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The taxonomic and systematic relationships of several salt marsh Fucus taxa (Heterokontophyta, Phaeophyceae) within the Gulf of Maine and Ireland examined using microsatellite markers

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THE TAXONOMIC AND SYSTEMATIC RELATIONSHIPS OF SEVERAL SALT MARSH *FUCUS* TAXA (HETEROKONTOPHYTA, PHAEOPHYCEAE) WITHIN THE GULF OF MAINE AND IRELAND EXAMINED USING MICROSATELLITE MARKERS

BY

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ABSTRACT

THE TAXONOMIC AND SYSTEMATIC RELATIONSHIPS OF SEVERAL SALT MARSH *FUCUS* TAXA (HETEROKONTOPHYTA, PHAEOPHYCEAE) WITHIN THE GULF OF MAINE AND IRELAND EXAMINED USING MICROSATELLITE MARKERS

by

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The University of New Hampshire, May, 2005

The present thesis utilizes microsatellite markers to examine genetic affinities between several salt marsh *Fucus* ecads in order to ascertain their relationships with attached parental species. Chapter I provides an introduction to the genus *Fucus* and discusses morphological plasticity, systematic difficulties, and studies of hybridization between different *Fucus* species.

Chapter II describes my development of microsatellite markers for *Fucus*. Four polymorphic microsatellite loci were used to determine the origin of a dwarf muscoides-like *Fucus* from the Brave Boat Harbor (ME) salt marsh. Similar forms were originally described in Europe, and appear to be derived from *F. vesiculosus* L. However, my results indicate that dwarf *Fucus* populations from Brave Boat Harbor are largely comprised of hybrids between *F. vesiculosus* and *F. spiralis* L., and differ from morphologically similar European plants.

Chapter III presents the results of a larger Gulf of Maine survey that examined the genetic affinities of several estuarine taxa. My results support the hypothesis that the smallest limicolous muscoides-like forms are generally hybrids of *F. vesiculosus* and *F. spiralis*. However, somewhat larger muscoides-like forms that grade into the ecad *F. spiralis* ecad *lutarius* (Kützing) Sauvageau are composed of a mixture of hybrid and 'pure' genotypes, largely from *F. spiralis*. The relationships between *F. vesiculosus*, its variety *spiralis* Farlow and ecad *volubilis* (Hudson) Turner are also examined.

Chapter IV examines the affinities of the European dwarf taxon *Fucus cottonii* Wynne et Magne. Samples were collected from Rosmuc, Ireland, near the type location for this species, and genetic relationships were examined between *F. cottonii, F. vesiculosus*, *F. spiralis*, and a putative *F. vesiculosus x F. spiralis* hybrid. My results suggest that *F. cottonii* from Rosmuc is not of hybrid origin, but is affiliated with *F. vesiculosus*. In addition, the putative hybrid was genetically indistinguishable from *F. vesiculosus*, and may be equivalent to *F. vesiculosus* var. *spiralis* from the Gulf of Maine or *F. vesiculosus* var. *volubilis* (Hudson) Turner from Europe. Taken together, the work described in this thesis helps shed light on the relationships between several problematic groups of algae and resolves some taxonomic confusions that have plagued the genus *Fucus*.

CHAPTER I

A SURVEY OF TAXONOMIC PROBLEMS WITHIN THE GENUS *FUCUS* (HETEROKONTOPHYTA, PHAEOPHYCEAE) AND THE USE OF MOLECULAR APPROACHES FOR SYSTEMATICS STUDIES OF MARINE MACROALGAE

Summary

Traditionally, marine algae have been classified according to the morphological species concept (MSC), by which morphological discontinuities between groups are used to delineate species boundaries. However, the high levels of morphological plasticity displayed by many seaweeds have resulted in a large degree of taxonomic confusion under the MSC. The present chapter summarizes the difficulties caused by morphological plasticity within the genus *Fucus*, particularly as it applies to those forms lacking a holdfast (commonly termed ecads). Systematic difficulties at the inter- and intraspecific level within the genus are also discussed. Hybridization and introgression between different species of *Fucus* are believed to be the causes of some taxonomic problems within the genus based upon field and laboratory experiments. The environmental factors that give rise to ecad morphologies are discussed, and the taxonomic treatments that have been applied to these forms are summarized. Next, the advantages and limitations of microsatellite markers in resolving intra- and interspecific relationships are outlined, and the results of microsatellite developments for several groups of marine algae are presented. Finally, a description of all taxa studied in this thesis is provided.

Introduction

The ability to identify taxon boundaries has important practical implications for understanding ecosystems and biodiversity in terms of species management and conservation. Further, it is impossible to study processes such as speciation, hybridization, and gene flow without some understanding of the systematic relationships between the taxa involved. Within the algae (particularly macroalgae and diatoms), the most common approach to systematics has been to differentiate taxa based upon morphological discontinuities between them [the morphotype approach or "morphological species concept (MSC)" – Mathieson *et al.* 1981, Guiry 1992, John and Maggs 1997, Wattier and Maggs 2001]. In their discussion of algal systematics Wattier and Maggs (2001) note that the bias towards morphological discontinuity as a basis for taxonomy is strong enough that "...as far as we can determine, newly discovered sibling species have never been formally described on the basis of non-morphological characters." Such reliance upon morphology in delineating algal taxa has several advantages. Firstly, the MSC is relatively easy to apply where a properly described herbarium specimen is regarded as the type for the species. Secondly, the MSC is applicable in cases where the biological species concept of Mayr (1963) fails, such as the relatively large number of algal species that reproduce asexually or by selfing. Finally, it is comparatively easy to gather morphological data from a large number of individuals and sampling is not an intrinsically destructive process (Mathieson *et al.* 1981). However, the morphological species concept has several drawbacks. For example, it fails to distinguish cryptic species, even when they may be reproductively isolated from other conspecifics (Guiry 1992, Sosa and Lindstrom 1999, Wattier and Maggs 2001). Problems also arise with species that display bi- or triphasic life histories, each possessing their own distinctive morphology (Dring 1974, Moss 1974, Mathieson *et al.*, 1981). Further, phenotypic responses to environmental or genetic factors can mask diagnostically important characters within the same species. Conversely, the high levels of phenotypic plasticity displayed by some species can make it difficult to distinguish morphological differences that are assumed to separate species under this concept (Russell 1978, Mathieson *et al.* 1981, Norton and Mathieson 1983, Prud'Homme van Reine *et al.* 1996, Wattier and Maggs 2001, Durand *et al.* 2002, Schaeffer *et al.* 2002). Partly for these reasons, there has been a trend towards the use of biochemical and genetic techniques to delineate taxonomic and systematic relationships among many groups of algae. While molecular approaches have become increasingly popular, several workers have pointed out that they should complement rather than replace other investigative methods, such as culture studies, common garden and reciprocal transplant studies, and morphometric analyses (Mathieson *et al.* 1981, Norton and Mathieson 1983, John and Maggs 1997, Wattier and Maggs 2001). However, with this important caveat in mind, there is no doubt that the application of molecular methods has greatly increased our understanding of algal systematics, particularly at the generic and intraspecific levels.

The current chapter gives a general introduction to the research presented in this thesis. First, several problems in the taxonomy and systematics of the brown algal genus *Fucus* L. are discussed, with particular emphasis to those resulting from the large degree of phenotypic plasticity within the genus. The results of several morphological and genetic experiments related to the intra- and interspecific systematics of *Fucus* are also summarized. Secondly, the ecology and systematics of salt marsh forms are discussed, and descriptions of the taxa examined in this study are provided. Finally, the advantages and limitations of microsatellite markers as tools for population genetic research are considered, along with a summary of their use in marine algae.

Morphological Variation and its Causes in Fucus

The genus *Fucus* (Heterokontophyta: Phaeophyceae) is comprised of at least six species (Powell 1963) that occur commonly within the intertidal and shallow subtidal zones as well as in salt marshes throughout the northern hemisphere. Fucoid algae are important components of their constituent ecosystems; thus, several studies of *Ascophyllum* Stackh. (a sister genus of *Fucus)* have estimated their biomass and primary productivity (Brinkhuis 1976, Chock and Mathieson 1976, Cousens 1984). In addition, both open coastal and salt marsh fucoids provide food and shelter for other marine organisms (Fritsch 1959). Peters *et al.* (2004) note that *Fucus* species are economically important and commonly used in genetic, ecological, and cell biology studies. In addition, Prud'Homme Van Reine *et al.* (1996) suggest that *Fucus* may be well suited as an indicator species for biodiversity studies.

Species of *Fucus* are differentiated by a variety of morphological and sexual criteria (Fritsch 1959, Powell 1963, Rice and Chapman 1985, Leclerc *et al.* 1998, Serrão *et al.* 1999a – also cf. Table 1). Different species also display distinct ecologies, such as their zonation along the shore. For example, *F. vesiculosus* L. tends to occur within the mid intertidal to shallow subtidal zones, whereas *F. spiralis* L. is generally found within the upper intertidal zone. The differences in zonation appear largely due to variable resistance to desiccation (Fritsch 1959) and the differing abilities of species to regain full photosynthetic activity after periods of emersion (Dring and Brown 1982, Chapman 1995). Species of *Fucus* share a common body plan, consisting of a discoid holdfast that serves to attach the plant to rocks or other solid substrata and a thallus with a central midrib that branches into individual fronds. Terminal reproductive structures (receptacles) are present seasonally. Figures 1a and 1b provide photographs of *F. vesiculosus* and *F. spiralis*.

As with many seaweeds, species of *Fucus* display high levels of morphological plasticity. For this reason, and despite the existence of diagnostic morphological characters, various authors have noted much taxonomic confusion associated with *Fucus* at both the inter- and intra-specific level (Baker and Bohling 1916, Burrows and Lodge 1951, Fritsch 1959, Powell 1963, Chapman and Chapman 1973, Russell 1978, Marsden *et al.* 1983, Pérez-Ruzafa and García 2000). The extensive levels of morphological variation found in *Fucus* are due in part to environmental factors, including variability of wave exposure, salinity, desiccation, temperature, and light availability (Baker and Bohling 1916, Naylor 1936, Fritsch 1959, Russell 1979, 1986, 1987, Mathieson *et al.* 1981, Norton *et al.* 1982, Norton and Mathieson 1983, Kalvas and Kautsky 1993, 1998, Chapman 1995, Munda and Kremer 1997, Ruuskanen and Bäck 1999, 2002). Probably the most widely accepted species taxonomy for *Fucus* was created by Powell (1963), who grouped more than one hundred previously described taxa into six species (*F. ceranoides* L., *F. serratus* L., *F. spiralis* L., *F. vesiculosus* L., *F. distichus* L. and *F. virsoides J.* Ag.). Several other taxa have since been accorded species status by various authors [for example *F. cottonii* Wynne *et* Magne 1991[1], and *F. evanescens* C. Ag. (1820) by Rice and Chapman 1985].

Intraspecific morphological variation in *Fucus* may be considerable. While some studies have found a direct correlation between environmental gradients and phenotypic response (Kalvas and Kautsky 1993, 1998, Chapman 1995, Ruuskanen and Bäck 1999, 2002), it also appears that genetic factors are involved. For example, Munda and Kremer (1997) used morphometric characters and population analysis to examine relationships within *F. vesiculosus* on Helgoland Island. They found that vesiculated and evesiculated forms grew side by side in a patchwork mosaic and concluded that ecological factors alone were not sufficient to explain the observed morphological differences. Rice and Chapman (1985) observed a similar patchwork of morphologies based upon the analysis of thirty-nine characters in *F. distichus*; hence, they argued that these populations consisted of two distinct species, *F. distichus* and *F. evanescens* C. Ag., with the latter often being regarded as a subspecies of the former. Several studies have examined the presence of two distinct morphologies of *F. spiralis* occurring as mixed stands [*F. spiralis* and *F. spiralis* f. *nanus* (Stackhouse) Børgesen] using both morphological characters and pyrolysis mass spectrometry (Anderson and Scott 1998, Scott *et al.* 2000, 2001). Such studies have concluded that the distinct morphologies found in these stands were best described taxonomically as formae (implying genetic differences) rather than environmentally induced morphotypes.

Morphological plasticity has also resulted in taxonomic confusion at the interspecific level within *Fucus*. The common occurrence of 'intermediate' morphologies

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[1] *F. cottonii* Wynne *et* Magne = *F. muscoides* Feldmann *et* Magne (Feldmann and Magne 1964).

where different species of *Fucus* grow together has long been recognized. Such individuals are often regarded as interspecific hybrids (Thuret 1854, Sauvageau 1908a, Kniep 1925, Burrows and Lodge 1951, Boney 1966, Niemeck and Mathieson 1976, Bolwell *et al.* 1977, Evans *et al.* 1982, Scott and Hardy 1994, Hardy *et al.* 1998, Coyer *et al.* 2002a), although the high degree of variation found within *Fucus* species has made confirmation using morphological characters difficult. Hybrid plants tend to be found in narrow bands between different species of *Fucus* or in areas subject to recent environmental disturbance where the previous fucoid species have been displaced (Burrows and Lodge 1951, Boney 1966). Based upon these observations several workers have hypothesized that hybrids may be unable to effectively compete with parental forms under ordinary circumstances (Sauvageau 1909, Kniep 1925, Burrows and Lodge 1951, Boney 1966).

Although difficult to demonstrate morphologically, several lines of evidence support the likelihood of natural hybridization between different species of *Fucus*. Thuret (1854) produced hybrids by crossing *F. serratus* (male) and *F. vesiculosus* (female), and a variety of other workers have since carried out interspecific crosses with varying success (see Mathieson *et al.* 1981 and Coyer *et al.* 2002b for specific details). A cytological analysis of several *Fucus* species by Evans (1962) concluded that "… uniformity in chromosome number, size, and appearance in the various species may help to explain the ease with which hybrids are formed". In this case, it would be expected that chromosomal similarities would aid in introgression rather than hybridization *per se* (also see Lewis 1996). Culture experiments by McLachlan *et al.* (1971) using four species of *Fucus* [*F. distichus, F. serratus, F. edentatus* (synonymous with *F. evanescens*), and *F.*

vesiculosus] led these workers to conclude that morphological variation in *Fucus* was largely due to interspecific hybridization. In contrast to earlier workers (Stomps 1911, Burrows and Lodge 1951), Bolwell *et al.* (1977) found that interspecific barriers to fertilization between *Fucus* species exist. However, such barriers apparently weaken with the age of the egg. In addition, several studies have noted that the eggs of *Fucus* release a sperm attractant that can act inter-specifically (Boney 1966, Green *et al.* 1990). Serrão *et al.* (1996) and Berndt *et al.* (2002) found that gamete release in some species of *Fucus* is inhibited by turbulent conditions, while Berndt *et al.* (2002) speculated that delayed gamete release due to stormy conditions could promote hybridization.

Within the last decade several studies have attempted to examine hybridization in *Fucus*. Scott and Hardy (1994) conducted morphological analyses similar to Burrows and Lodge (1951) and reported the presence of hybrids between *F. vesiculosus* and *F. spiralis* in a narrow band between the zones occupied by each species. Hardy *et al.* (1998) confirmed the identity of putative hybrids between *F. vesiculosus* and *F. spiralis* using pyrolysis mass spectrometry (also see Russell 1995). Finally Coyer *et al.* (2002a) confirmed the presence of hybrids between *F. serratus* and *F. evanescens* in a zone of secondary contact using microsatellite markers[2]. In addition, hybridization has now been documented for several groups of brown algae (particularly the Laminariales) using several approaches, including artificial crosses (Lewis and Neushul 1994, 1995), and genetic markers such as the internal transcribed spacer (ITS) region of the ribosomal

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[2] Secondary contact is probably the most widely accepted mechanism for the formation of hybrid zones. In this scenario, a population becomes geographically subdivided and one or more of the subpopulations undergo allopatric speciation. The geographic regions in which the new species subsequently co-occur after speciation are thus referred to as zones of secondary contact. Reviewed in Hewitt (1988).

cistron (Liptak and Druehl 2000, Kraan and Guiry 2000a) and the ribulose bisphosphate carboxylase/oxygenase (RuBisCo) spacer region (Kraan and Guiry 2000b).

Interestingly, the presence of a large number of transitional forms and their apparent ability to hybridize led Stomps (1911) to propose that *F. spiralis*, *F. vesiculosus*, and *F. ceranoides* were simply different forms of the same species. However, this view has not gained widespread acceptance (Burrows and Lodge 1951, Powell 1963). Nonetheless, Burrows and Lodge (1951) acknowledged that the ability of these species to interbreed implied that barriers to the creation of a single polymorphic species were largely due to ecological differentiation (a hypothesis that was subsequently called into question by Bolwell *et al.* 1977, see above). More recently, Serrão *et al.* (1999a) confirmed the close evolutionary relationship between *F. ceranoides, F. spiralis*, and *F. vesiculosus* using the ITS region; they also found that these three species formed a well-supported clade along with *F. virsoides* J. Ag., but that sequence variation in the ITS region was not able to resolve relationships between them (also see Leclerc *et al.* 1998). Other studies have distinguished between closely related *Fucus* species using several approaches, including pyrolysis mass spectrometry (Hardy *et al.* 1998), cellulose acetate isozyme electrophoresis (Hull *et al.* 2001), and a combination of nuclear, mitochondrial, and chloroplast markers (Coyer *et al.* 2002a).

Causes of Morphological Variation Amongst Salt Marsh Fucus

All of the *Fucus* species initially classified by Powell (1963) are saxicolous (i.e., they grow attached to rock). However, two species (*F. spiralis* and *F. vesiculosus*) may give rise to detached salt marsh forms lacking a holdfast. Such detached forms are often referred to as ecads, which emphasizes the strong influence of environmental factors upon their morphology (Baker and Bohling 1916).[3] For the present discussion, an ecad may be defined in the sense of Clements (1905) as morphological variability due to habitat (see Chapter IV for further details). Salt marsh ecads may be either entangled or loose-lying (i.e. lying directly on the muddy banks of estuaries or entangled among the bases of marsh plants such as *Spartina* spp.) or embedded (with the base of the plant being partly buried by mud or silt).[4] Ecads may arise *via* fragmentation of attached plants by several means, including ice rafting, herbivory, and wave action, etc. (Chock and Mathieson 1976, Norton and Mathieson 1983). Fragments deposited in a suitable habitat will grow, proliferate, and potentially produce more fragments. Alternatively, a zygote may become attached to an object that is insufficient to provide anchorage (a small stone or shell fragment) or is ephemeral (i.e., a rotting piece of wood or the roots or rhizomes of other salt marsh flora), leading to subsequent loss of a holdfast and detachment (Baker and Bohling 1916, Fritsch 1959, Boney 1966, Chapman and Chapman 1973, Norton and Mathieson 1983). Finally, a study by Torrey and Galun (1970) using *F. vesiculosus* demonstrated that rhizoid formation was inhibited by high salinities; Norton and Mathieson (1983) have suggested that this could also give rise to detached forms in salt marshes.

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[3] *Fucus ceranoides* is a species that is restricted to inner estuarine sites near freshwater sources (Chapman 1995). While it also gives rise to some ecad forms (Baker and Bohling 1916), it does not occur within the Gulf of Maine and is therefore not treated here.

[4] The term embedded is synonymous with the term limicolous (buried within mud), and they are used interchangeably in this thesis.

While it has been emphasized that fucoid ecads are all derived from attached forms (Fritsch 1959), their morphologies are often very different than those of 'parental' species. Fucoid ecads are characterized by a lack of a holdfast, spiraling of the thallus, extensive proliferation, a trend towards reduction in size, a loss of sexual reproduction, and enhanced vegetative proliferation (Baker and Bohling 1916, Fritsch 1959, Chapman and Chapman 1973, Boney 1966, Norton and Mathieson 1983). The possible causes of detachment (lack of a holdfast) of *Fucus* ecads were reviewed in the preceding paragraph, while the environmental factors that may result in the other morphological alterations described are discussed below.

A high degree of spiraling or curling of the thallus tends to be commonly observed in *Fucus* ecads that grow entangled amongst the bases of salt marsh plants such as *Spartina* Schreb. [e.g., *F. vesiculosus* ecad *volubilis* (Hudson) Turner], while spiraling is less pronounced or absent in smaller embedded forms (i.e. muscoides-like or dwarf limicolous *Fucus*). Baker and Bohling (1916) concluded that spirality was caused by salinity, differential nutrient access, and growth by the side of the plant actually in contact with the underlying muddy substrata. Alternatively, in their review of detached seaweeds Norton and Mathieson (1983) propose spirality as a response by a plant to a constantly changing orientation. Proliferation (extensive branching of the thallus) may result from damage to the plant (Norton and Mathieson 1983). Baker and Bohling (1916) observed that proliferation tended to occur where the thallus was subject to some degree of burial as well as during the winter. Dahl (1971) showed that burial and damage to the brown algae *Zonaria farlowii* Setchell and Gardner resulted in enhanced proliferation.

A trend towards reduction in size has been very generally observed for *Fucus* ecads (Baker and Bohling 1916, Fritsch 1959, Boney 1966, Chapman and Chapman 1973, Niell *et al.* 1980, Norton and Mathieson 1983). As noted by Norton and Mathieson (1983), *Fucus* ecads tend to be found in the high intertidal zone, where many seaweeds (including those with a holdfast) exhibit slow growth and reduction in size (Norton *et al.* 1981) due to factors such as increased exposure to air. Baker and Bohling (1916) stated that reduction in thallus length was caused solely by exposure, whereas narrowing of the thallus was caused by low salinities. They hypothesized that the combination of these two factors produced a morphological/ecological gradation from entangled to embedded forms along these two environmental gradients. Similarly, Brinkhuis (1976) observed that gradation in size between two ecads of *Ascophyllum* correlated with vertical position on shore, with smaller forms occurring higher within the intertidal. Reduction in size is especially frequent among embedded forms where gradual sedimentation results in partial burial of the detached thallus followed by basal decay and rotting. When such burial reaches a dichotomy it will ultimately separate the thallus into two plants, a process sometimes referred to as dichotomic splitting (Den Hartog 1972, Norton and Mathieson 1983). When combined with extensive proliferation, dichotomic splitting can give rise to the dense carpet-like patches that are a common feature of the smallest *Fucus* ecads (Figure 1).

The loss of sexual reproduction is nearly universal among *Fucus* ecads. While several workers have reported receptacles on detached *Fucus* plants (Sauvageau 1908a, 1915, Cotton 1912, Baker and Bohling 1916, Skrine *et al.* 1932, Lynn 1935), others note that the gametes from these plants are often inviable (Gibb 1957, Norton and Mathieson

1983). Further, several workers (Gibb 1957, Chock and Mathieson 1976) have reported that receptacles are often found on recently detached plants, and conclude that receptacle initiation in these cases likely occurred prior to detachment. Thus, it appears that initiation of receptacle formation is inhibited in detached ecads. While the specific causes of reduced sexual reproduction have not been identified, various causal factors have been suggested, including high humidity (Baker and Bohling 1916), decreased nutrient uptake or irradiance, exposure to biologically produced inhibitors, and reversion of the plant to a juvenile state (reviewed in Norton and Mathieson 1983). However, the latter authors point out that the clonal reproduction of ecads is an efficient way to propagate copies of a genotype with proven survival ability for a particular environment (also see Smith and Walters 1999).

Systematic and Taxonomic Difficulties of Salt Marsh Fucus Ecads

Previous studies of *Fucus* ecads have been largely confined to Europe, where there have been conflicting treatments of their taxonomy (Sauvageau 1908a, b, 1923, Baker and Bohling 1916, Feldmann and Magne 1964, Niell *et al.* 1980, Wynne and Magne 1991). Difficulties in developing a generally acceptable taxonomy are largely the result of four factors: 1) The somewhat arbitrary delineation of taxa resulting from a morphological continuum between different forms; 2) The possibility that similar ecad morphologies may be derived from multiple parental species (i.e., convergence of morphology); 3) Disagreements over the significance of specific character states, and; 4) Failure to provide evidence to justify changes in taxonomic ranking. All four problems are discussed below.

The morphological gradations of *Fucus* ecads are even more pronounced than those found in saxicolous taxa; hence, they are more difficult to treat taxonomically. The most comprehensive attempt to simplify conflicting taxonomies was carried out by Baker and Bohling (1916) during an extensive survey of detached/embedded *Fucus* forms in British salt marshes. They regarded all estuarine *Fucus* taxa that lacked a holdfast to be derived from *F. vesiculosus*, with the sole exception of *F. ceranoides*. Their conclusions were based upon the presence of a morphological continuum connecting even the smallest embedded fragments to attached *F. vesiculosus via* intermediate entangled plants and the usual dioecious state of the receptacles when present. For convenience, Baker and Bohling established three taxa, *F. vesiculosus* ecads *volubilis, caespitosus,* and *muscoides* Baker *et* Bohling. Differences between these ecads were based largely on decreases in size, spirality, and vesiculation of the thallus, as well as a loss of receptacles, grading from ecad *volubilis* through ecad *muscoides*. However, transitional morphologies exist at the boundaries of these designated groups that cannot be easily classified. Mathieson and Dawes (2001) reported a similar situation in a Maine estuary, with numerous transitional forms linking a muscoides-like dwarf *Fucus* to *F. spiralis* ecad *lutarius* (Kützing) Sauvageau (Figure 1). The importance of morphological gradation as it affects ecad systematics has also played a role in disputes over the taxonomic status of the dwarf limicolous *Fucus* described from European salt marshes, which has been regarded by various workers as a variety (*F. vesiculosus* var. *muscoides*, Cotton 1912), ecad (*F. vesiculosus* ecad *muscoides*, Baker and Bohling 1916, Niell *et al.* 1980) or distinct species (*F. cottonii*, Feldmann and Magne 1964, Parke and Dixon 1976, Wynne and Magne 1991).

Convergence of morphology is also an issue in ecad systematics. For example, the muscoides-like *Fucus* reported by Mathieson *et al.* (2001) and Mathieson and Dawes (2001) is very similar to the ecad *muscoides* of Baker and Bohling (1916), although the two forms are apparently derived from different species. Further, the channel wrack *Pelvetia canaliculata* L. also appears to give rise to dwarf morphologies resembling ecad *muscoides* and the muscoides-like *Fucus* (Skrine 1928, Carter 1933).

Disagreements regarding the value of specific character states have also plagued the classification of *Fucus* ecads. For example, in his studies of *Fucus* ecads, Sauvageau recognized two species, *F. lutarius* Kützing (which lacked vesicles) and *F. volubilis* Hudson (a vesiculated form) and argued that the former was most likely affiliated with *F. spiralis* (Sauvageau 1908a, b, 1923). However, Baker and Bohling (1916) placed *F. lutarius* in their ecad *volubilis*. Both arguments hinged largely on the weight given to the sexual state of the receptacles. Baker and Bohling (1916) argued that the dioecious state of the receptacle favored an origin from *F. vesiculosus*. By contrast, Sauvageau (1907, 1908a, 1915) argued that as the receptacles were always female, this condition probably resulted from reduction of the receptacle from a hermaphroditic to a dioecious state due to environmental factors with a consequent loss of males. As several workers have proposed that the sexual state of the receptacles in the only conclusive diagnostic trait between *F. vesiculosus* and *F. spiralis* (Burrows and Lodge 1951), this debate highlights the difficulty of classifying such variable forms and demonstrates the need for systematic assessments based upon combinations of morphological, non-morphological (i.e. chemical and/or molecular), and transplant data where applicable (see below).

Finally, transfers in taxonomic ranking are sometimes carried out with little or no supporting evidence. Russell (1987) and Norton and Mathieson (1983) cite several instances of such transfers in fucoid ecads, such as the elevation of Baker and Bohling's ecad *volubilis* to ecotype status by Davy de Virville (1944, reviewed in Norton and Mathieson 1983) or the confusion over the taxonomic status of the dwarf limicolous *Fucus* discussed by Norton and Mathieson.[5] In the latter example, Norton and Mathieson point out that transplant experiments that might have shed light on the issue were never carried out. The potential value of transplant experiments for algal taxonomy was discussed by Wattier and Maggs as well (2001) who also noted the current unpopularity of this approach. The utility of transplant and common garden experiments for the taxonomy of *Fucus* ecads has been recently demonstrated by Mathieson and Dawes (2001).

Microsatellite Markers and their Utility in Marine Algae

Microsatellites are short, tandemly repeated nucleotide motifs (often defined as ≤ 8 bases in length). Mutation generally occurs by polymerase slippage during DNA replication that results in the gain or loss of repeat units, although mutations in the region flanking the repeat or within the repeat itself (leading to 'interrupted' motifs) may also occur (cf. Jarne and Lagoda 1996, Ellegren 2000, Schlötterer 2000, Ortí *et al.* 1997). Thus, loci containing microsatellites tend to be hypervariable as compared to conventional genetic markers; hence, microsatellites are often preferred for the study of

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[5] In the case discussed by Norton and Mathieson (1983), the ecad *Fucus vesiculosus* ecad *muscoides* was raised to species status without comment by Parke and Dixon (1976).

population structure and other microevolutionary processes. The utility of microsatellite markers in resolving species and population level questions has been demonstrated for many organisms, including insects (Freiburger *et al.* 2004), gymnosperms (Vendramin *et al.* 1998, Marquardt and Epperson 2004), fish (Lu *et al.* 2001, Bernatchez *et al.* 2002, Shaw *et al.* 2004), fungi (Bucheli *et al.* 2001), and mammals (Domingo-Roura 2002).

The potential utility of microsatellite markers for resolving taxonomic and systematic issues within marine algae is increasingly recognized (cf. Wattier and Maggs 2001). Microsatellites possess several advantages over other molecular techniques commonly used for population or species level studies such as restriction fragment length polymorphism (RFLP), randomly amplified polymorphic DNAs (RAPDs), or amplified fragment length polymorphism (AFLP). Microsatellite markers are co-dominant, simple to score, permit high-throughput analysis, and results are easily reproducible within and between laboratories. Also, unlike RFLP, RAPDs, and AFLP they are unaffected by the small amounts of contaminating DNA often present in DNA isolations from marine algae (Wattier and Maggs 2001). However, there are also several potential problems associated with microsatellites, most of which may result in the underestimation of the actual number of heterozygotes. For example, stutter effects may arise from DNA polymerase slippage during PCR, making it difficult to detect heterozygotes whose alleles differ by only one or two repeats.[6] Stutter effects tend to be most severe with dinucleotide repeats and become progressively less obtrusive as the repeat length increases. A second problem

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[6] "Stutter" refers to artificial peaks seen during an electrophoresis run that are ± 1-2 repeat units < or > the actual allele size. It is caused by slippage of DNA polymerase as it moves along a microsatellite repeat, resulting in a gain or loss of repeats. If an individual is heterozygous for alleles that differ by only 1-2 repeats, then the resulting pattern may mistakenly be interpreted as a homozygous genotype displaying stutter.

may arise due to 'binning' effects.[7] Muller (1991) has pointed out that discrepancies between bin boundaries and the actual range of variation present in the allele scoring process may produce an apparent heterozygote deficit. The presence of non-amplifying alleles (or nulls) will also produce an apparent heterozygote deficit. Several possible causes may exist for null alleles, including point mutations or indels in the primer-binding sites within the flanking regions (Ede and Crawford 1995, Paetkau and Strobeck 1995). In addition, short allele dominance, or the preferential exponential amplification of short alleles over longer ones during PCR, may cause null alleles and apparent heterozygote deficits. In fact, short allele dominance has been documented for one microsatellite locus in the red alga *Gracilaria* (Wattier *et al.* 1998). Finally, the nature of mutation in microsatellites may cause size homoplasy (i.e., alleles are identical by state but not by decent) and this possibility should be borne in mind when analyzing data from such loci.

Currently, microsatellite markers have been developed for several groups of marine algae; including the red algal genera *Gracilaria* (Wattier *et al.* 1997, Luo *et al.* 1999) and *Porphyra* (Teasdale 2004), the green algae *Cladophoropsis* (Van Der Strate *et al.* 2000) and *Enteromorpha* (Alström-Rapaport and Leskinen 2002), and within the brown algal orders Laminariales (Billot *et al.* 1998, Whitmer 2002) and Fucales (Coyer *et al.* 2002c, Olsen *et al.* 2002, Engel *et al.* 2003). Table 2 provides a summary of several microsatellite isolations for algae. The average number of alleles per locus observed in

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[7] "Binning" is a way of resolving size-calling difficulties both within and between polyacrylamide gels. In reality each allele is some whole number of bases in size; however, the 'assigned' size on a gel may vary continuously over a given range. Bins designate cutoff points between which an allele will always be assigned a given size. For example, given an allele size of X bp, a bin might be defined as X±1 bp, and any allele in this size range will then be called as X.

these studies is often <10, and it has been noted that microsatellite loci in algae tend to be less polymorphic than those in higher plants and animals (Wang *et al.* 1994, Tóth *et al.* 2000, Wattier and Maggs 2001). A striking example of this was found by Teasdale (2004) who saw no polymorphism at ten microsatellite loci in *Porphyra umbilicalis* (L.) J. Agardh. Interestingly, primers for these loci even supported cross-species amplification by PCR, while not displaying size polymorphism between species. Low levels of polymorphism and genetic diversity in marine algae are borne out by other studies as well. For example, Lindstrom (1993) found no intraspecific polymorphism for two species of *Porphyra* over a range of1000 kilometers using isozyme analysis. Hull *et al.* (2001) reported low levels of genetic diversity in four Fucales species using cellulose acetate electrophoresis, while other workers have also suggested that low genetic diversity may be common within the brown algae as a whole (Williams and Di Fiori 1996)

A further general observation is that the proportion of microsatellite loci within algal nuclear genomes appears to be less than in other eukaryotes (Wattier and Maggs 2001). The difference in proportion does not simply appear to be due to genome size. The haploid genome sizes of several algal taxa have been determined, including the red algal genera *Porphyra* (≈ 300 Mbp Kapraun *et al.* - 1991) and *Chondrus* (≈ 150 Mbp - Peters *et al.* 2004), as well as the heterokont orders Laminariales (580-720 Mbp), Fucales (1095-1271 Mbp), and Ectocarpales (127-290 Mbp – all from Peters *et al.* 2004). While the haploid genome sizes of gymnosperms and angiosperms are generally estimated to be larger than those of algae (4120-76,900 Mbp and 50-125,000 Mbp, respectively; data from Li 1997), the range of genome size in algae is comparable to groups such as insects

(98-7350 Mbp) and fungi (8.8-1470 Mbp; data from Li 1997) from which numerous microsatellite loci have been reported (Tóth *et al.* 2000). Although Wattier and Maggs (2000) do not offer an explanation for the observation of low proportions of microsatellites in algal nuclear genomes, it may be simply a result of the relatively small numbers of taxa studied to date.

While most of the papers cited in Table 2 simply report the development of microsatellites, a new generation of algal studies is beginning to apply these markers towards analyses of population structure, genetic diversity, hybridization, phylogeography, and paternity and fertilization within marine algae. For example, Engel *et al.* (1999) used two microsatellite loci to conduct paternity studies in *Gracilaria*, demonstrating that male fertilization efficiency was not a simple function of spermatic dispersal distance. Van der Strate *et al.* (2002, 2003) used eight microsatellite loci to examine population structure and the presence of cryptic species within *Cladophoropsis*. Coyer *et al.* (2003) used seven microsatellite loci to examine population structure in northern European populations of *Fucus serratus*. In addition, Coyer *et al.* (2002a) used five nuclear microsatellite loci, along with mitochondrial and chloroplast markers, to demonstrate the presence of hybrids between *F. serratus* and *F. evanescens* in a zone of secondary contact. Studies such as those described above as well as the research presented here demonstrate the utility of microsatellite analyses in combination with other genetic and morphological approaches to enhance our understanding of marine algae and ecosystems.

Description of Taxa Examined in this Thesis

Within the Gulf of Maine, which extends along the northwest Atlantic from Nova Scotia to Cape Cod, MA, *Fucus* is represented by four species (*F. serratus*, *F. distichus, F. spiralis,* and *F. vesiculosus*), along with several infraspecific taxa (Mathieson and Hehre 1986, Sears 2002). The present study examines some aspects of the taxonomy and systematics of several *Fucus* taxa distributed throughout the Gulf of Maine and Ireland using microsatellite markers. The taxa involved are *F. spiralis*, *F. spiralis* ecad *lutarius* (Kützing) Sauvageau, a muscoides-like *Fucus* (from the Gulf of Maine), *F. cottonii* Wynne et Magne (from Ireland), *F. vesiculosus*, *F. vesiculosus* ecad *volubilis* (Hudson) Turner, and *F. vesiculosus* var. *spiralis* Farlow. Pictures and descriptions of all taxa can be found in Figure 1 and Table 1, respectively. A brief summary of each taxon is provided below.

Fucus spiralis L. is a perennial cosmopolitan species occurring along the open coast and (occasionally) within estuaries throughout much of the northern Hemisphere. Along the coast, it tends to occur within the upper intertidal zone, where it may grow up to >30 cm depending upon environmental conditions (Sears 2002). Stunted or dwarf forms (sometimes referred to as f. *nanus*) may be found within the extreme upper intertidal zone of exposed sites or high up on muddy banks of inner estuarine sites. Its' reproductive period is between June-September (Mathieson 1989).

Fucus vesiculosus L. has a geographic distribution similar to that of *F. spiralis*, although it is more common within salt marshes than is the latter species. It appears to display a greater tolerance for reduced salinity than *F. spiralis* (Niemeck and Mathieson 1978), and it is the only species of *Fucus* widely distributed throughout the Baltic Sea

(Serrão *et al.* 1999b). *Fucus vesiculosus* is also perennial and generally occurs within the mid to lower intertidal. Reproduction occurs from March to June (Mathieson 1989).

Fucus vesiculosus var. *spiralis* Farlow is a perennial taxon characterized by a very spiraled thallus that may or may not display vesicles. It is a mid-intertidal plant that commonly occurs in salt marshes along tidal channels or on heavily sedimented rocky shores (Sears 2002). Although *F. vesiculosus* var. *spiralis* possesses a holdfast, Taylor (1957) notes its resemblance to *F. vesiculosus* var. *volubilis* (Hudson) Turner and speculates that these two taxa may be identical. Baker and Bohling (1916) also note the resemblance of *F. vesiculosus* var. *spiralis* from the United States with *F. vesiculosus* var. *volubilis* from British estuaries. Receptacles may be present from March through June (Niemeck and Mathieson 1976, Mathieson 1989), but to date no studies have examined the viability of gametes in this taxon.

As noted above, *Fucus vesiculosus* ecad *volubilis* (Hudson) Turner resembles *F. vesiculosus* var. *spiralis*, except that it is a detached form that occurs in salt marshes entangled amongst *Spartina* species. It is generally heavily spiraled and proliferous and ranges in size from very small fragments (several centimeters) to plants that may be longer than average attached forms of *F. vesiculosus* (i.e., over a meter). While this taxon is probably perennial, I am unaware of any studies that have determined its average lifespan.

Fucus spiralis ecad *lutarius* (Kützing) Sauvageau is an entangled or loose lying ecad that has been reported from salt marshes in Europe and the Northwest Atlantic (Sauvageau 1923, Chapman 1939, Taylor 1957, Mathieson and Dawes 2001, Mathieson *et al.* 2001). Chapman (1939) considered it a "low marsh plant" affiliated with *Spartina* *alterniflora* Loisel. It is distinguished from *F. vesiculosus* ecad *volubilis* in that the fronds are narrower and show little spiraling. The average size of this ecad is also considerably smaller than *F. vesiculosus* ecad *volubilis*. Receptacles are rare, but when present they are dioecious (Sauvageau 1907).

