen} is the product of genotype frequencies at each locus expected from Hardy-Weinberg equilibrium, or equivalently, it can be expressed as the product of allele frequencies at each locus:

$$\prod_{i=1}^l (p_i q_i) 2^h$$

where l is the number of loci forming the multilocus genotype, p_i and q_i are the frequencies of alleles p and q at i th locus (p^2 if locus is homozygous), h is the number of loci that are heterozygous in a given multilocus genotype. P_{sex} is found as

$$P_{\text{sex}} = \sum_{x=n}^N \frac{N!}{x!(N-x)!} (P_{\text{gen}})^x (1 - P_{\text{gen}})^{N-x}$$

The morphometric variables were analyzed for their relative significance in species classification by discriminant analysis. The outcome was validated by reclassifying all individuals to their most probable populations, based on these two morphological variables, by the obtained classification function and jack-knifing as implemented in SYSTAT 10.2, SYSTAT Software Inc., Richmond, CA, USA.

Separate analyses of each morphological variable were performed by one-way analysis of variance with planned comparisons to test for differences between 1) the two dwarf morph populations, 2) the two common morph populations with highest difference in the concerned variable, and 3) the dwarf and common morph populations most similar in the concerned variable.

To achieve equal sample sizes, the morphological analyses were performed on subsets of the 20 tallest individuals from populations D1, DM, and CM. This also decreased the risk of ontogenetic effects on the results, because only reproductive individuals were included in the reference material. The variable *frond length* was used as a proxy for reproductive maturity, because most of the individuals from D1, DM, and CM did not

have any reproductive structures left at the time of sampling in late summer. Reproductive maturity is known to occur at a certain size in *Fucus* (Chapman 1995), usually from 15 to 20 cm length in marine *F. vesiculosus* (Knight and Parke 1950). No data on size at receptacle initiation was obtainable for Baltic *Fucus*, but the smallest individuals observed to still carry receptacles in the current D1, DM, and CM data sets had frond lengths of 17, 20, and 28 cm, respectively. The smallest individuals included in each subset of 20 had frond lengths of 20, 20, and 24 cm, respectively. This indicates that reproductively equivalent material was included in all comparisons.

RESULTS

Fucus radicans Lena Bergström et Lena Kautsky sp. nov.

Fucus vesiculosus L. *similis*, differt a haec species fronde brevior et tenuiore (2–5 mm); costa tenuiore (0.8–1.5 mm); cryptostomatibus paucis, in seriebus unicus insolitis distributis; habitu ramosiore; interdichotomis brevioribus (10–20 mm); vesiculis nullis.

Fucus radicans is similar to *F. vesiculosus* L. but is shorter with a thinner frond (2–5 mm wide); a thinner midrib (0.8–1.5 mm wide); cryptostomata few, randomly distributed in single rows; a more bushy appearance; a shorter distance between dichotomies (10–20 mm); no vesicles.

Habitat: Sublittoral, brackish water.

Distribution: Gulf of Bothnia (Baltic Sea).

Holotype: Sweden, Ångermanland, Nordmaling par., Järnäs Udde, depth 3 m, 30 August 2003, coll. Lena Bergström J16, 3 sheets (Swedish Museum of Natural History).

Paratypes: Sweden, Uppland, Öregrund, Smedjegatan: 16 June 2004, coll. L. Bergström IDS1; 8 July 2004, coll. L. Bergström 1–2 (Swedish Museum of Natural History).

Etymology: Named in recognition of its capacity of vegetative propagation.

Genetic differentiation. *Fucus vesiculosus* and *F. radicans* were highly genetically differentiated where they grew completely mixed (populations DM and CM of Fig. 3), with allele frequencies differing at all five microsatellite loci studied (exact probability test, all $P < 0.001$). Factorial correspondence analysis confirmed that *F. radicans* and *F. vesiculosus* represented separate gene pools, in that individuals from the two *F. radicans* populations (DM and D1), although geographically separated by 400 km, were genetically more similar to each other than sympatric individuals of the two different species (DM and CM) (Fig. 4).

An exception to the general pattern was that one individual, classified by morphology as belonging to *F. radicans*, grouped very nicely with *F. vesiculosus* on basis of the microsatellite genotype in the FCA (Fig. 4). For example, this individual was homozygous for alleles at loci L20 (171/171) and L94 (178/178), which were not encountered in any other individual classified as *F. radicans* but were common alleles in *F. vesiculosus*. The individual was not included in the original discri-

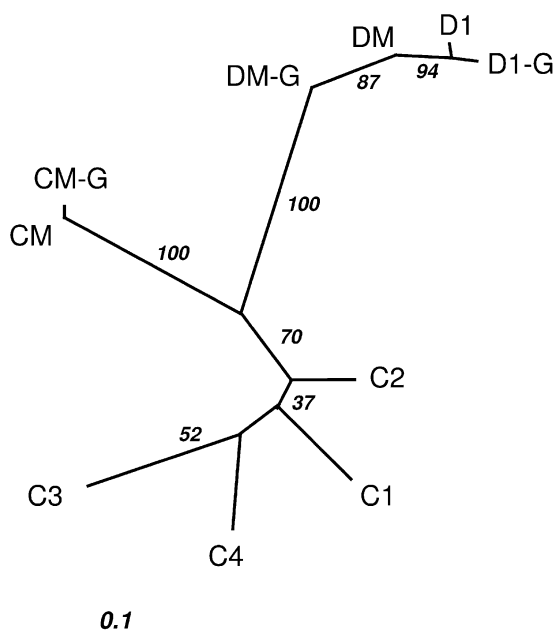


FIG. 3. Neighbor-joining tree showing genetic distances between studied populations. The position of populations with a high frequency of genetically identical individuals is shown for calculations based on all individuals (CM, DM, and D1) and when considering different genotypes only (CM-G, DM-G, and D1-G). D, Dwarf morphotype (*Fucus radicans*); C, common morphotype (*Fucus vesiculosus*).