Fucus cottonii Wynne et Magne has been described from several locations in Europe (Cotton 1912, Baker and Bohling 1916, Niell 1980) as well as Alaska[8] (Ruiz *et al.* 2000) where these small (≤5-6 cm) embedded plants occur in dense carpets on well-drained areas of salt marshes, generally in the extreme upper intertidal and associated with *Spartina alterniflora* or *S. patens* (Aiton) Muhl. Receptacles are rare, but when present they are dioecious. As discussed later, in Europe this species has generally been regarded as arising from *F. vesiculosus via* intermediates such as *F. vesiculosus* ecad *volubilis*. The muscoides-like forms are similar in morphology and ecology to *F. cottonii,* and have been reported from multiple estuarine sites throughout the Gulf of Maine (Mathieson and Dawes unpublished). A comparison of muscoides-like plants from the Gulf of Maine with *Fucus cottonii* collected near the type location in Ireland has shown some morphological differences between the two ecads (the Ireland samples are on average smaller – Mathieson and Dawes unpublished). In addition, Mathieson and Dawes (2001 and unpublished) have shown that muscoides-like forms from the Gulf of Maine differ in origin from *F. cottonii.* Determining the origins of the muscoides-like forms and their affinities to *F. cottonii* is a primary goal of this thesis (cf. Chapters II-IV). Various taxonomic difficulties arise when discussing *F. cottonii* and the muscoides-like *Fucus*. Throughout this thesis, both taxa may be variously referred to as 'dwarf *Fucus*' or 'dwarf

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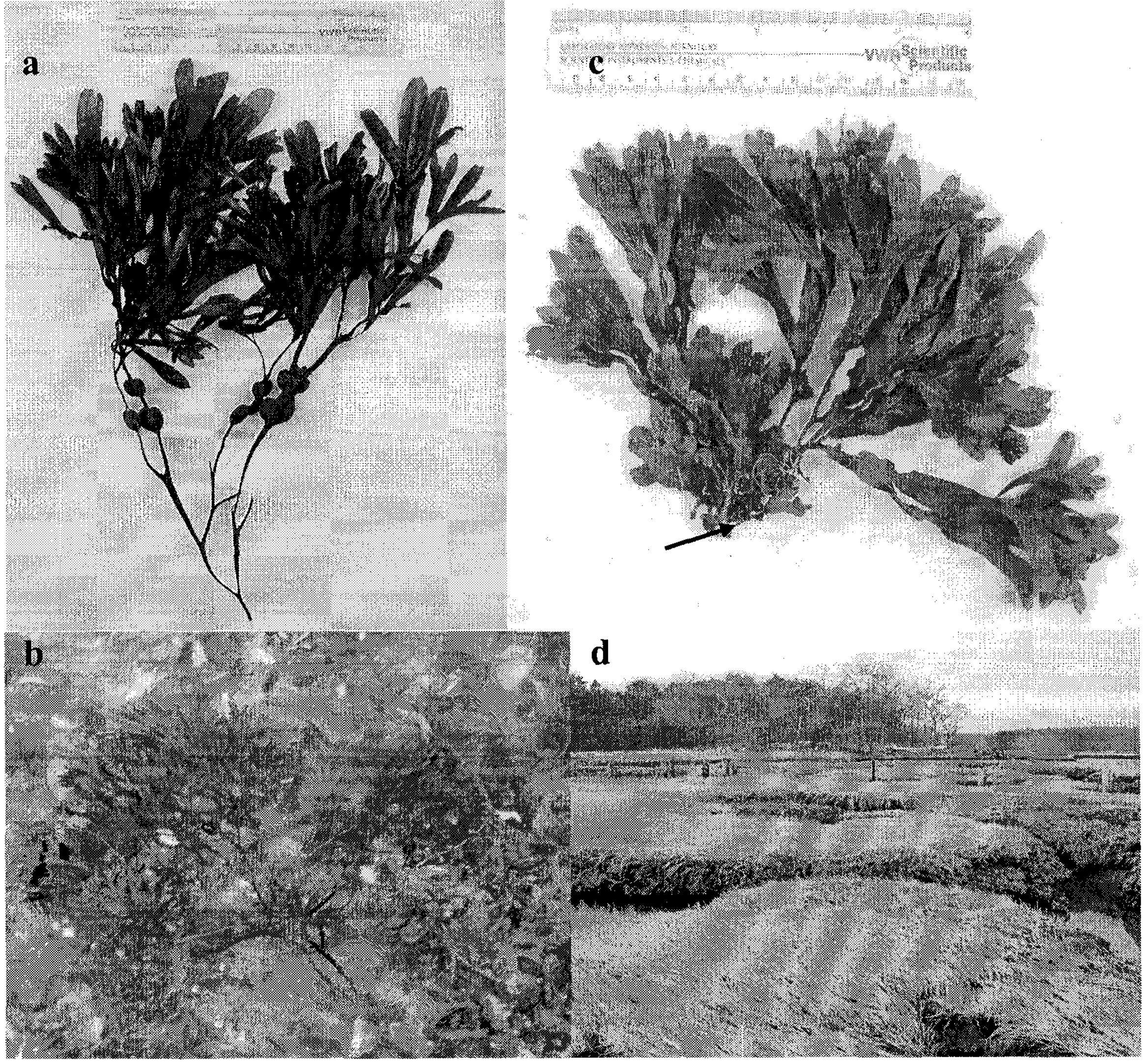
[8] Although Ruiz *et al.* referred to the Alaskan forms as *F. cottonii,* it would probably be better to avoid such a designation pending further morphological, ecological and genetic studies.

limicolous *Fucus*'. However, the term *F. cottonii* is only used for dwarf forms in Europe, whereas muscoides-like is used for such forms in the Northwest Atlantic or (to a lesser extent) Alaska.

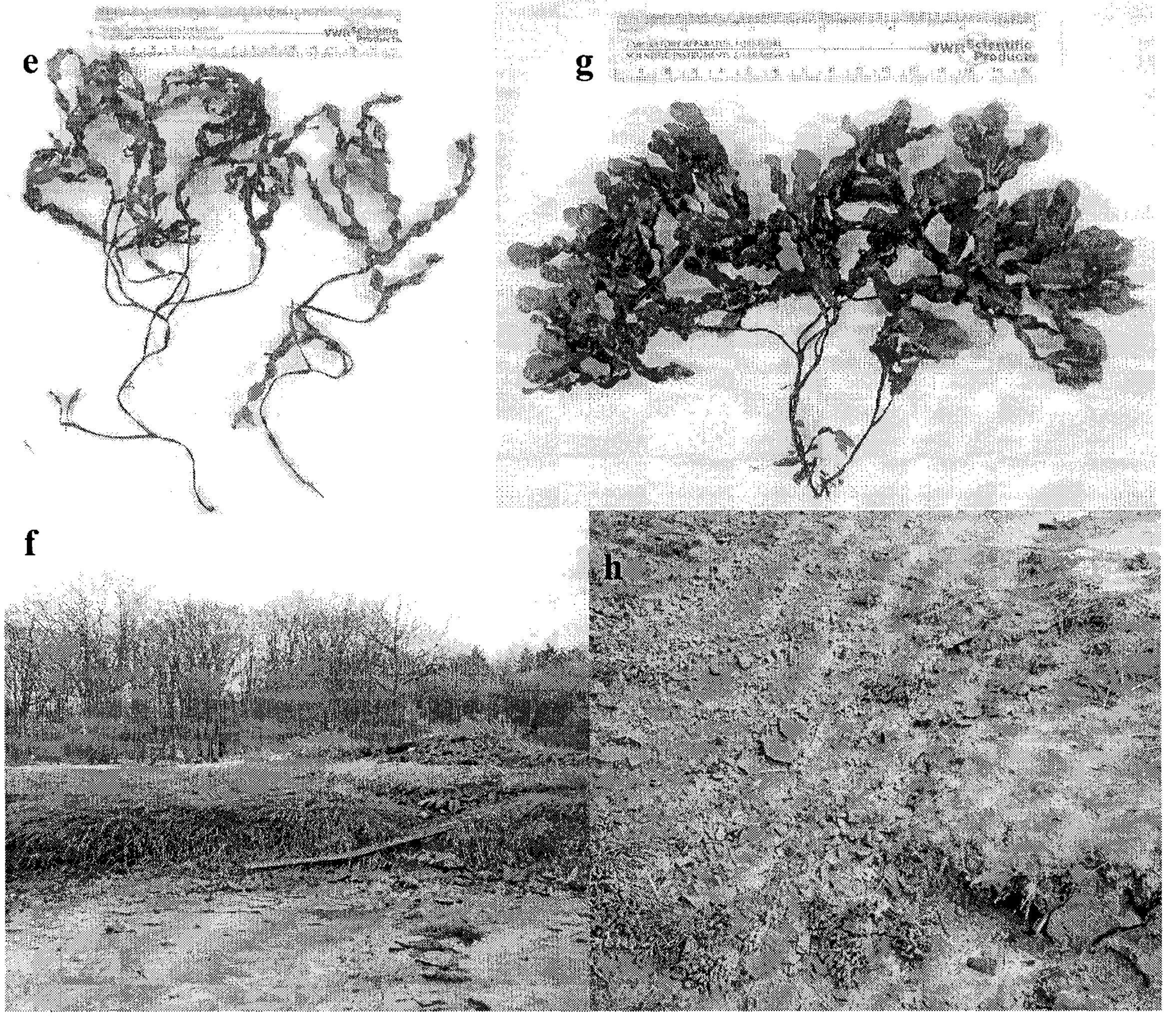
In his discussion of environment and form in the Phaeophyta, Russell (1978) distinguishes between an ecotype ("a phenotypic expression determined to some extent by genetic differences") and an ecad ("morphological differences which are due to direct environmental effects") and criticizes the application of these terms without sufficient evidence. By providing a better understanding of the population genetics of estuarine *Fucus* within the Gulf of Maine, this study will provide some of the evidence needed to resolve the confusing systematics of this group.

Figure1. Morphology and habitat of those *Fucus* taxa examined in this study.



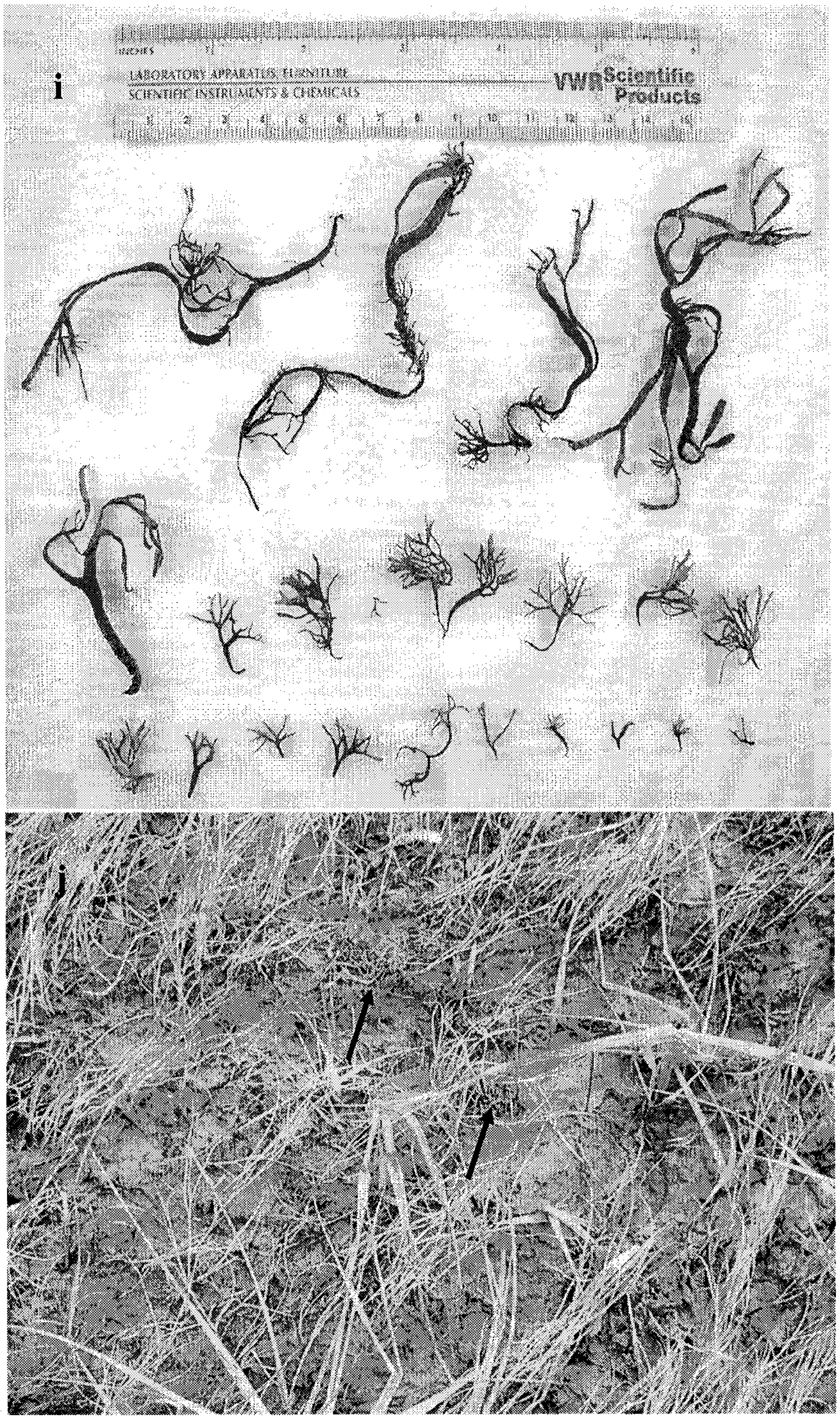
(a) *F. vesiculosus*. Note paired vesicles along midrib. (b) Habitat picture of attached *F. vesiculosus* taken at low tide. (c) Attached *F. spiralis*. Arrow indicates holdfast. (d) Photo showing channels and embankments within Brave Boat Harbor, ME. Within this estuary, *F. spiralis* is generally found near the top of these embayments. Figure 1 cont. on next page.

Figure1 cont. Morphology and habitat of those *Fucus* taxa examined in this study.



(e) *F. vesiculosus* ecad *volubilis*. Note the lack of a holdfast, the loss of the frond from the midrib in the basal part of the plant, and the narrow, spiraled fronds. (f) Habitat picture for *F. vesiculosus* ecad *volubilis*, which grows on banks within salt marshes such as that pictured here. (g) *F. vesiculosus* var. *spiralis*. Note loss of frond towards the base of the plant, and the relatively spiraled fronds as well. Vesicles are also present, as is a holdfast. (h) Habitat picture for *F. vesiculosus* var. *spiralis*, which grows attached within salt marsh habitats within the low intertidal. Figure 1 cont. on next page.

Figure1 cont. Morphology and habitat of those *Fucus* taxa examined in this study.



(i) Larger *F. spiralis* ecad *lutarius* (top row) grading into smaller muscoides-like forms (bottom row). (j) Habitat picture for muscoides-like forms in Brave Boat Harbor, ME. Several clumps of the dwarf *Fucus* are shown here (arrows indicate two such clumps) growing underneath *Spartina* on the very tops of embayments such as those pictured in Figure 1d.

Table 1 Summary of characters used to distinguish *Fucus* taxa in this study. Modified from Wallace *et al.* 2004.

Character	*F. vesiculosus*	*F. vesiculosus* var. *spiralis*	*F. vesiculosus* ecad *volubilis*	*F. spiralis*	*F. spiralis* ecad *lutarius*[1]	Dwarf *Fucus* from the Gulf of Maine	*F. cottonii* Wynne *et* Magne
Holdfast	present	present	absent	present	absent	absent	absent
Growth habit	attached & upright	attached & upright	entangled to partially embedded	attached & upright	entangled to partially embedded	embedded	embedded
Thallus shape:	flat, sometimes spiraled	spirally twisted	spirally twisted & proliferous	flat, sometimes spiraled	narrow, flat & proliferous	narrow, flat to terete & proliferous	narrow, flat to terete & proliferous
Vesicles:	present	present	present but uncommon	absent	absent	absent	absent
Cryptostomata:	uncommon & scattered on flat surface	? - absent	uncommon & scattered on flat surface	present & scattered on flat surface	uncommon & scattered on flat surface	mostly marginal on terete fronds; some scattered on flat surfaces	marginal
Receptacle occurrence:	present	present	usually absent	present	usually absent	absent	very rare
Receptacle shape:	pointed or ellipsoidal with 1-2 dichotomies	variable	pointed or ellipsoidal with 1-2 dichotomies	rounded & with no dichotomies	usually absent	absent	very small
Sterile Rim:	no	no	no	yes	usually vegetative	vegetative	no
Sexual reproduction:	dioecious	dioecious	dioecious but rare	hermaphroditic	hermaphroditic to unisexual	absent	dioecious/absent

Table 2. Summary of microsatellite development within marine algae. With the exception of Wallace *et al.*, the Average no. of alleles/ polymorphic locus and H_E and H_O over all loci are estimates based upon the arithmetic mean using all loci. Continued on next page.

	Wallace *et al.* 2004	Coyer *et al.* 2002c	Engel *et al.* 2003	Olsen *et al.* 2002	Billot *et al.* 1998
Species of origin	*Fucus spiralis*[1], *F.vesiculosus*[2] (Phaeophyta)	*F. serratus*[1] *F.evanescens* (Phaeophyta)	*F.vesiculosus*[1], *F. serratus*[2], *A. nodosum*[3] (Phaeophyta)	*A. nodosum* (Phaeophyta)	*Laminaria digitata* (Phaeophyta)
No. clones sequenced	183	?	96	≈ 300	216
No. of individuals sampled	[1]= 113 [2]= 33	[1] =120	[1] = 12 [2] = 12 [3] =12	1200	50
No. monomorphic microsatellites	[1] = 4 [2] = N/A	?	[1] = 4 [2] = 3 [3] = 3	64	32
No. polymorphic microsatellites	[1] = 4 [2] = 4	[1] = 10	[1] = 6 [2] = 8 [3] = 2	6	10
Average no. of alleles/polymorphic locus	[1]= 8 [2]= 6.75	[1] = 13.4	[1] = 5 [2] = 4.9 [3] = 4	15.5	6.7
H_E over all loci	[1]= 0.386 [2]= 0.567	[1] = 0.77	[1] = 0.67 [2] = 0.63 [3] = 0.54	0.5616	0.571
H_O over all loci	[1]= 0.183 [2]= 0.384	[1] = 0.67	[1] = 0.58 [2] = 0.53 [3] = 0.0.58	0.4013	0.555

Table 2 cont. Summary of microsatellite development within marine algae.

	Whitmer 2002	Wattier *et al.* 1997	Luo *et al.* 1999	Alström-Rappaport and Leskinen 2002	van der Strate *et al.* 2000
Species of origin	*Postelsia palmaeformis* (Phaeophyta)	*Gracilaria gracilis* (Rhodophyta)	*Gracilaria gracilis* (Rhodophyta)	*Enteromorpha intestinalis* (Chlorophyta)	*Cladophoropsis membranacea* (Chlorophyta)
No. clones sequenced	?	66	225	6	174
No. of individuals sampled	76-32	123 haploid genotypes	94-71	800	120
No. monomorphic microsatellites	0	2	7	1	17
No. polymorphic microsatellites	6	2	9	5	8
Average no. of alleles/polymorphic locus	3.5	>10	4.55	13.4	5.75
H_E over all loci	0.597	?	0.407	0.544	Haploid species, $\Delta H_O = H_E$
H_O over all loci	0.054	?	0.415	0.178	0.645

CHAPTER II

DETERMINING THE AFFINITIES OF SALT MARSH FUCOIDS USING MICROSATELLITE MARKERS: EVIDENCE OF HYBRIDIZATION AND INTROGRESSION BETWEEN TWO SPECIES OF *FUCUS* (PHAEOPHYTA) IN A MAINE ESTUARY[1]

Abstract

The high degree of morphological plasticity displayed by species of the brown algal genus *Fucus* L. is well documented. Such variation is especially pronounced for those estuarine taxa lacking holdfasts (termed ecads) that often bear little resemblance to the attached species from which they are derived. To better understand the systematics of salt marsh fucoids, we developed a suite of four microsatellite-containing loci capable of distinguishing between *F. vesiculosus* L. and *F. spiralis* L.. The genetic markers were used to determine the relationships of the fucoid ecads *F. vesiculosus* ecad *volubilis* (Hudson) Turner and a muscoides-like *Fucus* in the Brave Boat Harbor (ME, USA) estuary. Ecad populations had 2-3 fold higher levels of heterozygosity than attached populations of *F. vesiculosus* and *F. spiralis*. Further, ecads were 'intermediate' between *F. vesiculosus* and *F. spiralis* in their allele frequencies and genotype composition. Our data indicate that populations of muscoides-like *Fucus* in Brave Boat Harbor mainly

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consist of F_1 hybrids between *F. vesiculosus* and *F. spiralis,* whereas *F. vesiculosus* ecad *volubilis* may arise through backcrosses between *F. vesiculosus* and other fertile hybrids. Finally, our data support the hypothesis that introgression has occurred between attached populations of *F. vesiculosus* and *F. spiralis*.

Introduction

The genus *Fucus* (Heterokontophyta, Phaeophyceae) is a major constituent of intertidal and shallow subtidal zones throughout much of the northern hemisphere, where it typically grows attached to rocks and other solid substrata (i.e. as saxicolous or attached forms). At least eight species of *Fucus* are currently recognized (Powell 1963) based upon a variety of morphological and ecological criteria (Fritsch 1959, Powell 1963). Considerable morphological variation exists within this genus, which has led to many taxonomic problems (Norton and Mathieson 1983). The morphological plasticity of *Fucus* is probably due to a combination of factors, including variability of wave exposure, salinity, temperature, sunlight, and desiccation (Baker and Bohling 1916, Naylor 1936, Fritsch 1959, Russell 1979, 1987, Kalvas and Kautsky 1993, 1998, Chapman 1995, Munda and Kremer 1997, Ruuskanen and Back 1999). Genetic differences also seem to play a role. For example, Anderson and Scott (1998) observed several distinct morphotypes of *F. spiralis* L. in a single British population and hypothesized that they represented genetically distinct forms rather than phenotypic variants. Munda and Kremer (1997) noted similar patterns for German populations of *F.*

vesiculosus L., with an evesiculate or poorly vesiculated form occurring together with a more heavily vesiculated one.

Because different species of *Fucus* often grow together, another explanation for the observed morphological variation may be inter-specific hybridization. As noted by Evans (1962), the chromosome numbers of different species of *Fucus* appear to be indistinguishable (2n=64), which may contribute to successful introgression (Evans 1962, Scott and Hardy 1994). Further, a chemotactic sperm attractant released by the eggs appears to act inter-specifically in *Fucus* (Boney 1966, Green *et al.* 1990). Numerous workers have carried out cross-fertilization studies in *Fucus* (reviewed in Mathieson *et al.* 1981, also see Coyer *et al.* 2002a, b). Cross-fertilization studies carried out under laboratory conditions led Bolwell *et al.* (1977) to conclude that there were barriers preventing hybridization, but that these became less effective with the age of the egg. Several workers (Serrão *et al.* 1996, Berndt *et al.* 2002) found that gamete release in some *Fucus* species is inhibited under turbulent conditions, and Berndt *et al.* (2002) noted the potential for hybridization when these over-ripe gametes were finally released. In a culture study of four *Fucus* species McLachlan *et al.* (1971) concluded that morphological variation in natural populations was largely due to hybridization rather than environmental influences. Several investigators also examined hybridization between *Fucus* species in nature using morphological (Burrows and Lodge 1951, Scott and Hardy 1994, Anderson and Scott 1998), chemical (Hardy *et al.* 1998), and genetic approaches (Coyer *et al.* 2002a). Despite these studies, the frequency of hybridization and the extent of gene flow between different *Fucus* species are still largely unknown.

Salt marshes represent one of the most challenging habitats for fucoid seaweeds, with populations being exposed to wide fluctuations in environmental conditions, particularly salinity (Chapman 1995). Fucoids lacking a holdfast are common in such environments, and are sometimes referred to as ecads (Baker and Bohling 1916, Niell *et al.* 1980), a term proposed by Clements (1905) to indicate variability in morphology due to habitat. Fucoid ecads are generally distinguished from attached species by their lack of a holdfast, curling and proliferation of thalli, dwarf morphology, and vegetative reproduction (Baker and Bohling 1916, Boney 1966, Chapman and Chapman 1973, Mathieson *et al.* 1981, Norton and Mathieson 1983, Mathieson and Dawes 2001). As discussed by Norton and Mathieson (1983), entangled ecads, such as *Fucus vesiculosus* ecad *volubilis* (Hudson) Turner and *F. spiralis* ecad *lutarius* (Kützing) Sauvageau tend to be highly branched and occur intertwined or coiled around other plants (e.g., *Spartina* spp.). Typically, their fertility is reduced and they primarily reproduce, like most other unattached plants, by vegetative propagation. Embedded seaweeds, such as the muscoides-like forms of *Fucus*, lack holdfasts but their bases are embedded in mud or sand; hence, they are fixed in place although they lack a holdfast. Decay resulting from burial in mud spreads progressively up their thalli and, upon reaching a dichotomy, such burial separates the plant into multiple individuals. Such vegetative proliferation has been referred to as dichotomic splitting (Den Hartog 1972). The range of morphologies displayed by salt marsh ecads is considerable, with some appearing similar to the attached species from which they have been derived; by contrast the relationships of others are nearly impossible to determine based upon morphology alone (Figure 2).

The embedded *Fucus* taxon that has been referred to as *Fucus cottonii* M.J. Wynne *et* Magne in Europe (Wynne and Magne 1991) or simply as a muscoides-like *Fucus* in New England (Mathieson and Dawes 2001) lies at the far end of the morphological spectrum in detached fucoids (Figure 2). This dwarf *Fucus* occurs in well-drained areas at the extreme high tide mark, forming a moss-like turf. Such forms were first described by Cotton (1912) in the salt marshes of Clare Island, Ireland and have since been reported from several locations in Europe (Baker and Bohling 1916, Skrine 1928, Carter 1933, Lynn 1935, Den Hartog 1959, Feldmann and Magne 1964, Niell *et al.* 1980, Mathieson and Dawes 2001), the northwest Atlantic (Mathieson and Dawes 2001, Mathieson *et al.* 2001), and the northeastern Pacific (Ruiz *et al.* 2000).

The origins and taxonomy of muscoides-like *Fucus* plants have been the subject of much controversy. In his survey of seaweeds from Clare Island, Ireland, Cotton (1912) originally described these embedded forms as *Fucus vesiculosus* var. *muscoides*. Subsequently, Baker and Bohling (1916) included var. *muscoides* within their megaecad *limicola*, which contained all salt marsh fucoids without holdfasts, designating them as *F. vesiculosus* ecad *muscoides*, a name later used by Niell *et al.* (1980). These dwarf *Fucus* plants have also been designated as a distinct species, namely *Fucus muscoides* (Cotton) Feldmann *et* Magne (Feldmann and Magne 1964), later renamed *Fucus cottonii* M.J. Wynne *et* Magne (Wynne and Magne 1991). In addition, it appears that species other than *F. vesiculosus* may give rise to embedded dwarf morphologies, including *F. spiralis* (Mathieson and Dawes 2001) and *Pelvetia canaliculata* L. (e.g., *P. canaliculata* ecad *muscoides*, cf. Skrine 1928). Further, plants designated as *F. cottonii* are recorded from Alaska (Ruiz *et al.* 2000; S. Lindstrom pers. com.) where the only epilithic Fucaceae are

Fucus gardneri P.C. Silva, *Pelvetia siliquosa* Tseng and Chang, and perhaps *F. spiralis* (Ruiz *et al.* 2000; S. Lindstrom pers. com.). The high levels of morphological variation commonly seen in fucoid ecads raise several interesting questions. Foremost, is this variability soley due to ecological factors or is there an underlying genetic basis? Second, should unusual forms such as the embedded dwarf *Fucus* be treated taxonomically as a single species, an ecad derived from a single species, or as multiple ecads derived from different species? Because of the difficulties in resolving such questions, Mathieson and Dawes (2001) suggested that molecular tools would be helpful to complement conventional morphological and taxonomic approaches in studies of fucoid ecads.

Several chemical and molecular approaches to fucoid systematics have been developed. Pyrolysis mass spectrometry has been used successfully to study intra- and inter-specific relationships within *Fucus* (Russell 1995, Hardy *et al.* 1998, Scott *et al.* 2001). Several studies examined the utility of the internal transcribed spacer (ITS) region of the rDNA repeat to determine relationships in *Fucus* (Leclerc *et al.* 1998, Serrão *et al.* 1999a, Coyer *et al.* 2002a, b) and the Laminariales (Liptak and Druehl 2000, Coyer *et al.* 2001). Coyer *et al.* (2002b) confirmed the identity of artificial hybrids between *F. serratus* L. and *F. evanescens* C. Agardh using single stranded conformational polymorphisms within the ITS region and demonstrated the existence of natural hybrids between the two species using nuclear, mitochondrial, and chloroplast markers (Coyer *et al.* 2002a). In their phylogeny of the Fucaceae using combined ITS1 and ITS2 sequence data, Serrão *et al.* (1999a) found several examples of species-level clustering for *F. vesiculosus* and *F. spiralis*, although both species were part of an unresolved clade that also contained *F. gardneri*, *F. distichus* L., and *F. evanescens*. However, these authors noted that the presence of shared polymorphisms between individuals of *F. vesiculosus* and *F. spiralis* for the ITS1 and ITS2 regions, as well as the high levels of intra-specific and intra-individual variation of these repeats in both species, may place a limit on the utility of these loci to discriminate between the two species.

Microsatellite markers have become increasingly common as tools for population genetic analysis and have been developed for several groups of seaweeds, including, among others, *Enteromorpha* (Alstrom-Rapaport and Leskinen 2002), *Fucus* (Coyer *et al.* 2002c, Engel *et al.* 2003), *Gracilaria* (Wattier *et al.* 1997), and several members of the Laminariales (Billot *et al.* 1998, Whitmer 2002). Microsatellite markers are well suited for a variety of applications, ranging from population genetics and ecology to phylogeographic studies (Jarne and Lagoda 1996, Coyer *et al.* 2002a, 2003).

The goal of this study was to determine the affinities of two salt marsh ecads, *Fucus vesiculosus* ecad *volubilis* (entangled) and a muscoides-like *Fucus* (embedded), to populations of *F. vesiculosus* and *F. spiralis* within the Brave Boat Harbor salt marsh on the York-Kittery border in Maine, USA (Figure 3). Several factors recommend this marsh as an ideal site for molecular studies of *Fucus* ecads. The ecology and flora of Brave Boat Harbor have been well documented, and the only two attached *Fucus* species present in the marsh are *F. vesiculosus* and *F. spiralis* (Mathieson *et al.* 2001). In addition, Mathieson and Dawes (2001) conducted detailed transplant and morphological studies in Brave Boat Harbor, concluding that the muscoides-like *Fucus* represented a phenotypic variant of *F. spiralis* caused by detachment, extensive proliferation, and subsequent degeneration of detached fragments (i.e. dichotomic splitting). Hence, because the dwarf *Fucus* in Brave Boat Harbor probably differs in origin compared with *F. cottonii* in

Europe (Cotton 1912, Baker and Bohling 1916, Niell *et al.*1980) we use the terms muscoides-like or dwarf *Fucus* to describe this taxon. Here we describe the development of four polymorphic dinucleotide microsatellite markers for *Fucus* and use these markers to examine genetic differentiation between the four *Fucus* taxa in this salt marsh.

Materials and Methods

Isolation of Microsatellite-Containing Loci

To obtain DNA for the isolation of microsatellite-containing loci, multiple individuals of *Fucus spiralis* were collected from nearby Fort Stark (Newcastle, NH) and returned to the laboratory. To reduce diatom load, specimens were incubated for two weeks at 4° C in artificial seawater (Instant Ocean® from Aquarium Systems Inc., Mentor, OH) containing 0.18 $\mathrm{mg} \bullet \mathrm{L}^{-1}$ germanium dioxide (Markham and Hagmeier 1982). After incubation, samples were thoroughly rinsed in distilled water and DNA was extracted from fresh apical tissue and purified according to the method of Apt and Grossman (1993).

Microsatellites were isolated using a hybridization-capture protocol following the methods of Carleton *et al.* (2001). Whole genomic DNA (4 μg) was restricted with *Dpn*II and DNA fragments from 0.4-0.9 kilobase pairs were isolated to create a size-selected partial genomic library. Oligonucleotide adaptors were ligated to both ends of the DNA fragments using T4 DNA ligase and used as annealing sites for PCR primers (adaptor sequences: A1 = 5′-gatcgtcgacggtaccgaattct-3′; A2 = 5′-gtcaagaattcggtaccgtcga-3′). An initial round of PCR increased the copy number of all size-selected DNAs. The initial procedure was followed by a microsatellite capture step in which microsatellite-containing sequences were hybridized to biotinylated probes containing complimentary dinucleotide motifs (either $[GT]_{15}$, $[AT]_{15}$, or $[GA]_{15}$). Hybridized probe/microsatellite complexes were isolated and purified from background DNAs using streptavidin magnetic beads (Dynal, Oslo, Norway) according to the manufacturers instructions. A second round of PCR increased the copy number of putative microsatellite-containing DNA. Products from the second round of PCR were cloned using the pGEM®-T cloning kit from Promega (Madison, WI, USA) and blue/white screening identified bacterial colonies containing inserts. Plasmids were isolated using the QIAprep® Spin Miniprep Kit (Qiagen, Valencia, CA, USA).

Plasmid inserts were sequenced using the ABI DYEnamic™ terminator cycle sequencing kit on an ABI377 automated sequencer (Applied Biosystems, Foster City, CA, USA) at the University of New Hampshire's Hubbard Center for Genome Studies. Both forward and reverse M13 primers, as well as T7 and SP6 primers were used. Sequences were analyzed using the ABI software SeqEd version 1.0.3 (Applied Biosystems) and those containing >16 uninterrupted repeats and sufficient flanking sequence for PCR primer design were selected for further analysis. Primers were designed for these sequences using OLIGO® and ordered from MWG-Biotech AG (High Point, NC, USA). Preliminary tests for polymorphism were carried out using unlabeled primers with approximately 10 individuals each of *F. vesiculosus* and *F. spiralis,* with PCR products being separated on 3% MetaPhor® agarose gels (BioWhittaker Molecular Applications, Rockland, ME, USA). Labeled forward primers were ordered for all primer sets showing polymorphism (Table 3), as well as four primer pairs that appeared to amplify monomorphic loci. Plasmid insert sequences for the four polymorphic microsatellite loci obtained are available in GenBank (http://www.ncbi.nlm.nih.gov/) as accessions AY484939-AY484942.

Although most analyses of microsatellites assume that variation is due to a simple expansion or contraction of repeats, several authors showed that this assumption is often violated (Ortí *et al.* 1997, Hale *et al.* 2004). Therefore, to examine the nature of variation in alleles between *Fucus* species, DNA sequences were determined for the most common alleles of loci F227 and F300 for both *F. vesiculosus* and *F. spiralis* from Brave Boat Harbor. The two loci were selected because they displayed relatively species-specific distributions with widely separated allele size classes. The DNA from two individuals of each species that had been scored as homozygous at each locus was used as template for PCR using unlabeled primers as described above. Amplicons were purified using the QIAquick® PCR Purification Kit (Qiagen), then cloned and sequenced as described above. Sequences were aligned using the Clustal W algorithm in Megalign (DNASTAR, version 3.72, Madison, WI, USA) and deposited in GenBank as accessions AY455794-AY455797.

Field Sampling and Identification of Taxa

Whenever possible, sampling of *Fucus vesiculosus* and *F. spiralis* was carried out during the fruiting period of each species (Mathieson 1989) when receptacles were present (March-September). Field identifications were based on the presence or absence of vesicles and receptacular morphology (e.g., shape, presence or absence of a sterile rim, etc.; Table 4). If required, the reproductive status of the receptacle was determined by sectioning and microscopic observation. As outlined in Table 4, *F. spiralis* from Brave Boat Harbor possess several characters typical of this species (i.e., spiraled thalli, absence of vesicles, hermaphroditic reproduction, the occurrence of cryptostomata, and a sterile receptacular rim), whereas at the same time their stature, branching pattern, and ecology are somewhat different from that generally seen for this species. In particular, the plants are somewhat smaller, less spiraled, exhibit reduced branching, and grow on sandy rather than rocky substrata (Newton 1931, Taylor 1957, Niemeck and Mathieson 1976, Mathieson *et al.* 2001). Similar morphologies have been observed for *F. spiralis* growing on sandy substrata in other salt marshes (A.C. Mathieson, personal obs.).

Table 4 lists the characters used to distinguish between the four *Fucus* taxa in this study. The identification of entangled/embedded samples was carried out according to the criteria of Mathieson and Dawes (2001) and Mathieson *et al.* (2001) with the following exceptions: *F. spiralis* ecad *lutarius* and the dwarf *Fucus* described in those studies were grouped together and are here referred to as muscoides-like *Fucus* (Table 4 provides a separate morphological description of *F. spiralis* ecad *lutarius* and the dwarf *Fucus* as given by Mathieson and Dawes 2001). The grouping is justified because these two groups are closely linked ecologically and form a morphological continuum in this salt marsh (Mathieson and Dawes 2001). In the present study, only the smallest *F. spiralis* ecad *lutarius* as described by Mathieson and Dawes (2001) were examined (i.e., plants displaying a high degree of morphological similarity to the dwarf *Fucus* – Figure 2, Table 4). In addition, the muscoides-like *Fucus* had a clumped distribution within sites, and it is possible that individuals within each clump originated from a single "founder" plant as a result of vegetative proliferation followed by dichotomic splitting (Den Hartog 1959, 1972, Norton and Mathieson 1983). Therefore, to minimize potential sampling errors, a patch of muscoides-like *Fucus* was treated as a single sample for collection purposes. Samples were collected at 17 sites within the outer marsh of Brave Boat Harbor (Figure 3) from September 2000 through the summer of 2002. The four taxa collected were *Fucus vesiculosus, F. vesiculosus* ecad *volubilis*, *F. spiralis*, and the muscoides-like *Fucus*. The numbers of each taxon collected per site and the total sample number of each taxon are given in Table 5. Most sites were approximately 5 m^2; however, collection sites for *F. vesiculosus* were larger (e.g., sites 7 and 10), because attached plants were relatively scarce. In addition, all site 9 collections were made along a 400 m transect line as shown in Figure 3.

After collections, samples were returned to the laboratory where approximately 5-10 mg of fresh vegetative tissue were excised, washed in deionized water, flash frozen in liquid nitrogen, and stored at -80° C until DNA extraction. Representative voucher specimens are deposited in the Albion R. Hodgdon Herbarium at the University of New Hampshire as accession numbers 77003-77081.

DNA Extraction and Amplification

The DNA was extracted following the protocol of Serrão *et al.* (1999a) or by using the Gentra Puregene™ DNA Isolation Kit (Gentra, Minneapolis, MN, USA) with the following modifications: 1) polyvinyl-pyrrolidone (PVP-40 from Sigma, Steinheim, Germany) was added to the cell lysis buffer to a final concentration of 20 mg•mL^{-1}; 2) 1.5 μL Proteinase K (Sigma, St. Louis, MO, USA) was added to every 300 μL cell lysis buffer (0.1 mg•mL^{-1} final concentration) and; 3) samples were incubated on ice for one hour during the protein precipitation step. In both protocols, precipitated DNA was resuspended in 100 μL Tris-EDTA (TE: 10 mM Tris, 1 mM EDTA, pH = 8.0). Working stocks were diluted an additional 50-100 fold with TE before use.

All PCR reactions were carried out in a PTC-100™ thermocycler using labeled forward primers and 1 μL (approximately 0.1-2 ng) template DNA. With primers F90 and F227, reactions contained 50 mM KCl, 10 mM Tris-HCl, 0.1% Triton, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.4 μM of each primer, and 0.8 U *Taq* DNA polymerase. Amplification reactions for F227 also contained 200 $\mu g \bullet mL^{-1}$ BSA. With primers F26II and F300, reactions contained 50 mM KCl, 10 mM Tris-HCl, 0.1% Triton, 2 mM $MgCl_2$, 200 $\mu g \bullet mL^{-1}$ BSA, 0.2 mM of each dNTP, 0.2 μM primer, and 0.8 U *Taq* DNA polymerase. Total reaction volume was 20 μL and all reactions were performed using a standard Hot Start protocol (D'Aquila *et al.* 1991). Cycling parameters included an initial denaturation step of five minutes at 94° C, followed by 40 cycles of 30 s at the annealing temperature (Table 3), a 30 s extension at 72° C, and denaturation for 30 s at 94° C. A final extension at 60° C for 90 minutes was used to promote uniform A-tailing of amplicons (Applied Biosystems, 1995).

After PCR, 10 μL from each reaction was loaded onto a 3% agarose gel and separated by electrophoresis at approximately 4 V/cm for 1.5 hours. Based upon the brightness of each band, samples were diluted from 0-5 fold with TE. To size alleles, 1.25 μL from each diluted reaction was mixed with 1.75 μL 5:1 deionized formamide: loading dye and 0.25 μL GeneScan™-500 ROX™ size standard (Applied Biosystems, Warrington, UK), denatured for 2 minutes at 94° C, and immediately placed on ice. One microliter of each sample mixture was loaded onto a 6% denaturing polyacrylamide gel.

Electrophoresis was carried out for six hours on an ABI373A automated DNA sequencer. Gels were analyzed using ABI GeneScan™ software version 3.1 (Applied Biosystems).

Simulating a Population of F_1 Hybrids

To estimate the properties of a hybrid population between *Fucus spiralis* and *F. vesiculosus*, a simulated population of 61 F_1 hybrids (F_1HYB) was randomly generated based on the allele frequencies calculated from both parental species in Brave Boat Harbor. A random number generator was used to generate numbers from 1-100. For each parental species, alleles were assigned "bins" between 1-100, with the size of the bin defined by the frequency of that allele. Each F_1HYB individual was then assigned two random numbers per locus and received the appropriate allele from each "parent" based on the bins those numbers fell into. Alleles within each genotype were independently assigned (i.e., no linkage was present) and random mating was assumed. Data from the F_1HYB population were then analyzed identically to that obtained from field collections.

Data Analysis

The mean numbers of alleles per locus, the observed heterozygosity (H_o), and Nei's (1987) unbiased estimate of the expected heterozygosity (H_E) over all loci were determined separately for all taxa and the F_1HYB population using the software program GDA (Lewis and Zaykin 2001). Tests of Hardy-Weinberg equilibrium (HWE) at each locus were performed using 1000 permutations in FSTAT v.2.9.3 (Goudet 2001) and the significance of the *p*-values was adjusted using sequential Bonferroni corrections (Rice 1989). Specifically, this analysis tested the significance of the inbreeding coefficient F_{IS}, where a positive value indicates a heterozygote deficit and a negative value a heterozygote excess within the population being analyzed. Where significant heterozygote deficits were found, the possible presence of null alleles and their frequencies were estimated according to Brookfield (1996) using the software program Micro-checker (van Oosterhout *et al.* 2003). Departures from genotypic equilibrium for each taxon were also determined using FSTAT. A significance level of $p \leq 0.05$ was used for all tests.

To visualize genetic relationships between *Fucus vesiculosus*, *F. spiralis*, *F. vesiculosus* ecad *volubilis*, and the muscoides-like *Fucus* all samples of a given taxon were considered to represent a single population. A factorial correspondence analysis (FCA) was carried out to generate a graph of genotypes from all taxa (including the F_1HYB population) using GENETIX v.4.0 (Belkhir 1999), with this multivariate analysis providing a qualitative visualization of the variation between objects based upon discrete variables (Benzécri 1973). For microsatellite data in a diploid species, a contingency table can be constructed in which each individual is scored as possessing zero, one, or two copies of each allele over all loci. The table may then be used to generate a cloud of points within a multi-dimensional space, that is, where the number of dimensions equals the total number of alleles, assuming that the number of alleles is less than the number of individuals genotyped. Within this space, variation within the cloud of points is partitioned among orthogonal axes such that the first axis describes the most variation, with each subsequent axis accounting for progressively less of the total variation present (Lebart *et al.* 1984, She *et al.* 1987). In this analysis, each point represents a single genotype, meaning that individuals with identical genotypes will be represented by a single point. Individuals with incomplete genotypes were excluded from this analysis, as were alleles occurring <5 times in the whole dataset (Kotulas 1989).

Results

Isolation of Microsatellite-Containing Loci

A total of 293 putative microsatellite-containing plasmid inserts were sequenced. Of these, 57% contained a dinucleotide microsatellite motif ≥5 repeats in length, whereas 21.5% (63) had a motif of ≥16 uninterrupted repeats. Primers were developed for 12 loci where clones possessed sufficient flanking sequence on either side of the dinucleotide repeat. Two primer sets supported amplification of a PCR product when the insert-containing plasmid was used as template, but they failed to amplify when genomic DNA from the original sample was used, suggesting that these clones represented chimeric sequences (i.e., they were artifacts of the development process). Two primer sets failed to support good amplifications despite extensive attempts at optimization; these were discarded. Eight primer sets amplified clear bands in multiple samples. Four of these primer sets amplified monomorphic loci and four polymorphic loci. The last four loci were used in all subsequent analyses (Table 3).

Sequence alignments showing allelic variation between *Fucus vesiculosus* and *F. spiralis* at loci F227 and F300 (GenBank accessions AY455794-AY455797) demonstrated that the differences between these alleles was not simply due to a gain or loss of repeats (alignments not shown). At locus F227, the most common allele in *F. vesiculosus* was 126 bp in length (including primers), whereas in *F. spiralis* it was 144 bp. No difference was seen in the flanking sequence around each repeat. Differences between F227 alleles within the repeat region consisted of a 9-repeat indel and a single point mutation from G to A in the 126 bp allele, thereby producing an interrupted motif. At locus F300, the size of the most common allele for *F. vesiculosus* and *F. spiralis* was 228 and 137 bp, respectively. At least one point mutation appeared to be present within the flanking sequence on both sides of the repeat. The 228 bp allele was distinguished from the 137 bp allele by an additional 67 bp of flanking sequence on one side of the repeat motif. In addition, the motifs themselves were somewhat different between alleles. The general motif for the 228 bp allele was $A(CA)_6TA(CA)_{10}(GA)_{17}A$, while that of the 137 bp allele was $TA(GA)_{28}A$.

Intra-Taxon Analysis

A summary of general population statistics for all taxa and the simulated F_1HYB population is provided in Table 6. The mean number of alleles across all loci was fairly low, ranging from 5.75 in *Fucus vesiculosus* ecad *volubilis* to 8.25 in the F_1HYB population, with only two groups (*F. vesiculosus* and the F_1HYB population) exceeding 10 alleles at any locus (F26II). Despite relatively few alleles per locus, two taxa (*F. vesiculosus* and *F. vesiculosus* ecad *volubilis*) plus the F_1HYB population contained a fairly high number of genotypes relative to the overall sample size. Conversely, *F spiralis* and the muscoides-like *Fucus* had fairly low numbers of genotypes.

Two taxa (*Fucus spiralis* and *F. vesiculosus* ecad *volubilis*) showed significant genotypic disequilibrium between loci. In *F. spiralis,* the pair F300/F26II showed significant linkage. In *F. vesiculosus* ecad *volubilis* significant linkage was observed between F227/F90 and F227/F300, whereas no linkage was observed in *F. vesiculosus,* the muscoides-like *Fucus*, or the F_1HYB population.

A global test of HWE over all loci indicated that all taxa except *Fucus vesiculosus* ecad *volubilis* deviated from their expected genotypic proportions, as did the F_1HYB population (Table 6). A significant heterozygote deficit (positive F_{IS}) was found for *F. vesiculosus* and *F. spiralis*, with the largest deficits seen in the latter species, whereas a significant heterozygote excess was found in the muscoides-like *Fucus* and the F_1HYB population. The presence of putative null alleles was detected at loci F26II (in *F. vesiculosus* and *F. spiralis*) and F227 (*F. spiralis*).

Differentiating Taxa Using Microsatellites

Several important differences were observed between the four *Fucus* taxa and the F_1HYB population. In general, the muscoides-like *Fucus*, *F. vesiculosus* ecad *volubilis*, and the F_1HYB population displayed an intermediate trend between the two attached species. Both *F. vesiculosus* and *F. spiralis* displayed distinct differences in allele size and frequency, with the most common alleles in one species being present at very low frequencies (if at all) in the other species across all four loci (Table 6). The results differed for the muscoides-like *Fucus*, the F_1HYB population, and to a lesser extent *F. vesiculosus* ecad *volubilis,* as these groups shared the same alleles as both attached species, albeit at intermediate frequencies.

When comparing genotypes, both ecads and the F_1HYB population were distinguished from the attached species in two ways: (1) they displayed much higher heterozygosities than *Fucus vesiculosus* and *F. spiralis* (Table 6); (2) they were largely composed of heterozygous genotypes created by a combination of the most common alleles of both attached species. The total number of genotypes per individuals sampled differed considerably between taxa. *Fucus spiralis* and the muscoides-like *Fucus* had a much lower percentage of different genotypes relative to sample number (33% and 34%, respectively) compared to *F. vesiculosus* (82%), *F. vesiculosus* ecad *volubilis* (75%), and the F_1HYB population (90%).

Despite the presence of few taxon-specific alleles and some overlap between different taxa, a comparison of all taxa using FCA showed that it was often possible to assign an individual to the correct *Fucus* taxon based upon genotypic information (Figure 4). Clouds representing *F. vesiculosus* and *F. spiralis* were separated from each other along the X-axis (representing 59.63% of the total variation), with no overlap between them. The muscoides-like *Fucus* and the F_1HYB clouds showed a strong degree of overlap with each other and were located primarily in the middle of the attached taxa along both the X and Y axes. In addition, they showed some overlap with both *F. vesiculosus* and *F. spiralis*, particularly the latter species. However, the muscoides-like *Fucus* and the F_1HYB population were mainly comprised of a number of unique 'intermediate' genotypes. Genotypes of *F. vesiculosus* ecad *volubilis* showed the greatest level of variation, with some genotypes appearing to be 'intermediate' between the two attached species, whereas others grouped more closely with *F. vesiculosus.* In addition, three genotypes from this taxon defined most of the variation along the Y-axis (19.06% of the total variation); these fell between *F. vesiculosus* and the muscoides-like *Fucus* along the X-axis and were not closely aligned with *F. spiralis* on any axis.

Discussion

Dinucleotide Microsatellites in Fucus

Numerous studies have shown that the mutation rate of dinucleotide microsatellites is greater than that of tri- and tetranucleotide motifs (reviewed in Ellegren 2000, Schlotterer 2000), leading in turn to greater variability in this class of repeat. Overall, the levels of polymorphism obtained across these four loci in *Fucus* were fairly low (commonly <10 alleles/locus) compared with other eukaryotes, but they were comparable to those found in other seaweeds (Wattier *et al.* 1997, Billot *et al.* 1998, Van Der Strate *et al.* 2000, Wattier and Maggs 2001, Coyer *et al.* 2003).

In general, we found that the four microsatellite markers provided sufficient resolution to distinguish between all four fucoid taxa examined. More markers may be required to resolve detailed population structure. However, our results demonstrate that low levels of polymorphism may actually mean that fewer loci are required to distinguish taxa; that is, provided that the groups in question differ significantly in their allele frequencies.

Population Analysis: *Intra-Taxon Analysis*

In our analysis of population parameters for the four *Fucus* taxa and the F_1HYB population, the most significant trend observed was a departure from HWE. All taxa except *Fucus vesiculosus* ecad *volubilis* departed from HWE at some loci. Conformity to HWE depends on multiple assumptions, including random mating in a sexually reproducing population and the absence of selection or gene flow. Several of these assumptions are probably invalid for the *Fucus* taxa from Brave Boat Harbor. For example, *Fucus* ecads usually lack receptacles, and vegetative reproduction violates the HWE assumptions of random mating and sexual reproduction. Probably of more importance, however, are the observed similarities between these detached/embedded taxa and the simulated F_1HYB population. The F_1HYB population showed a heterozygote excess despite the applied conditions of random, sexual mating. Similar patterns of heterozygote excess were seen in both ecad taxa (though not statistically significant in *F. vesiculosus* ecad *volubilis*). These similarities suggest that departures from HWE in the detached/embedded taxa within Brave Boat Harbor may well be due to the presence of a relatively large proportion of hybrid individuals in those taxa (see below).

The significant heterozygote deficits observed in *Fucus vesiculosus* and *F. spiralis,* as indicated by fairly large positive F_{IS} values, may be due to several factors. Artifactual causes, such as null alleles or the grouping together of genetically different populations (the Wahlund effect, Hartl and Clark 1997), may produce such deficits. However, it seems unlikely that the observed deficits are entirely artifactual because they were observed over several loci, not simply those with putative null alleles. In addition, most samples of *F. vesiculosus* were collected from a single site (site 7), which would argue against the presence of a Wahlund effect for this taxon. Heterozygote deficits may occur naturally as a result of inbreeding, gene flow between genetically distinct populations, and selection against heterozygotes (Hedrick 2000, Jiggins and Mallet 2000). In the case of *F. spiralis* the potential for high levels of self-fertilization has been demonstrated (Müller and Gassmann 1985). Consequently, partial selfing may contribute to deviations from HWE in this species. Selfing might also contribute to the low numbers of genotypes observed in this species. Another factor contributing to inbreeding may be the ecophysiology of reproduction in both species. Studies of gamete release and dispersal in fucoid algae indicate that dispersal is relatively limited (Pearson and Brawley 1996, Serrão *et al.* 1996, 1997, Pearson *et al.* 1998) and that the heaviest recruitment of germlings occurs within 1 m^2 of the parents (Chapman 1995). Limited dispersal could therefore result in inbreeding for both species, although Coyer *et al.* (2003) found no spatial clustering of alleles over distances of 0-100 meters in populations of *F. serratus* L. from northern Europe. Heterozygote deficits may also result from introgression between *F. vesiculosus* and *F. spiralis* at Brave Boat Harbor (She *et al.* 1987, Roques *et al.* 2001). Finally, the potential for selection on *Fucus* populations occupying fringe ecological niches has been noted by several workers (Russell 1979, 1987, Sideman and Mathieson 1983, 1985, Hardy *et al.* 1998, Scott *et al.* 2001). Although the role of selection was not examined in this study, the genotypic, morphological, ecological, and genetic differences between attached and ecad taxa in Brave Boat Harbor suggest that selection may be an important factor in ecad recruitment and establishment.

Inter-Taxon Comparisons: Hybridization and Introgression

Two important conclusions can be drawn from comparisons of microsatellite data between the four *Fucus* taxa studied. First, some level of introgression has occurred between *F. vesiculosus* and *F. spiralis*. Second, differences between attached taxa and their related ecads in Brave Boat Harbor have a genetic basis, with the muscoides-like *Fucus* plants consisting largely of hybrids derived from attached populations of *F.*

vesiculosus and *F. spiralis*. Our data are also consistent with an origin for *F. vesiculosus* ecad *volubilis* via hybridization followed by introgression.

The shared pattern of allele size distributions observed within *Fucus vesiculosus* and *F. spiralis* is best explained as resulting from introgression. Thus, alleles found at high frequencies in one species often occurred in the other species at much lower frequencies. Convergent evolution is extremely improbable because identically sized alleles are found in both species and sequence comparisons at loci F227 and F300 show that a simple expansion and/or contraction of dinucleotide repeats does not explain the differences between alleles at these loci. An alternative hypothesis to explain the pattern of allele frequencies is that as *Fucus* appears to have undergone a recent evolutionary radiation (LeClerc *et al.* 1998, Serrão *et al.* 1999a), both species may be diverging towards fixation for alternate alleles inherited from a common ancestor. However, if this were true, then the process of fixation towards a given allele at each locus should occur independently between species instead of displaying a frequency-dependent pattern across all loci. The existence of such a pattern strongly implies introgression.

The identification of hybrids between *Fucus vesiculosus* and *F. spiralis* also provides support for gene flow between the two species. Our data present several arguments in favor of a hybrid status for the muscoides-like *Fucus*. First, these dwarf plants are intermediate but distinct from *F. vesiculosus* and *F. spiralis* in allele frequency and genotype; second, this taxon strongly resembles the F_1HYB simulated population in allele frequency, heterozygosity, and genotype composition. Furthermore, our data also suggests that this dwarf *Fucus* population contains a relatively high proportion of F_1 hybrids rather than a “mixed” hybrid swarm containing numerous backcrosses. Such findings are similar to those of Coyer *et al.* (2003), who investigated a swarm of hybrids apparently dominated by F_1 individuals between *F. serratus* and *F. evanescens.*

The probable hybrid status of at least some individuals of *Fucus vesiculosus* ecad *volubilis* may be inferred using similar arguments to those for the muscoides-like *Fucus* (i.e., comparisons of allele frequencies and heterozygosities and its clustering pattern in the FCA analysis). The present results also suggest that, if this taxon is indeed of hybrid origin, it is mostly comprised of backcrosses from a fertile *F. spiralis x F. vesiculosus* hybrid with *F. vesiculosus*. Based upon the reproductive strategies of *F. vesiculosus* (dioecious and outcrossing) and *F. spiralis* (hermaphroditic and partially selfing), it has been hypothesized that backcrosses between fertile hybrids and *F. vesiculosus* may be more frequent than those between hybrid forms and *F. spiralis* (Scott and Hardy 1994). Similarly, asymmetrical hybridization has been observed between some species of *Fucus* (Coyer *et al.* 2003), although our data clearly supports the hypothesis that gene flow is bidirectional between *F. vesiculosus* and *F. spiralis.* An alternative possibility for the origin of some individuals of *F. vesiculosus* ecad *volubilis* is that selection for individuals able to survive the environmental stresses imposed on ecads may have produced unique combinations of alleles among relatively pure individuals of *F. vesiculosus*. Although selection may well help maintain genetic boundaries between ecads and attached species, our data suggest that hybridization has played the major role in the origin of these ecads in Brave Boat Harbor.

In addition to the molecular evidence, ecological factors are also consistent with a hybrid origin of ecads in Brave Boat Harbor. Salt marshes may present a favorable environment for the formation and successful establishment of hybrids between *Fucus* *vesiculosus* and *F. spiralis* for several reasons. Foremost, the fruiting period of *F. vesiculosus* generally occurs from March-June, whereas that of *F. spiralis* is from June-September (Mathieson 1989, Mathieson and Guo 1992), but the timing and duration of reproduction in both species depends on several environmental factors, including salinity (Burrows and Lodge 1951, Munda 1964, Niemeck and Mathieson 1976, Berndt *et al.* 2002). Niemeck and Mathieson (1976) noted that reproductive responses to reduced salinity may enhance the degree of overlap between the fruiting periods of *F. vesiculosus* and *F. spiralis,* thus increasing the probability of hybridization between these two species.

In addition to reproductive factors, fucoid hybrids may also be more successful in competition with parental species in some salt marsh environments. Observations of potential *Fucus* hybrids among attached populations indicate that they are most likely to occur in zones between the parental species within the intertidal zone (Baker and Bohling 1916, Kniep 1925) or in disturbed and recently cleared areas (Burrows and Lodge 1951, Boney 1966), implying that they are outcompeted by parental types under normal conditions. Considering the correlation between hybridization and environmental disturbance in *Fucus* it may be significant that the muscoides-like *Fucus* in Brave Boat Harbor tend to grow in high narrow bands along banks subject to extensive erosion, whereas *F. vesiculosus* ecad *volubilis* is found at lower levels along such banks (Mathieson and Dawes 2001). In addition, various studies have shown that hybrids are often most successful near the fringes of a species ecological niche (Lewontin and Birch 1966, Barton 2001, Burke and Arnold 2001, Milne *et al.* 2003), such as within salt marshes and the extreme upper intertidal limits for *Fucus*. The "Bounded Hybrid

Superiority" model (Moore 1977) holds that hybrid zones are maintained through exogenous (i.e., environmental) selection favoring hybrid forms over parental types in certain habitats, whereas parental genotypes remain most fit throughout most of the species range (Arnold and Bennett 1993, Arnold and Hodges 1995, Arnold 1997, Burke and Arnold 2001). In his discussion of hybridization in *Fucus,* Boney (1966) hypothesized that hybrid swarms might dominate parts of the shore if they were better adapted to local environmental conditions than either parental species. In Brave Boat Harbor, both ecad forms display habitat differentiation from *F. vesiculosus* and *F. spiralis*. The muscoides-like *Fucus* is generally found higher up in the intertidal zone than either attached species. *Fucus vesiculosus* displays a circumscribed distribution based upon the presence of solid substrata, whereas *F. vesiculosus* ecad *volubilis* is a fairly cosmopolitan plant entangled amongst *Spartina alterniflora* Loisel. along coarse or sandy banks (Mathieson and Dawes 2001, Mathieson *et al.* 2001). The separation between taxa seen in the FCA combined with the ecological differences between ecads and attached *Fucus* species are consistent with the hypothesis that habitat-related fitness plays a role in taxon boundaries in these groups.

In their study of the origins of muscoides-like *Fucus* in Brave Boat Harbor, Mathieson and Dawes (2001) concluded that it was derived from *F. spiralis*, whereas the present study supports a hybrid origin. At least two reasons may exist for this discrepancy. First, our data show that several "*F. spiralis"* genotypes are present within the muscoides-like *Fucus* cloud, which suggests that individuals with these genotypes may have been F_1 hybrids bearing a strong morphological resemblance to *F. spiralis*. In that case, it would have been difficult to avoid using some hybrid individuals when

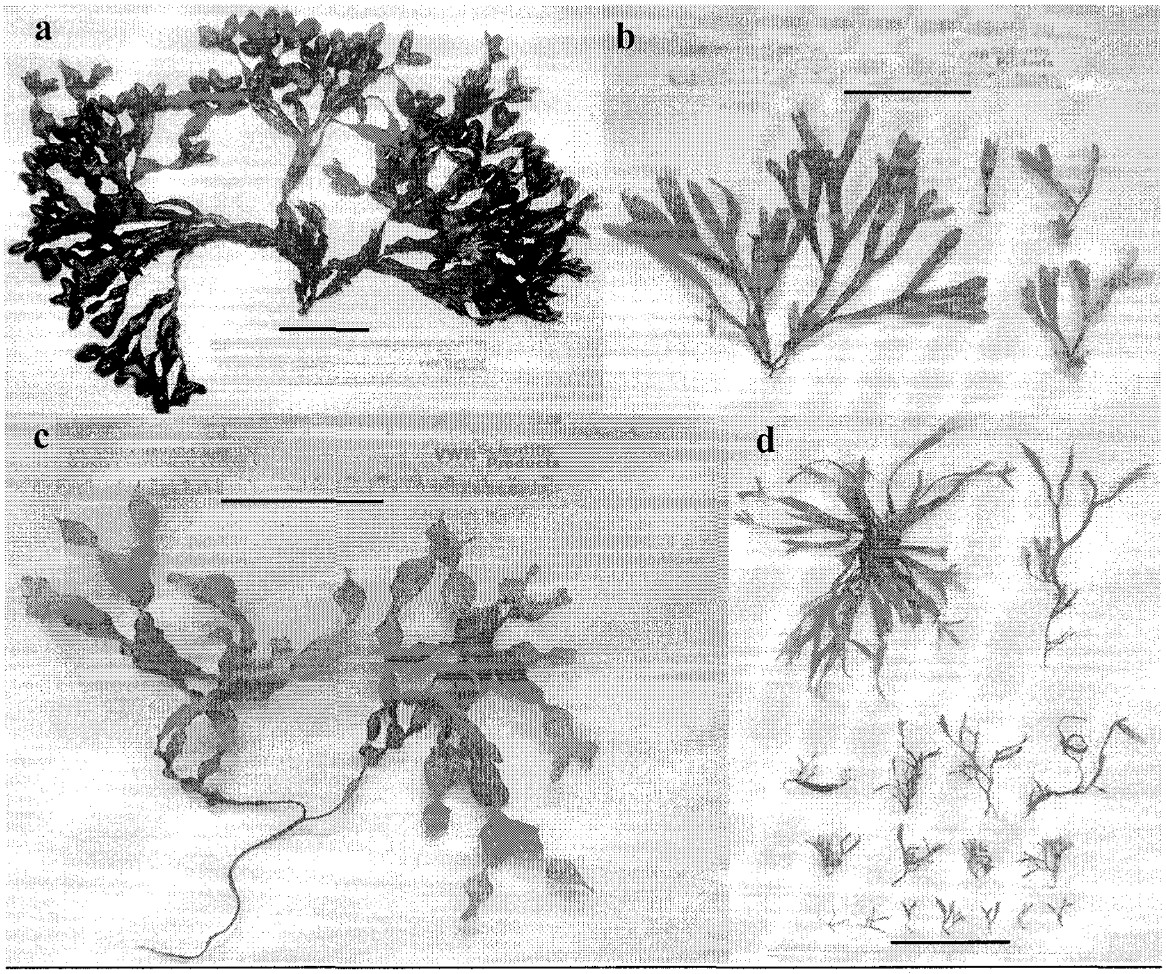
Mathieson and Dawes (2001) carried out reciprocal transplant experiments. Second, it was impossible for them to take into account long-term differences in survivorship that might lead to a bias towards hybrids over true bred individuals in ecad-producing environments.

Finally, it would be of some interest to determine the frequency of fertile F_1 hybrids between *Fucus vesiculosus* and *F. spiralis* and to ascertain the actual level of gene flow between the two species. Several factors may act to limit gene flow between *Fucus* species, including hybrid inferiority (e.g. selection), hybrid breakdown and sterility, prezygotic barriers, and the relative frequency of fertile F_1 hybrids. The first three factors that represent post-zygotic barriers to gene flow (e.g. hybrid inferiority, breakdown, and sterility) are commonly observed within hybrid zones and/or at their borders, and their mechanisms have been widely investigated (Arnold and Bennett 1993, Arnold and Hodges 1995, Rieseberg and Carney 1998, Burke and Arnold 2001).

Introgression is dependent upon the presence of reproductive F_1 individuals and successful, fertile backcrosses. Mathieson and Dawes (2001) were unable to find any receptacles in Brave Boat Harbor muscoides-like *Fucus* samples transplanted to zones where *F. spiralis* dominates. Receptacles are sometimes present on larger individuals of *F. spiralis* ecad *lutarius* in Brave Boat Harbor (A.C. Mathieson personal obs.), but none has been found on the smaller plants used in this study. Further, Norton and Mathieson (1983) and Mathieson and Guo (1992) note that the receptacles of fucoid ecads are often inviable. Such observations suggest that these dwarf *Fucus* are unlikely to provide a vehicle for gene flow between *F. vesiculosus* and *F. spiralis*. The loss of receptacles in ecads appears to be due to environmental influences, and this study provides evidence that some hybrids are present within attached *Fucus* populations in Brave Boat Harbor (discussed above). Several morphological studies have concluded that hybridization is probably frequent where populations of *F. vesiculosus* and *F. spiralis* are found together (Kniep 1925, Burrows and Lodge 1951, Scott and Hardy 1994), and the possibility of introgression has also been raised by Serrão *et al.* (1999a) based on their ITS analysis. Coyer *et al.* (2002a) reported that eggs from *F. serratus* x *F. evanescens* hybrids were capable of attracting sperm, and in a recolonization study of a cleared shoreline along the Isle of Man, Burrows and Lodge (1951) found that gametes of individuals morphologically intermediate between *F. vesiculosus* and *F. spiralis* were "fully formed and active". If these intermediate forms were in fact hybrids, then the identification of several putative hybrid genotypes among both attached species in Brave Boat Harbor suggests a potential vector for gene flow. However, further work is required to determine the relative frequency of hybrids within attached populations of *Fucus*.

In conclusion, our results indicate that populations of *Fucus vesiculosus* ecad *volubilis* and the dwarf *Fucus* within Brave Boat Harbor have arisen primarily through hybridization of *F. vesiculosus* and *F. spiralis*. The only formal taxonomic description of muscoides-like *Fucus* material is based upon type specimens from Clare Island, Ireland, which is currently recognized as a distinct species (*Fucus cottonii* Wynne *et* Magne, Wynne and Magne 1991); our results highlight the need to make molecular comparisons between *F. cottonii* from the type location and embedded dwarf *Fucus* specimens from other locations to resolve taxonomic confusions in this group. Finally, our data shows that the application of molecular techniques to the identification of detached fucoids will prove useful in elucidating relationships of taxa that vary markedly in both morphology and ecology.

Figure 2. Morphological variation of attached species and detached (i.e., embedded and entangled) ecads of *Fucus* populations from Brave Boat Harbor, York/Kittery, Maine, USA.



Scale bar, 5 cm. (a) attached individual of *F. vesiculosus* having paired air bladders, conspicuous midrib and wide wings; (b) attached juvenile and adult plants of *F. spiralis*, showing conspicuous cryptostomata on adult specimens, dichotomous branching and receptacles; (c) A detached/entangled fragment of *F. vesiculosus* ecad *volubilis*, with upper parts being evesiculate, spiraled, foliose, and having midribs, and the basal portion lacking wings and only composed of residual midribs; (d) transitional ecad populations ranging from large detached/entangled and proliferous fragments of *F. spiralis* ecad *lutarius* that grade into smaller proliferous fragments, and ultimately into dwarf muscoides-like *Fucus* plants; note thinner, more parallel margins on the larger fragments of *F. spiralis* ecad *lutarius* (d) versus broader, more spiraled fronds of *F. vesiculosus* ecad *volubilis* (Table 4). Only the smallest plants were used in this study (i.e., the bottom two rows of d).

Figure 3. Map of the Brave Boat Harbor estuary showing collection sites and 400 m transect line running southeast from site nine.

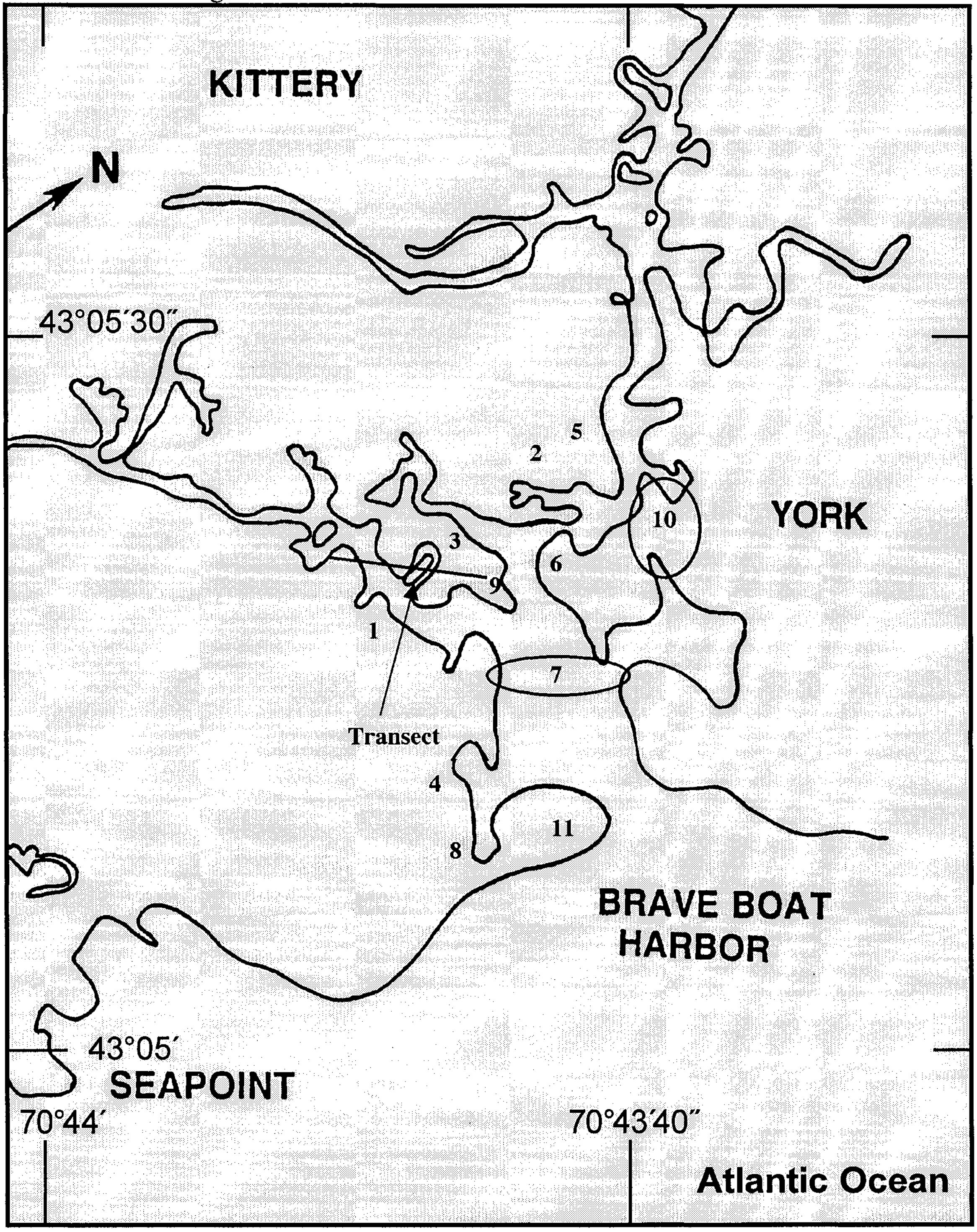
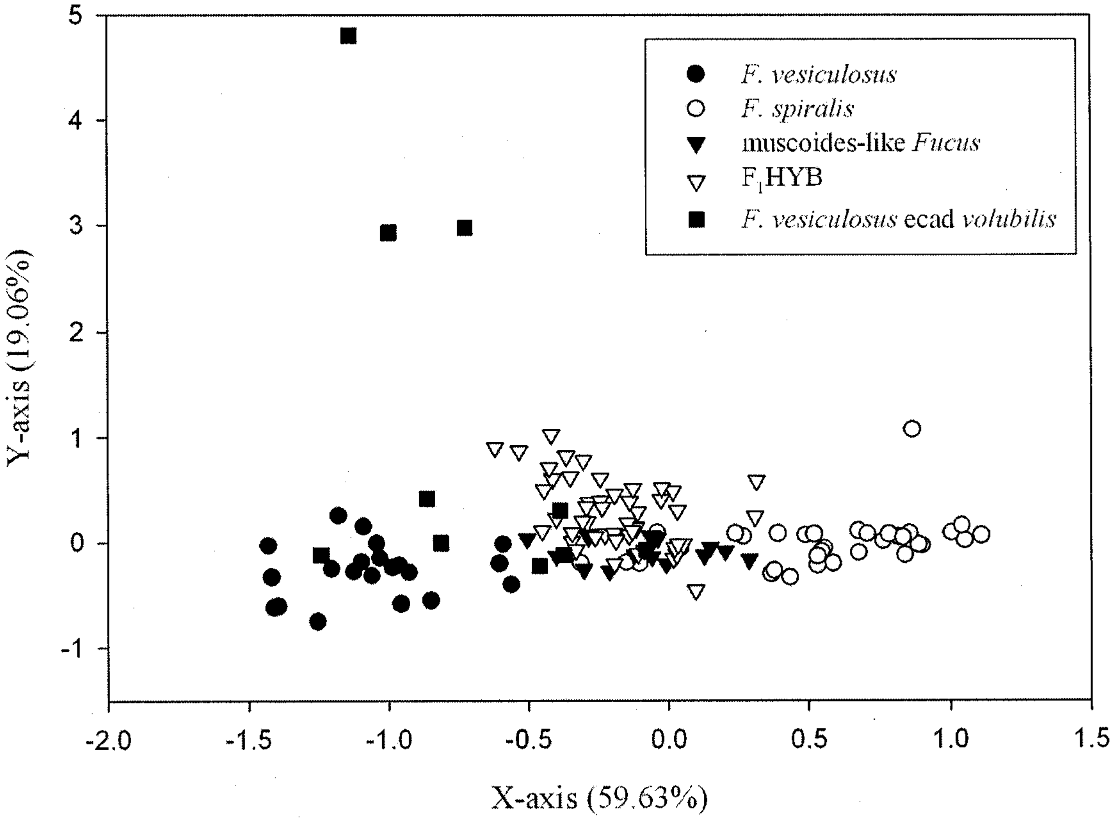


Figure 4. Factorial correspondence analysis of all *Fucus* taxa and the F$_1$HYB population.



The graph shows genotypes within each taxon and the F$_1$HYB population. Percent of the total variation explained by each of the two axes is given.

Table 3. Primer sequences for polymorphic loci and annealing temperatures.

Primer	Sequence (5′→3′)	Annealing temperature (°C)
F26IIF	TGCGAGTGGCATATAATGACTA	54.2
F26IIR	GCAACGCCGAAGTATCTATTG	
F90F	CTTCGCTTCTGCTATGATATG	46
F90R	AATCGGGTTCATGTGAAGAAA	
F227F	CGGCAGAGACATCCACCAT	52.6
F227R	TTCTTGGCACTACAGCGAAAC	
F300F	GCATGTGGCGTATAATGACTG	61.5 for 1st 30 cycles,
F300R	CCGCTCACAATCCTTCCCTGG	56.5 for final 10 cycles

Table 4. Summary of characters used to distinguish *Fucus* taxa. From Mathieson *et al.* (2001) and Mathieson and Dawes (2001).

Character	*Fucus vesiculosus*	*Fucus spiralis*	*Fucus vesiculosus* ecad *volubilis*	*Fucus spiralis* ecad *lutarius*[1]	Dwarf *Fucus*[1]
Holdfast	present	present	Absent	absent	absent
Growth habit	attached & upright	attached & upright	entangled to partially embedded	entangled to partially embedded	embedded
Thallus shape:	flat, sometimes spiraled	flat, sometimes spiraled	Spirally twisted & proliferous	narrow, flat & proliferous	narrow, flat to terete & proliferous
Vesicles:	present	absent	present but uncommon	absent	absent
Cryptostomata:	uncommon & scattered on flat surface	present & scattered on flat surface	uncommon & scattered on flat surface	uncommon & scattered on flat surface	mostly marginal on terete fronds; some scattered on flat surfaces
Receptacle occurrence:	present	present	Usually absent	usually absent	absent
Receptacle shape:	pointed or ellipsoidal with 1-2 dichotomies	rounded & with no dichotomies	pointed or ellipsoidal with 1-2 dichotomies	usually absent	absent
Sterile Rim:	no	yes	no	usually vegetative	vegetative
Sexual reproduction:	dioecious	hermaphroditic	dioecious but rare	hermaphroditic to unisexual	absent

[1] Refers to those taxa classified as muscoides-like *Fucus* in the present study.

Table 5. Key to collection sites in Brave Boat Harbor, ME.

Site	Number of samples of each species/ecad per site			
	F. spiralis	*F. vesiculosus*	muscoides-like *Fucus*	*F. vesiculosus* ecad *volubilis*
1	5	--	--	--
2	17	--	30	12
3	20	5	3	5
4	11	5	4	3
5	7	--	12	--
6	5	--	10	--
7	--	19	--	--
8	5	--	6	--
9	5	--	--	--
9 (10 m)	5	--	--	--
9 (25 m)	5	--	--	--
9 (50 m)	5	--	--	--
9 (100 m)	5	--	--	--
9 (200 m)	5	--	--	--
9 (300 m)	4	--	--	--
9 (400 m)	5	--	--	--
10	--	4	--	--
11	4	--	--	--
Total:	**113**	**33**	**65**	**20**

A 400M transect line was run beginning from site 9 (See Figure 3). Each site 9 (X m) represents a site along the transect line X meters from site 9.

Table 6. Summary of population genetic data for all *Fucus* taxa and the F_1HYB population.

	F. vesiculosus	F. spiralis	muscoides-like *Fucus*	F_1HYB	*F. vesiculosus* ecad *volubilis*
		Allele frequencies			
Locus F26II Allele Size (bp)					
340	---	---	0.129	---	---
353	0.031	---	---	0.008	---
363	---	0.041	0.026	0.033	0.053
365	0.156	0.009	0.009	0.115	0.105
367	0.031	---	---	---	0.026
369	0.297	0.018	0.233	0.164	0.342
371	0.016	---	0.009	0.008	---
380	0.203	0.032	0.198	0.123	0.132
382	---	0.027	---	---	0.053
384	0.047	0.698	0.388	0.352	0.263
386	0.141	0.126	0.009	0.115	0.026
389	0.016	0.036	---	0.041	---
391	0.047	---	---	0.025	---
394	0.016	---	---	0.016	---
400	---	0.014	---	---	---
Null	0.142	0.289	---	---	---
F_{IS}	**0.29**[1]	**0.855**[1]	**-0.159**	**-0.155**[1]	**-0.191**
H_O	**0.563**	**0.063**	**0.862**	**0.934**	**0.947**
H_E	**0.849**	**0.666**	**0.745**	**0.81**	**0.799**
F90 Allele Size (bp)					
120	---	0.062	---	0.016	---
122	---	0.062	---	0.049	---
124	---	0.004	---	0.016	---
140	---	0.009	0.016	---	---
142	0.226	0.848	0.5	0.533	0.45
146	0.419	0.013	0.09	0.246	0.1
148	0.032	---	---	---	---
151	0.274	---	0.393	0.107	0.35
153	0.048	---	---	0.033	0.05
155	---	---	---	---	0.05
F_{IS}	**0.18**	**0.021**	**-0.418**[1]	**-0.273**[1]	**-0.341**
H_O	**0.581**	**0.268**	**0.836**	**0.82**	**0.9**
H_E	**0.747**	**0.281**	**0.593**	**0.646**	**0.677**
F227 Allele Size (bp)					
116	---	---	---	---	0.125
118	---	---	---	---	0.025
122	0.015	0.005	---	0.008	---
124	0.045	0.014	---	0.016	---
126	0.773	0.099	0.484	0.41	0.625
138	---	0.014	0.008	0.008	---

Tabe 6 continued.