minant analysis because it was not among the 20 tallest individuals in its population, but its morphological classification within *F. radicans* was verified by including it in a repeated analysis (data not shown). Remarkably, however, it had wounds similar to what is typically

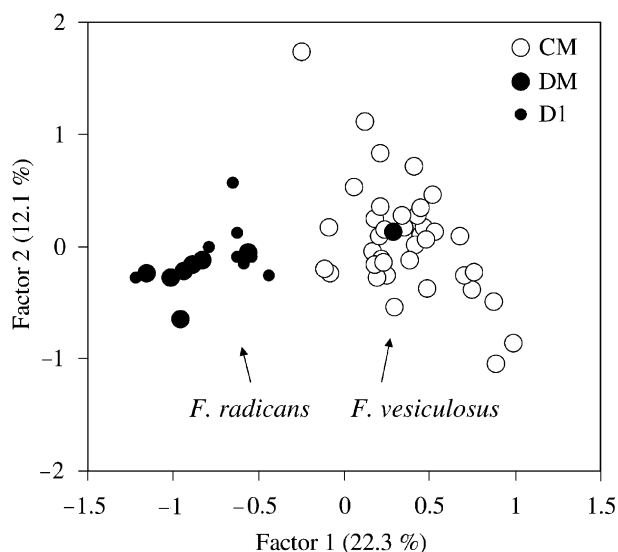


FIG. 4. Result of FCA based on allele frequencies of populations CM and DM (sympatric *Fucus vesiculosus* and *F. radicans*) and of population D1 (northern population of *F. radicans*). Loci used = L20, L38, L58, L85, and L94. Plot of individual genotypes only ($n = 57$).

the result of the shedding of receptacles in common *F. vesiculosus*, as were frequently observed on individuals of *F. vesiculosus* at the sympatric site but not on individuals classified as *F. radicans*. Additionally, one dwarf individual from population D1 was genetically unusual possessing alleles L58¹³² and L85¹¹⁴, which were not found in the other individuals included in the FCA analysis and which were rare or absent in the reference material of *F. vesiculosus* (Table 1). This individual caused compression of the remaining data points and was removed from the final FCA (Fig. 4) but kept in all other analyses. Its original position was at the far negative end of the two first axes.

A more detailed examination of the allele composition within populations showed that allele L38¹⁸⁷, with a frequency of 50% in the sympatric *F. radicans* population (DM), was not found in the population of *F. vesiculosus* with which it was sympatric. Similarly, allele L20¹⁶⁵ with a frequency >10% in *F. vesiculosus* was not detected in the *F. radicans* population of the same site, further supporting a conclusion of a gene flow barrier between the two taxa (Table 1). Overall, high genetic differentiation was observed among populations of *F. vesiculosus*, especially with respect to population CM at the mixed site, which represents the innermost limit of *F. vesiculosus* in the Baltic Sea (Fig. 3).

An unexpected result was the observation of clonality, that is, we found individuals that had identical multilocus genotypes. Clonality was most widespread in *F. radicans*, where 73% of the individuals in D1 and 83% in DM all had identical genotypes at all five loci, and of these, four were heterozygous. The probability of observing such high numbers of identical genotypes in sexually reproducing randomly mating populations is extremely low ($P < 10^{-10}$), which suggests that these are all members of the same clone. More detailed results on the observed clonality are presented by Tatarenkov et al. (2005). When multiply sampled clones were taken into account and only different genotypes were included in the analysis, genetic differentiation between *F. radicans* and *F. vesiculosus* at the sympatric site was significant at four loci (Table 2).

Analysis of the RUBISCO region showed that all six analyzed individuals (two *F. vesiculosus* and four *F. radicans* from the sympatric populations) had identical 627-bp long sequences (GenBank accession number AY878074). In comparison with GenBank sequences of *F. vesiculosus*, they differed at only two nucleotide positions from AF132474 (Lee et al. 1999) in the spacer region and at one nucleotide position from AF346700 (Pearson et al. 2001) in the small subunit gene (amino acid substitution Asp → Asn). The sequences were also similar to *F. spiralis* (four differences) and were markedly more distinct from *F. gardineri*, *F. evanescens*, and *F. serratus* (Fig. 5).

Morphological characterization. The general morphology of *F. radicans* is pictured in Figure 1. It is also pictured by Wærn (1952, fig. 75 Ab, Ba, Bb, pp. 170–1, as *F. vesiculosus* L.). Individuals of *F. radicans*

TABLE 1. Allele frequencies by microsatellite locus (L20, L38, L58, L85, L94) for the studied populations.