140	---	0.009	0.008	0.008	---
144	0.167	0.766	0.459	0.508	0.225
146	---	0.095	0.041	0.041	---
Null	---	0.173	---	---	---
F_{IS}	**0.121**	**0.592**[1]	**-0.624**[1]	**-0.283**[1]	**-0.36**
$\mathbf{H_O}$	**0.333**	**0.162**	**0.901**	**0.738**	**0.75**
$\mathbf{H_E}$	**0.379**	**0.401**	**0.558**	**0.585**	**0.556**
F300 Allele Size (bp)					
118	---	---	---	---	0.158
123	---	0.01	---	---	---
131	---	---	0.016	---	---
133	---	---	0.016	---	---
135	---	0.042	0.016	---	0.026
137	0.03	0.896	0.615	0.475	0.263
142	0.03	---	---	0.008	---
146	---	0.01	---	0.008	---
188	---	---	---	---	0.053
222	0.03	---	0.008	0.016	---
224	---	0.005	---	---	---
226	0.03	0.016	0.025	0.033	---
228	0.803	0.021	0.295	0.385	0.447
230	0.045	---	---	0.049	---
232	0.03	---	---	0.025	0.053
235	---	---	0.008	---	---
F_{IS}	**0.831**[1]	**0.747**[1]	**-0.252**	**-0.578**[1]	**0.198**
$\mathbf{H_O}$	**0.06**	**0.052**	**0.672**	**0.984**	**0.579**
$\mathbf{H_E}$	**0.38**	**0.213**	**0.618**	**0.81**	**0.751**
Total number of alleles per taxa per locus					
F26II	11	9	8	11	8
F90	5	6	4	7	5
F227	4	7	5	7	4
F300	7	7	8	8	6
Mean # Alleles	**6.75**	**8**	**6.25**	**8.25**	**5.75**
Total number of genotypes per taxa/number of individuals sampled					
	27/33	**37/113**	**22/65**	**55/61**	**15/20**
Tot $\mathbf{H_O}$	0.384	0.183	0.821	0.869	0.794
Tot $\mathbf{H_E}$	0.567	0.386	0.608	0.665	0.688
Tot F_{IS}	0.313[1]	0.596[1]	-0.349[1]	-0.311[1]	-0.159

Allele sizes are in base pairs; *Null* = estimated frequency of null allele; F_{IS} = inbreeding coefficient; $\mathbf{H_O}$ = observed heterozygosity; $\mathbf{H_E}$ = expected heterozygosity; Mean #Alleles = mean number of alleles over all four loci for each taxa; **Tot** $\mathbf{H_O}$ = total observed heterozygosity over all loci; **Tot** $\mathbf{H_E}$ = total expected heterozygosity over all loci; **Tot** $\mathbf{F_{IS}}$ = inbreeding coefficient over all four loci. [1]Significant deviation from Hardy-Weinberg equilibrium ($p<0.05$).

CHAPTER III

A SURVEY OF THE GENETIC AFFINITIES OF SALT MARSH *FUCUS* FROM THE GULF OF MAINE USING MICROSATELLITE MARKERS

Abstract

While studies of limicolous dwarf *Fucus* plants from Europe suggest that these forms are derived from *F. vesiculosus* L., morphological, ecological, and genetic studies from Brave Boat Harbor, Maine, USA, indicate that plants with similar morphologies and ecologies may be derived from *F. spiralis* L. or arise via hybridization between *F. vesiculosus* and *F. spiralis*. Therefore, the present study was undertaken to provide a broader understanding of the genetic affinities of salt marsh fucoids throughout the Gulf of Maine. Microsatellite markers were used to evaluate relationships between several *Fucus* taxa from Wells Harbor, ME, and these results were compared to those previously obtained from Brave Boat Harbor. In addition, general collections were made from fourteen sites throughout the Gulf of Maine to permit a 'broad scale' determination of genetic relationships between estuarine *Fucus* taxa. Overall, attached plants of *F. vesiculosus* and *F. spiralis* were well differentiated genetically. My results indicate that the smallest limicolous *Fucus* within the Gulf of Maine are generally hybrid in origin, but that the ratio of hybrid to species-specific genotypes decreases as plants become larger. Thus, *F. spiralis* ecad *lutarius* (Kützing) Sauvageau (a morphological intermediate between *F. spiralis* and the smaller dwarf plants) is comprised of a mixture of hybrid and 'pure' genotypes. *Fucus vesiculosus* variety *spiralis* Farlow, a common attached salt marsh form, appears to be little differentiated from *F. vesiculosus*. By contrast, *F.*

vesiculosus and its ecad *volubilis* (Hudson) Turner appear to be genetically differentiated. The results of this study suggest that the term ecad is inappropriate for entangled/embedded *Fucus* taxa in the Gulf of Maine, as morphological and ecological differences between these forms and attached parental populations appear to reflect underlying genetic differences.

Introduction

Although many studies of the ecology, reproduction, and physiology of *Fucus* have been conducted within the Gulf of Maine (Niemeck and Mathieson 1976, 1978, Mathieson 1989, Mathieson *et al.* 1977, 1983, Mathieson and Hehre 1982), these would be greatly enhanced by an understanding of genetic variation within and between species of *Fucus*. Recently, several workers have carried out genetic studies of *Fucus* examining phylogenetic relationships (Leclerc *et al.* 1998, Serrão *et al.* 1999a), hybridization, nuclear and organelle inheritance patterns (Coyer *et al.* 2002a, b), and population structure (Coyer *et al.* 2003). In addition, other workers have inferred that genetic variation underlies differences in morphology between conspecific plants growing close together along the shore (Anderson and Scott 1998, Munda and Kremer 1997, Rice and Chapman 1985).

In Chapter II the origin of a muscoides-like *Fucus* from Brave Boat Harbor (ME) was examined, and it was concluded that hybridization (and probably introgression) played a major role in the derivation of entangled and limicolous (= embedded) *Fucus* ecads at Brave Boat Harbor. As the present study (Chapter II) concluded that dwarf *Fucus* populations from Brave Boat Harbor were primarily hybrid in origin, a determination of how widely applicable this conclusion is throughout the Gulf of Maine as a whole is an important consideration. To this end, Mathieson and Dawes (unpublished) have recently evaluated dwarf limicolous *Fucus* populations from twenty-two salt marshes within the Gulf of Maine. Based upon detailed ecological, morphological, and transplant studies they concluded that dwarf *Fucus* plants were either derived from *F. vesiculosus* L or *F. spiralis* L., and that extreme environmental conditions were primarily responsible for the morphological convergence observed. Mathieson and Dawes (2001) have also suggested that molecular and genetic techniques could complement more traditional approaches (morphological and ecological) in resolving problems of ecad systematics and genesis. The utility of such a combined approach has been previously demonstrated (Chapter II) dealing with muscoides-like *Fucus* plants from Brave Boat Harbor.

The present study attempts to examine genetic affinities of several salt marsh fucoid taxa throughout the Gulf of Maine using microsatellite markers. The taxa examined included *Fucus vesiculosus*, its variety *spiralis* and ecad *volubilis*, *F. spiralis* and its ecad *lutarius*, and a dwarf limicolous muscoides-like *Fucus*. Initially, detailed microsatellite analyses were conducted in Wells Harbor, ME, on three taxa (*F. spiralis, F. vesiculosus* ecad *volubilis*, and a muscoides-like dwarf *Fucus*), a site similar in terms of exposure and ecology to Brave Boat Harbor. Allele frequency data and genotypic relationships were analyzed as done previously (Chapter II) and then compared to those from Brave Boat Harbor. Thereafter, a broader assessment of fucoid taxa was made at fourteen salt marshes throughout the Gulf of Maine stretching from northern Maine to southern Massachusetts in order to test the applicability of conclusions drawn from Wells and Brave Boat Harbors.

Aside from the above studies I also examined whether hybridization played a role in the origin of muscoides-like forms throughout the Gulf of Maine. In my previous studies at Brave Boat Harbor only the smallest dwarf limicolous *Fucus* plants were evaluated and the 'intermediate' taxon *F. spiralis* ecad *lutarius* was not assessed. Therefore, the affinities of *F. spiralis* ecad *lutarius* were also evaluated as well as those of *F. vesiculosus* var. *spiralis* and *F. vesiculosus* ecad *volubilis*. Although *F. vesiculosus* var. *spiralis* possesses a holdfast, it bears a strong morphological resemblance to *F. vesiculosus* ecad *volubilis*. Thus, I hypothesized that *F. vesiculosus* var. *spiralis* was the primary source of *F. vesiculosus* ecad *volubilis*, and tested this hypothesis using microsatellites to genotype the various species and ecads. Finally, the taxonomic status of the *volubilis*-type plants was examined. Entangled forms of *F. vesiculosus* lacking a holdfast have previously been designated ecads based upon morphological and ecological criteria (Baker and Bohling 1916). However, no genetic studies had been carried out to verify this designation. The results of this study will enhance our understanding of the genetic variation within and between estuarine *Fucus*.

Materials and Methods

Sample Collection and Identification

Samples of various *Fucus* taxa were collected during April-May 2003 from fourteen sites throughout the Gulf of Maine, with these representing five distinct geographic regions (Figure 5): Downeast ME, from the northeastern Maine coast to Penobscot Bay (Sites 1-4); the indented coastline from Penobscot Bay to Boothbay Harbor, Maine (Sites 5-6); southern ME from Boothbay Harbor to Kittery (Sites 7-9); the New Hampshire coastline to Cape Ann near Gloucester, MA (Sites 10-12); and the southernmost sites in Massachusetts (Sites 13-14). Following collection, samples were returned to the laboratory and stored at 4 °C. Table 7 summarizes the latitude and longitude of each site as well as the number and morphological identifications of samples collected. The identifications of samples were based upon diagnostic characters previously outlined in Chapter I (Table 7). At each site only a single sample was taken from a given patch of the dwarf limicolous *Fucus* in order to prevent a sampling bias. A similar approach was applied to *F. spiralis* ecad *lutarius*. In some cases it was difficult or impossible to identify samples morphologically, particularly with germlings and vegetative samples (Table 7).

DNA Extraction and Genotyping

To obtain clean tissue for DNA extractions, samples were rinsed thoroughly in distilled water and 5-10 mg tissue was excised from portions of the thallus with no visible epiphytes. The tissue was then flash frozen in liquid nitrogen and stored at –80 °C until DNA extraction using the Gentra Puregene™ DNA Isolation Kit (Gentra, Minneapolis, MN), employing modifications previously described (Chapter II). All samples were diluted 100-fold prior to use.

Genotyping was carried out using the microsatellite markers developed as part of this research (Chapter II). All PCR amplifications were performed using fluorescently labeled primers and the reaction conditions outlined in Chapter II. In addition, the marker L94 developed by Engel *et al.* (2003) was used for samples collected throughout the Gulf of Maine. The locus contains a complex interrupted/compound repeat with a motif of $(GCA)_3GACGAT(GCA)_5ACA(CCA)_5$ $[CCT(\mathbf{V}CA)_6]_2(ACA)_2$, where **V** = G, A, or C. The primer sequences for this locus were: F-5′-TTAGGAATGGGCGGGATG-3′; R-5′-CATTTCCTCACCCTCCTTCA-3′, with the forward primer being fluorescently labeled. All PCR reactions for L94 contained 1 μL template DNA (≅ 0.2 ng), 0.2 μg/μL BSA, 10 mM Tris-HCl, 50 mM KCl, 2.0 mM $MgCl_2$, 0.25 μM of each primer, 200 μM of each dNTP, and 0.5 U *Taq* DNA polymerase. The final reaction volume was 20 μL. Amplification conditions involved an initial denaturation step at 95 °C for 5 min followed by a 'touchdown' (Don *et al.* 1991) series of five cycles consisting of 30 s at 95 °C, 30 s at 61 °C (-1 °C per cycle), and 30 s at 72 °C, followed by 35 cycles of 95 °C for 30 s, 56°C for 30 s, and 72 °C for 30 s, with a final extension step at 60 °C for 90 min to promote uniform A-tailing (Applied Biosystems, 1995). Following amplification, alleles were separated by polyacrylamide gel electrophoresis using an ABI373A automated DNA sequencer (Foster City, CA, USA) and gels were analyzed using ABI GeneScan™ software version 3.1.

Using Microsatellite Markers for Species Identification of Ambiguous Samples

A combination of intra- and inter-site analyses were carried out to determine if any large discrepancies existed between morphological and genetic identifications for saxicolous taxa, as well as to determine the identities of a few samples whose species was uncertain. While assignment tests are often used to determine the source of individual genotypes, these generally assume that populations are in both Hardy-Weinberg and linkage equilibrium (Waser and Strobeck 1998, Davies *et al.* 1999). As equilibrium conditions probably do not apply to salt marsh *Fucus* in the Gulf of Maine (see Chapter II and present Results), assignment tests were not used. Instead, taxon identifications were confirmed as follows: (1) Allele frequency distributions between all taxa from a given site were compared; (2) factorial correspondence analysis (FCA) was performed using all samples from that site. A second identical set of analyses were then carried out using the same data but including samples of known identity from other sites. The patterns and relationships within and between these analyses were compared to determine species-level identifications.

Population Analysis of Multiple Fucus Taxa from Wells Harbor and Brave Boat Harbor, Maine.

Forty samples each of *Fucus spiralis, F. vesiculosus* ecad *volubilis,* and the dwarf *Fucus* were collected at Wells Harbor in order to compare population parameters from Brave Boat Harbor (Chapter II) and Rosmuc, Ireland (Chapter III). Owing to their extreme rarity, saxicolous *F. vesiculosus* plants were not collected from Wells Harbor. Estimation of allele frequencies and file formatting were carried out using CONVERT (Glaubitz 2004). Estimates of the observed heterozygosity and Nei's (1987) unbiased estimate of the expected heterozygosity (H_O and H_E, respectively) per taxon were obtained for each locus separately as well as globally over all loci using GDA (Lewis and Zaykin 2001). Estimates of the inbreeding coefficient F_{IS} were obtained for all taxa at each locus separately in FSTAT (Goudet 2001) according to the method of Weir and

Cockerham (1984). Significant departures from Hardy-Weinberg equilibrium for each locus/taxon were analyzed using GENEPOP (Raymond and Rousset 1995). Global estimates of F_{IS} were obtained using GDA, and the presence of global heterozygote deficits or excesses within each taxon were tested using GENEPOP. Tests for the presence of linkage disequilibrium within each taxon were performed using FSTAT.

To examine population structuring within and between sites, pairwise F_{ST} estimates were obtained using GENEPOP between all taxa analyzed from Wells Harbor as well as the Brave Boat Harbor salt marsh over all loci except L94 that was excluded as it was not used in the earlier Brave Boat Harbor study. Pairwise tests of population differentiation were performed using GENEPOP as well. Bonferroni corrections (Rice 1989) were applied to the significance values of all tests as appropriate, and all probabilities were evaluated using a significance level of $p \leq 0.05$.

Relationships between taxa were visualized using FCA in two ways. The first analysis was carried out using all genotypes, including those with missing data (or putative null alleles) and low frequency alleles. However, since both missing data and low frequency alleles may introduce bias into correspondence analyses (Kotulas 1989), a second FCA was carried out for comparison using only those individuals with complete genotypes and alleles with a frequency of 0.05 for the dataset as a whole. The first FCA contained genotypes from 40 samples each of *F. spiralis, F. vesiculosus* ecad *volubilis*, and the muscoides-like *Fucus*. The second FCA contained 19 *F. spiralis*, 14 *F. vesiculosus* ecad *volubilis*, and 33 muscoides-like *Fucus*.

Comparisons Between Salt Marsh Fucus Taxa Within the Gulf of Maine

In order to compare the parameters of *Fucus spiralis* ecad *lutarius* and the muscoides-like *Fucus* to those expected from a hybrid taxa, a simulated hybrid taxa (F_1HYB) was created using only the allele frequencies of saxicolous *F. vesiculosus* and *F. spiralis* combined over all fourteen sites from the Gulf of Maine (cf. Chapter II). The F_1HYB simulation was generated from populations of *F. vesiculosus* and *F. spiralis* after the completion of genetic identifications. The computer-generated simulation was then included in analyses involving comparisons between all taxa collected from the Gulf of Maine as described below.

To compare genetic affinities and differences between taxa, all individuals of a given taxa from the fourteen sites sampled were combined into a single population. Allele frequencies over all loci for each taxon were estimated using the software program Convert (Glaubitz, 2004), which was also used to generate file formats compatible with several other software programs used in this analysis (e.g., GDA, GENEPOP, and PHYLIP). The software program GDA (Lewis and Zaykin 2001) was utilized to determine the mean number of alleles per locus and the observed heterozygosity (H_o) for each taxon (including the F_1HYB simulation). As samples from the entire Gulf of Maine were pooled but were not expected to represent interbreeding populations, the expected heterozygosity was not reported.

Relationships between taxa were visualized in two ways. A factorial correspondence analysis was carried out using the program GENETIX v.4.0 (Belkhir 1999) for all taxa as well as the F_1HYB simulation. While previous FCA analyses excluded genotypes containing alleles with a frequency of <0.05 in the entire dataset, in the present case over 70% of all individuals were excluded when this criteria was applied, leading to a significant loss of resolution. Therefore, only those genotypes that contained alleles with a frequency of <0.05 in any taxon were excluded from this analysis. In addition, an unrooted neighbor-joining tree was constructed by successively using the programs SEQBOOT>GENDIST>NEIGHBOR> CONSENSE >DRAWGRAM (part of the PHYLIP software package, Felsenstein 2004). Only those genotypes with data for at least four loci (out of the five used in this study) were used in the analysis. Bootstrap values were estimated based upon one thousand replicates.

Results

Typing Samples to Species

Table 7 summarizes morphological and genetic identifications of samples from fourteen sites in the Gulf of Maine. The microsatellite data helped to resolve identifications of saxicolous *Fucus spiralis* and *F. vesiculosus*, as well as ambiguous samples, such as germlings or vegetative plants lacking species-specific morphological traits. Morphological and genetic identifications were always identical when samples had clear-cut morphological traits, such as the presence of vesicles or hermaphroditic/ dioecious receptacles. In Table 7, therefore, two sets of numbers are distinguished. The number of individuals determined genetically are given, as well as the number of individuals identified morphologically (in parenthesis) should it differ. Although it would be impractical to discuss the results in detail from every site, two examples illustrate the utility as well as drawbacks of the present approach.

The first example is particularly striking because of the discrepancy between the original morphological identifications and subsequent genetic results. That is, a set of samples from Barnstable Harbor, MA (site 14) was originally identified as *F. spiralis*; morphologically they were evesiculate germlings and were vegetative. However, this set grouped genetically with *F. vesiculosus* from Barnstable Harbor and Scarborough Marsh (i.e., samples with obvious *F. vesiculosus* morphologies) and they were reclassified as *F. vesiculosus* (see Table 7, Figure 6).

The above example demonstrates the utility of allele comparisons and FCA to type samples provided that all of the samples in question were misclassified as a group. However, when the 'sample set' from a given site consisted of a mixture of several taxa, the FCA approach to species identification used here can run into some difficulties. For example, at Hampton Harbor, a set of samples morphologically classified as *F. spiralis* appeared to contain a mixture of *F. spiralis*, *F. vesiculosus*, and several 'intermediate' genotypes. The file formatting process made it extremely difficult to determine which individual genotype corresponded to a specific sample. Therefore, several samples from this site remained listed as *F. spiralis*, despite the strong likelihood that they were in fact *F. vesiculosus*. Accordingly, small subsets of samples were left as 'misidentified' (discussed below). While some of the difficulties encountered above could be overcome with a different formatting process where each genotype retained a unique identifier throughout the analysis, the initial morphological identifications were sufficient to show a good correlation between morphological and genetic identifications, except for unusual circumstances. All subsequent analyses were carried out after genetic confirmation of species identifications.

Analysis of Fucus Taxa within the Wells Harbor and Brave Boat Harbor Salt Marshes

Table 8 provides a summary of intra-taxon population genetic parameters for multiple *Fucus* taxa from Wells Harbor.[1] Overall, the mean number of alleles per polymorphic locus for each taxa was fairly low, with high and low values of 5.0 (*F. vesiculosus* ecad *volubilis*) and 3.2 (*F. spiralis*). Significant deviations from Hardy-Weinberg equilibrium (HWE) were detected in all three taxa. *Fucus spiralis* displayed significant deviation from HWE at loci F300 and F26II. *Fucus vesiculosus* ecad *volubilis* showed significant deviation from HWE at all loci except L94, while the dwarf *Fucus* deviated at all loci. Pairwise tests for departure from linkage equilibrium showed no significant associations between loci in *F. spiralis* and a single significant pairwise association between loci F90 and F26II in the dwarf *Fucus*. The situation was very different for *F. vesiculosus* ecad *volubilis*, where significant linkage disequilibrium was found between three of the five loci (the exceptions being F90 and F300).

Estimates of the inbreeding coefficient F_{IS} for all taxa are provided in Table 8. *Fucus spiralis* displayed significant heterozygote deficits at two loci (F300 and F26II) as well as globally. *Fucus vesiculosus* ecad *volubilis* also displayed significant heterozygote deficits at two loci (F227 and F300) and globally. By contrast, the dwarf *Fucus* displayed a significant heterozygote excess at all loci with the exception of F300, which showed a significant heterozygote deficit. In the case of F300, the 137 bp allele was present at high frequencies in both *F. spiralis* and the muscoides-like *Fucus*. The dwarf *Fucus* taxon was also marked by a significant global heterozygote excess.

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[1] As the terms ecad and saxicolous are morphological designations, no samples were reclassified at Wells Harbor based upon genotype.

An FCA using all genotypes from Wells Harbor showed a separation of the three taxa (Figure 7a), with 14.59% of the variation being explained by the X-axis and 8.54% by the Y-axis. Genotypes of the muscoides-like *Fucus* were situated more closely to the *F. spiralis* cloud than *F. vesiculosus* ecad *volubilis* but overlapped with both taxa to some degree. *Fucus vesiculosus* ecad *volubilis* was the most variable taxon in the FCA and appeared to consist of three fairly distinct groups; the first was distinguished by its position along the X-axis and overlap with the muscoides-like *Fucus*, while groups two and three were distinguished by their positions along the Y-axis.

An FCA containing only complete genotypes and alleles with a frequency of ≥ 0.05 for the entire dataset is shown in Figure 7b. Whereas overlap between taxa was observed for Figure 7a, this was not present in Figure 7b. In this case, 38.04% of the variation was explained by the X-axis and 18.06% by the Y-axis. As shown in Figure 7b, *F. vesiculosus* ecad *volubilis* was responsible for most of the variation along the Y-axis.

Pairwise estimates of F_{ST} within and between taxa at Wells Harbor and Brave Boat Harbor are given in Table 9. Two taxa displayed fairly low levels of genetic partitioning between sites, these being *F. spiralis* ($F_{ST} = 0.020$) and the muscoides-like *Fucus* ($F_{ST} = 0.039$). In addition, two other estimates were <0.1, with these being between the muscoides-like *Fucus* and *F. vesiculosus* ecad *volubilis* from Brave Boat Harbor ($F_{ST} = 0.054$) and *F. vesiculosus* and *F. vesiculosus* ecad *volubilis* from Brave Boat Harbor. The largest estimate was between *F. spiralis* and *F. vesiculosus* ecad *volubilis* from Wells Harbor (0.409). As would be predicted, estimates between *F. spiralis* and *F. vesiculosus* were also large. Both *F. vesiculosus* taxa from Brave Boat Harbor were genetically much more similar to each other than either was to *F.*

vesiculosus ecad *volubilis* from Wells Harbor. Pairwise tests of population differentiation showed significant differences between all taxa and populations.

Comparisons Between Salt Marsh Fucus Taxa Within the Gulf of Maine

Several obvious species- and taxon-specific trends in allele frequencies were evident throughout the Gulf of Maine (Figure 8a-e). At locus F90 (Figure 8b), three alleles (142, 146, and 151 bp) were by far the most common and were found in all taxa, albeit at differing frequencies. *Fucus spiralis* had a high frequency of the 142 bp allele (frequency = 0.86), while all *F. vesiculosus* taxa displayed a more uniform frequency across the three alleles. Generally, the muscoides-like dwarf *Fucus* and *F. spiralis* ecad *lutarius* were similar in allele frequency distribution to each other and the F_1HYB simulation.

Three loci (F227, F300, and L94) were each largely defined by two alleles (Figures 8c-e). At locus F300, the 137 bp allele was most frequent in *Fucus spiralis* (0.63), whereas the 228 bp allele was most frequent in all *F. vesiculosus* taxa (*F. vesiculosus* = 0.72, var. *spiralis* = 0.63, ecad *volubilis* = 0.62). The muscoides-like *Fucus* tended to resemble *F. spiralis* in allele frequencies at this locus, while *F. spiralis* ecad *lutarius* fell between the former taxon and the F_1HYB simulation. As with locus F300, both F227 and L94 displayed species-specific patterns of allele frequencies between *F. spiralis* and all *F. vesiculosus* taxa. However, the allele frequencies of the muscoides-like *Fucus* and *F. spiralis* ecad *lutarius* were much more similar to the F_1HYB simulation at F227 and L94 than was the case for locus F300. Locus F26II (Figure 8) was more difficult to interpret, as the differences in frequencies between allele size classes were not as great as those seen at the other loci. Nonetheless, even at F26II, some differences in allele frequencies were observed between *F. vesiculosus* and *F. spiralis*. Although most sample sizes were too small to permit a quantitative analysis, there appeared to be no obvious trends toward significant changes in allele frequencies or the appearance of distinct allele sizes between even the most geographically separated sites for *F. vesiculosus* and *F. spiralis* in the Gulf of Maine (cf. Appendix 2).

Comparisons of observed heterozygosities for all taxa throughout the Gulf of Maine are provided in Table 10.The highest heterozygosities were found in *Fucus spiralis* ecad *lutarius* and the muscoides-like *Fucus* (0.731 and 0.872, respectively), both of which were similar to the F_1HYB (0.862). *Fucus vesiculosus*, its variety *spiralis*, and ecad *volubilis* had similar frequencies (0.401, 0.478, and 0.431, respectively), being intermediate between those of the dwarf *Fucus* and *F. spiralis* (0.2).

Factorial correspondence analysis was used to examine affinities between taxa within estuarine *Fucus* populations throughout the Gulf of Maine (Figure 9a-h). In this analysis, 64.06% of the variation was explained by the X-axis and 14.66% by the Y-axis. As would be predicted, *F. vesiculosus* and *F. spiralis* formed discrete clouds with very little overlap. However, the presence of several *F. vesiculosus* genotypes within the *F. spiralis* cloud (and vice versa) indicates that several samples from both taxa were incorrectly typed to species for some of the reasons discussed above (cf. Figures 9b-c and Discussion). Considerable overlap was observed between *F. spiralis* ecad *lutarius* and the muscoides-like *Fucus* genotype clouds (Figure 9a). In addition, both taxa displayed some overlap with *F. spiralis* (greater overlap was observed with *F. spiralis* ecad *lutarius*, Figures 9d-e). No significant overlap was found between the muscoides-like *Fucus* and

F. vesiculosus; however, while the muscoides-like *Fucus* cloud was fairly compact, *F. spiralis* ecad *lutarius* was more widely distributed, with several genotypes representing a single site present within the *F. vesiculosus* cloud (Figure 9d). Using FCA *F. vesiculosus* var. *spiralis* appeared to be very similar to *F. vesiculosus,* although one genotype was situated in the FCA within the region between the clouds of the two attached species (Figure 9f). By contrast, *F. vesiculosus* ecad *volubilis* appeared to be the most variable taxon of the *F. vesiculosus* clade and represented most of the variation explained by the Y-axis (Figure 9g). Interestingly, while many genotypes of ecad *volubilis* appeared to be similar to those of the other *F. vesiculosus* taxa examined, other genotypes appeared to be specific to ecad *volubilis*.

To determine if either the muscoides-like *Fucus* or *F. spiralis* ecad *lutarius* displayed a hybrid character, a computer simulation of F_1 hybrid genotypes (F_1HYB) was added to the correspondence analysis (Figure 9h). The overlap between the F_1 hybrid and muscoides-like *Fucus* clouds in the FCA is consistent with the hypothesis that the latter taxon is largely comprised of F_1 hybrids (Figures 9e, h). However, the range of variation found in *F. spiralis* ecad *lutarius* compared to the F_1HYB suggests that while some samples may have been hybrid in origin, others were probably not (cf. Figures 9d, 9h).

A neighbor-joining analysis of all taxa (including the F_1HYB simulation) is provided in Figure 10, where branch length is proportional to genetic distance. All *Fucus vesiculosus* taxa were much closer genetically to each other than to any other taxa, and in this analysis both *F. vesiculosus* ecad *volubilis* and var. *spiralis* grouped with each other and away from *F. vesiculosus* with moderate bootstrap support (76.1). Interestingly, all *F. vesiculosus* taxa grouped away from all other taxa examined as well as the F_1HYB simulation with bootstrap support of 100. Bootstrap analysis provided very weak support for the tree topology grouping *F. spiralis* and the muscoides-like *Fucus* away from ecad *lutarius* (50.5) and only somewhat better support for the position of the F_1HYB (67.4).

Discussion

Genetic Identification of Samples

Microsatellite markers are often used to assign genotypes to their probable source population, with the most common approach being the assignment test (Paetkau *et al.* 1995, Rannala and Mountain 1997). However, as explained previously, the application of assignment tests is dependent upon certain assumptions – i.e., that populations are in Hardy-Weinberg and linkage equilibrium (Cornuet *et al.* 1999, Davies *et al.* 1999). The approach used in this study combined comparisons of allele frequency data and FCA between morphologically questionable samples with similar data from known samples to determine taxon identity. For the most part, this approach confirmed the morphological identifications made previously for both saxicolous species and ecads as verified by the clustering of genotypes within Figure 9; however, as discussed above it worked best for groups of questionable samples from a given site and it was not always possible to apply this procedure to individual samples. It is important to emphasize again that morphological misidentifications were limited to plants that failed to display species-specific traits of characters. Therefore, 'outlier' genotypes in Figure 9b and 9c for both *F. vesiculosus* and *F. spiralis* are best interpreted as samples morphologically typed to the wrong species rather than being representative of actual intra-specific genetic variation.

Thus, taxon assignment using microsatellite markers shows potential for *Fucus* species, particularly for saxicolous juvenile plants or those lacking distinctive species-specific morphological characters. As any entangled or embedded sample was designated as an ecad the correct taxonomic delineation is based solely upon morphology. Even so, ecads also clustered fairly well within the FCA (Figure 9), suggesting that the five loci used in this study were sufficient to permit genetic identifications of ecads and to determine their affinities with parental taxa.

Population Analysis of Fucus *from Wells and Brave Boat Harbor*

Analysis of *Fucus* populations from Wells Harbor (WH) showed several similarities to Brave Boat Harbor (Chapter II), especially for *F. spiralis* and the muscoides-like *Fucus*. For example, estimates of F_{IS} over all loci for *F. spiralis* were 0.529 (WH) and 0.596 (BBH), while estimates for the muscoides-like *Fucus* were –0.453 (WH) and –0.349 (BBH). Similarly, the observed heterozygosity (H_O) of *F. spiralis* was 0.12 (WH) and 0.183 (BBH); that of the muscoides-like *Fucus* was 0.789 (WH) and 0.821 (BBH). The only taxon that differed significantly in these parameters between the two sites was *F. vesiculosus* ecad *volubilis*, which displayed a significant heterozygote deficit over all loci at WH and an excess at BBH. Lastly, allele frequencies were generally similar for all three taxa between sites. The observations above are of interest as they suggest that the conclusions from these studies are generally applicable to estuarine *Fucus* within the Gulf of Maine.

While no attached forms of *F. vesiculosus* were examined at Wells Harbor (and consequently no computer simulation of an F_1 hybrid population was generated), genetic analysis of the muscoides-like dwarf forms at Wells Harbor is nonetheless consistent with the hypothesis that they are primarily hybrid in origin. As with the dwarf *Fucus* from Brave Boat Harbor, a hybrid origin is suggested by allele frequency patterns (Table 8) as well as FCA (Figure 7a). Interestingly, at several loci the dwarf *Fucus* possessed alleles at relatively high frequencies that were absent from both *F. spiralis* and *F. vesiculosus* ecad *volubilis* (Table 8). The most likely explanation for this is that these alleles are present within attached *F. vesiculosus* populations, and further evaluations of Wells Harbor with saxicolous *F. vesiculosus* plants are planned. Also of interest was the presence of several putative hybrid genotypes of *F. vesiculosus* ecad *volubilis* (Group 1, figure 7a). While it proved difficult to make a one-to-one match between specific samples and points on the FCA, several alleles were shared in common between group one and the muscoides-like *Fucus* that were not found in groups two or three of *F. vesiculosus* ecad *volubilis*, including the 124 bp allele at locus F227 and the 137 bp allele at locus F300. While there seems to be little genetic evidence directly connecting ecad *volubilis* to dwarf forms in Brave Boat Harbor, the presence of hybrid genotypes amongst the former taxon may indicate that some muscoides-like forms have arisen *via F. vesiculosus* ecad *volubilis* at Wells Harbor.

While comparisons of genetic partitioning between taxa (F_{ST}) should be used cautiously (as interspecific comparisons generally represent phylogenetic rather than population analyses), estimates between putative hybrids and parental populations can be compared between sites or relative to the partitioning found at the infraspecific level. In general, it would be expected that ecads (as the term is applied to detached *Fucus* taxa) would differ little or not at all genetically from their parent species at the same site.

Taxonomic varieties, in turn, should display greater differentiation from the 'parent'. Lastly, should a group presently regarded as an infraspecific taxon display partitioning as great or greater than that found between its 'parent' and a closely related species (such as *F. vesiculosus* and *F. spiralis*), then this may be grounds for elevating that group to species status.

The F_{ST} estimates between Wells Harbor and Brave Boat Harbor suggests that the muscoides-like *Fucus* from these sites is not a true ecad to the extent that they are genetically different from their parent species (Table 9). While the muscoides-like *Fucus* from both sites showed little genetic partitioning ($F_{ST} = 0.039$), such a result cannot be due to interbreeding within this group. Instead, it must be a consequence of relatively little genetic partitioning between populations of *F. vesiculosus* and *F. spiralis* between the two sites. In fact, *F. spiralis* displayed very little difference between the two salt marshes ($F_{ST} = 0.020$): genetic evaluations of *F. vesiculosus* from Wells Harbor are planned.[2] The hybrid status of a majority of the dwarf *Fucus* from Brave Boat Harbor has already been demonstrated (Chapter II), and the low F_{ST} estimates between this taxon from both sites suggests that muscoides-like forms from Wells Harbor may well also be hybrid in origin.

Estimates of F_{ST} between *F. vesiculosus* from Brave Boat Harbor and its ecad *volubilis* from both sites ($F_{ST} = 0.075$ for Brave Boat Harbor and $F_{ST} = 0.145$ for Wells Harbor, respectively) are smaller than those between saxicolous species and the muscoides-like *Fucus*. However, the presence of some genetic differentiation between *F.*

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[2] Thus, two predictions can be made regarding *F. vesiculosus* from Wells Harbor; (1) estimates of F_{ST} between it and *F. vesiculosus* from Brave Boat Harbor should be low; (2) private alleles found at relatively high frequencies in the muscoides-like *Fucus* from Wells Harbor should be present in *F. vesiculosus* from that site.

vesiculosus ecad *volubilis* and *F. vesiculosus* within Brave Boat Harbor is inconsistent with a strict classification of these forms as ecads as well. It would be of particular interest to include populations of *F. vesiculosus* ecad *spiralis* from one or both sites in this analysis assuming it is present. Combined with the results of genetic analysis from sites throughout the Gulf of Maine (see below), the results from both Brave Boat Harbor and Wells Harbor indicate that a taxonomic reevaluation of the dwarf *Fucus* and *F. vesiculosus* ecad *volubilis* recognizing the genetic differences displayed by these taxa may be appropriate; these issues are discussed in more detail below.

Saxicolous Populations of Fucus vesiculosus *and* F. spiralis *Within the Gulf of Maine*

Much uncertainty exists regarding the appropriate taxonomic status of several salt marsh fucoids. By carrying out a study using samples from throughout the Gulf of Maine, I hoped to provide a genetic perspective on the systematic relationships of some of these taxa and to see how these results compared to more detailed studies from specific salt marshes (discussed above). In addition, the results of this study give some insights into the genetics underlying ecad formation. In terms of attached (saxicolous) *F. vesiculosus* and *F. spiralis*, the most significant observations from the present study are the presence of putative hybrid genotypes (meaning recent hybrids, i.e., F_1 or F_1 x parental backcrosses) within both species. The existence of putative hybrids and several related issues are discussed below.

Analysis using microsatellite markers showed that *Fucus vesiculosus* and *F. spiralis* are genetically well differentiated from each other, despite a small subset of misclassified samples (Figures 9b-c, 10). The degree of overlap between *F. vesiculosus* or *F. spiralis* with the hybrid simulation within the FCA is much less than that seen for ecad *lutarius* and the muscoides-like *Fucus*, and both saxicolous species generally displayed very different allele frequencies than the F_1HYB simulation (Figures 9b-c, h; also see below). However, any genotype of either species that overlaps with the computer simulation of F_1 hybrids (Figure 9h) may represent a putative hybrid (discounting misclassifications –discussed above). Therefore, the present research supports the idea that hybrids are present in saxicolous populations of both *F. vesiculosus* and *F. spiralis* in salt marshes throughout the Gulf of Maine, though it seems likely that hybrids are more frequent at some sites than others. In a phylogenetic study using the ribosomal internal transcribed spacer (ITS) region Serrão *et al.* (1999a) found that individuals of both *F. vesiculosus* and *F. spiralis* were sometimes more closely related to members of the opposite species from the same site than to conspecifics from other sites; hence, they hypothesized that introgressive hybridization was occurring and was responsible for their ITS observations. As most fucoid ecads do not reproduce sexually, putative hybrids amongst attached "parental" populations represent the most likely source of gene flow between the two species. Further studies to document such gene flow are obviously warranted.

Despite the presence of putative hybrids in both attached species as well as some misidentifications, *Fucus spiralis* was strongly differentiated from the *Fucus vesiculosus* grouping in neighbor-joining analysis (Figure 10). The F_1HYB simulation also grouped away from *F. vesiculosus* with 100% bootstrap support. Obviously, Figure 10 cannot be interpreted as implying that the F_1HYB simulation is more closely related to *F. spiralis* than *F. vesiculosus*, as it received exactly half of its alleles from both parents. Rather, it indicates that the average genetic distance between groups within those *F. vesiculosus* taxa examined is much less than that which separates *F. vesiculosus* from a hybrid taxon. Conversely, the genetic distances between *F. spiralis*, ecad *lutarius*, and the muscoides-like forms are much larger than those found between the *F. vesiculosus* taxa, and the ecad *lutarius*, muscoides-like forms, and F_1HYB simulation are poorly resolved relative to each other. In general, the distribution of genotypes present in the various ecads relative to parental forms as well as their affinities (particularly in Figure 9) are of particular interest, as these data suggest hypotheses for the origins of several of these limicolous forms within the Gulf of Maine.

Genetic Affinities of Fucus spiralis *and Associated Ecads Within the Gulf of Maine*

A comparison of the genotypic distribution of *Fucus spiralis* ecad *lutarius* (Figure 9d) with the F_1HYB computer simulation (Figure 9h) shows that while the former taxon has numerous genotypes that are probably recent hybrids (i.e., F_1 or F_2), other genotypes are outside the range of variation displayed by the hybrid simulation. Thus, *F. spiralis* ecad *lutarius* appears to be comprised of a mixture of hybrids and relatively "pure" genotypes of both parental species (with *F. spiralis* being much better represented than *F. vesiculosus*). The situation is quite different for muscoides-like forms, which show a range of genotypic variation almost entirely within the range expected for a population of F_1 hybrids (Figure 9e). In addition, *F. spiralis* ecad *lutarius* and the muscoides-like form are most similar to the hybrid simulation in terms of allele frequencies (Figures 8a-d) as well as observed heterozygosity (Table 10).

In determining the origins of *Fucus* ecads, three general considerations must be recognized. First, all entangled and embedded *Fucus* ecads are originally derived from attached plants (Fritsch 1959). Secondly, the mechanisms responsible for the loss of a holdfast and subsequent limicolous habit discussed in Chapter I (e.g. wave action, ice rafting, loss of substrate; also see Norton and Mathieson 1983) appear to be largely extrinsic to the plant. Finally, ecological differences exist between habitats occupied by *Fucus* ecads (particularly the dwarf embedded *Fucus*) and attached saxicolous forms (Baker and Bohling 1916, Wynne and Magne 1991, Mathieson and Dawes 2001). The above considerations, as well as the genetic data previously discussed, are consistent with the following hypothesis for the origin of *F. spiralis* ecad *lutarius* and the muscoides-like *Fucus*. First, hybrid plants may be found within attached populations of *F. spiralis*, albeit at low frequencies (discussed above). Next, subsequent recruitment of limicolous plants occurs largely at random relative to genotype, meaning that new recruits consist of a mixture of relatively 'pure' *F. spiralis* as well as a smaller number of hybrid plants. Over time, proliferation, burial, basal decay, and fragmentation lead to production of *F. spiralis* ecad *lutarius* (Mathieson and Dawes 2001). However, in the process hybrid genotypes display increased survivorship over 'pure' genotypes, leading to a relatively larger proportion of the former in populations of ecad *lutarius*. As further proliferation and decay produce muscoides-like forms from *F. spiralis* ecad *lutarius*, the ratio of hybrid to 'pure' genotypes further increases, until the smallest muscoides-like forms tend to be almost entirely dominated by F_1 hybrids or recent backcrosses. While under this scenario the ratio of hybrids to 'pure' genotypes is correlated with size, the change in this ratio is presumably caused by selection for genotypes best able to survive the stresses associated with detachment combined with exposure to extreme habitat conditions (see Chapters I and II). Thus, this hypothesis presumes that exogenous selection (i.e., selection largely imposed by a particular habitat as opposed to competition within and between organisms) is responsible for the observed ratios of putative hybrid to 'pure' genotypes amongst *F. spiralis* ecad *lutarius* and the muscoides-like *Fucus*.

The role of exogenous selection in the establishment and maintenance of hybrid zones has been discussed by numerous authors (Moore 1977, Moore and Price 1993, Arnold and Hodges 1995, Rieseberg and Carney 1998, Barton 2001, Burke and Arnold 2001). As discussed in Chapter II, the 'Bounded Hybrid Superiority' (BHS) model (Moore 1977, Moore and Price 1993) predicts that hybrid genotypes will tend to be favored in habitats representing environmental extremes for the species. In this regard, the muscoides-like *Fucus* tend to occur in fringe habitats high within the intertidal zone, whereas *F. spiralis* ecad *lutarius* is generally found somewhat lower in the intertidal (Baker and Bohling 1916, Fritsch 1959, Wynne and Magne 1991, Mathieson and Dawes 2001, Mathieson *et al.* 2001). Both taxa must tolerate longer periods of emersion than *F. vesiculosus* or *F. spiralis*.[3] More generally, the BHS model is consistent with the observations of various workers (Sauvageau 1909, Kniep 1925, Burrows and Lodge 1951, Boney 1966) who conclude that hybrid *Fucus* populations tend to be most successful in areas where they do not directly compete with parental forms, such as recently cleared areas or areas subjected to environmental disturbance. The preceding observation is consistent with my results (granting the hybrid status of the muscoides-like

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[3] Mathieson and Dawes (2001) document the muscoides-like *Fucus* and *F. spiralis* occurring at the same elevation at some sites in Brave Boat Harbor. It would be of interest to determine if some of these *F. spiralis* plants are hybrid.

Fucus), since in addition to growing high within the intertidal, this taxon commonly occurs along banks subject to relatively rapid erosion (Cotton, 1912, Wynne and Magne 1991, Mathieson and Dawes 2001).

While the role of selection was not explicitly examined in this study, selective effects on the origins of *Fucus spiralis* ecad *lutarius* and the muscoides-like *Fucus* within the Gulf of Maine as predicted by the BHS model are amenable to investigation in future studies. Mathieson and Dawes (unpublished) have demonstrated that environmental extremes exert a significant effect on the morphology of *Fucus*; in any event, examining the selective pressures imposed by such environmental extremes would be a logical direction for future studies.

It is worth comparing the results of the present study with those obtained by Mathieson and Dawes (2001, unpublished) at Brave Boat Harbor, who transplanted *Fucus spiralis* from the lower to the upper marsh (in terms of vertical zonation along the shore) and muscoides-like forms from the upper to lower marsh. Their results showed that *F. spiralis* and the muscoides-like forms often converged upon an intermediate morphology (i.e., ecad *lutarius*) and from this they concluded that the muscoides-like *Fucus* at Brave Boat Harbor was largely derived from *F. spiralis via F. spiralis* ecad *lutarius*. A subsequent study by Mathieson and Dawes (unpublished) involving multiple sites throughout the Northwest Atlantic as well as sites from Ireland and Alaska, has concluded that muscoides-like forms may be derived from either *F. vesiculosus* or *F. spiralis*, depending upon the dominant species within a given salt marsh.

While the conclusions of Mathieson and Dawes are somewhat different than those presented in Chapters I and II of this thesis, I believe that they are complementary to my own. Mathieson and Dawes documented the 'short term' effects of transplantation on saxicolous and ecad forms (i.e., ≈ 1 year), and several workers point out that unattached seaweeds may live for years (Cotton 1912, Norton and Mathieson 1983). In this regard, Norton and Mathieson (1983) report that *Ascophyllum nodosum* (L.) Le Jol. (which belongs to a sister genus of *Fucus*) may take several years to form *A. nodosum* ecad *mackaii* (Turner) Cotton following the loss of a holdfast, a plant that is similar in many ways to the muscoides-like *Fucus.* Assuming that selection favors hybrids in the formation of muscoides-like forms as a function of time, it would be difficult to distinguish between the morphological plasticity of *Fucus* and the genetic potential for long-term survivorship in transplant studies without careful monitoring of individual plants over several years. Due to factors such as erosion, wave action, ice scouring, and fragmentation of the plants themselves, it is extremely difficult to conduct such long-term monitoring. Alternatively, the population genetic analysis of *F. spiralis* ecad *lutarius* and particularly the smaller muscoides-like forms in the present study provides a method to look at rough changes in genotypic proportions amongst ecads over time, as the latter taxon appears to be largely derived from the former in the Gulf of Maine.

As my results suggest that *Fucus spiralis* ecad *lutarius* may often be a hybrid, this taxon requires further study; thus, it may be misleading to regard it strictly as being derived from *F. spiralis*, despite the fact that it often appears morphologically similar to this species. One explanation for the observed morphological similarity might be that hybrid plants generally resemble *F. spiralis* rather than *F. vesiculosus.* In a study of reciprocal transplants in kelp, Druehl and Collens (2003) found that hybrids tended to resemble the female parent. However, hybrid resemblance to the female parent seems unlikely to explain a trend towards *F. spiralis*-like morphologies for an *F. spiralis* x *F. vesiculosus* hybrid, because *F. spiralis* is hermaphroditic and partially selfing and most eggs may be fertilized prior to release (Chapman 1995, Scott and Hardy 1994). Therefore, it would be predicted that most hybrids would be produced from *F. vesiculosus* eggs. Further, the present study suggests that hybrid genotypes are likely present within both attached species, although they seem to occur at a higher frequency in *F. spiralis*. Whatever the ultimate cause, the relatively large number of apparent hybrids in a taxon associated with *F. spiralis* on morphological ground warrants further study (see below).

To summarize the origin of muscoides-like *Fucus* within the Gulf of Maine, it appears to be linked to saxicolous populations of *F. spiralis via* the entangled ecad *lutarius*. However, the morphological continuum that connects these distinct forms appears to reflect an underlying genotypic continuum in which F_1 hybrids or recent backcrosses tend to become increasingly frequent in the smallest limicolous *Fucus* populations. Finally, regardless of how this situation is addressed taxonomically, the apparent hybrid component of the limicolous *Fucus* taxa (discussed above) in the Gulf of Maine should be recognized, and these forms should not be confused taxonomically with similar forms that lack this hybrid character.

Genetic Affinities of the Fucus vesiculosus Species Complex Within the Gulf of Maine

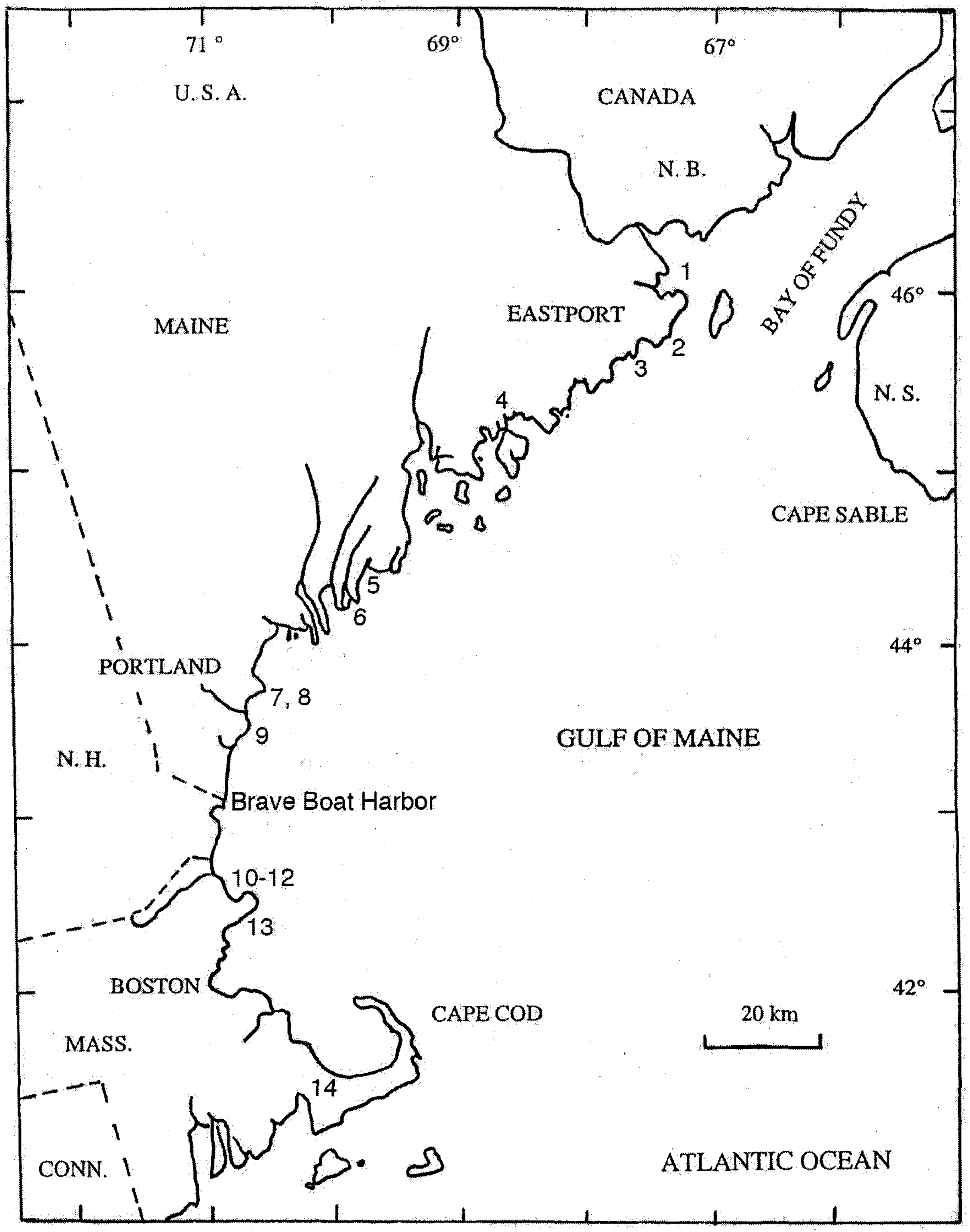
Genetic analyses of taxa within the *Fucus vesiculosus* species complex in the Gulf of Maine were intended to resolve several questions within this group, including: (1) the affinities of *F. vesiculosus* and its ecad *volubilis* with muscoides-like forms; and (2) the affinities of *F. vesiculosus* var. *spiralis*. Both issues are discussed below.

As muscoides-like *Fucus* from the Gulf of Maine appears to be primarily hybrid in origin, the preceding discussion regarding the BSH model is applicable to *Fucus vesiculosus* as well as *F. spiralis*. To the extent that ecad *lutarius* is composed of hybrid genotypes, they are equally related to both species. However, most 'pure' genotypes of *F. spiralis* ecad *lutarius* examined here were clearly derived from *F. spiralis*. While several 'pure' *F. vesiculosus* genotypes were present in ecad *lutarius*, these were mainly from a "*gracillimum*-like" form [i.e., an elongate form bearing some resemblance to *F. vesiculosus* f. *gracillimus* Collins, a saxicolous estuarine taxon only reported from Massachusetts (Collins 1900, Taylor 1957)] collected from a single site (Conomo Point, MA – Figure 5, Table 7).

Originally, I hypothesized that *Fucus vesiculosus* ecad *volubilis* might represent an intermediate between *F. vesiculosus* and muscoides-like forms within the Gulf of Maine. However, this taxon displayed relatively few genotypes that overlapped with the F_1HYB simulation (Figures 9g-h) and was much closer genetically to saxicolous *F. vesiculosus* than to either the muscoides-like *Fucus* or the F_1HYB simulation (Figure 10). *Fucus vesiculosus* ecad *volubilis* is quite variable morphologically and the plants examined here were generally larger and lacked extensive proliferation. However, smaller and very proliferous forms do exist at some sites. Mathieson and Dawes (unpublished) have suggested that these smaller forms may give rise to muscoides-like morphologies in the event of further proliferation and basal decay, and it would be interesting to conduct genetic evaluations of these forms as well. Therefore, despite the presence of a small number of putative *F. vesiculosus* ecad *volubilis* hybrids in this study (for example at Wells Harbor), little evidence exists that the plants I examined were likely to give rise to muscoides-like forms. Instead, *F. vesiculosus* ecad *volubilis* contained a number of unique genotypes that were well separated from *F. vesiculosus* along the Y-axis of Figure 9. In the case of the Brave Boat Harbor study (Chapter II) ecad *volubilis* also displayed several unusual genotypes, and the allele frequency patterns of this taxon in Brave Boat Harbor were consistent with a hypothesis of introgression. However, when analyzed over the Gulf of Maine as a whole the allele frequency pattern displayed by *F. vesiculosus* ecad *volubilis* was fairly similar to that of *F. vesiculosus*. Despite the close genetic similarity of all *F. vesiculosus* taxa examined (Figure 10), *F. vesiculosus* ecad *volubilis* was the most genotypically diverse taxon and contained the most unusual genotypes (Figure 9g). Hence, it would prove informative to carry out further genetic and ecological studies of this taxon.

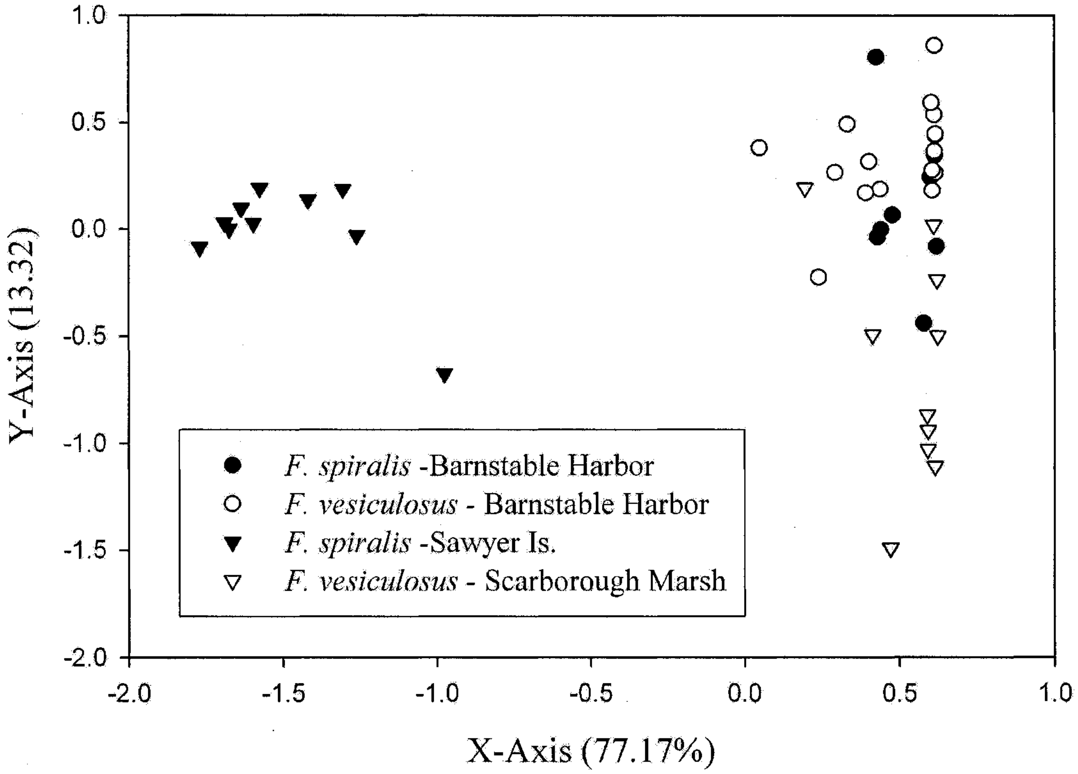
A second hypothesis examined as part of this study was that *Fucus vesiculosus* ecad *volubilis* might be derived from *F. vesiculosus* var. *spiralis*, to which it is morphologically similar. Further, *F. vesiculosus* var. *spiralis* displays the greatest tolerance to low salinity of all saxicolous taxa examined and is well represented in estuarine habitats (Niemeck and Mathieson 1978). The results presented here provide some evidence in favor of this hypothesis. Although the range of genotypic variation seen in *F. vesiculosus* var. *spiralis* was similar to that of *F. vesiculosus* using FCA (Figure 9b, f), *F. vesiculosus* var. *spiralis* and ecad *volubilis* both grouped with each other and away from *F. vesiculosus* in a neighbor- joining tree (Figure 10), albeit with only moderate bootstrap support. While my results do not disprove the hypothesis that ecad *volubilis* is derived from var. *spiralis*, they are at least as consistent with the alternative hypothesis that it is derived from *F. vesiculosus*. The present study is therefore entirely consistent with the general consensus as discussed by Baker and Boiling (1916) and Neil *et al.* (1980) that *F. vesiculosus* ecad *volubilis* is derived from *F. vesiculosus* in most cases. In addition, the position of *F. vesiculosus* ecad *volubilis* in the *F. vesiculosus* group in Figure 10 suggests that the designation "ecad" may be appropriate for this taxon (or at least for the larger forms examined in this study), since it is less distinct genetically from normal *F. vesiculosus* than *F. vesiculosus* var. *spiralis*. Further genetic evaluations of the forms and varieties of *F. vesiculosus* should be conducted to further clarify the taxonomic relationships within this species complex.

Figure 5. A map of the Gulf of Maine showing all collection sites used in the present study.



*Note that site fourteen occurs within a harbor on the northern side of Cape Cod, MA.

Figure 6. FCA showing the genotypic affinities of a set of germlings from Barnstable Harbor.



FCA showing the genotypic affinities of a set of germlings from Barnstable Harbor originally designated *F. spiralis* (represented by the black circles in the FCA) based upon morphology. Note that these samples clearly group with confirmed samples of *F. vesiculosus* and away from confirmed *F. spiralis*. Based upon this analysis, the set of Barnstable Harbor *F. spiralis* samples were subsequently reclassified as *F. vesiculosus*.

Figure 7. FCA from Wells Beach

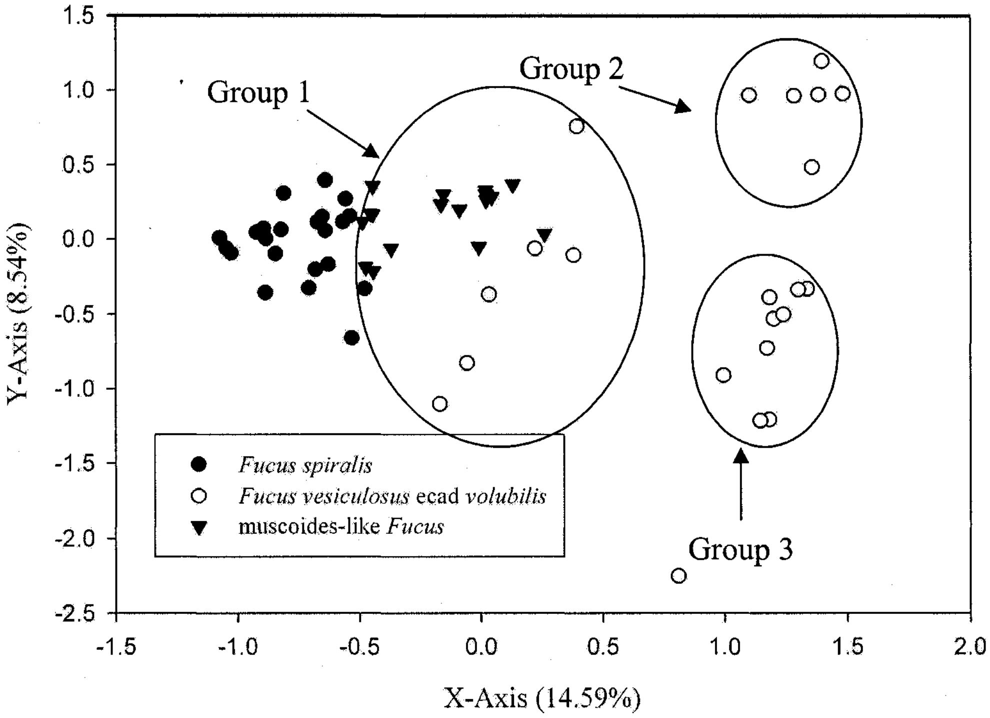


Figure 7a. FCA using all genotypes from all taxa. For each taxon, N = 40.

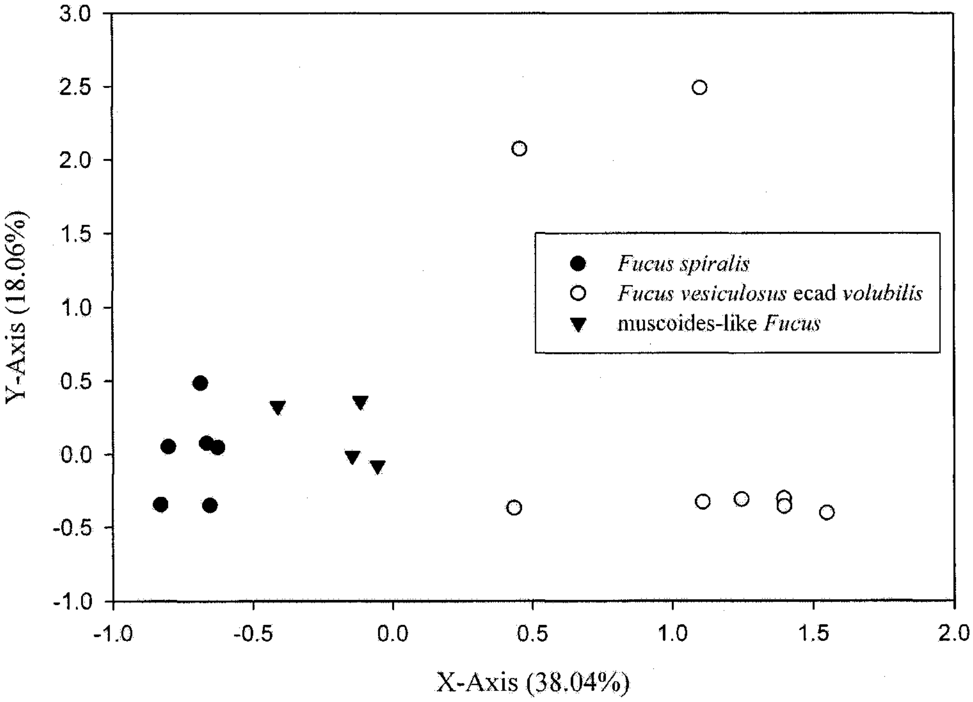


Figure 7b. FCA from Wells Beach using only genotypes with no missing data and alleles with a frequency ≥ 0.05 in the entire dataset. FCA contained 19 *F. spiralis*, 14 *F. vesiculosus* ecad *volubilis*, and 33 muscoides-like *Fucus*.

Figure 8. Allele frequencies for all taxa throughout the Gulf of Maine.

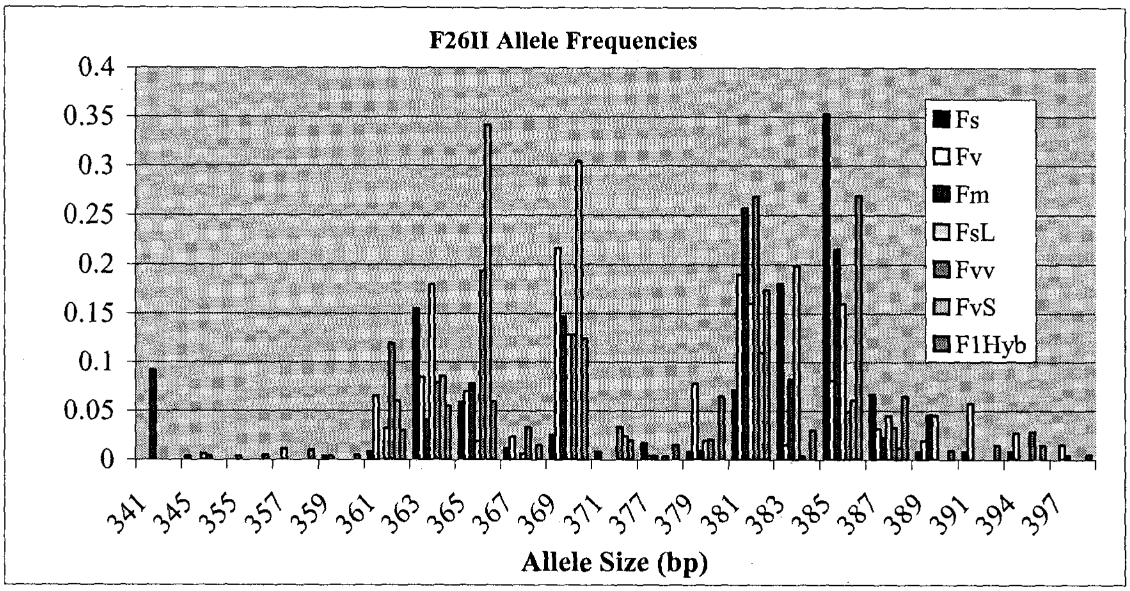


Figure 8a. For Figures a-e, Fs = *F. spiralis*; Fv = *F. vesiculosus*; mlF = muscoides-like or dwarf *Fucus*; Fsl = *F. spiralis* ecad *lutarius*; Fvv = *F. vesiculosus* ecad *volubilis*; Fvs = *F. vesiculosus* var. *spiralis*; F1Hyb = F_1 *F. vesiculosus* x *F. spiralis* computer simulated population.

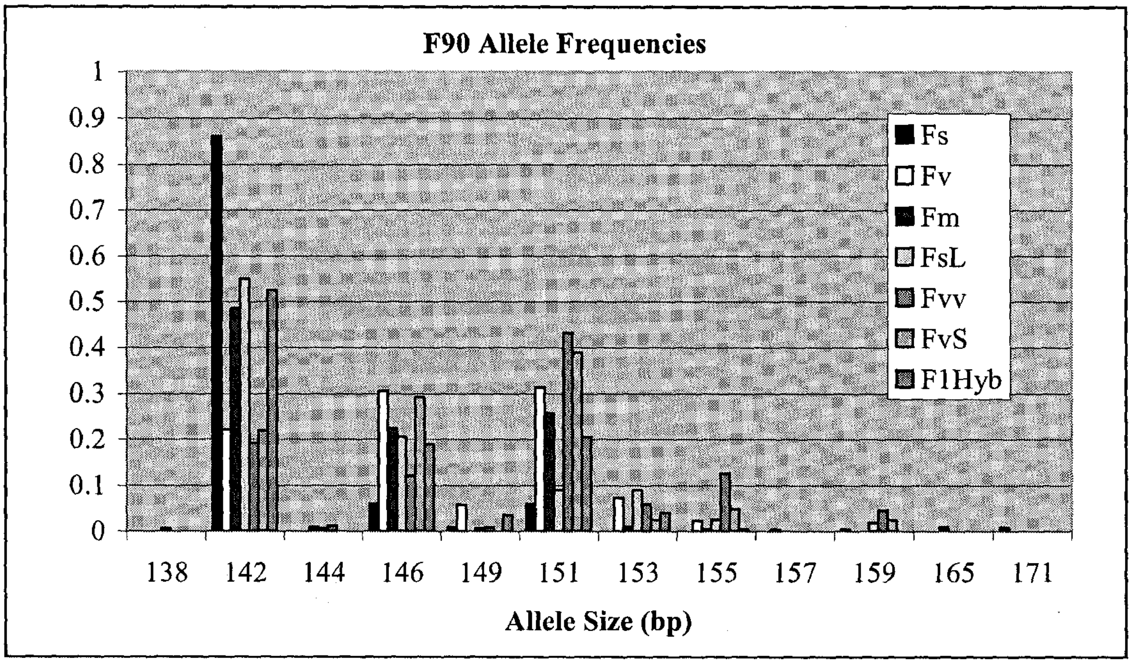


Figure 8b.

Figure 8 cont. Allele frequencies for all taxa throughout the Gulf of Maine.

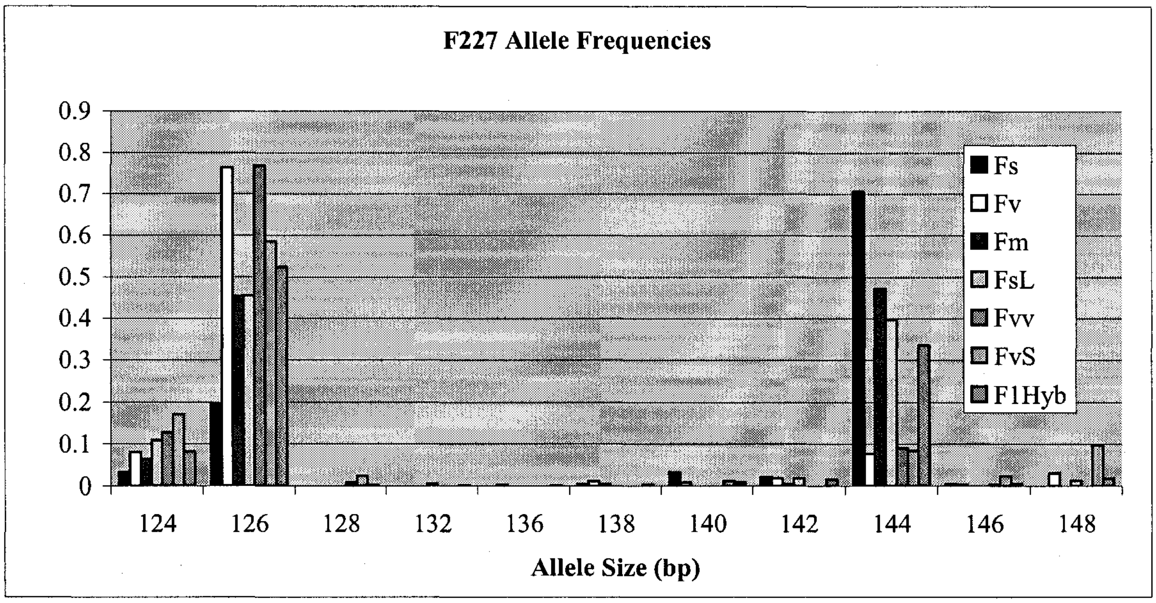


Figure 8c.

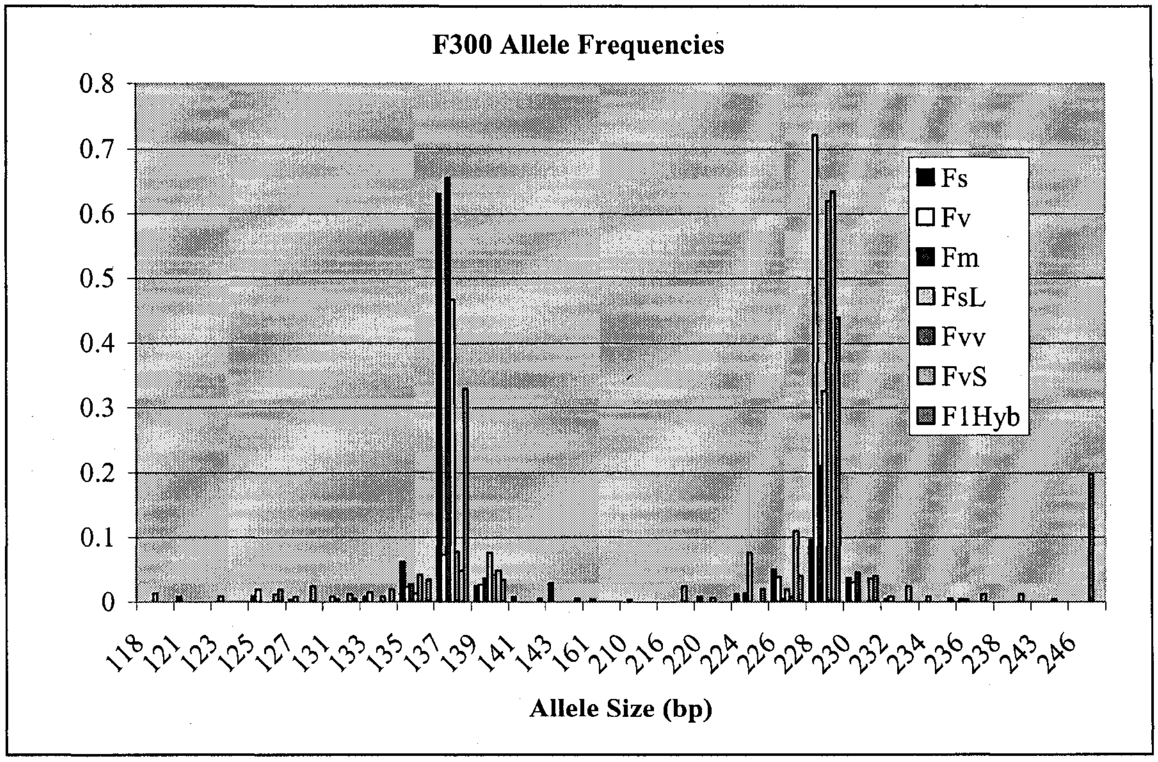


Figure 8d.

Figure 8 cont. Allele frequencies for all taxa throughout the Gulf of Maine.

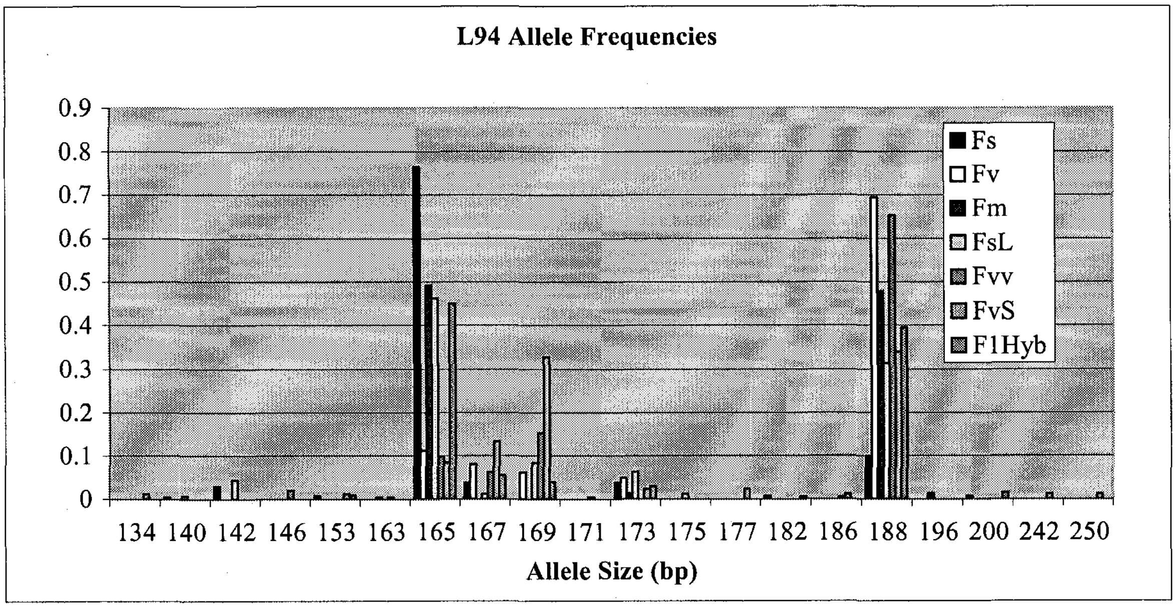


Figure 8e.

Figure 9. FCA of all *Fucus* taxa sampled throughout the Gulf of Maine

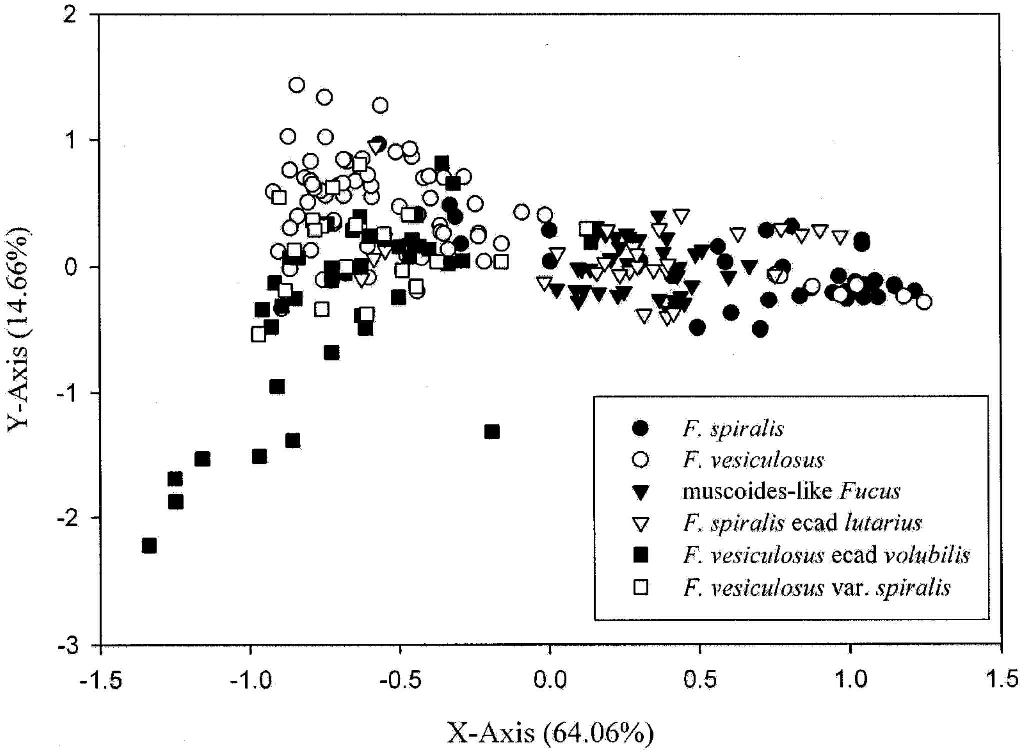


Figure 9a. For clarity, Figure 9a is reproduced as Figures 9b-g, each highlighting a single taxon compared to all others. All orientations and points correspond to Figure 9a. In this figure, a single point may represent more than one sample, and only those individuals with alleles displaying a frequency >0.05 in at least one taxon are shown. The number of individuals sampled (corresponding to those given in Table 7) is provided for each taxon below.

Figure 9 cont. FCA of all *Fucus* taxa sampled throughout the Gulf of Maine

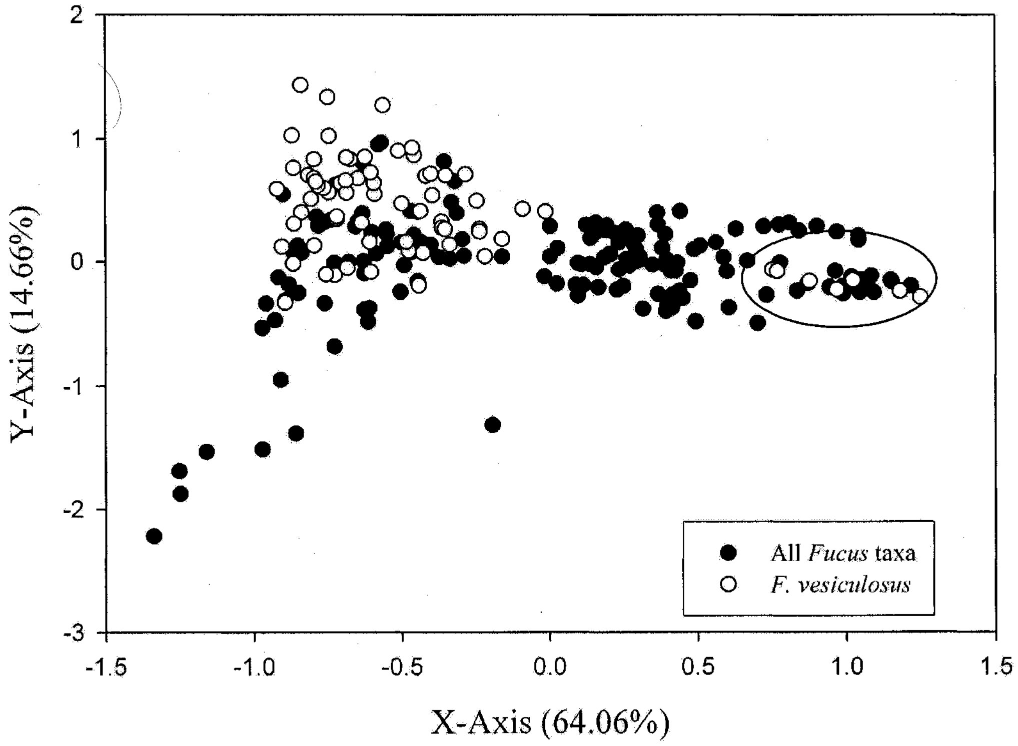


Figure 9b. FCA highlighting the distribution of *F. vesiculosus* in Figure 9a (N = 79). The circled white genotypes at the right represent misidentified *F. spiralis* within the data set, while several white genotypes in the center of the FCA may represent putative hybrids.