Allele	<i>Fucus vesiculosus</i>						<i>Fucus radicans</i>			
	C4	C3	C2	C1	CM	CM-G	DM	D1	DM-G	D1-G
L20										
N	45	45	41	40	44	34	48	48	8	11
138	0.044	0.000	0.000	0.000	0.091	0.029	0.000	0.000	0.000	0.000
150	0.056	0.011	0.024	0.000	0.000	0.000	0.000	0.000	0.000	0.000
156	0.178	0.100	0.220	0.038	0.068	0.074	0.438	0.531	0.188	0.591
159	0.011	0.000	0.085	0.113	0.000	0.000	0.010	0.021	0.063	0.091
162	0.089	0.278	0.232	0.088	0.216	0.177	0.531	0.448	0.625	0.318
165	0.156	0.322	0.012	0.163	0.102	0.132	0.000	0.000	0.000	0.000
168	0.044	0.044	0.049	0.163	0.000	0.000	0.000	0.000	0.000	0.000
171	0.022	0.078	0.329	0.288	0.523	0.588	0.021	0.000	0.125	0.000
174	0.400	0.167	0.049	0.138	0.000	0.000	0.000	0.000	0.000	0.000
177	0.000	0.000	0.000	0.013	0.000	0.000	0.000	0.000	0.000	0.000
L38										
N	45	45	49	46	46	36	48	45	8	11
184	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.000
187	0.400	0.256	0.367	0.457	0.000	0.000	0.500	0.433	0.500	0.313
208	0.556	0.578	0.245	0.250	0.467	0.403	0.010	0.000	0.063	0.000
211	0.000	0.000	0.020	0.022	0.000	0.000	0.000	0.000	0.000	0.000
217	0.000	0.011	0.143	0.217	0.000	0.000	0.000	0.000	0.000	0.000
220	0.000	0.011	0.000	0.011	0.000	0.000	0.000	0.000	0.000	0.000
226	0.044	0.144	0.214	0.043	0.533	0.597	0.490	0.567	0.438	0.688
L58										
N	47	48	50	50	47	37	48	48	8	11
122	0.000	0.000	0.010	0.010	0.000	0.000	0.000	0.000	0.000	0.000
124	0.032	0.000	0.080	0.000	0.096	0.122	0.000	0.031	0.000	0.091
126	0.298	0.760	0.290	0.450	0.745	0.770	0.490	0.510	0.438	0.545
128	0.074	0.177	0.140	0.260	0.074	0.014	0.000	0.010	0.000	0.045
130	0.596	0.063	0.320	0.270	0.085	0.095	0.510	0.438	0.563	0.273
132	0.000	0.000	0.150	0.010	0.000	0.000	0.000	0.010	0.000	0.045
136	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.000
L85										
N	44	48	48	48	48	38	48	48	8	11
114	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.000	0.045
116	0.250	0.000	0.198	0.073	0.010	0.013	0.000	0.000	0.000	0.000
118	0.227	0.188	0.188	0.313	0.740	0.750	0.510	0.458	0.563	0.364
120	0.489	0.760	0.615	0.469	0.198	0.171	0.490	0.531	0.438	0.591
122	0.034	0.052	0.000	0.146	0.052	0.066	0.000	0.000	0.000	0.000
L94										
N	27	43	45	48	48	38	47	46	7	9
157	0.000	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.000
172	0.296	0.023	0.422	0.573	0.438	0.368	0.979	1.000	0.857	1.000
178	0.667	0.919	0.511	0.292	0.563	0.632	0.021	0.000	0.143	0.000
181	0.037	0.000	0.056	0.083	0.000	0.000	0.000	0.000	0.000	0.000
187	0.000	0.058	0.011	0.042	0.000	0.000	0.000	0.000	0.000	0.000

For populations DM, CM, and D1, which had high frequencies of genetically identical individuals, allele frequencies are also shown when considering unique genotypes only (CM-G, DM-G, D1-G).

and *F. vesiculosus* were clearly morphologically separated by discriminant analysis, whereas populations of the same taxon overlapped in morphology (Fig. 6). Jackknifed reclassification correctly reassigned all individuals to species but was not able to distinguish populations within each species (Table 3). All studied morphological variables differed significantly between *F. radicans* and *F. vesiculosus* when considered separately, although some of them were obviously correlated (Fig. 7, Table 4). The first discriminant function reflected aspects of width, most importantly frond width (Table 5), which was a diagnostic trait to distinguish *F. radicans* and *F. vesiculosus* of all studied populations. The total range in frond width was 2.1–

TABLE 2. Genetic differentiation.

Locus	<i>Fr vs. Fv</i>	<i>Fucus radicans</i>	<i>F. vesiculosus</i>
L20	<0.001	0.024	<0.001
L38	<0.001	0.283	<0.001
L58	<0.001	0.281	<0.001
L85	0.195	0.408	<0.001
L94	0.003	0.187	0.092

Result of exact probability tests of differentiation between *Fucus radicans* and *F. vesiculosus* at the sympatric site (*Fr vs. Fv*), between the two studied populations of *F. radicans*, and between two populations of *F. vesiculosus* geographically separated by comparable distance. Analyses were based on unique genotypes, excluding clones, and were performed separately for each locus (values = *P*).