Figure 9 cont. FCA of all *Fucus* taxa sampled throughout the Gulf of Maine

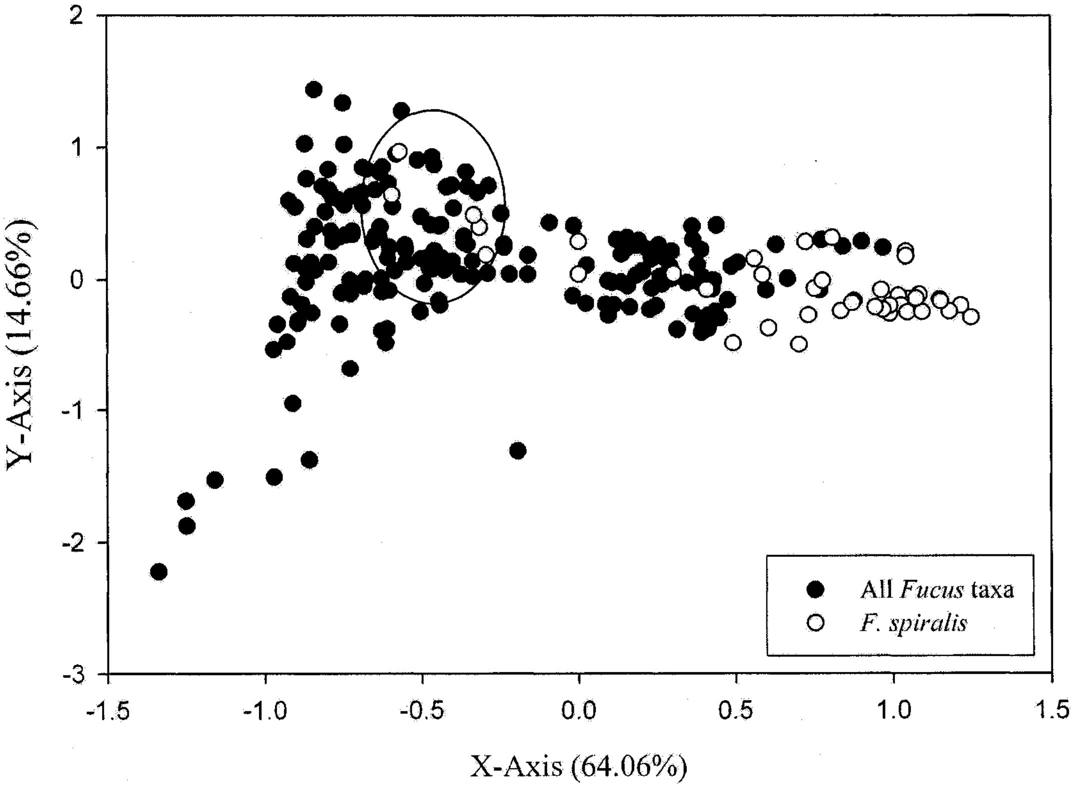


Figure 9c. FCA highlighting the distribution of *F. spiralis* in Figure 9a (N = 79). As in Figure 9b, circled white genotypes represent misidentified *F. vesiculosus* within the data set, while several white genotypes within the center of the FCA may represent hybrids.

Figure 9 cont. FCA of all *Fucus* taxa sampled throughout the Gulf of Maine

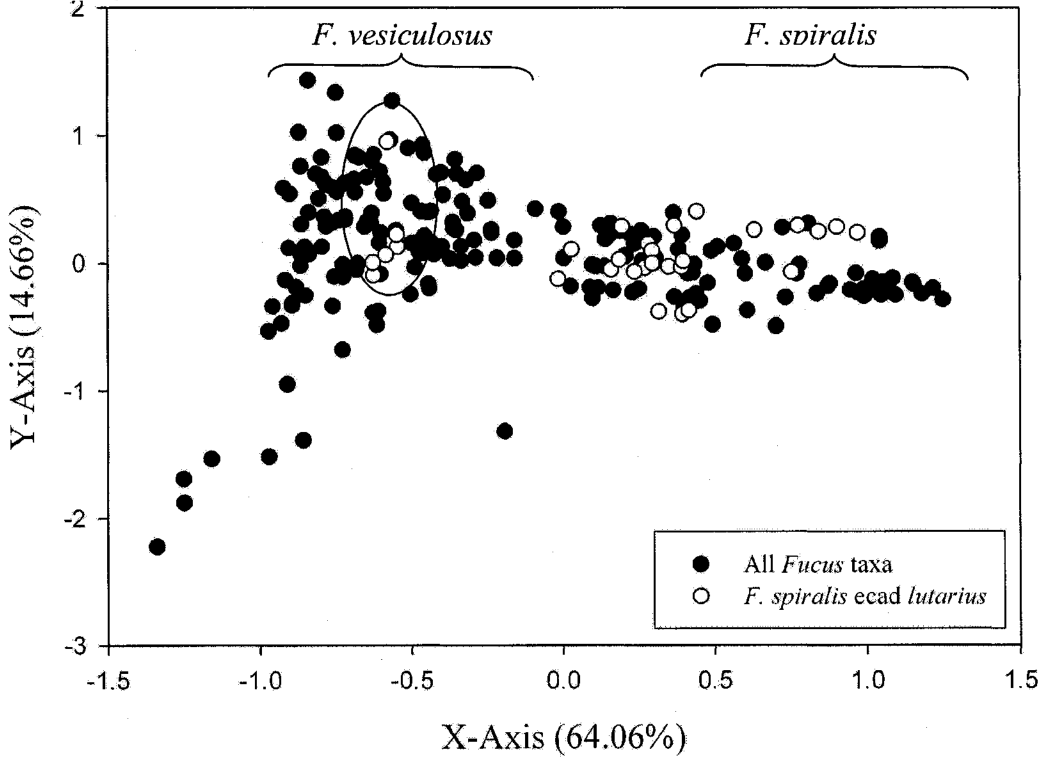


Figure 9d. FCA highlighting the distribution of *F. spiralis* ecad *lutarius* (N = 50) in Figure 9a. While white genotypes towards the center of the FCA are likely hybrid, white genotypes within the *F. vesiculosus* and *F. spiralis* clouds represent limicolous plants that are probably not hybrid. In the case of the white genotypes on the left (i.e., circled within the *F. vesiculosus* cloud, these were collected from Conomo Point, MA (Site 12), and displayed morphological affinities with *F. vesiculosus*.

Figure 9 cont. FCA of all *Fucus* taxa sampled throughout the Gulf of Maine

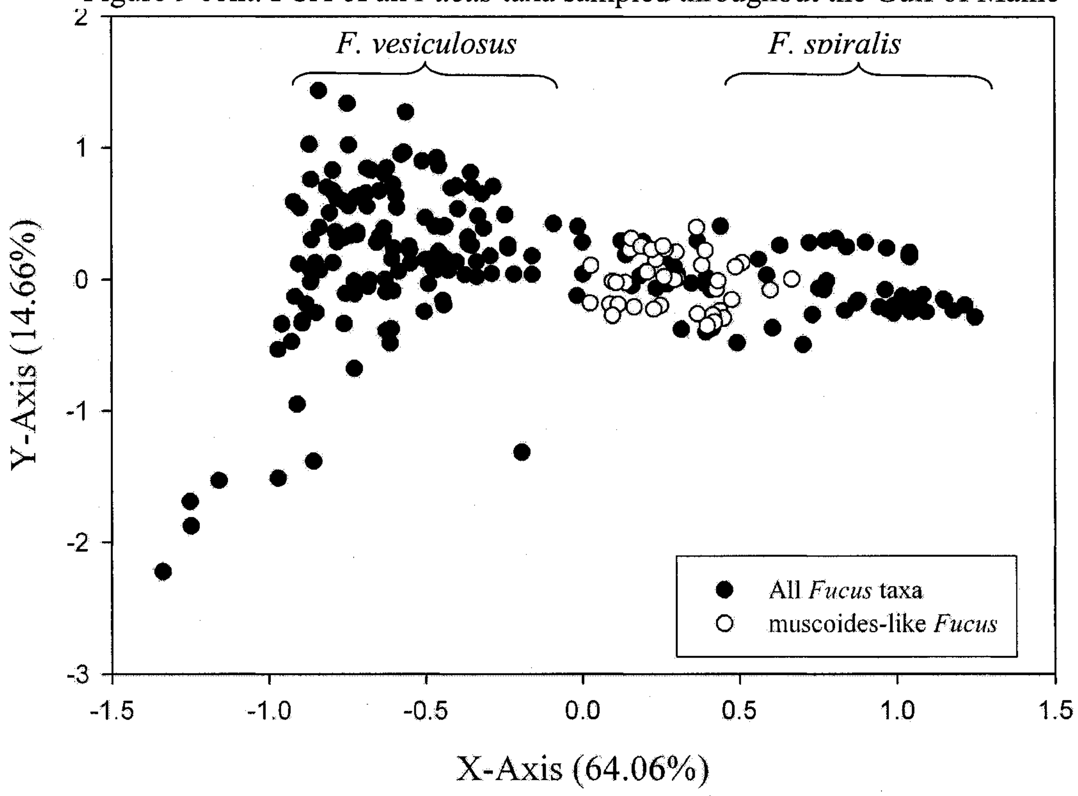


Figure 9e. FCA highlighting the distribution of the muscoides-like *Fucus* (N = 81) in Figure 9a.

Figure 9 cont. FCA of all *Fucus* taxa sampled throughout the Gulf of Maine

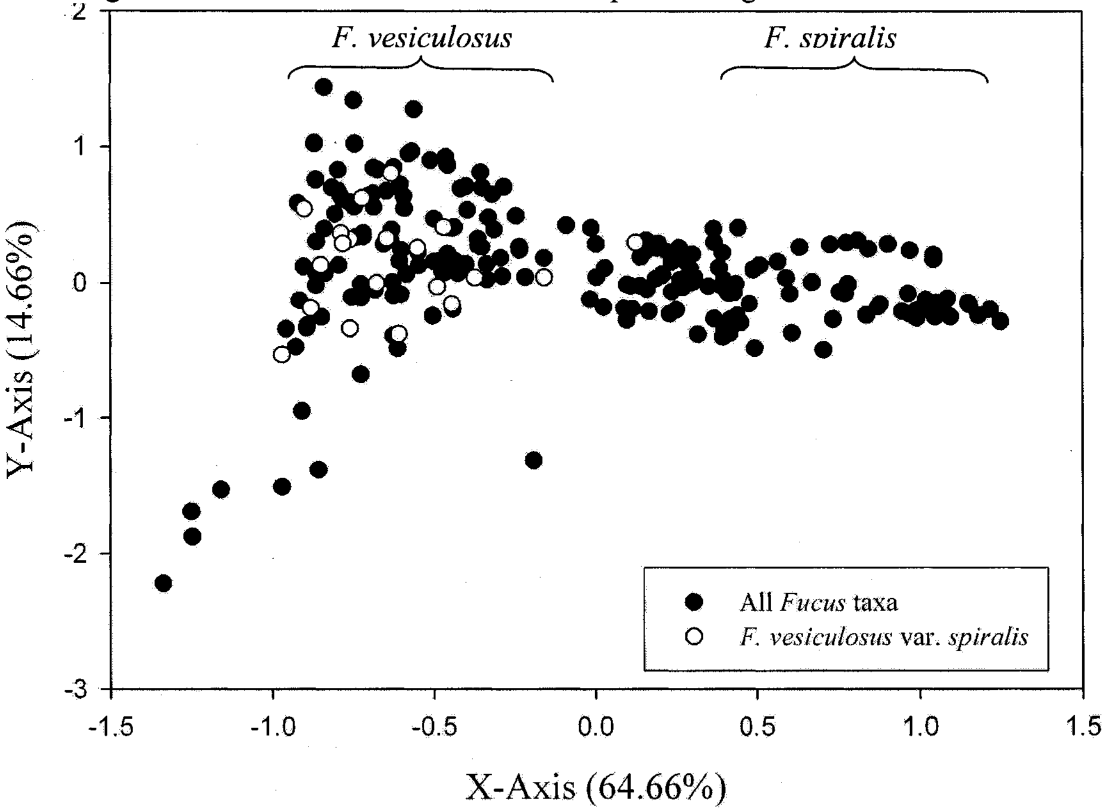


Figure 9f. FCA highlighting the distribution of *F. vesiculosus* var. *spiralis* (N = 26) in Figure 9a.

Figure 9 cont. FCA of all *Fucus* taxa sampled throughout the Gulf of Maine

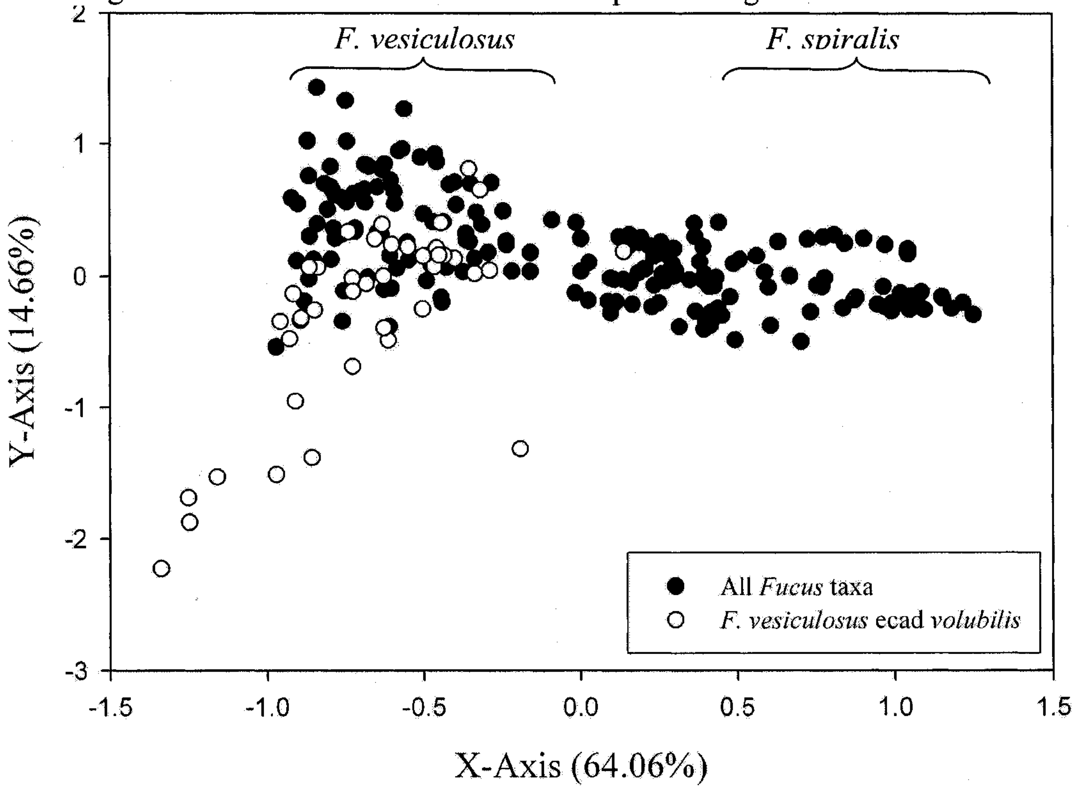


Figure 9g. FCA highlighting the distribution of *F. vesiculosus* ecad *volubilis* (N = 81) in Figure 9a.

Figure 9 cont. FCA of all *Fucus* taxa sampled throughout the Gulf of Maine

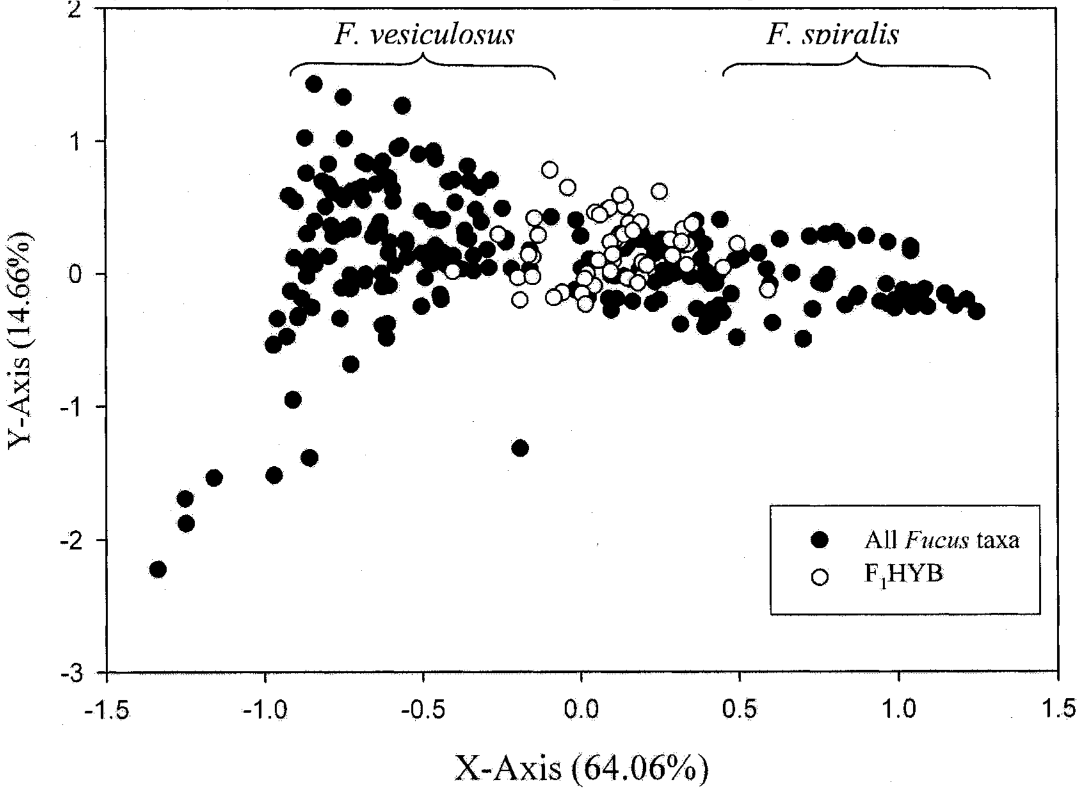


Figure 9h. Identical to Figure 9a, but showing the distribution of a population of *F. vesiculosus* x *F. spiralis* F_1 hybrid computer simulation (F_1HYB; N = 54).

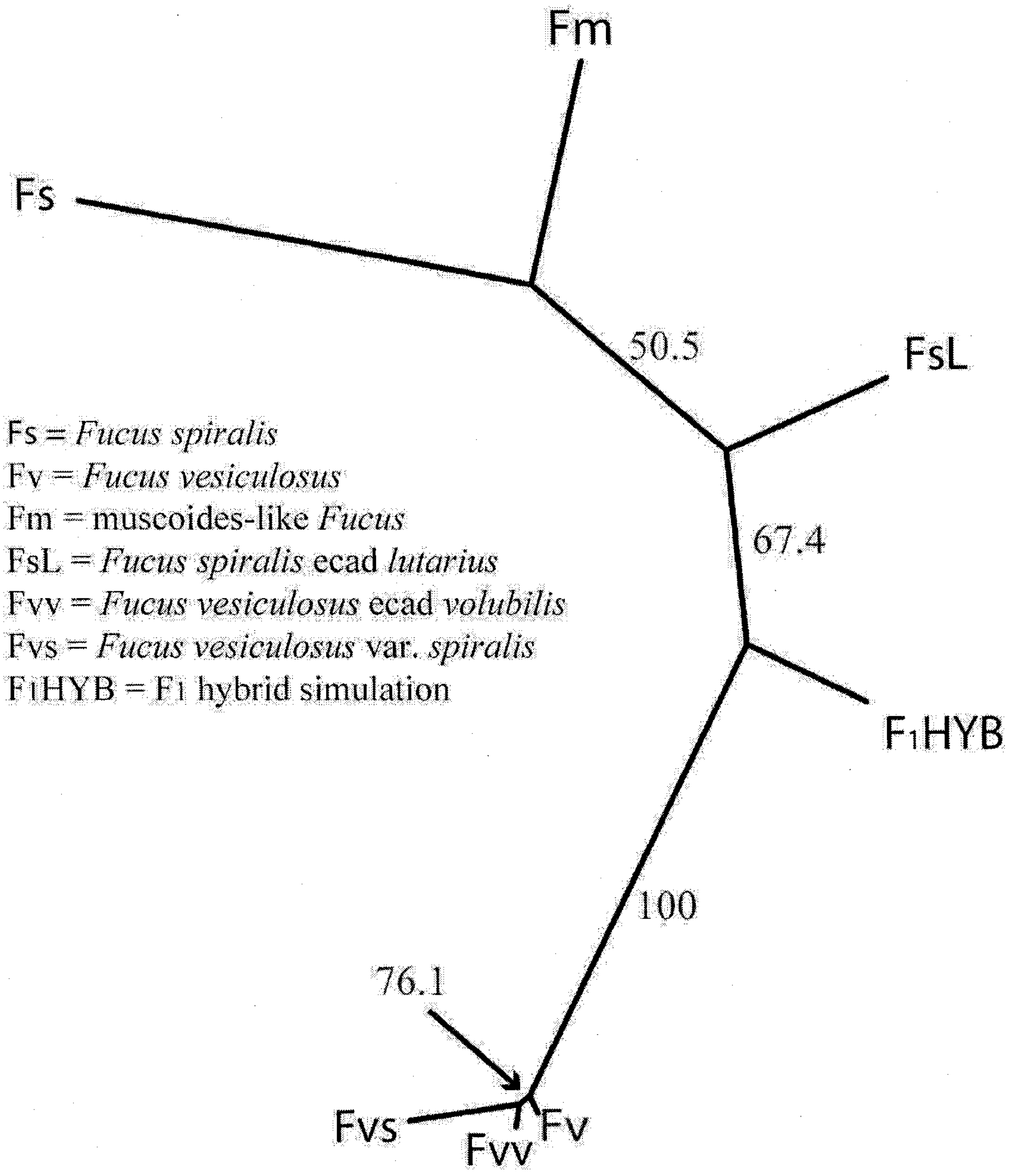


Figure10. Neighbor-Joining analysis of all *Fucus* taxa examined from the Gulf of Maine. The F_1 hybrid simulation is also included. Bootstrap values are based upon 1000 replicates.

Table 7. Morphological and genetic identifications of all samples from all sites.

Collection Site Locations	*Fucus vesiculosus*	*Fucus spiralis*	*Fucus vesiculosus* ecad *volubilis*	*Fucus vesiculosus* variety *spiralis*	*Fucus spiralis* ecad *lutarius*	muscoides-like *Fucus*
1. Reversing Falls, Pembroke, ME 44°53.1'N, 67°07.8'W	9	12 **(0)**[1]	12	3 **(15)**	5	7
2. Moose Cove, Whiting Bay, South Trescott, ME 44°44'N, 67°07.5'W	0	23 **(12)**	0	1 **(12)**	0	17
3. Sandy River Beach, Jonesport, ME 44°34.67'N, 67°34.17'W	0	0	12	0	0	3
4. Marlboro Beach Boat Ramp at Racoon Cove, Lamoine, ME 44°28.23'N, 68°16.9'W	11	0	0	1	0	0
5. Kitzi Colbi Preserve, Newcastle, ME 43°59.5'N, 69°33.9'W	0	0	0	16	0	12
6. Back River, Sawyer Island, Boothbay Harbor 43°50.2'N, 69°40'W	12	12 (vegetative)	0	2	12	0
7. Higgins Beach near Spurwink River, Scarborough, ME 43°33.7'N, 70°16.55'W	15	0	14	13	0	0
8. Scarborough Marsh near Jones Creek, Scarborough, ME 43°32.83'N, 70°22.98'W	16	0	0	0	5	15
9. Wells Harbor, ME 43°19.94'N, 70°41.89'W	0	40	40	0	0	40
10. Hampton-Seabrook Marsh, Hampton, NH 42°53.68'N, 70°50.13'W	0	24	12	7	24	0
11. Eagle Hill Public Boat Ramp, Ipswitch, MA 42°42.91'N, 70°48.80'W	19	1	18	0	0	0
12. Conomo Point, mouth of Essex River at Concord Lane, Essex, MA 42°38.1'N, 70°43.15'W	20	0	17	0	20 (*gracillimum*-like)	10

Table 7 continued.

13. Cohasset Harbor Marsh, MA 42°14.46'N, 70°46.7'W	0	15	15	0	0	0
14. Barnstable Harbor, the end of Bone Hill Road, Barnstable, MA 41°42.58'N, 70°16.57'W	25 **(15)** *F. vesiculosus*; 15 *F. vesiculosus* forma *gracillimus*	0 **(10)**	0	0	0	0
Total Number of Sites Represented:	6	10	8	7	5	7
Total Number of Samples:	88	119	130	43	66	104

Numbers in parentheses represent the numbers of each taxon collected per site based upon morphology if those numbers differed from the genetic identifications. Thus, at Site 1, out of fifteen samples of *F. vesiculosus* identified morphologically, twelve were identified as *F. spiralis* using microsatellite screening.

Table 8. Population Genetic data from Wells Harbor

Species/taxa	*F. spiralis*	muscoides-like *Fucus*	*F. vesiculosus* ecad *volubilis*
	F227 Allele Frequencies		
Allele Size (bp)			
124	0.092	0.077	0.329
126	0.092	0.41	0.513
128	---	---	0.027
144	0.816	0.513	0.132
F_{IS}	**0.019**	**-0.725**[1,2]	**0.578**[1,3]
H_E	**0.321**	**0.57**	**0.619**
H_O	**0.316**	**0.974**	**0.263**
	F90 Allele Frequencies		
142	0.908	0.5	0.171
144	---	0.026	---
146	---	0.141	---
149	0.013	---	---
151	0.066	0.333	0.474
155	---	---	0.237
159	0.013	---	0.118
F_{IS}	**-0.064**	**-0.441**[1]	**-0.076**[1]
H_E	**0.173**	**0.626**	**0.685**
H_O	**0.184**	**0.897**	**0.737**
	F300 Allele Frequencies		
133	---	---	0.026
135	0.033	---	0.04
137	0.767	0.846	0.158
139	---	0.103	---
141	0.033	---	---
143	0.117	---	---
224	---	0.026	---
226	0.033	---	---
228	---	0.026	0.408
230	0.017	---	---
246	---	---	0.368
F_{IS}	**0.754**[1,3]	**0.631**[1,3]	**0.422**[1,3]
H_E	**0.402**	**0.276**	**0.68**
H_O	**0.1**	**0.103**	**0.395**
	F26II Allele Frequencies		
341	---	0.256	---
361	---	---	0.118
365	---	---	0.026
369	---	0.154	0.013
371	---	---	0.105

Table 8 continued.

	F26II Allele Frequencies		
381	---	0.333	0.5
383	---	0.013	---
385	0.765	0.231	0.118
387	0.235	0.013	0.04
394	---	---	0.079
F_{IS}	**1.0**[1,3]	**-0.294**[1,2]	**0.3**[1]
H_E	**0.365**	**0.756**	**0.712**
H_O	**0.0**	**0.974**	**0.5**
	F94 Allele Frequencies		
165	1.0	0.5	0.158
167	---	---	0.132
169	---	---	0.197
188	---	0.5	0.513
F_{IS}	**N/A**	**-1.0**[1,2]	**-0.151**
H_E	**0**	**0.506**	**0.664**
H_O	**0**	**1**	**0.763**
	Population parameters over all loci		
Mean # of Alleles/polymorphic locus	3.2	3.8	5
F_{IS}	**0.529**[3]	**-0.453**[2]	**0.211**[1,3]
H_E	**0.252**	**0.547**	**0.672**
H_O	**0.12**	**0.789**	**0.531**

[1] = Significant deviation from Hardy-Weinberg equilibrium
[2] = Significant heterozygote excess
[3] = Significant heterozygote deficit

Table 9. Pairwise F_{ST} comparisons between *Fucus* taxa from Wells Harbor (MA) and Brave Boat Harbor (ME).

		Wells Harbor			Brave Boat Harbor		
		Fs	mlF	*Fvv*	*Fs*	mlF	*Fvv*
Wells Harbor	*Fs*	0					
	MlF	0.213	0				
	Fvv	0.448	0.241	0			
Brave Boat Harbor	*Fs*	0.020	0.212	0.488	0		
	mlF	0.178	0.039	0.176	0.198	0	
	Fvv	0.320	0.146	0.112	0.358	0.054	0
	Fv	0.481	0.289	0.145	0.516	0.192	0.075

Table 10. Observed heterozygosity of all taxa from the Gulf of Maine.

Taxon	*F. vesiculosus*	*F. spiralis*	muscoides-like *Fucus*	*F. spiralis* ecad *lutarius*	*F. vesiculosus* ecad *volubilis*	*F. vesiculosus* var. *spiralis*	F_1HYB
H_O	0.401	0.2	0.872	0.731	0.431	0.478	0.862

CHAPTER IV

THE GENETIC AFFINITIES OF *FUCUS COTTONII* WYNNE *ET* MAGNE FROM ROSMUC, IRELAND

Abstract

The taxonomic status of the limicolous fucoid taxon *Fucus cottonii* Wynne et Magne has been the subject of much controversy. To help provide insight into the origins of this unusual taxon, samples were collected from populations of four *Fucus* taxa from a salt marsh at Rosmuc, Ireland, and genotyped at four microsatellite-containing loci. The taxa sampled were *F. cottonii*, *F. vesiculosus*, *F. spiralis*, and a putative *F. vesiculosus x F. spiralis* hybrid (PH). The observed heterozygosity of *F. cottonii* at Rosmuc was much lower than that seen in muscoides-like *Fucus* from the northwest Atlantic. In addition, factorial correspondence analysis showed that *F. cottonii* displayed the most genetic similarity to *F. vesiculosus* and was not strongly affiliated with *F. spiralis*. Whereas studies within the Gulf of Maine indicate that muscoides-like forms are often derived from hybridization between *F. vesiculosus* and *F. spiralis*, my data suggests that *F. cottonii* from Rosmuc is likely derived from *F. vesiculosus* and is not of hybrid origin. However, genetic differences between *F. cottonii* and *F. vesiculosus* also are present, and the designation of the former taxon as an ecad of the latter may therefore be inappropriate. A putative hybrid previously identified by Dr. Robert Wilkes based on morphological criteria also did not appear to be a genetic hybrid. Estimates of F_{ST} between Rosmuc *Fucus* populations and those found in the Gulf of Maine indicate that

intra-site genetic variation between Rosmuc *Fucus* is less than intraspecific variation between Irish *Fucus* and populations from the Northwest Atlantic.

Introduction

The limicolous (= embedded) dwarf *Fucus* currently referred to as *F. cottonii* Wynne *et* Magne[1] was first reported from Clare Island by Cotton (1912). However, Cotton himself did not regard this unusual form as a distinct species. Instead, he noted that it appeared to be connected *via* a morphological continuum to saxicolous forms of *F. vesiculosus*. Further, although receptacles were rarely present on dwarf plants, Cotton observed that both taxa were dioecious. Therefore, he designated this dwarf plant as *Fucus vesiculosus* var. *muscoides* based upon its unique morphology and ecology. While the modern definition of a variety assumes that phenotypic differences have an underlying genetic basis, it is not clear that Cotton envisioned var. *muscoides* as genetically distinct from saxicolous forms of *F. vesiculosus*. In a subsequent taxonomic study of British salt marsh fucoids Baker and Bohling (1916) classified the dwarf plant as *F. vesiculosus* ecad *muscoides*, following Clements (1905) definition of an ecad as "...a new form which results from adaptation or a change in morphology due to a new habit." Thus, their delineation of an ecad did not imply genetic differentiation between the ecad *muscoides* and its saxicolous parental material.

Feldmann and Magne (1964) elevated this dwarf fucoid plant to *Fucus muscoides* based upon its unique morphology and ecological niche, while Niell *et al.* (1980) argued

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[1] Refer to Chapter I for a description of the morphology and ecology of this taxon.

that the existence of a morphological cline connecting it to *F. vesiculosus* indicated that they were not distinct. Thus, Niell *et al.* (1980) reaffirmed Baker and Bohling's delineation of the plant as *F. vesiculosus* ecad *muscoides*. Most recently Wynne and Magne (1991) noted that the name *F. muscoides* was illegitimate as it was a basionym of the red alga *Acanthophora muscoides* (L.) Bory de Saint-Vincent. Thus, they renamed the taxon *F. cottonii* in honor of A. D. Cotton.

To date the criteria for assigning the limicolous dwarf *Fucus* to the specific, varietal, or ecad level have been either morphological and/or ecological in nature. However, given the high levels of morphological plasticity found in *Fucus*, (Powell 1963, Russell 1978, Mathieson *et al.* 1981), morphological and ecological criteria for taxon designation leave important questions unresolved. For example, what is the relationship of *F. cottonii* to *F. vesiculosus*? Is there a genetic basis for recognizing *F. cottonii* as a distinct taxon? Further, can species other than *F. vesiculosus* give rise to *cottonii*-like morphologies? The latter question is of particular importance in light of evidence that a muscoides-like *Fucus* found in the Gulf of Maine may arise from *F. spiralis* (Mathieson and Dawes 2001) or by hybridization of *F. vesiculosus* (a dioecious taxon) and *F. spiralis* (a hermaphroditic and potentially selfing species; Chapters II and III); further, the occurrence of muscoides-like forms in Alaska where *F. vesiculosus* is absent (S. Lindstrom, pers. com.) should be noted.

As outlined in previous Chapters, microsatellite analyses of estuarine *Fucus* populations demonstrate considerable potential to determine systematic relationships between taxa, both at the inter- and intra-specific levels. The goal of the present study is to examine the affinities of an *F. cottonii* population within an estuary at Rosmuc, Ireland

(Figure 11) using microsatellite markers. While the type location (Clare Island, Ireland) is nearby, Rosmuc was selected for a genetic study for several reasons. First, it is very near the type location and *F. cottonii* from this location is morphologically and ecologically identical to populations from the type location (M.D. Guiry, per. comm.). Hence, it is reasonable to assume that the dwarf *Fucus* from Rosmuc is representative of *F. cottonii.* In addition, Wilkes (2003) has carried out morphometric and genetic studies of a putative *F. vesiculosus* x *F. spiralis* hybrid (Figure 12) from the same location. Accordingly, my molecular study of *Fucus* from Rosmuc should allow comparisons with his results.

In the present study, the genetic parameters of *F. cottonii* are compared to those of three other *Fucus* taxa from Rosmuc, including *F. vesiculosus*, *F. spiralis*, and a putative attached *F. vesiculosus* x *F. spiralis* hybrid. The specific questions that this study seeks to answer are as follows: (1) Is *F. cottonii* at Rosmuc genetically distinct from *F. vesiculosus* and *F. spiralis* within this salt marsh? (2) How does Rosmuc *F. cottonii* compare to the muscoides-like *Fucus* from the Gulf of Maine in terms of their genetic affinities? (3) Assuming that Rosmuc *F. cottonii* represents a genetically distinct population, what taxonomic rank might be most appropriate? (4) Since muscoides-like forms from the Gulf of Maine often appear to be hybrid in origin, the presence of a putative *Fucus* hybrid population at Rosmuc suggests that this taxon might be the primary source for *F. cottonii* within this estuary. Therefore, the affinities and taxonomic status of this population are assessed. While the present study does not attempt to present a final resolution of the taxonomic status of *F. cottonii*, it does provide a genetic perspective on the origins and affinities of this taxon which together with morphological and ecological criteria may provide the basis for subsequent systematic assessments of various salt marsh *Fucus* taxa.

Material and Methods

Sample Collections

Samples were collected from Galway Bay at Rosmuc near Roundstone, Ireland (53°21'40"N, 9°37' 30"W – Figure 10) on May 20th, 2004 by Dr. Christopher Neefus and Dr. Robert Wilkes. The taxa sampled included *Fucus cottonii* (n=32), *F. spiralis* (n=50), *F. vesiculosus* (n=44), and a putative *F. vesiculosus x F. spiralis* hybrid[2] (n=53)[3]. For all taxa except *F. cottonii,* sampling involved collecting a clean vegetative tip which was stored in a plastic bag for transportation. For *F. cottonii,* small clumps or individuals were collected and placed in separate plastic bags. All samples were stored at 4 °C or on ice until subsequent processing within one week at the University of New Hampshire. Each sample was thoroughly rinsed with distilled water and ≅5-10 mg of tissue was then excised. Excised tissue was placed in a 1.5 mL micro-centrifuge tube and flash frozen in liquid nitrogen, followed by storage at -80 °C until DNA extraction.

DNA Extraction, Microsatellite Amplification, and Genotyping

The DNA was extracted as previously described (Chapters II and III) using the Gentra Puregene™ DNA isolation kit (Gentra, Minneapolis, MN, USA). The present

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[2] Hereafter referred to a putative hybrid or PH

[3] For descriptions and figures of all taxa except PH, refer to Chapter I.

study used two loci for which markers were developed as part of this thesis research (F26II and F90)[4] and two markers (L94 and L58) developed by Engel *et al.* (2003). Marker L94 was used previously to analyze taxa affinities between *Fucus* in the Gulf of Maine (Chapter III), and its motif, amplification and cycling parameters are described there. Marker L58 contains a (GA) dinucleotide repeat motif (primer sequences 5′→3′: F-AAACGAAAATGGCACAGTGA; R-CCTTGCATGTAGGAGGGAAC). The amplification conditions and profile of this locus are identical to those given previously for L94, except that the annealing temperature of L58 was 59 °C.[5] Following amplification, samples were genotyped as described previously (Chapters II and III) using an ABI377 automated DNA sequencer (Applied Biosystems, Foster City, CA, USA) at the Hubbard Center for Genome Studies at the University of New Hampshire. Allele sizing was carried out by eye using GeneScan Analysis® software version 3.1.2 (Applied Biosystems).

Data Analysis

In a study of the population structure of *Fucus serratus* in Northern Europe, Coyer *et al.* (2003) found that the minimum distance over which a panmictic unit existed was from 0.5-2 kilometers. All sampling for the present study was carried out over distances less than two kilometers; thus, each taxon is treated here as a single potentially interbreeding population. Also, in order to compare the population genetic parameters of

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[4] Primers for loci F227 and F300 (which were used in Chapters II and III) produced a very faint band upon PCR or failed to amplify in a majority of samples collected from Ireland (>60%) and were therefore not used in this study.

[5] Marker L58 was not used in Chapter III because it proved to be monomorphic for *F. spiralis* and *F. vesiculosus* within the Gulf of Maine.

Fucus cottonii and PH to those that would be expected from a population of F_1 hybrids, a simulated population of *F. vesiculosus x F. spiralis* hybrids (F_1HYB) containing forty individuals was randomly created as described in Chapter's II and III using the estimated allele frequencies from *F. vesiculosus* and *F. spiralis*, but not the putative hybrid. As in previous chapters, this simulated population was then analyzed identically to the Rosmuc *Fucus* taxa.

Prior to analysis, allele size data from all loci were tested for the effects of stutter and short allele dominance[6] using MicroChecker (van Oosterhout *et al.* 2003). File formatting and estimation of allele frequencies were carried out using CONVERT (Glaubitz 2004). Global estimates of the observed and expected heterozygosity (H_O and H_E, respectively) for each taxon were obtained using GDA (Lewis and Zaykin 2001). As the inbreeding coefficient F_{IS} provides an estimate of heterozygote deficiency or excess within a population (e.g. a positive value indicates a heterozygote deficit, and a negative value a heterozygote excess), the significance level of these values serves as a test of departure from Hardy-Weinberg equilibrium. Estimates of F_{IS} were obtained for all taxa at each locus separately in GENEPOP version 1.2 (Raymond and Rousset 1995) using the method of Weir and Cockerham (1984). The significance of the F_{IS} estimates was also tested using a Markov Chain method in GENEPOP (100 batches, 1000 iterations per batch). Global estimates of F_{IS} were obtained using GDA, and the presence of global heterozygote deficits or excesses within each taxon were tested using a score test (or U test) in GENEPOP. Where significant heterozygote deficits were found, the frequencies of putative null alleles (assuming Hardy-Weinberg equilibrium) were estimated using

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[6] Short allele dominance refers to the preferential exponential amplification of short alleles over longer ones during PCR, and may cause null alleles and apparent heterozygote deficits.

MicroChecker according to the method of Brookfield (1996). The probability of pairwise linkage disequilibria was tested within each taxon and pairwise estimates of multi-locus F_{ST} were calculated between taxa according to Weir and Cockerham (1984) using FSTAT (Goudet 2001). An exact test of population differentiation was also performed in FSTAT using 1000 permutations, with Bonferroni corrections (Rice 1989) being applied to the significance values of these tests as necessary. All probabilities were evaluated using a significance level of $p \leq 0.05$.