	RbcL->	80
Consensus(this study)	TCACCCTGATGGTATACAAGCAGGTGCTACAGCGAATCGTGTGCGTTAGAAGCTATGGTTTTAGCTCGTAATGAAGGTC	
vesiculosus-AF132474	
vesiculosus-AF346700	-----	
spiralis- AY246553	
evanescens-AY044263	-----	
gardneri-AF106516	
serratus-AY044264	-----	
Ascophyllum-AF106515G.....C.....G....	
		160
Consensus(this study)	GTGATTATGTTGGTGAAGTCTCTGAAATTTACGTACAGCTGCTAGTACTTGTGGACCATTAAAAGCAGCTTTAGATTTA	
vesiculosus-AF132474	
vesiculosus-AF346700	-----	
spiralis- AY246553C.....	
evanescens-AY044263	-----	
gardneri-AF106516G.....G.....G.....	
serratus-AY044264	-----	
Ascophyllum-AF106515G.....G.....	
		240
	spacer->	
Consensus(this study)	TGGAAAGATATTACTTTTGAATATACTTCAACAGATACACCTGATTTCACTGAAGTGGCAACTGAAAGTAACTAAATAAT	
vesiculosus-AF132474	
vesiculosus-AF346700	
spiralis- AY246553	
evanescens-AY044263	-----GT.....	
gardneri-AF106516	-----GT.....	
serratus-AY044264	-----GT.....	
Ascophyllum-AF106515	
		320
Consensus(this study)	ATATTAAAAGTAGTATAATGTTTACTATAAAGAAGATCAAAGTTAAGTTTAAATAAGATTTTCTTTATAGTAAAATTCT	
vesiculosus-AF132474	
vesiculosus-AF346700	
spiralis- AY246553	
evanescens-AY044263	-----	
gardneri-AF106516	-----	
serratus-AY044264	-----	
Ascophyllum-AF106515	...AGT...T...A...A...G.....C.....T.AG.....AAT.	
		400
Consensus(this study)	ATTAATTTTAAAAAATTTTAATACTTTACATTAAAGTTAAATAAATAAATGGTTTAGTAGTTAACTAAAAACAAAAAT	
vesiculosus-AF132474G.....	
vesiculosus-AF346700	
spiralis- AY246553T.....	
evanescens-AY044263A.....CA.....	
gardneri-AF106516A.....CA.....	
serratus-AY044264A.....CA.....	
Ascophyllum-AF106515	...G.A...T.....AA.....G.....	
		480
	RbcS->	
Consensus(this study)	TTCAGATTTATATTAAATAACATATTTGAAGAGTGATGAGACTTACACAAGGATGTTTTTCATTTTACCAGATTTAAGT	
vesiculosus-AF132474A.....	
vesiculosus-AF346700	
spiralis- AY246553A.....	
evanescens-AY044263A.....	
gardneri-AF106516A.....	
serratus-AY044264A.....	
Ascophyllum-AF106515	...T.....A.....	
		555
Consensus(this study)	GATGAGCAAATTAATAATTAATTATGCTATTTCAAAAAATGGGCTGTTAGTGTGAATGGACAGATGAT	
vesiculosus-AF132474	
vesiculosus-AF346700	A.....	
spiralis- AY246553	
evanescens-AY044263	-----	
gardneri-AF106516	-----	
serratus-AY044264	-----	
Ascophyllum-AF106515GG.....A.....	

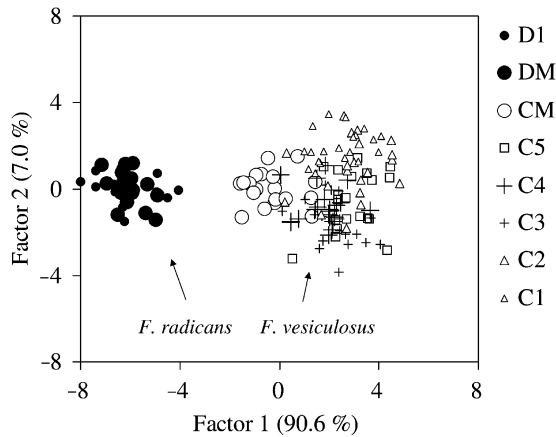


FIG. 6. Result of discriminant analysis of all populations based on five morphometric variables. Plot of discriminant function scores for each individual on the first two axes. Pillai's trace = 1.780, approx. $F = 11.9$, $df = 35, 755$, $P < 0.001$.

4.7 mm in *F. radicans* and 5.4–18.0 mm in *F. vesiculosus*. This also included the smaller individuals at sites D1, DM, and CM that were not used in the statistical analyses. The smallest individuals included in the total material had a frond length of 10.2, 6.5, and 7.5 cm and a minimum number of dichotomous branchings of 6, 5, and 1 (populations D1, DM, and CM, respectively).

The second function had the highest loading for the variable distance between dichotomies. This variable was correlated with frond length but was less variable among individuals within each population. Overall, the two populations of *F. radicans* were morphologically similar to each other, whereas the populations of *F. vesiculosus* were more variable (Figs. 6 and 7). Frond length was maximally 26 cm in *F. radicans* as compared with a maximum length of 83 cm in the *F. vesiculosus* populations included in this study. The presence of vesicles on the investigated fronds varied considerably among the populations of *F. vesiculosus* and was 100 % in C1–C2, 0% in C3–C4, 50% in C5, and 10% in CM. Vesicles were never observed in *F. radicans*.

We observed a striking difference in sex ratio between the two species. The sex ratio was even in *F. vesiculosus*, with 47% females (total $n = 116$), but the population of *F. radicans* consisted of 100% females (total $n = 98$). In fact, the only male individual of *F. radicans* that we observed at the Öregrund site was collected detached and adrift during a period of strong northern winds and probably was derived from some

TABLE 3. Jackknifed reclassification of populations by morphology, according to the classification functions obtained in a discriminant analysis.

Population	<i>F. vesiculosus</i>						<i>F. radicans</i>		
	C1	C2	C3	C4	C5	CM	D1	DM	% Correct
C1	17	2	0	1	0	0	0	0	85
C2	5	8	2	3	1	1	0	0	40
C3	0	0	13	4	1	2	0	0	65
C4	1	2	4	7	4	2	0	0	35
C5	4	2	3	1	10	0	0	0	50
CM	1	0	0	5	0	14	0	0	70
D1	0	0	0	0	0	0	14	6	70
DM	0	0	0	0	0	0	7	13	65
Total	28	14	22	21	16	19	21	19	60

more distant population. We did not study the sex ratio of the Järnäs population, but previous studies revealed a strong female bias also in the most northern populations, with 80%–86% females (Serrão et al. 1999a). The size range of *F. radicans* receptacles was $3.5\text{--}5.1 \times 2.6\text{--}4.4 \times 1.7\text{--}3.0$ mm (length \times width \times thickness). Corresponding values for sympatric *F. vesiculosus* were $4.9\text{--}9.3 \times 3.5\text{--}8.3 \times 1.0\text{--}2.7$ mm. Thus, the receptacles of *F. radicans* were shorter, less wide, but more spherical. The average proportions of the receptacles were 1.7:1.5:1 in *F. radicans* and 4.0:3.1:1 in *F. vesiculosus* (length:width:thickness). There was no difference in egg size, which was $14.2 \pm 1.7 \mu\text{m}$ in *F. radicans* and $14.3 \pm 1.5 \mu\text{m}$ in *F. vesiculosus* (mean diameter \pm SD, $n = 67$ in both species).

DISCUSSION

We observed a substantial genetic differentiation between the dwarf morphotype and common *F. vesiculosus* at the sympatric Baltic Sea site, indicating the presence of a gene-flow barrier between the two taxa. Furthermore, populations of the same morph revealed a coherent pattern of genetic variation, although widely dispersed populations of common *F. vesiculosus* were influenced by isolation-by-distance effects. Even if the dwarf morphotype to a large extent reproduces asexually, 16% and 22% of the individuals of the two populations had unique genotypes produced by sexual reproduction. From this we conclude that the dwarf morphotype represents an evolutionary lineage that is reproductively isolated from common *F. vesiculosus* and consequently should be considered a separate species (*Fucus radicans* sp. nov.).

The finding of clonality is exciting in itself but may somewhat complicate the taxonomic interpretation of

FIG. 5. Sequence alignment of RUBISCO region in species of *Fucus* and in the related fucoid *Ascophyllum nodosum*. First sequence obtained in this study is consensus of four individuals of *F. radicans* and two individuals of *F. vesiculosus*, which were identical. The remaining sequences are from GenBank; accession numbers are shown together with species names. Dots represent nucleotides identical to the first sequence; dashes indicate indels (insertions and deletions) and missing data. RUBISCO spacer is shown in italic. *RbcL* and *RbcS* are genes coding for large and small subunits, respectively. Only part of the nucleotide sequence that we obtained is shown, corresponding to the region studied by Lee et al. (1999). In the remaining part of the consensus there are no additional differences from *F. vesiculosus*, and there is one additional amino acid difference from *F. spiralis*.