The affinities between *Fucus* taxa at Rosmuc were visualized in two ways. A factorial correspondence analysis (FCA) of all taxa and the F_1HYB simulation was performed using the software program GENETIX v. 4.03 (Belkhir 1999). In addition, a consensus tree was constructed based upon 1000 neighbor joining trees (i.e. 1000 bootstrap replicates) by sequential use of the software programs SEQBOOT>GENDIST> NEIGHBOR>CONSENSE>DRAWTREE. All of these programs are part of the PHYLIP software package (Felsenstein 2004). Individuals whose genotypes contained missing data were excluded from the analyses.

Results

Allele and Genotype Frequency Data

Allele frequencies at all four loci are given in Figure 13a-d, and the mean number of alleles per taxon and the number of observed alleles per locus per taxon are provided in Table 11. The mean number of alleles varied considerably between taxa: *Fucus spiralis* had the smallest mean number of alleles (6.5), by comparison with three groups (*Fucus vesiculosus*, PH, and the F_1HYB) that each displayed a mean value approximately double that of *F. spiralis*. The mean number of alleles for *F. cottonii* (8.0) was closer to that found in *F. spiralis* than in the other taxa and F_1HYB. When comparing allele frequencies between taxa, several general trends were observed. For the most part, a fairly high degree of allele size overlap was observed for all taxa. At locus F90, all taxa as well as F_1HYB showed their highest frequency for the 141 bp allele, with three groups (*F. spiralis, F. cottonii*, and F_1HYB) displaying this allele at frequencies >0.5. At F26II, the highest frequency alleles were 350 (*F. spiralis*), 352 (*F. cottonii* and PH), or 354 (*F. vesiculosus*) bp in size. Locus L94 showed a clear difference in frequencies between taxa; the highest-frequency allele for *F. spiralis* was 165 bp, whereas that for *F. vesiculosus, F. cottonii,* and PH was 173 bp. As expected, F_1HYB displayed both alleles at approximately equal frequencies. Finally, at L58, all taxa except *F. cottonii* possessed the125 bp allele at the highest frequency (for *F. cottonii*, the 129 bp allele was most frequent). Private alleles (i.e., those restricted to one taxon) were observed at all loci (except the F_1HYB), but always at a low frequency (<0.1).

When comparing the number of observed genotypes to the total number of individuals sampled (Table 11, 2nd column), two taxa (*Fucus vesiculosus* and PH) displayed unique genotypes across all four loci for all individuals, as did F_1HYB. The lowest number of genotypes relative to individuals sampled was found in *F. spiralis*, while several identical genotypes were also found in *F. cottonii*. It is important to note that this comparison provides the most conservative estimate of genotype identity, as individuals with seven alleles out of a possible eight in common are not considered any more similar than individuals who share no alleles at all. Individual genotypes showing identical genotypes across three loci (six alleles in common) or with seven alleles over four loci in common were often present in *F. spiralis* and to a lesser extent in *F. vesiculosus*.

Table 12 provides a summary of potential size-scoring problems and an estimate of null allele frequencies assuming Hardy-Weinberg equilibrium. Analysis with MicroChecker found no evidence of large allele dropout at any locus in any taxon. Several possible instances of stutter that might have affected the scoring of allele sizes were noted (for loci F26II and F90 in some taxa); however, potentially misleading stutter effects were not generally observed in chromatograms of F90 and were only sometimes present for F26II chromatograms.

Population Genetic Analysis

Comparisons of observed versus expected heterozygosity and Hardy-Weinberg equilibrium showed a significant heterozygote deficit (F_{IS}) for all *Fucus* taxa, including *F. cottonii* and PH (Table 11). Global estimates of F_{IS} ranged from 0.679 (*F. spiralis*; $H_O \approx 68\% < H_E$) to 0.364 (*F. vesiculosus*; $H_O \approx 36\% < H_E$). All loci also displayed a significant heterozygote deficit with the exception of L94 in *F. vesiculosus* and *F. spiralis*. By contrast F_1HYB displayed a significant global heterozygote excess, with these also being observed at all individual loci (though only significantly at L94). Significant pairwise linkage disequilibria were found between L94 and L58 for *F. cottonii* and F26II and L58 for *F. vesiculosus*.

Tests of population differentiation between *Fucus* taxa at Rosmuc showed that all taxa were significantly different from each other at the $p = 0.05$ level, with the exception of *F. vesiculosus* and PH that showed no significant genetic differences (data not shown). As the relationships of *F. cottonii* and PH to either *F. vesiculosus* or *F. spiralis* were unknown, pairwise F_{ST} estimates were obtained between these groups (Table 13). All comparisons gave fairly large estimates ($F_{ST} > 0.05$), again with the exception of *F. vesiculosus* and PH as well as *F. vesiculosus* and the F_1HYB simulation. Tests of population differentiation comparing Rosmuc *Fucus* to the F_1HYB showed significant differences between the simulated population and all taxa. However, F_{ST} estimates involving the F_1HYB were on average smaller than those found between the Rosmuc *Fucus* taxa. A factorial correspondence analysis (Figure 14) showed that along the X-axis (which accounted for 53.89% of the total variation) *F. spiralis* was essentially separated from all other Rosmuc *Fucus* taxa. *Fucus vesiculosus* and PH displayed a large degree of overlap along both the X- and Y-axes, whereas *F. cottonii* differed from both taxa primarily by its position along the Y-Axis (which accounted for 29.02% of the total variation). The F_1HYB cloud was chiefly situated between *F. vesiculosus* and *F. spiralis* along the X-Axis, and overlapped to some extent with both species. In addition, one genotype of *F. cottonii* was located directly in the center of the F_1HYB cloud, while one *F. vesiculosus* genotype was clearly associated with *F. spiralis.* A neighbor-joining analysis of all taxa (Figure 15) showed that *F. vesiculosus, F. cottonii,* and PH grouped together with strong bootstrap support (97.7) away from *F. spiralis* and the F_1HYB. Within the *F. vesiculosus* clade, *F. vesiculosus* and PH grouped together away from *F. cottonii* with weak bootstrap support (48.3%).

Pairwise estimates of F_{ST} between two sites in the Gulf of Maine and Rosmuc using two shared loci (F90 and F26II) are provided in Table 14. In general, the Irish

Fucus showed greater partitioning relative to sites within the Gulf of Maine than sites within the Gulf of Maine displayed between themselves. The estimate of F_{ST} between *F. spiralis* from Wells Harbor (WH) and Brave Boat Harbor (BBH) was 0.010, whereas that between Rosmuc and WH and Rosmuc and BBH was 0.53 and 0.525, respectively. By contrast, the F_{ST} estimate between *F. vesiculosus* and *F. spiralis* at Rosmuc was only 0.125. Similarly, F_{ST} between Rosmuc and BBH *F. vesiculosus* was estimated at 0.149, and between Rosmuc *F. vesiculosus* and *F. vesiculosus* ecad *volubilis* 0.190 (WH) and 0.161 (BBH). These F_{ST} estimates were similar to those between PH from Rosmuc and *F. vesiculosus* ecad *volubilis* from WH (0.174) and BBH (0.155). *Fucus cottonii* from Rosmuc was also differentiated from muscoides-like forms within the Gulf of Maine, with F_{ST} estimates of 0.343 (BBH) and 0.332 (WH). Thus, genetic partitioning between the same species/ecads from Ireland and the Gulf of Maine was greater than that found between taxa at Rosmuc.

Discussion

A Comparison of Rosmuc Fucoids to Those Within the Gulf of Maine

The population genetic data for Rosmuc *Fucus* show both similarities and differences to those within populations of the same species in salt marshes in the Gulf of Maine. The putative hybrid (PH) identified at Rosmuc also appears to be very similar genetically to *F. vesiculosus* and does not appear to be of hybrid origin. Lastly, *F. cottonii* from Rosmuc appears to be more closely associated with *F. vesiculosus* than *F. spiralis* and also does not appear to be of hybrid origin.

The allele frequency distributions for the Irish *Fucus* were different than those found in the northwest Atlantic populations. In both the Brave Boat Harbor study (Chapter II) and the larger Gulf of Maine study (Chapter III), allele frequencies tended to differ between *F. vesiculosus* and *F. spiralis* in a species-specific manner across all four loci. However, within Irish populations both species shared their most common allele at three loci (F90, F26II and L58). The only exception to this pattern was at locus L94, where each species was close to fixation for alternate alleles (Figure 13c). The observed pattern does not appear to be simply due to the fact that different markers were used, as two markers (F90 and F26II) were common to all three studies. It is possible that greater gene flow exists between *F. vesiculosus* and *F. spiralis* at Rosmuc, but further studies would be required to evaluate this possibility. Thus, while it was easily possible to separate estuarine *Fucus* taxa from the northwest Atlantic based upon differences in allele frequency between species and ecads, allele frequency patterns from Rosmuc *Fucus* populations were not so easily interpreted.

Comparisons of the inbreeding coefficient F_{IS} between Rosmuc *Fucus* populations and those in the Gulf of Maine also revealed similarities and differences. Populations of *F. vesiculosus* and *F. spiralis* were characterized by heterozygote deficits in all studies. In the case of the Rosmuc *Fucus,* both *F. spiralis* and *F. cottonii* displayed multiple instances of identical genotypes from different plants (Table 11). A similar situation was also observed for *F. spiralis* from Brave Boat Harbor (Chapter II). In these instances, it is possible that reproduction *via* selfing or vegetative reproduction may give rise to the observed deficits. Heterozygote deficits have been observed in several asexual or selfing groups of algae (reviewed in Sosa and Lindstrom 1999) and these authors discuss the advantages to clonal reproduction of locally adapted genotypes (also see Norton and Mathieson 1983, Smith and Walters 1999). However, Sosa and Lindstrom (1999) also point out that the conditions required for Hardy-Weinberg equilibrium are often violated in seaweed populations, and they caution against adopting a particular hypothesis to explain heterozygote deficits in algal populations unless such interpretations are explicitly warranted by the sampling strategy employed (also see Russell 1986, Pearson and Murray 1997, Valero *et al.* 2001). For example, although several workers have found large heterozygote deficits in clonally reproducing or hermaphroditic species (Innes and Yarish 1984, Williams and Di Fiori 1996, Pearson and Murray 1997, Sosa *et al.* 1998), such deficits are sometimes observed in outcrossing species with the potential for long-range dispersal (Lu and Williams 1994). Caution in speculating about the causes of heterozygote deficits are particularly applicable to the present thesis, as the primary goal was to analyze genetic affinities between taxa rather than examining genetic structuring or deviations from Hardy-Weinberg equilibrium.

As seen in Chapters II and III, despite similarities in allele sizes and frequencies between populations of both species from Rosmuc, *F. vesiculosus* and *F. spiralis* were readily distinguished using factorial correspondence analysis and comprised two well-separated groups in a neighbor-joining tree. However, both PH and *F. cottonii* were clearly associated with *F. vesiculosus* and neither appeared to be of hybrid origin. The affinities of these two taxa are discussed below.

The Affinities of the Putative Hybrid (PH) from Rosmuc

The putative hybrid analyzed in the present study is one of several such populations occurring within salt marshes along the Irish west coast that have been studied by Wilkes (2003). The reasons for including the Rosmuc population in the present study were twofold. Firstly, I wished to verify or disconfirm the hybrid status of this population. Secondly, if this population as well as *Fucus cottonii* were in fact of hybrid origin, then it seemed possible that the former taxon might be the progenitor of the latter and might therefore permit the identification of a specific source of *F. cottonii* at Rosmuc.

The study by Wilkes (2003) combined morphometric and molecular analyses using the ribulose bisphosphate carboxylase/oxygenase (*rbc*L) spacer region to examine the affinities of the PH morphotype relative to that of *Fucus vesiculosus* and *F. spiralis.*[7] Morphometric analysis has been previously used to examine intra- and interspecific variation within *Fucus* (Rice and Chapman 1985, Munda and Kremer 1997, Anderson and Scott 1998, Kalvas and Kautsky 1998, Scott *et al.* 2001). In the study by Wilkes (2003), ten morphological characters were selected. At Rosmuc, principle component analysis showed that *F. spiralis* and *F. vesiculosus* could be grouped into well-separated clusters with the PH population being intermediate in position between them and showing some overlap with both species. The separation between taxa was primarily based upon significant differences in frond length and width as well as the number of plantlets growing from the holdfast. A similar separation of the three taxa was obtained using discriminant function analysis. Wilkes (2003) used the *rcb*L spacer region to analyze relationships between *Fucus* species (organelle inheritance in *Fucus* appears to be

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[7] While Wilkes (2003) carried out analysis of the putative hybrid at five sites, only the results from Rosmuc are discussed here.

maternal, although this has not been specifically demonstrated in *F. spiralis* or *F. vesiculosus*, cf. Coyer *et al.* 2002b). While Wilkes (2003) demonstrated that the *rbc*L spacer could be useful for intergeneric-level analysis (i.e., it provided resolution of genera within the Fucales), it did not provide the resolution required for species-level taxonomic discrimination between *F. vesiculosus* and *F. spiralis*. Thus, Wilkes demonstrated that PH was a true morphotype, but was unable to determine its genetic affinities using the spacer region.

The present study suggests that although PH represents a distinct morphotype, it differs little from *F. vesiculosus* at the four microsatellite loci examined and does not appear to be hybrid in origin based upon my results. Such a conclusion is supported by a comparison of PH to both *F. vesiculosus* as well as the F_1HYB simulation. For example, whereas the F_1HYB simulation displayed a significant excess of heterozygotes, both PH and *F. vesiculosus* displayed a significant heterozygote deficit (Table 11). Similarly, the observed heterozygosity of PH was much lower than that of the F_1HYB and closer to *F. vesiculosus*. By contrast, the dwarf muscoides-like forms from the Gulf of Maine (i.e. Chapters II and II) appear to be hybrid in origin, displaying heterozygote excesses and observed heterozygosities similar to those predicted by computer simulations of hybrid populations. Further, while no significant difference was seen between PH and *F. vesiculosus* in tests of population differentiation, both taxa were significantly different than the F_1HYB simulation. A factorial correspondence analysis (Figure 14) shows that PH displays extensive overlap with *F. vesiculosus*, only a slight overlap with F_1HYB, and no overlap with *F. spiralis*. Finally, a neighbor-joining analysis grouped PH and *F. vesiculosus* away from both *F. spiralis* and the F_1HYB simulation with a high degree of confidence (Figure 15). Such observations are quite different than those that would be predicted for a hybrid population.

If the PH population at Rosmuc is not a hybrid, then what if anything may be inferred about its taxonomic status? In terms of morphology, this morphotype appears to be similar to other infra-specific taxa such as *F. vesiculosus* ecad *volubilis* (Hudson) Turner and *F. vesiculosus* var. *spiralis* Farlow from the northwest Atlantic. Were the PH plants detached then it seems certain that they would be regarded (at least initially) as *F. vesiculosus* ecad *volubilis* as described by Baker and Bohling (1916); however, since PH individuals possess a holdfast they are more similar in this respect to *F. vesiculosus* var. *spiralis*. To my knowledge, this last taxon has only been described from the northwest Atlantic, though Taylor (1957) and Baker and Bohling (1916) state that this taxon may be identical to *F. vesiculosus* var. *volubilis* (Hudson) Turner, which was described from British salt marshes (cf. Chapter I, Pérez-Ruzafa and García 2000).

One potential way to gain a very rough estimate of the relative taxonomic status of PH is through comparisons of pairwise estimates of F_{ST}. Since F_{ST} provides an estimate of the partitioning of genetic variation between populations, and since some gene flow might reasonably be expected between adjacent populations of *Fucus* differing only at the infra-specific level, it is possible to compare such estimates provided they are regarded with caution. In the Brave Boat Harbor study the pairwise estimate of F_{ST} between *F. vesiculosus* and *F. vesiculosus* ecad *volubilis* was 0.075 (Chapter III) and 0.057 for loci F90 and F26II, respectively (Table 14, this chapter), whereas the estimate between *F. vesiculosus* and the simulated hybrid population from the same study was approximately twice that (0.145; estimate not provided in table). Based upon these criteria, then, the pairwise F_{ST} estimate between *F. vesiculosus* and PH in the present study (0.01 – Table 13) seems to fall well within the level of genetic partitioning found below the species level in *F. vesiculosus*. Such a conclusion is supported by the lack of significant population differentiation between *F. vesiculosus* and PH, as well as their obvious affinities in Figures 14 and 15. For all of these reasons it seems appropriate to tentatively regard PH as a morphological (as opposed to genetic) variant of *F. vesiculosus* pending further studies (morphometric, transplant, chemical, and genetic) on plants from a variety of sites.

The Affinities of Fucus cottonii from Rosmuc and its relation to the muscoides-like forms from the Gulf of Maine

Since Cotton's (1912) original description, European dwarf limicolous *Fucus* populations have generally been regarded as derived from saxicolous populations of *F. vesiculosus*, to which they are linked by numerous transitional morphologies (Niell *et al.* 1980). However, recent work in the Gulf of Maine has demonstrated that dwarf forms from northwestern Atlantic salt marshes may be derived from *F. spiralis* (Mathieson and Dawes 2001, unpublished) or may also be hybrids of *F. vesiculosus* and *F. spiralis* (Chapters II and III of this thesis). Thus, collections and genetic analysis of *F. cottonii* from sites near the type location in Ireland are of considerable interest in resolving taxonomic confusions within this species/ecad.

The present study suggests that *Fucus cottonii* plants from Rosmuc are not of hybrid origin. As with PH, *F. cottonii* differs from the F_1HYB in possessing a significant heterozygote deficit (positive F_{IS}) and in its observed heterozygosity (H_O). Similarly, both factorial correspondence and neighbor-joining analysis (Figures 14 and 15) support the hypothesis that *F. cottonii* groups relatively close to *F. vesiculosus* and generally away from *F. spiralis* and the F_1HYB simulation. Interestingly, there appear to be genetic differences between *F. cottonii* and *F. vesiculosus*, suggesting that populations of the former do not simply represent a genetically random sampling of the latter (see below). Such differences are also present between *F. cottonii* and the PH population and there appears to be no compelling reason to regard *F. cottonii* as being preferentially derived from PH as opposed to *F. vesiculosus.* It seems likely that the unusual ecology of both *F. cottonii* from Rosmuc and elsewhere in Europe as well as muscoides-like forms from the Gulf of Maine results from selection for genotypes best suited for survival in these fringe environments (see Chapter II). Future studies should test for the selection of certain *Fucus* genotypes in such extreme environments.

In light of the genetic analysis presented in this thesis, it may now be appropriate to consider the possible taxonomic status of both *F. cottonii* and the muscoides-like *Fucus*. The studies carried out within the Gulf of Maine and Ireland have demonstrated that the morphological variation found in both muscoides-like forms and *Fucus cottonii* is consistently associated with genetic differentiation from nearby parental populations, both in terms of allele frequencies and genotype composition. In fact, it appears that the genotypes represented by these dwarf forms are not simply a subset of all genotypes observed in attached species of *F. vesiculosus* and *F. spiralis*. Instead, while these dwarf genotypes are comprised of the same alleles found in their respective source species over all loci examined (with only minor exceptions), they are largely unique and are not found (or are found only rarely) in attached populations. Under these circumstances a separate taxonomic designation of these embedded ecad/species is appropriate genetically, morphologically, and ecologically. However, it must be recognized that any individual limicolous plant is only statistically likely to be genetically different from its saxicolous source population. For example, several genotypes of *F. cottonii* from Rosmuc appear similar to *F. vesiculosus* (Figure 14). Although this probability may be high, it implies that a taxonomy based upon morphological and ecological criteria will not be exactly identical to one based upon genetic identity (at least assuming that the initial recruitment of embedded plants from attached species occurs at random). Further, it is also clear that *F. cottonii* from Rosmuc is not the same entity genetically as the muscoides-like forms from the Gulf of Maine, despite extensive similarities in morphology and ecology. Hence, it would seem advisable to treat them as separate entities.

Pairwise F_{ST} comparisons (Table 13) show considerably less partitioning of genetic variation between *F. cottonii* and *F. vesiculosus* than between the latter species and *F. spiralis*. The relatively low levels of partitioning would seem to suggest that the designation of *F. cottonii* as a distinct species from *F. vesiculosus* may be unwarranted from a genetic perspective. Such a conclusion does not necessarily invalidate species recognition for this group. Wynne and Magne (1991) emphasized niche differentiation and morphological distinctiveness as the distinguishing characters of this species and made no reference to reproductive isolation or to other genetic criteria. Alternatively, *F. cottonii* has also been regarded as an ecad by various workers (Baker and Bohling 1916, Niell *et al.* 1980), but such a designation is not always easily applied. For example, in his review of variation in marine macroalgae, Russell (1986) discussed the proposed taxonomic system of Turesson (1922) and its utility for algal classification. Turesson

(1922) proposed several different taxonomic designations based primarily upon ecological considerations: (1) ecospecies, whose members were fully interfertile, and capable of producing partially fertile hybrids with other ecospecies; (2) ecotype, defined as ..."individuals occupying a particular habitat and forming an interbreeding population which differs genotypically from other such populations",[8] capable of forming fully fertile hybrids with other ecotypes; (3) ecophene, defined as ..."individuals occupying a particular habitat and adapted to it phenotypically but not genotypically", capable of forming fully formed hybrids with other ecophenes. Of these categories ecophene is synonymous with ecad and seems to be identical to the definition provided by Clements (1905) of an ecad as "... a new form which results from adaptation or a change in morphology due to a new habit (from Baker and Bohling 1916)".[9] In the case of both *F. cottonii* and the muscoides-like *Fucus*, the present research suggests that both taxa should be classified as ecotypes rather than ecads according to the system of Turesson (1922), although the issue is complicated by the lack of sexual reproduction in these groups. Other definitions of ecads present similar difficulties. Prud'Homme Van Reine *et al.* (1996) used ecad as a shorthand version of 'ecological phenotype', but did not specifically incorporate a genetic component into the term. One definition that does incorporate genetic criteria is that of Davis and Heywood (1963), who defined an ecad as

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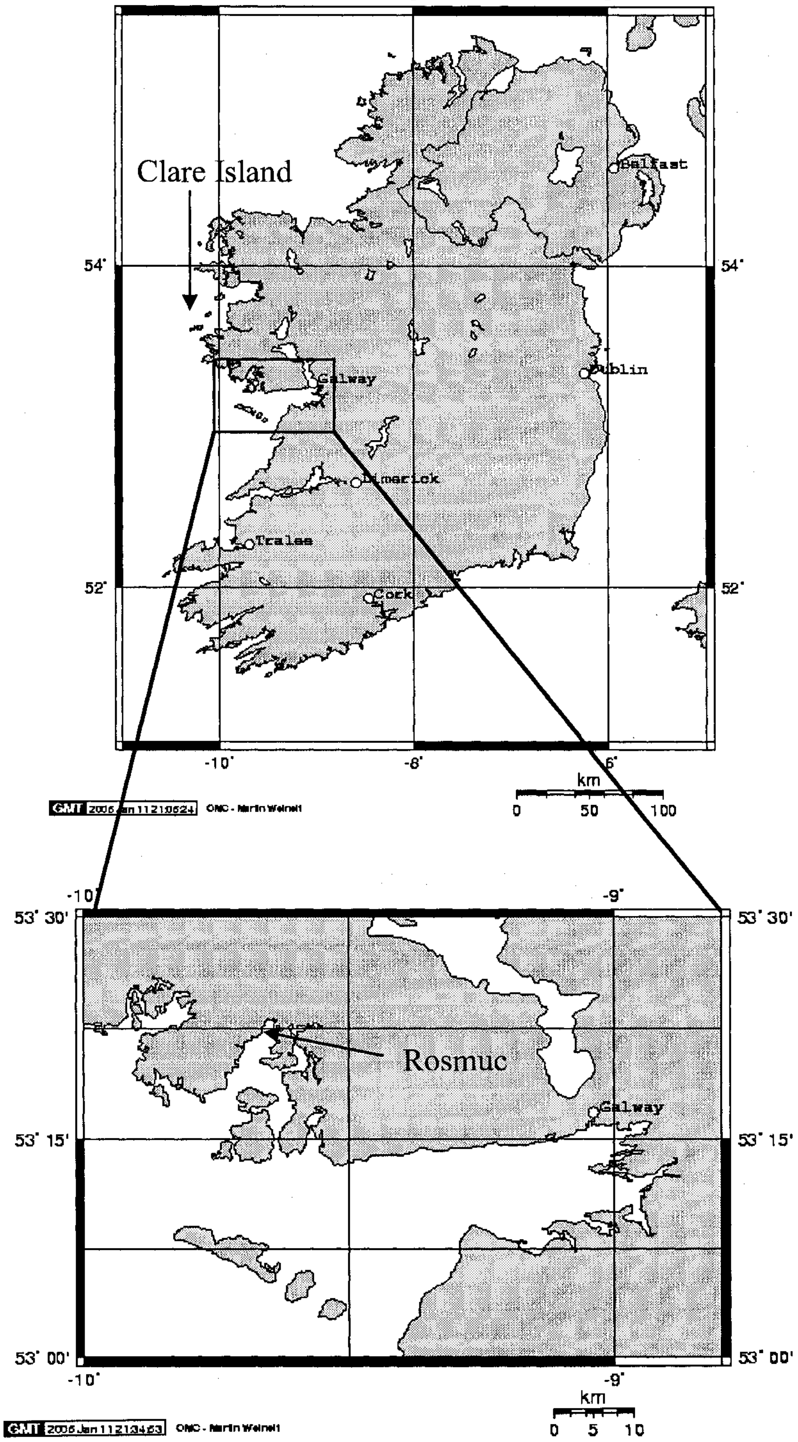
[8] All quotations and definitions are from Russell 1986.

[9] According to Russell, one problem with the definitions of ecotype and ecophene is that a given algal taxa may be both simultaneously depending upon the factors being used to make the classification. Further, it is often extremely difficult to determine whether a given morphological character represents a passive phenotypic response to environmental conditions or is due to specific genetic adaptations. Hence, while certain traits may be 'ecotypic' or 'ecophenic', Russell argues that it is difficult to apply the labels to entire organisms in many instances, particularly among marine macroalgae.

"...the phenotypical product of environmental selection of genotypes which are able to grow in a range of habitats by consequence of their wide range of tolerance." However, genotypes from embedded *Fucus* populations are not generally present within habitats occupied by attached populations, but rather seem to survive in ecological niches that are different from those ordinarily occupied by attached plants. Under these circumstances, Cotton's (1912) original designation of *F. cottonii* as a variety of *F. vesiculosus* might prove to be the most appropriate, as a variety is defined as an infra-specific taxon displaying some level of genetic and/or phenotypic difference from other members of the species (Mathieson 2002). Based upon the results presented in this thesis, it appears that *F. cottonii* is at least as distinct as *F. vesiculosus* var. *spiralis*, although it might be argued that this taxon is also in need of some reevaluation.

Regardless of the final status of *F. cottonii* from Ireland, it is clear that this taxon needs to be defined in such a way as to exclude morphologically similar hybrid forms as well as those that are not affiliated with *F. vesiculosus*. In addition, more detailed evaluations are required to determine the affinities of embedded fucoids from other locations, such as Alaska (Ruiz *et al.* 2000). Ultimately, the current research provides compelling evidence that genetic studies will greatly aid in our understanding of the systematics and ecological relationships, both intra- and inter-specifically, of estuarine *Fucus* populations.

Figure 11. Map of Ireland, showing Clare Island (the type location for *Fucus cottonii*) and Rosmuc (the site of the present study).



Maps were created using OMC at http://www.aquarius.geomar.de/omc/omc_intro.html.

Figure 12. A putative hybrid of *Fucus vesiculosus* and *F. spiralis* (referred to as PH) from Rosmuc, Ireland.

Note the presence of vesicles and the narrow, extensively spiraled thallus. Photograph by Dr. C. Neefus.

Figure 13. Allele frequency data for all taxa from Rosmuc.

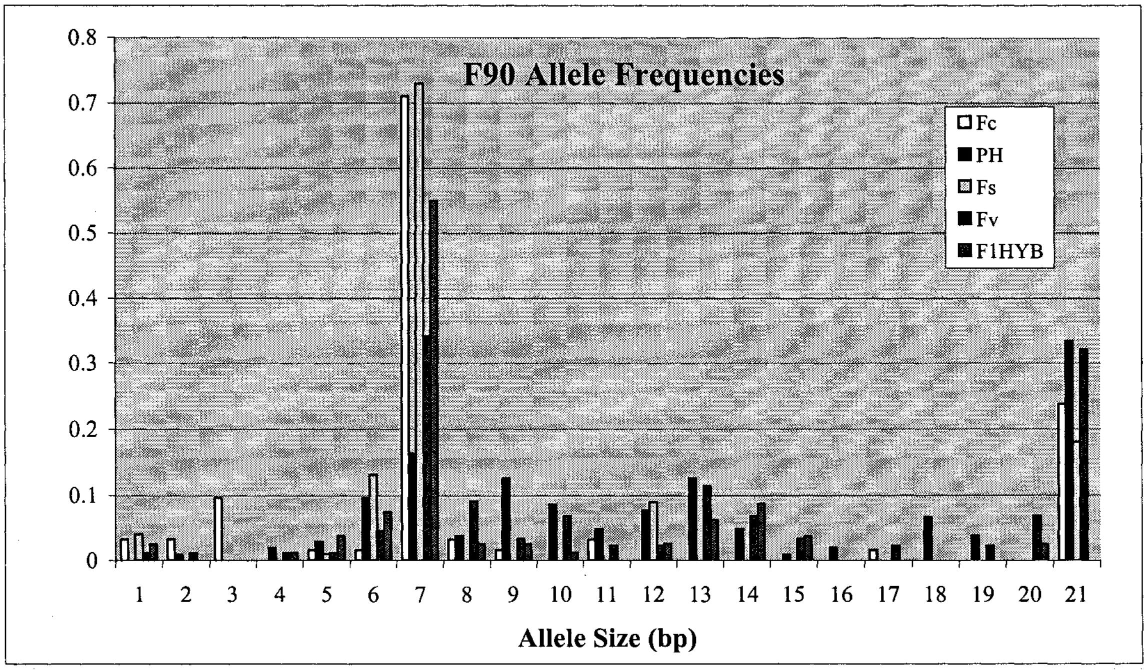


Figure 13a.

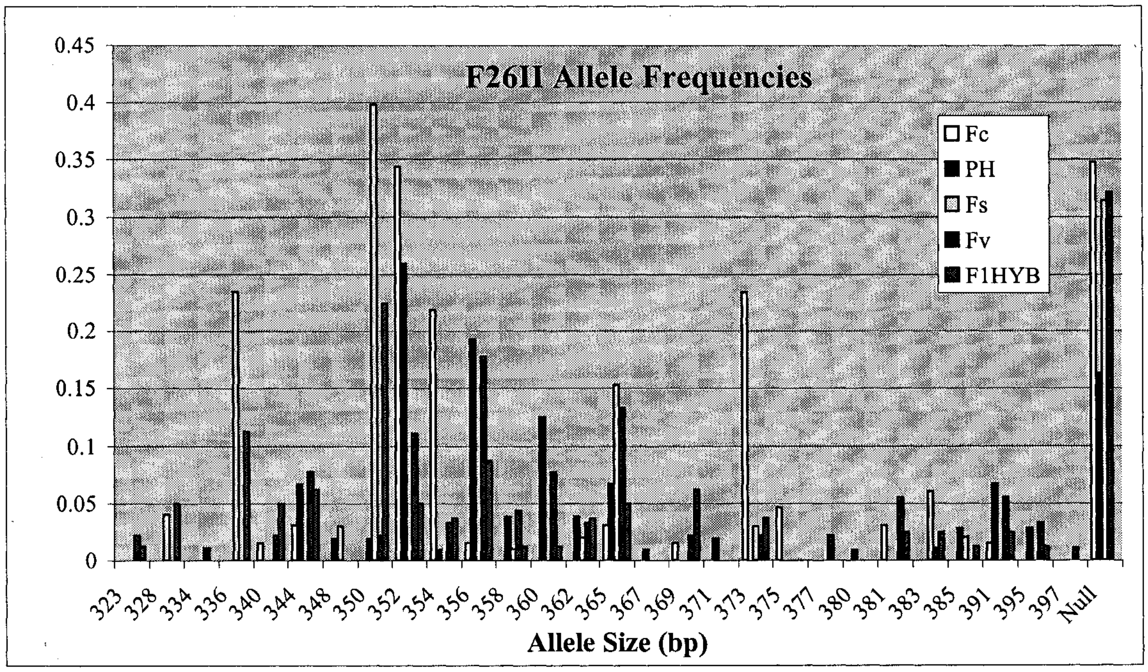


Figure 13b.

Figure 13 cont. Allele frequency data for all taxa from Rosmuc.

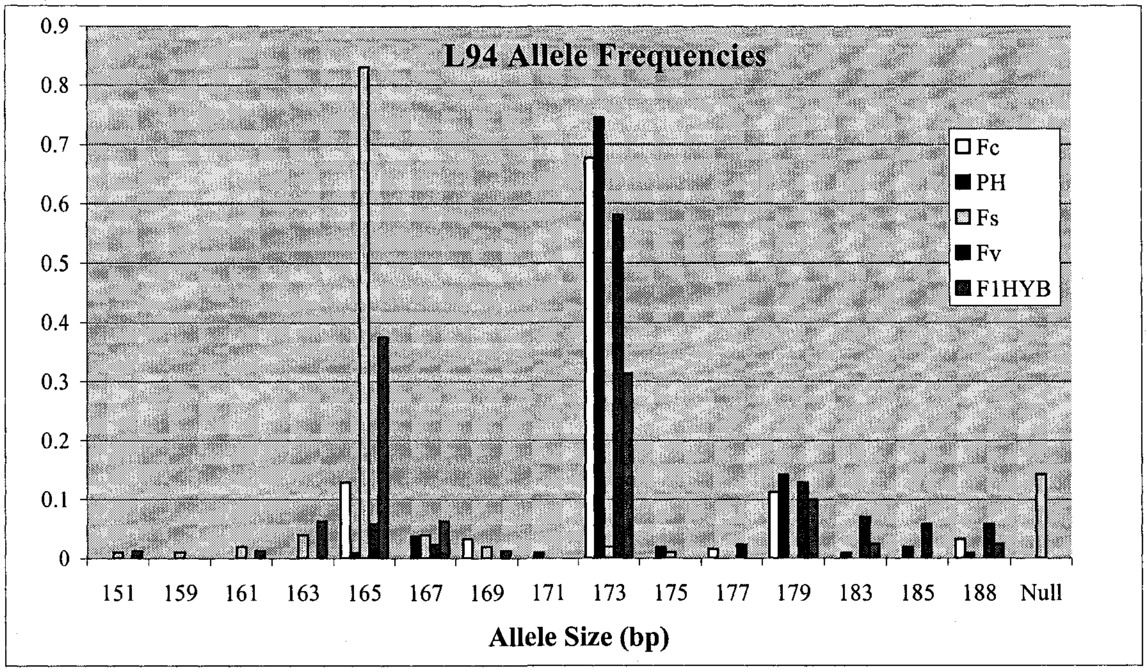


Figure 13c.

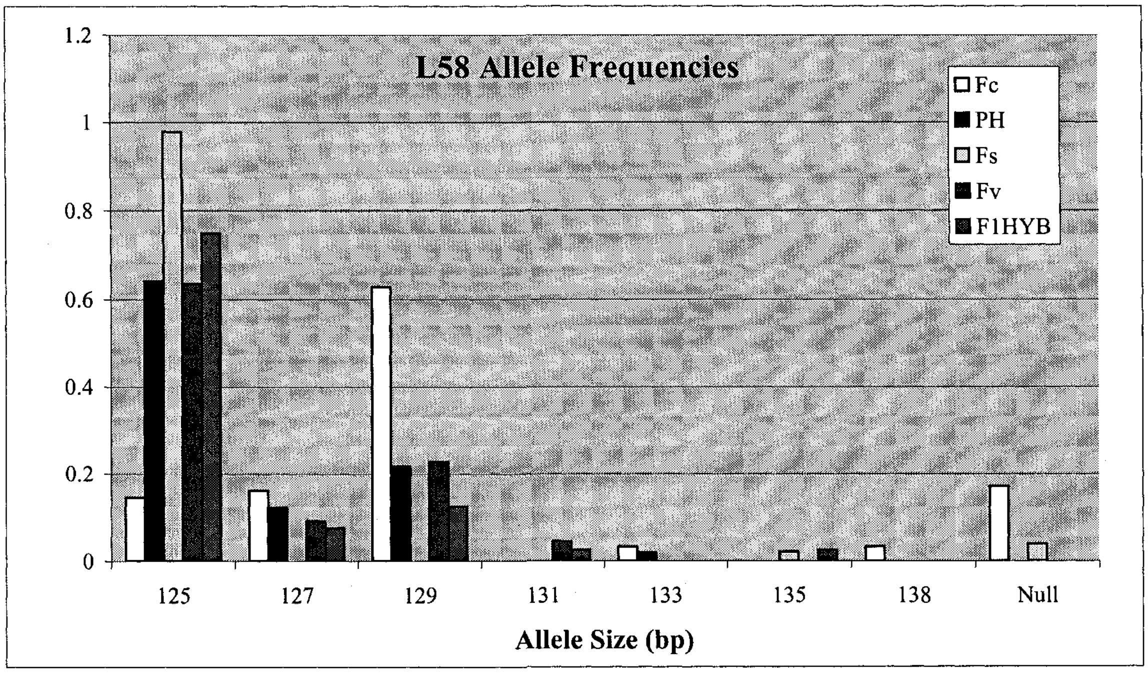


Figure 13d.

Figure 14. Factorial correspondence analysis of Rosmuc *Fucus*.

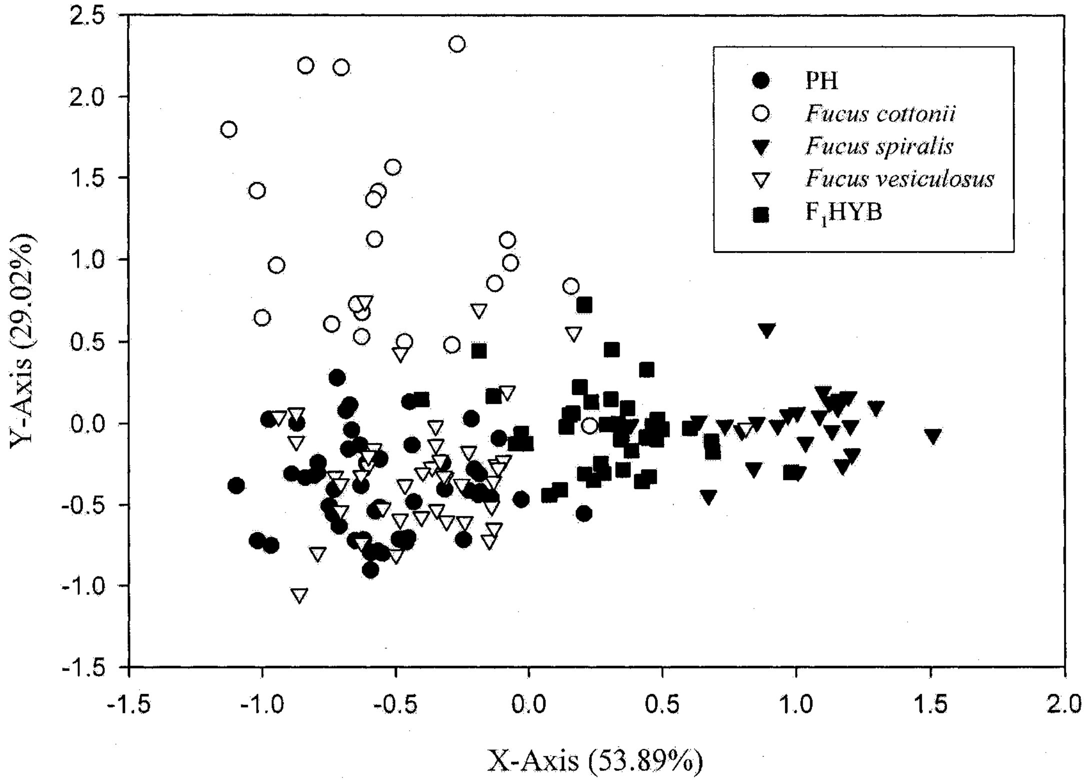


Figure 15. Neighbor-Joining analysis of Rosmuc *Fucus* taxa.