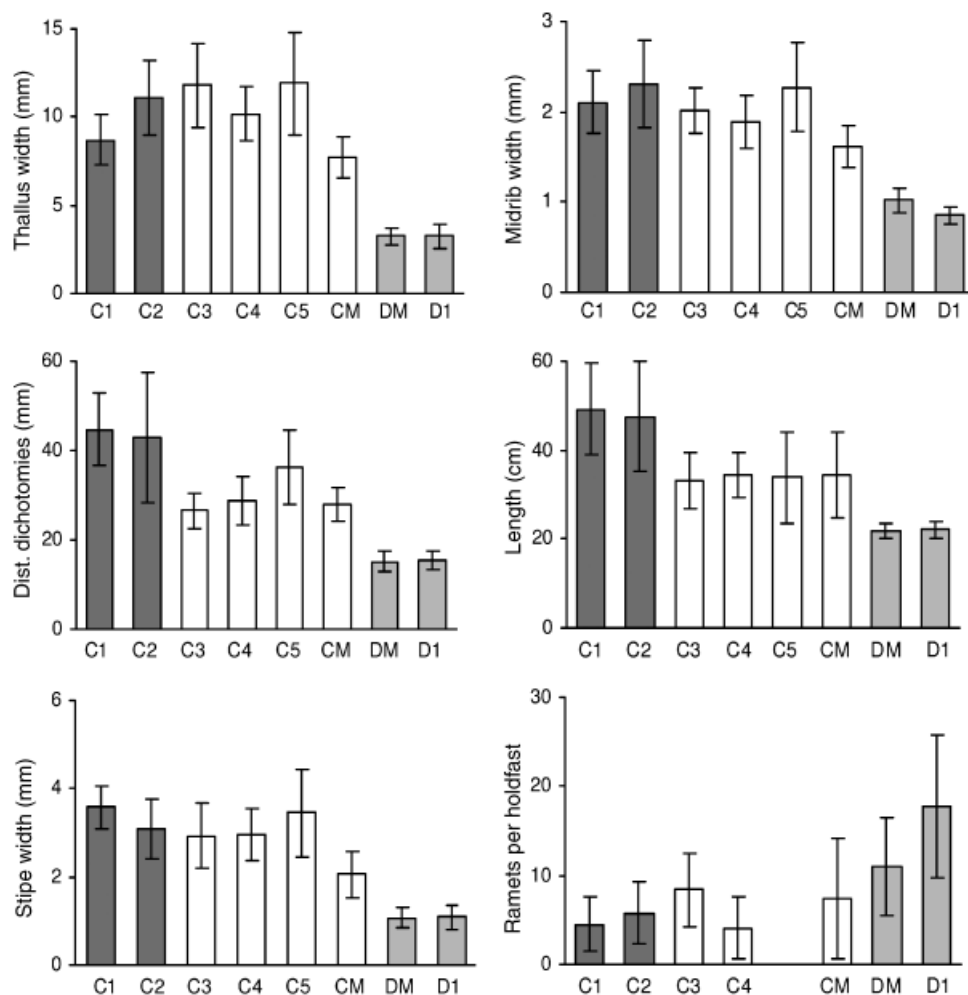


FIG. 7. Population means with SDs of the morphological variables. C1–C5 = *Fucus vesiculosus*, D1 = *F. radicans*, CM and DM = sympatric population of *F. vesiculosus* and *F. radicans*, respectively.

the results. An alternative explanation to the presence of two distinct species in the sympatric area would be that one or both morphs are recent immigrants from other populations that differ morphologically and genetically from the sympatric populations or from each other and that only asexual reproduction has occurred since they were established in the sympatric area. That is, if one or both of the morphs do not re-

produce sexually in the area of overlap, this would effectively prevent gene exchange between them. This “immigrant scenario” is, however, unlikely because it would involve massive recent migration from other sites, as mixed populations with approximately equal proportions of both morphs are observed over a distance of more than 200 km along the Swedish coast (Wærn 1952, Kautsky et al. 1992) and are also ob-

TABLE 4. Morphological differentiation.

	<i>Fv vs. Fr</i>			<i>Fucus radicans</i>			<i>F. vesiculosus</i>		
	Pop	F	P	Pop	F	P	Pop	F	P
Frond width	DM, CM	63.3	<0.01	D1, DM	0.00	1.00	C5, CM	56.0	<0.01
Midrib width	DM, CM	34.3	<0.01	D1, DM	2.84	0.09	C2, CM	46.0	<0.01
Stipe width	DM, CM	26.1	<0.01	D1, DM	0.04	0.84	C1, CM	60.2	<0.01
Dist. dichotomies	D1, C3	23.6	<0.01	D1, DM	0.01	0.91	C1, C3	62.7	<0.01
Frond length	D1, C3	19.0	<0.01	D1, DM	0.01	0.91	C1, C3	39.4	<0.01
Fronds per holdfast ^a	D1, C3	32.5	<0.01	D1, DM	16.75	<0.01	C3, C4	6.8	0.01

Result of one-way analysis of variance with planned comparisons of the morphologically most similar populations representing different species (*Fv vs. Fr*), the most dissimilar populations of *Fucus vesiculosus*, and the two studied populations of *F. radicans*. Analyses were performed separately for each variable DF, 1, 152; Pop, populations compared.

^aDF = 1, 133.

TABLE 5. Result of discriminant analysis.

Variable	Function 1	Function 2	Function 3
Fronde width	0.61	-0.68	0.51
Stipe width	0.49	-0.08	-0.67
Midrib width	0.30	0.19	-0.27
Dist. dichotomies	0.11	0.68	-0.33
Fronde length	0.18	0.40	0.89
Eigenvalue	14.11	1.09	0.20
Cum. % of total dispersion	0.906	0.976	0.989

Relative contribution of morphometric variables to the first three discriminant functions; coefficients standardized by mean variances. Analyses based on log-transformed data, $P < 0.001$.

served on the Finnish side of the Gulf of Bothnia (unpublished results). The FCA analysis also indicates that both morphs are clearly outlined genetically, which in case of the immigrant scenario would require that the origin of immigrants of each group was a single population. Therefore, according to the immigrant scenario, the influx of immigrants must be massive and from a localized area. The populations that got admixed must be well isolated before admixture to accumulate such high genetic differences. Moreover, at least one group should not be able at all to reproduce sexually in sympatry. Altogether, this seems a very unlikely scenario, and it is complicated by the presence of rather many unique genotypes ($> 16\%$). On the contrary, the assumption of two isolated gene pools (i.e. separate species) easily evades these dilemmas. Indeed, the observed pattern of morphological and genetic cohesiveness is expected if there is gene flow within but not between morphs.

The absence of intermediate genotypes or morphotypes in the studied material is, however, remarkable, because hybridization has long been observed within the genus *Fucus* (Burrows and Lodge 1953). Although an obvious deficiency of male individuals in *F. radicans* clearly may restrict the rate of sexual reproduction within this species, female *F. radicans* \times male *F. vesiculosus* crosses might be expected. Indeed, it has previously been observed that *F. radicans* does reproduce sexually up to its inner limit at 4 psu in the northern Baltic Sea; however, sexual reproduction is impeded by physiological problems due to an extremely low salinity (Serrão et al. 1999a). The occurrence of one individual morphologically classified as a *F. radicans* but genetically similar to *F. vesiculosus* may indicate weak hybridization between the two species. However, as this individual did not show the characteristics of a *Fucus* hybrid previously observed, such as intermediate morphology or genotype (Burrows and Lodge 1951, Coyer et al. 2002), it is more likely that it represented an artifact produced during analysis or an aberrant form of *F. vesiculosus*. Also, the absence of allele L38¹⁸⁷ in *F. vesiculosus* despite its high frequency (50%) in *F. radicans* in the mixed populations is in conflict with what would be expected even with a low level of hybridization. The mechanisms causing reproductive iso-

lation remain to be further clarified, because it is evident that both species occur completely mixed over large areas and are fertile at the same time in June and July. Even though the gametes of *F. radicans* are functional and fertilization may occur down to a salinity of 4 psu in the Baltic Sea, one explanation could be that hypoosmotic stress restricts the probability of interspecific hybridization (see also Serrão et al. 1999a).

The population of *F. vesiculosus* living in sympatry with *F. radicans* (CM) represents an ecologically as well as geographically marginal population of *F. vesiculosus* in the Baltic Sea. Marginal populations are commonly under stress from isolation, genetic drift, and odd selection regimes, leading to genetic divergence from central populations (Lesica and Allendorf 1995). It is thus not surprising that this population is strongly differentiated genetically from the other studied populations of *F. vesiculosus*. Indeed, the two other Baltic populations (C3 and C4) appeared genetically more similar to the west coast populations (C1 and C2) than to the geographically closer marginal population (CM). A decoupling of genetic and geographic distances may be further sustained if gene flow differs between areas, and this might be the case by a gradual decrease in sexual reproductive success with decreasing salinity in the Baltic Sea (Andersson et al. 1992, Serrão et al. 1996, unpublished data), also leading to increased genetic drift. This suggestion is supported by a lower genetic diversity in the marginal population of *F. vesiculosus* (CM) compared with the other populations (C1–C4). Thus, the apparent similar genetic distances between *F. radicans* and C1–C4 of *F. vesiculosus* on the one hand and CM and C1–C4 on the other hand is probably a consequence of strong genetic perturbation of the sympatric population of *F. vesiculosus* (CM) at the peripheral of the species' distribution and not a consequence of gene flow between the sympatric populations. Furthermore, if *F. radicans* is recently derived from a population of *F. vesiculosus* in the Baltic Sea, for some considerable time they will share common alleles (albeit at different frequencies) even though being reproductively isolated.

Sequences of the RUBISCO region in *F. radicans* were identical to *F. vesiculosus* from the sympatric site (Öregrund) and highly similar to marine *F. vesiculosus*, suggesting a very close phylogenetic relationship between *F. radicans* and Baltic *F. vesiculosus*. Previous studies of nuclear rDNA–internal transcribed spacer (Serrão et al. 1999b) have likewise found high similarity and paraphyletic relationships among nucleotide sequences representing *F. vesiculosus*, *F. spiralis*, *F. ceranoides*, *F. lutarius*, and *F. virsoides*, including *F. radicans* under the name of Northern Baltic Sea *F. vesiculosus* (AF 102914–8), which additionally suggests that *F. radicans* has a recent origin within this group. Also, sequences of a mitochondrial intergenic spacer from three individuals of *F. radicans* are identical to the sequence of *F. vesiculosus* individuals from the Finnish coast as well as to the main haplotype in *Fucus* populations from the European Atlantic coast (J. Coyer,

personal communication). Although the available information suggests that *F. radicans* is a result of a recent event of speciation from *F. vesiculosus*, a systematic study of sequence variation at highly variable genes is clearly necessary to determine time and place for the origin of *F. radicans* and the identification of the particular lineage of *F. vesiculosus* that gave rise to *F. radicans*.

We have excluded the possibility that *F. radicans* may be identical to any previously described species of *Fucus*, because its morphological and ecological characteristics do not conform to their species descriptions (von Linnaeus 1753, Powell 1963, Wynne and Magne 1991). The species that are potentially most similar to *F. radicans*, other than *F. vesiculosus*, are probably *F. spiralis* and *F. ceranoides*, which may both occur in areas of decreased salinity and are probably genetically close to *F. radicans* (Serrão et al. 1999b). *Fucus radicans* differs from these by always being dioecious, by having a distinctively thinner thallus, and by having rounded receptacles without a sterile rim (cf. *F. spiralis*) that are never pointed or dichotomized and a distinct midrib (cf. *F. ceranoides*). Further, these species do not occur in the Baltic Sea (Nielsen et al. 1995) and have probably been isolated from the *Fucus* populations of the inner Baltic Sea for a considerable period of time. The fact that *F. radicans* of the northern Baltic Sea is sympatric only with *F. vesiculosus* and is the only furoid present in large part of the Gulf of Bothnia also excludes the possibility that it may be a product of recurrent hybridization, which is increasingly recognized as an explanation to form variation in *Fucus* from more diverse areas (Wallace et al. 2004). *Fucus radicans* is neither similar to *F. cottonii* Wynne et. Magne (Wynne and Magne 1991) nor to the muscoides-like ecads of *F. vesiculosus* that appear in the Baltic Sea, sometimes referred to as *Fucus balticus* C. Agardh or *Fucus vesiculosus* var. *balticus* (Wærn 1952, Wynne and Magne 1991), as these have a much more minute morphology and do not have a holdfast. We do also not refer *F. radicans* to any of the taxonomic entities by Kjellman (1890), described for the Baltic Proper and represented within the reference populations of the present study. However, populations of *Fucus* that are potentially similar to *F. radicans* have been observed in the eastern Baltic Sea and in the White Sea (Ruuskanen and Bäck 2002).

In principle, two alternative origins may be considered for Baltic *F. radicans*. One is a pre-Baltic Sea origin followed by independent colonization of the Baltic Sea by *F. radicans* and *F. vesiculosus*, and the other is a much more recent origin within the Baltic Sea, from the Baltic Sea lineage of *F. vesiculosus*. If there is support for a pre-Baltic origin, the situation of Baltic *F. vesiculosus* and *F. radicans* may be similar to that of the two bivalves *Macoma baltica* and *Mytilus edulis*, which have had repeated transarctic invasions before the last (Weichselian) glaciation, and which both have genetic lineages present in the Baltic Sea that are separate from lineages dominating outside, or in the southern

part of the Baltic (Väinöla 2003). This alternative is supported by the fact that *F. radicans* in the Gulf of Bothnia shares habitat with two brown algae, *Sphacelaria arctica* Harv. and *Stictyosiphon tortilis* (Rupr.) Reinke sensu Rosenv., which have disjunct distribution patterns in the northeast Atlantic and are therefore conceived to be glacial relict taxa. That is, these populations would possibly represent early waves of colonization into the Baltic Sea by lineages with an arctic brackish-water affinity, now excluded from other northeast Atlantic areas (Wærn 1952, Snoeijs 1999). If, on the other hand, the dwarf species does have a Baltic Sea origin, this would demonstrate a very rapid rate of speciation (within the range of 8000 years) that is most likely accomplished by powerful forces of natural selection in a marginal environment and promoted by the presence of clonal reproduction fostering reproductive isolation. Available sequence data (RUBISCO gene) somewhat supports this alternative, because Baltic individuals of *F. vesiculosus* and individuals of *F. radicans* have identical haplotypes that are distinct from *F. vesiculosus* individuals outside the Baltic. Although the present data clearly show that two reproductively and morphologically distinct lineages of *Fucus* occur in the northern Baltic Sea, further studies are needed to clarify their evolutionary history in more detail. A parallel case might be seen in the red alga *Phycodrus rubens* (L.) Batters, which is represented by two reproductively isolated although morphologically similar sympatric species in the Baltic Sea and North Sea areas. These may have differentiated due to physical isolation during the last glaciation, by differential ecological selection along the salinity gradient, or by a combination of both factors (van Oppen et al. 1995).

The observation of clonal reproduction in *F. radicans* is remarkable, as fucoids have previously been considered to only reproduce sexually. Vegetative propagation is likely to occur by several means. First, new fronds may arise secondarily from an existing holdfast in both *F. vesiculosus* and *F. radicans* (Ruuskanen and Bäck 1999b, Malm and Kautsky 2003), and if such fronds are continually produced and get separated from each other in subsequent vegetative generations, this may plausibly result in physically separated clonal individuals on a scale of some meters. Second, adventive embryony was thoroughly documented in sporelings of *Fucus* by McLachlan and Chen (1972), who observed it to occur abundantly in their *Fucus distichus* L. subsp. *distichus*, hence giving rise to a bushy habit with no discernible main axis. This is a plausible development even for *F. radicans*, which is also characterized by a bushy habit, with up to 50 fronds observed to arise from the same holdfast in the present study. (We carefully avoided sampling from the same holdfast twice though, being aware of this possibility of clonal reproduction.) Third, vegetative propagation may be possible even over much longer distances, as loose vegetative parts of *F. radicans* may develop secondary rhizoids and reattach to a rocky substratum, thus acting as vegetative propagules (Tatarenkov et al.

2005). This capacity to reattach is mainly confined to adventitious branches, which are common in *F. radicans*. Interestingly, however, adventitious branches from northern populations of Baltic *F. vesiculosus* are also sometimes capable of reattaching to a substratum, although to a lesser extent (Tatarenkov et al. 2005). The observed shift to a predominantly asexual reproduction in *Fucus* toward areas of low salinity is in line with other observations in marginal environments for both plant and animal taxa (Eckert 2001, Billingham et al. 2003, Kearney 2003), including the Baltic Sea (von Wachtenfeldt 1969, Reusch et al. 2000, Gabrielsen et al. 2002, Bergström et al. 2003).

Our results suggest that fucoids of the Baltic Sea have evolved adaptive ecological characteristics to its low salinity environment by the advent of asexual reproduction in an environment where sexual reproduction is strongly impeded. In addition, asexual reproduction in itself is likely to enhance genetic isolation and possibly speciation. In future projects, there is a need to assess the reproductive and ecological characteristics of the dwarf species in much more detail, as well as its distribution and phylogeography. To date, *F. radicans* has only been tentatively subject to ecological studies (Raven and Samuelsson 1988, Serrão et al. 1996, Råberg 2004), and further characterization of its ecological function and importance is also of strong significance for management decisions in the species poor Baltic Sea ecosystem.

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