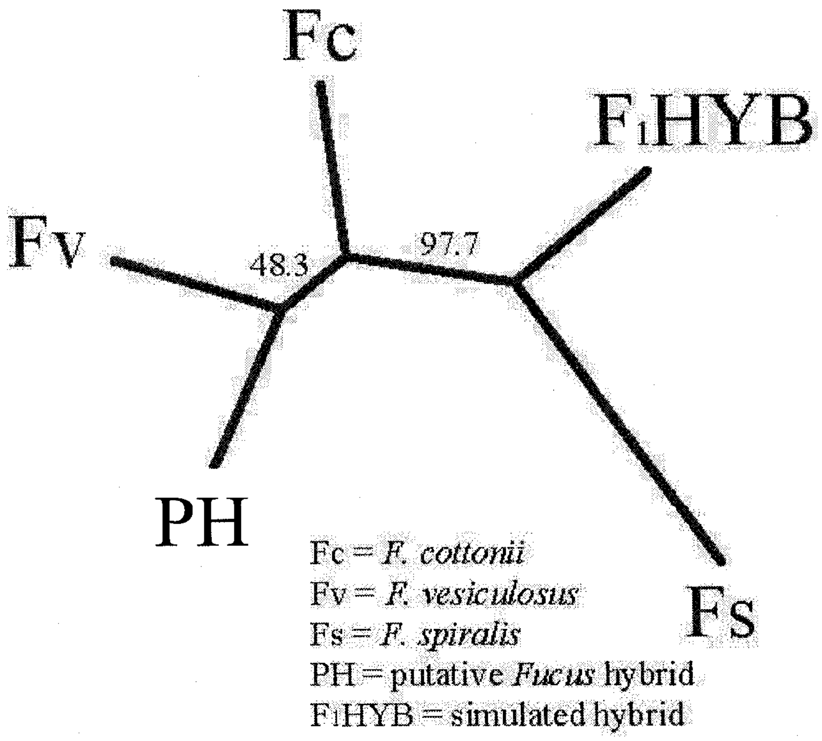


Table 11. General population parameters for all Rosmuc *Fucus* taxa (PH = putative hybrid) as well as the F_1HYB.

			Number of Alleles per Locus						Estimates of F_{IS}				
Taxa	total # of genotypes /N	Mean # Alleles	F26II	F90	L94	L58	H_E	H_O	F26II	F90	L94	L58	Global
F. vesiculosus	44/44	12.25	20	17	8	4	0.737	0.471	0.319[1]	0.705[1]	0.162	0.136[1]	0.364[1]
F. spiralis	29/50	6.5	10	5	9	2	0.39	0.126	0.716[1]	0.536[1]	0.505	1[1]	0.679[1]
F. cottonii	24/32	8	11	10	6	5	0.589	0.241	0.782[1]	0.793[1]	0.093[1]	0.407[1]	0.596[1]
PH	53/53	11.25	16	16	9	4	0.685	0.405	0.351[1]	0.703[1]	0.246[1]	0.16[1]	0.411[1]
F_1HYB	40/40	12	20	13	10	5	0.693	0.788	-.065	-0.1	-0.302[2]	-0.072	-0.139[2]

[1] Significant deviation from Hardy-Weinberg equilibrium showing a heterozygote deficit.

[2] Significant deviation from Hardy-Weinberg equilibrium showing a heterozygote excess.

Table 12. Possible scoring errors and estimates of putative null allele frequencies using MicroChecker.

	Potential scoring problems		Putative null allele frequencies			
	Stutter	Large Allele Dropout	F26II	F90	L94	L58
F. vesiculosus	N/A	N/A	0.321	0.147	N/A	N/A
F. spiralis	F26II	N/A	0.152	0.301	0.101	0.04
F. cottonii	F90	N/A	0.256	0.336	N/A	0.133
PH	F26II	N/A	0.332	0.159	0.069	N/A
F_1HYB	N/A	N/A	N/A	N/A	N/A	N/A

Table 13. Pairwise F_{ST} estimates for Rosmuc *Fucus* over all loci.

	PH	*F. spiralis*	*F. vesiculosus*	F_1HYB
F. cottonii	0.144	0.396	0.114	0.167
PH	0	0.32	0.01	0.099
F. spiralis	---	0	0.255	0.099
F. vesiculosus	---	---	0	0.047

Table 14. Pairwise F_{ST} estimates for all taxa examined from Wells Harbor, Brave Boat Harbor, and Rosmuc.

		Wells Harbor			Brave Boat Harbor				Rosmuc		
		Fs	Fvv	mlF	Fs	Fv	Fvv	mlF	Fs	Fv	PH
Wells Harbor	Fvv	0.416									
	mlF	0.269	0.116								
Brave Boat Harbor	Fs	0.010	0.417	0.254							
	Fv	0.388	0.141	0.091	0.387						
	Fvv	0.275	0.127	0.043	0.251	0.057					
	mlF	0.202	0.136	0.019	0.197	0.116	0.014				
Rosmuc	Fs	0.530	0.333	0.344	0.525	0.299	0.319	0.350			
	Fv	0.390	0.190	0.203	0.406	0.149	0.161	0.219	0.125		
	PH	0.378	0.174	0.193	0.396	0.145	0.155	0.208	0.182	0.011	
	Fc	0.532	0.327	0.332	0.523	0.288	0.308	0.343	0.147	0.087	0.127

Fs, *F. spiralis*; Fv, *F. vesiculosus*; Fvv, *F. vesiculosus* ecad *volubilis*; mlF, muscoides-like *Fucus*; Fc, *F. cottonii*; PH; putative *F. vesiculosus* x *F. spiralis* hybrid.

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APPENDIX I

TAXONOMIC SUMMARY

Taxonomic summary from Brave Boat Harbor, ME (Chapter II)

The study of *Fucus* populations within Brave Boat Harbor examined the genetic affinities of the smallest muscoides-like *Fucus* plants and the entangled taxon *F. vesiculosus* ecad *volubilis* to attached (saxicolous) populations of *F. vesiculosus* and *F. spiralis*. Based upon analysis with four microsatellite markers, I concluded that populations of the muscoides-like *Fucus* are primarily comprised of F_1 hybrids between *F. vesiculosus* and *F. spiralis*, whereas some *F. vesiculosus* ecad *volubilis* may have arisen *via* backcrosses between a fertile F_1 hybrid and *F. vesiculosus*. Both the muscoides-like *Fucus* and *F. vesiculosus* ecad *volubilis* forms appear to be genetically differentiated from the majority of attached plants. While both *F. vesiculosus* ecad *volubilis* and the muscoides-like forms have been regarded as ecads, such a designation may not be appropriate, as this term does not carry any connotations of underlying genetic differences between the forms it describes and saxicolous plants. In addition, while dwarf *Fucus* plants described from Europe are generally regarded as being derived from *F. vesiculosus*, the muscoides-like plants appear to be hybrids. Therefore, the European forms (i.e., *F. cottonii* Wynne et Magne) and the dwarf *Fucus* from the Brave Boat Harbor should not be regarded as a single taxonomic entity.

Taxonomic summary from the Gulf of Maine Survey (Chapter III)

Chapter III examined the genetic relationships between several salt marsh taxa using five microsatellite markers. The taxa studied were *Fucus vesiculosus*, its ecad *volubilis* and variety *spiralis*, the saxicolous taxon *F. spiralis*, its ecad *lutarius*, and a dwarf limicolous muscoides-like *Fucus*. My results indicate that *F. vesiculosus* and its affiliated taxa (var. *spiralis* and ecad *volubilis*) are genetically well differentiated from *F. spiralis*. Of the three *F. vesiculosus* taxa, *F. vesiculosus* ecad *volubilis* contained a number of unusual genotypes that distinguished it from the other two taxa. The genetic separation between *F. vesiculosus* ecad *volubilis* and both saxicolous *F. vesiculosus* taxa suggests that the designation ecad may be misleading for the entangled taxon *volubilis*. Hence, it would be informative to carry out further genetic and ecological studies of ecad *volubilis* (e.g. transplant studies, detailed population genetics studies, morphometric analyses, evaluation of possible selection pressures in ecad-forming environments, etc.) in order to better ascertain its relation to attached populations of *F. vesiculosus*.

Microsatellite genotypes of the muscoides-like *Fucus* taxon were compared to the genotypes of other fucoid ecads as well as saxicolous *F. vesiculosus* and *F. spiralis* from several salt marshes in the Gulf of Maine. The muscoides-like material appears to be linked to saxicolous populations of *F. spiralis via* the entangled ecad *lutarius*. Conversely, the morphological continuum between attached and dwarf *Fucus* taxa appears to reflect an underlying genotypic continuum in which F_1 hybrids or recent backcrosses into *F. spiralis* (and possibly *F. vesiculosus*) tend to become increasingly frequent in the smallest limicolous *Fucus* populations. Therefore, while hybrids are most common in the dwarf *Fucus*, the morphologically 'intermediate' taxon *F. spiralis* ecad *lutarius* also contains a mixture of hybrid genotypes as well as relatively 'pure' *F. spiralis* genotypes (and a small number of *F. vesiculosus* genotypes as well). The results of the Gulf of Maine survey support my conclusions from Brave Boat Harbor that muscoides-like forms from the Gulf of Maine are largely hybrid and thus differ genetically from similar forms in Europe.

Taxonomic summary from the Rosmuc, Ireland study (Chapter IV)

Whereas studies within the Gulf of Maine indicate that muscoides-like forms are often derived from hybridization between *F. vesiculosus* and *F. spiralis*, my study of three taxa from Rosmuc, Ireland (*F. cottonii, F. vesiculosus,* and *F. spiralis*) using microsatellite markers suggests that *F. cottonii* from Rosmuc is likely derived from *F. vesiculosus* and is not of hybrid origin. However, *F. cottonii* is also genetically distinct from *F. vesiculosus* and the designation of the former taxon as an ecad of the latter may be inappropriate. While further studies are required to establish an appropriate taxonomic ranking of *F. cottonii*, it is clear that this taxon is genetically distinct from the muscoides-like *Fucus* in the northwest Atlantic. A putative hybrid population at Rosmuc previously identified by Dr. Robert Wilkes based on morphological criteria did not appear to be a genetic hybrid. In this case, the close genetic similarity between the putative hybrid and *F. vesiculosus* suggests that the former taxon may simply be a morphotype of the latter species. Alternatively, Wilkes' putative hybrid may be taxonomically equivalent to *F. vesiculosus* var. *spiralis* from the Gulf of Maine or to *F. vesiculosus* var. *volubilis* (Hudson) Turner that has been described from salt marshes in Europe (Baker and Bohling 1916, Pérez-Ruzafa and García 2000).

APPENDIX II:

MICROSATELLITE GENOTYPE DATA FOR ALL SAMPLES

Collection Data and Genotype Information for All Samples at Brave Boat Harbor, ME (Chapter II, Figure 3)

Sample Number	Date Collected	Collection site	Morphological Identification	Diploid Allele Data (Sizes are in bp) Microsatellite Loci							
				F227		F90		F300		F26II	
116	12/1/01	Brave Boat Harbor Site 2	ecad *lutarius*/muscoides-like	126	144	142	151	137	228	369	384
117	12/1/01	Brave Boat Harbor Site 2	ecad *lutarius*/muscoides-like	126	144	142	151	137	228	369	384
118	12/1/01	Brave Boat Harbor Site 2	ecad *lutarius*/muscoides-like	126	144	142	151	137	228	384	384
119	12/1/01	Brave Boat Harbor Site 2	ecad *lutarius*/muscoides-like	126	144	142	151	137	228	369	384
120	12/1/01	Brave Boat Harbor Site 2	ecad *lutarius*/muscoides-like	126	144	142	151	137	228	369	384
121	12/1/01	Brave Boat Harbor Site 2	ecad *lutarius*/muscoides-like	126	144	142	151	137	228	369	384
122	12/7/01	Brave Boat Harbor Site 2	ecad *lutarius*/muscoides-like	126	144	151	151	137	228	363	384
123	12/7/01	Brave Boat Harbor Site 2	ecad *lutarius*/muscoides-like	126	144	142	151	137	228	363	384
124	12/7/01	Brave Boat Harbor Site 2	ecad *lutarius*/muscoides-like	126	144	142	142	135	228	?	?
125	12/7/01	Brave Boat Harbor Site 2	ecad *lutarius*/muscoides-like	126	144	142	151	137	137	340	380
126	12/7/01	Brave Boat Harbor Site 2	ecad *lutarius*/muscoides-like	126	144	142	151	137	137	340	380
127	12/7/01	Brave Boat Harbor Site 2	ecad *lutarius*/muscoides-like	126	144	142	151	137	228	369	384
128	12/7/01	Brave Boat Harbor Site 2	ecad *lutarius*/muscoides-like	126	144	142	151	137	228	369	384
129	12/7/01	Brave Boat Harbor Site 2	ecad *lutarius*/muscoides-like	?	?	?	?	?	?	?	?
130	12/7/01	Brave Boat Harbor Site 2	ecad *lutarius*/muscoides-like	126	144	142	151	137	137	340	380
131	12/7/01	Brave Boat Harbor Site 2	muscoides-like *Fucus*	126	144	142	151	137	228	369	384
132	12/7/01	Brave Boat Harbor Site 2	muscoides-like *Fucus*	126	144	142	151	137	228	384	384
133	12/7/01	Brave Boat Harbor Site 2	*A. nodosum* ecad *scorpiodes*	118	136	?	?	?	?	?	?
134	12/7/01	Brave Boat Harbor Site 2	muscoides-like *Fucus*	126	144	142	151	137	228	371	386
135	12/7/01	Brave Boat Harbor Site 2	*A. nodosum* ecad *scorpiodes*	?	?	?	?	?	?	?	?
136	12/7/01	Brave Boat Harbor Site 2	muscoides-like *Fucus*	126	144	142	151	137	137	340	380
137	12/7/01	Brave Boat Harbor Site 2	muscoides-like *Fucus*	126	144	142	151	137	228	369	384
138	12/7/01	Brave Boat Harbor Site 2	muscoides-like *Fucus*	126	144	142	151	137	228	369	384
139	12/7/01	Brave Boat Harbor Site 2	muscoides-like *Fucus*	126	144	142	151	137	137	340	380

Brave Boat Harbor Table continued.

Number	Date	Site	Morphological ID	F227		F90		F300		F26II	
140	12/7/01	Brave Boat Harbor Site 2	muscoides-like *Fucus*	126	144	142	151	137	228	369	384
141	12/7/01	Brave Boat Harbor Site 2	muscoides-like *Fucus*	126	144	142	151	137	228	369	384
142	12/7/01	Brave Boat Harbor Site 2	muscoides-like *Fucus*	126	144	142	151	137	228	369	384
143	12/7/01	Brave Boat Harbor Site 2	muscoides-like *Fucus*	126	144	142	151	137	228	369	384
144	12/7/01	Brave Boat Harbor Site 2	muscoides-like *Fucus*	126	144	142	151	137	228	369	384
145	12/7/01	Brave Boat Harbor Site 2	*A. nodosum* ecad *scorpiodes*	?	?	?	?	137	228	?	?
146	12/7/01	Brave Boat Harbor Site 2	muscoides-like *Fucus*	126	144	142	151	137	228	369	384
147	12/7/01	Brave Boat Harbor Site 2	muscoides-like *Fucus*	126	126	142	151	137	228	369	384
148	12/7/01	Brave Boat Harbor Site 2	muscoides-like *Fucus*	126	138	142	146	137	228	384	384
149	12/7/01	Brave Boat Harbor Site 2	*A. nodosum* ecad *scorpiodes*	?	?	?	?	?	?	?	?
150	12/7/01	Brave Boat Harbor Site 2	muscoides-like *Fucus*	126	140	142	151	137	228	369	384
151	12/7/01	Brave Boat Harbor Site 2	*F. spiralis*	144	144	142	142	137	137	384	384
152	12/7/01	Brave Boat Harbor Site 2	*F. vesiculosus* ecad *volubilis*	126	144	142	151	?	?	?	?
153	12/7/01	Brave Boat Harbor Site 2	*F. vesiculosus* ecad *volubilis*	126	144	142	151	232	232	369	380
154	12/7/01	Brave Boat Harbor Site 2	*F. vesiculosus* ecad *volubilis*	126	144	142	151	118	118	369	384
155	12/7/01	Brave Boat Harbor Site 2	*F. vesiculosus* ecad *volubilis*	126	126	142	151	228	228	363	369
156	12/7/01	Brave Boat Harbor Site 2	*F. vesiculosus* ecad *volubilis*	126	126	142	142	188	188	363	369
157	12/7/01	Brave Boat Harbor Site 2	*F. vesiculosus* ecad *volubilis*	126	144	142	146	137	228	365	384
158	12/7/01	Brave Boat Harbor Site 2	*F. vesiculosus* ecad *volubilis*	116	126	142	151	118	228	369	384
159	12/7/01	Brave Boat Harbor Site 2	*F. spiralis*	144	144	142	142	137	137	384	384
160	12/7/01	Brave Boat Harbor Site 2	*F. spiralis*	144	144	142	142	137	137	384	384
161	12/7/01	Brave Boat Harbor Site 2	*F. spiralis*	144	144	142	142	137	137	384	384
162	12/7/01	Brave Boat Harbor Site 2	*F. spiralis*	144	144	142	142	137	137	384	384
163	12/7/01	Brave Boat Harbor Site 2	*F. spiralis*	144	144	142	142	137	137	384	384
164	12/7/01	Brave Boat Harbor Site 2	*F. spiralis*	144	144	142	142	137	137	384	384
165	12/7/01	Brave Boat Harbor Site 2	*F. spiralis* (?)	144	144	142	142	137	137	384	384
166	12/7/01	Brave Boat Harbor Site 2	*F. spiralis*	144	144	142	142	?	?	384	384

Brave Boat Harbor Table continued.

Number	Date	Site	Morphological ID	F227		F90		F300		F26II	
167	12/7/01	Brave Boat Harbor Site 2	*F. spiralis*	126	144	142	142	?	?	386	386
168	12/7/01	Brave Boat Harbor Site 2	*F. spiralis*	126	126	142	142	137	137	384	384
169	12/7/01	Brave Boat Harbor Site 2	*F. spiralis*	126	144	142	142	137	137	384	384
170	12/7/01	Brave Boat Harbor Site 2	*F. spiralis*	144	144	142	142	137	137	384	384
171	12/7/01	Brave Boat Harbor Site 2	*F. spiralis*	124	144	142	142	137	137	386	386
172	12/7/01	Brave Boat Harbor Site 2	*F. spiralis*	126	144	142	142	137	137	384	384
173	12/7/01	Brave Boat Harbor Site 2	*F. spiralis*	126	144	142	142	137	137	384	384
174	12/7/01	Brave Boat Harbor Site 2	*F. spiralis*	124	144	142	142	137	137	384	384
175	12/7/01	Brave Boat Harbor Site 2	*F. vesiculosus* ecad *volubilis*	126	144	142	146	137	228	365	384
176	12/7/01	Brave Boat Harbor Site 2	*F. vesiculosus* ecad *volubilis*	126	144	142	146	137	137	365	384
177	12/7/01	Brave Boat Harbor Site 2	*F. vesiculosus* ecad *volubilis*	126	144	142	153	137	228	369	384
178	12/7/01	Brave Boat Harbor Site 2	*F. vesiculosus* ecad *volubilis*	126	144	142	153	137	228	369	384
179	12/7/01	Brave Boat Harbor Site 2	*F. vesiculosus* ecad *volubilis*	126	144	142	146	137	228	365	386
180	12/7/01	Brave Boat Harbor Site 2	*F. vesiculosus* ecad *volubilis*	?	?	?	?	?	?	?	?
181	4/3/02	Brave Boat Harbor Site B	*F. spiralis*	144	144	142	142	137	137	384	384
182	4/3/02	Brave Boat Harbor Site B	*F. spiralis*	144	144	120	142	135	135	386	386
183	4/3/02	Brave Boat Harbor Site B	*F. spiralis*	144	144	142	142	137	137	386	386
184	4/3/02	Brave Boat Harbor Site B	*F. spiralis*	144	144	142	142	?	?	386	386
185	4/3/02	Brave Boat Harbor Site B	*F. spiralis*	144	144	142	142	?	?	384	384
186	4/3/02	Brave Boat Harbor Site B+10	*F. spiralis*	144	144	120	142	135	135	386	386
187	4/3/02	Brave Boat Harbor Site B+10	*F. spiralis*	144	144	142	142	?	?	384	384
188	4/3/02	Brave Boat Harbor Site B+10	*F. spiralis*	144	144	122	142	?	?	384	384
189	4/3/02	Brave Boat Harbor Site B+10	*F. spiralis*	144	144	142	142	137	137	384	384
190	4/3/02	Brave Boat Harbor Site B+10	*F. spiralis*	144	144	120	142	?	?	384	384
191	4/3/02	Brave Boat Harbor Site B+95	*F. spiralis*	144	144	142	142	137	137	386	386
192	4/3/02	Brave Boat Harbor Site B+95	*F. spiralis*	144	144	122	142	137	137	384	384
193	4/3/02	Brave Boat Harbor Site B+95	*F. spiralis*	144	144	142	142	137	137	384	384

Brave Boat Harbor Table continued.

Number	Date	Site	Morphological ID	F227		F90		F300		F26II	
194	4/3/02	Brave Boat Harbor Site B+95	*F. spiralis*	144	144	120	142	?	?	384	384
195	4/3/02	Brave Boat Harbor Site B+95	*F. spiralis*	144	144	142	142	137	137	384	384
196	4/3/02	Brave Boat Harbor Site B+50	*F. spiralis*	144	144	122	142	137	137	386	386
197	4/3/02	Brave Boat Harbor Site B+50	*F. spiralis*	144	144	120	142	137	137	?	?
198	4/3/02	Brave Boat Harbor Site B+50	*F. spiralis*	122	144	142	142	137	137	386	386
199	4/3/02	Brave Boat Harbor Site B+50	*F. spiralis*	144	144	120	142	137	137	384	384
200	4/3/02	Brave Boat Harbor Site B+50	*F. spiralis*	144	144	122	142	137	137	384	384
201	4/3/02	Brave Boat Harbor Site B+200	*F. spiralis*	144	144	142	142	137	137	384	384
202	4/3/02	Brave Boat Harbor Site B+200	*F. spiralis*	144	144	122	142	137	137	384	384
203	4/3/02	Brave Boat Harbor Site B+200	*F. spiralis*	144	144	120	142	137	137	389	389
204	4/3/02	Brave Boat Harbor Site B+200	*F. spiralis*	144	144	122	142	137	137	384	384
205	4/3/02	Brave Boat Harbor Site B+200	*F. spiralis*	144	144	142	142	137	137	384	384
206	4/3/02	Brave Boat Harbor Site B+400	*F. spiralis*	144	144	122	142	137	137	384	384
207	4/3/02	Brave Boat Harbor Site B+400	*F. spiralis*	144	144	120	142	?	?	400	400
208	4/3/02	Brave Boat Harbor Site B+400	*F. spiralis*	144	144	122	142	?	?	384	384
209	4/3/02	Brave Boat Harbor Site B+400	*F. spiralis*	144	144	120	142	137	137	384	384
210	4/3/02	Brave Boat Harbor Site B+400	*F. spiralis*	126	144	120	140	137	137	384	384
211	4/3/02	Brave Boat Harbor Site B+25	*F. spiralis*	144	144	120	142	137	224	380	389
212	4/3/02	Brave Boat Harbor Site B+25	*F. spiralis*	144	144	122	142	137	226	386	386
213	4/3/02	Brave Boat Harbor Site B+25	*F. spiralis*	144	144	142	142	137	137	384	384
214	4/3/02	Brave Boat Harbor Site B+25	*F. spiralis*	144	144	142	142	137	137	389	389
215	4/3/02	Brave Boat Harbor Site B+25	*F. spiralis*	144	144	142	142	137	137	384	384
216	4/3/02	Brave Boat Harbor Site B+300	*F. spiralis*	144	144	122	142	137	137	384	384
217	4/3/02	Brave Boat Harbor Site B+300	*F. spiralis*	144	144	120	142	137	137	384	384
218	4/3/02	Brave Boat Harbor Site B+300	*F. spiralis*	144	144	122	142	?	?	384	384
219	4/3/02	Brave Boat Harbor Site B+300	*F. spiralis*	144	144	122	140	137	137	384	384
220	4/3/02	Brave Boat Harbor Mouth	*F. spiralis*	144	144	122	142	228	228	365	380

Brave Boat Harbor Table continued.

Number	Date	Site	Morphological ID	F227		F90		F300		F26II	
221	4/3/02	Brave Boat Harbor Mouth	*F. spiralis*	144	144	120	142	137	137	363	363
222	4/3/02	Brave Boat Harbor Mouth	*F. spiralis*	126	126	142	142	137	137	363	363
223	4/3/02	Brave Boat Harbor Mouth	*F. spiralis*	146	146	120	142	228	228	369	380
224	4/3/02	Brave Boat Harbor Site A	*F. spiralis*	146	146	122	142	137	137	384	384
225	4/3/02	Brave Boat Harbor Site A	*F. spiralis*	146	146	142	142	137	137	384	384
226	4/3/02	Brave Boat Harbor Site A	*F. spiralis*	146	146	142	142	137	137	384	384
227	4/3/02	Brave Boat Harbor Site A	*F. spiralis*	146	146	142	142	137	137	384	384
228	4/3/02	Brave Boat Harbor Site A	*F. spiralis*	144	144	142	142	137	137	386	386
229	7/16/02	Brave Boat Harbor Site 5	*F. spiralis* ecad *lutarius*	126	144	142	146	137	228	380	384
230	7/16/02	Brave Boat Harbor Site 5	*F. spiralis* ecad *lutarius*	126	144	142	146	137	137	384	384
231	7/16/02	Brave Boat Harbor Site 5	*F. spiralis* ecad *lutarius*	126	144	142	146	137	137	380	384
232	7/16/02	Brave Boat Harbor Site 5	*F. spiralis* ecad *lutarius*	126	144	142	146	137	228	380	384
233	7/16/02	Brave Boat Harbor Site 5	*F. spiralis* ecad *lutarius*	126	144	142	146	137	228	380	384
234	7/16/02	Brave Boat Harbor Site 5	*F. spiralis* ecad *lutarius*	126	144	142	146	137	137	380	384
235	7/16/02	Brave Boat Harbor Site 5	ecad *lutarius*/muscoides-like	126	144	142	146	137	228	380	384
236	7/16/02	Brave Boat Harbor Site 5	ecad *lutarius*/muscoides-like	126	144	142	142	137	228	369	384
237	7/16/02	Brave Boat Harbor Site 5	*F. spiralis* ecad *lutarius*	126	144	142	151	137	137	340	380
238	7/16/02	Brave Boat Harbor Site 5	*F. spiralis* ecad *lutarius*	144	144	142	142	137	137	380	380
239	7/16/02	Brave Boat Harbor Site 5	muscoides-like *Fucus*	126	144	151	151	137	137	380	380
240	7/16/02	Brave Boat Harbor Site 5	*F. spiralis*	126	138	142	142	146	146	384	384
241	7/16/02	Brave Boat Harbor Site 5	*F. spiralis*	144	144	142	142	135	135	382	382
242	7/16/02	Brave Boat Harbor Site 5	*F. spiralis*	126	144	142	142	137	137	384	384
243	7/16/02	Brave Boat Harbor Site 5	*F. spiralis*	138	138	142	146	137	137	389	389
244	7/16/02	Brave Boat Harbor Site 5	*F. spiralis*	126	144	142	142	137	137	384	384
245	7/16/02	Brave Boat Harbor Site 5	*F. spiralis*	126	144	146	146	123	123	?	?
246	7/16/02	Brave Boat Harbor Site 5	*F. spiralis*	144	144	124	142	?	?	386	386
247	7/16/02	Brave Boat Harbor Site 5	muscoides-like *Fucus*	144	144	140	140	133	133	340	340

Brave Boat Harbor Table continued.

Number	Date	Site	Morphological ID	F227		F90		F300		F26II	
248	7/16/02	Brave Boat Harbor Site 2	*F. vesiculosus*	126	144	142	142	228	228	369	369
249	7/16/02	Brave Boat Harbor Site 2	*F. vesiculosus*	126	144	142	142	142	142	?	?
250	7/16/02	Brave Boat Harbor Site 2	*F. spiralis*	?	?	142	142	137	137	384	384
251	7/16/02	Brave Boat Harbor Site 2	*F. spiralis*	144	144	142	142	137	226	386	386
252	7/16/02	Brave Boat Harbor Site 2	*F. spiralis*	144	144	142	142	?	?	382	382
253	7/16/02	Brave Boat Harbor Site 2	*F. spiralis*	144	144	142	142	137	137	384	384
254	7/16/02	Brave Boat Harbor Site 2	*F. spiralis*	144	144	142	142	137	137	384	384
255	7/16/02	Brave Boat Harbor Site 2	*F. spiralis*	144	144	142	142	137	137	384	384
256	7/16/02	Brave Boat Harbor Site 2	*F. spiralis*	144	144	142	142	137	137	384	384
257	7/16/02	Brave Boat Harbor Site 2	*F. spiralis*	144	144	142	142	137	137	384	384
258	7/16/02	Brave Boat Harbor Site 2	*F. spiralis*	144	144	142	142	137	137	384	384
259	7/16/02	Brave Boat Harbor Site 2	*F. spiralis*	144	144	142	142	137	226	384	384
260	7/16/02	Brave Boat Harbor Site 2	*F. spiralis*	144	144	142	142	137	137	389	389
261	7/16/02	Brave Boat Harbor Site 2	*F. spiralis*	144	144	142	142	137	137	384	384
262	7/16/02	Brave Boat Harbor Site 2	*F. vesiculosus* ecad *volubilis*	116	126	142	151	118	228	367	382
263	7/16/02	Brave Boat Harbor Site 2	*F. vesiculosus* ecad *volubilis*	126	126	151	151	228	228	369	380
264	7/16/02	Brave Boat Harbor Site 2	*F. vesiculosus* ecad *volubilis*	116	126	142	151	118	228	369	384
265	7/16/02	Brave Boat Harbor Site 2	*F. vesiculosus* ecad *volubilis*	116	126	142	151	118	228	369	384
266	7/16/02	Brave Boat Harbor Site 2	*F. vesiculosus* ecad *volubilis*	116	126	142	151	137	228	369	382
267	7/16/02	Brave Boat Harbor Site 2	*F. spiralis*	144	144	142	142	137	137	384	384
268	7/16/02	Brave Boat Harbor Site 2	*F. spiralis*	144	144	142	142	?	?	384	384
269	7/16/02	Brave Boat Harbor Site 2	*F. spiralis*	144	144	142	142	?	?	384	384
270	7/16/02	Brave Boat Harbor Site 2	*F. spiralis*	126	144	142	142	?	?	384	384
271	7/16/02	Brave Boat Harbor Site 2	*F. spiralis*	124	144	142	142	137	137	384	384
272	7/16/02	Brave Boat Harbor Site 2	*F. spiralis*	144	144	142	142	137	137	384	384
273	7/16/02	Brave Boat Harbor Site 2	muscoides-like *Fucus*	126	144	142	142	137	137	?	?
274	7/16/02	Brave Boat Harbor Site 2	muscoides-like *Fucus*	?	?	?	?	?	?	?	?

Brave Boat Harbor Table continued.

Number	Date	Site	Morphological ID	F227		F90		F300		F26II	
275	7/16/02	Brave Boat Harbor Site 2	muscoides-like *Fucus*	126	144	142	151	135	235	369	384
276	7/16/02	Brave Boat Harbor Site 2	*F. spiralis*	144	144	142	142	137	137	384	384
277	7/16/02	Brave Boat Harbor Site 2	*F. spiralis*	?	?	?	?	135	135	382	382
278	7/16/02	Brave Boat Harbor Site 2	muscoides-like *Fucus*	126	144	151	151	131	131	369	384
279	7/16/02	Brave Boat Harbor (Pilings)	*F. vesiculosus*	126	144	151	151	228	228	380	380
280	7/16/02	Brave Boat Harbor (Pilings)	*F. vesiculosus*	126	126	142	151	228	228	369	369
281	7/16/02	Brave Boat Harbor (Pilings)	*F. vesiculosus*	126	126	151	151	228	228	380	380
282	7/16/02	Brave Boat Harbor (Pilings)	*F. vesiculosus*	124	126	146	146	228	228	380	380
283	7/16/02	Brave Boat Harbor (Pilings)	*F. vesiculosus*	122	126	148	153	228	228	365	380
284	7/16/02	Brave Boat Harbor (Pilings)	*F. vesiculosus*	124	126	146	146	228	228	380	380
285	7/16/02	Brave Boat Harbor (Pilings)	*F. vesiculosus*	124	126	146	151	228	228	365	391
286	7/16/02	Brave Boat Harbor (Pilings)	*F. vesiculosus*	126	126	142	146	228	228	384	384
287	7/16/02	Brave Boat Harbor (Pilings)	*F. vesiculosus*	126	126	142	146	228	228	369	380
288	7/16/02	Brave Boat Harbor Site 6	muscoides-like *Fucus*	126	144	142	151	137	137	340	380
289	7/16/02	Brave Boat Harbor Site 6	muscoides-like *Fucus*	126	144	142	151	137	137	340	380
290	7/16/02	Brave Boat Harbor Site 6	muscoides-like *Fucus*	126	126	146	146	137	137	?	?
291	7/16/02	Brave Boat Harbor Site 6	muscoides-like *Fucus*	126	144	142	151	137	137	340	380
292	7/16/02	Brave Boat Harbor Site 6	muscoides-like *Fucus*	126	146	142	151	137	226	340	380
293	7/16/02	Brave Boat Harbor Site 6	muscoides-like *Fucus*	126	146	142	151	137	226	340	380
294	7/16/02	Brave Boat Harbor Site 6	muscoides-like *Fucus* (?)	126	146	142	151	137	137	340	380
295	7/16/02	Brave Boat Harbor Site 6	muscoides-like *Fucus*	126	146	142	151	137	226	363	384
296	7/16/02	Brave Boat Harbor Site 6	muscoides-like *Fucus*	126	146	142	151	137	137	340	380
297	7/16/02	Brave Boat Harbor Site 6	*F. spiralis*	144	144	142	142	137	137	384	384
298	7/16/02	Brave Boat Harbor Site 6	*F. spiralis*	144	144	142	142	137	137	384	384
299	7/16/02	Brave Boat Harbor Site 6	*F. spiralis*	144	144	142	142	137	137	386	386
300	7/16/02	Brave Boat Harbor Site 6	*F. spiralis*	146	146	142	142	137	137	384	384
301	7/16/02	Brave Boat Harbor Site 6	*F. spiralis*	146	146	142	142	137	137	384	384

Brave Boat Harbor Table continued.

Number	Date	Site	Morphological ID	F227		F90		F300		F26II	
302	7/16/02	Brave Boat Harbor Site 6	muscoides-like *Fucus*	144	144	142	146	137	228	384	384
303	7/16/02	Brave Boat Harbor Site 6	*F. spiralis*	?	?	?	?	?	?	?	?
304	7/16/02	Brave Boat Harbor Site 4	*F. vesiculosus*	126	126	142	146	228	228	369	369
305	7/16/02	Brave Boat Harbor Site 4	*F. vesiculosus*	126	126	146	153	228	228	365	380
306	7/16/02	Brave Boat Harbor Site 4	*F. vesiculosus*	126	126	142	153	228	228	365	369
307	7/16/02	Brave Boat Harbor Site 4	*F. vesiculosus*	126	126	142	146	228	228	369	369
308	7/16/02	Brave Boat Harbor Site 4	*F. vesiculosus*	126	126	146	146	228	228	365	369
309	7/16/02	Brave Boat Harbor Site 4	*F. vesiculosus* ecad *volubilis*	126	126	151	155	228	228	380	380
310	7/16/02	Brave Boat Harbor Site 4	*F. vesiculosus* ecad *volubilis*	118	126	142	151	137	137	369	384
311	7/16/02	Brave Boat Harbor Site 4	*F. vesiculosus* ecad *volubilis*	126	126	151	155	135	228	369	380
312	7/16/02	Brave Boat Harbor Site 4	*F. spiralis*	144	144	142	142	137	137	384	384
313	7/16/02	Brave Boat Harbor Site 4	*F. spiralis*	144	144	142	142	137	137	384	384
314	7/16/02	Brave Boat Harbor Site 4	*F. spiralis*	144	144	142	142	137	137	384	384
315	7/16/02	Brave Boat Harbor Site 4	*F. spiralis*	146	146	142	142	137	137	384	384
316	7/16/02	Brave Boat Harbor Site 4	*F. spiralis*	140	140	142	142	137	137	384	384
317	7/16/02	Brave Boat Harbor Site 4	*F. spiralis*	146	146	142	142	137	137	384	384
318	7/16/02	Brave Boat Harbor Site 4	*F. spiralis*	144	144	142	142	?	?	384	384
319	7/16/02	Brave Boat Harbor Site 4	*F. spiralis*	146	146	142	142	137	137	384	384
320	7/16/02	Brave Boat Harbor Site 4	*F. spiralis*	144	144	142	142	137	137	363	363
321	7/16/02	Brave Boat Harbor Site 4	*F. spiralis*	126	146	142	142	137	137	363	363
322	7/16/02	Brave Boat Harbor Site 4	*F. spiralis*	144	144	142	142	137	137	384	384
323	7/16/02	BBH Site 3 Clam Netting	*F. vesiculosus*	126	126	146	146	137	228	365	369
324	7/16/02	BBH Site 3 Clam Netting	*F. vesiculosus*	126	126	146	151	228	228	367	394
325	7/16/02	BBH Site 3 Clam Netting	*F. vesiculosus*	126	126	146	146	228	228	365	369
379	11/14/02	Brave Boat Harbor (Pilings)	*F. vesiculosus*	126	126	151	151	226	226	365	369
380	11/14/02	Brave Boat Harbor (Pilings)	*F. vesiculosus*	126	126	146	151	232	232	386	386
381	11/14/02	Brave Boat Harbor (Pilings)	*F. vesiculosus*	126	126	146	151	228	228	386	386

Brave Boat Harbor Table continued.

Number	Date	Site	Morphological ID	F227		F90		F300		F26II	
382	11/14/02	Brave Boat Harbor (Pilings)	*F. vesiculosus*	126	126	151	151	228	228	386	386
383	11/14/02	Brave Boat Harbor (Pilings)	*F. vesiculosus*	126	144	146	146	228	228	386	386
384	11/14/02	Brave Boat Harbor (Pilings)	*F. vesiculosus*	126	144	142	146	228	228	386	386
385	11/14/02	Brave Boat Harbor (Pilings)	*F. vesiculosus*	126	144	?	?	137	230	353	369
386	11/14/02	Brave Boat Harbor (Pilings)	*F. vesiculosus*	126	126	?	?	222	222	365	369
387	11/14/02	Brave Boat Harbor (Pilings)	*F. vesiculosus*	126	126	142	148	228	228	365	369
388	11/14/02	Brave Boat Harbor (Pilings)	*F. vesiculosus*	126	126	146	151	228	228	353	369
389	11/14/02	Brave Boat Harbor, Site 9	*F. spiralis*	126	126	142	142	137	137	380	380
390	11/14/02	Brave Boat Harbor, Site 9	*F. spiralis*	126	144	142	142	137	137	369	400
391	11/14/02	Brave Boat Harbor, Site 9	*F. spiralis*	126	144	142	142	137	137	369	380
392	11/14/02	Brave Boat Harbor, Site 9	*F. spiralis*	126	126	142	142	137	137	363	380
393	11/14/02	Brave Boat Harbor, Site 9	*F. spiralis*	126	144	142	142	137	137	365	369
394	11/14/02	Brave Boat Harbor, Site 9	muscoides-like *Fucus*	126	144	142	142	137	222	365	369
395	11/14/02	Brave Boat Harbor, Site 9	muscoides-like *Fucus*	126	144	142	151	137	228	369	384
396	11/14/02	Brave Boat Harbor, Site 9	muscoides-like *Fucus*	126	144	142	151	137	228	369	384
397	11/14/02	Brave Boat Harbor, Site 9	muscoides-like *Fucus*	126	144	142	151	137	228	369	384
398	11/14/02	Brave Boat Harbor, Site 9	muscoides-like *Fucus*	126	144	142	151	137	228	369	384
399	11/14/02	Brave Boat Harbor, Site 9	muscoides-like *Fucus*	144	144	142	151	137	228	369	384
400	11/14/02	Brave Boat Harbor, Site 10	*F. vesiculosus*	144	144	146	151	230	230	371	389
401	11/14/02	Brave Boat Harbor, Site 10	*F. vesiculosus*	144	144	146	151	228	228	380	391
402	11/14/02	Brave Boat Harbor, Site 10	*F. vesiculosus*	126	144	142	142	228	228	367	369
403	11/14/02	Brave Boat Harbor, Site 10	*F. vesiculosus*	126	126	146	151	228	228	380	391

Collection Data and Genotype Information for all Samples from the Gulf of Maine (Chapter III, Figure 5)

Diploid Allele Data (sizes are in bp)

Microsatellite Loci

Number	Location	Morphological Identification	F227		F90		F300		F26II		F-L94	
456	Site 6	*F. vesiculosus* (large attached)	116	126	142	142	137	230	363	386	165	165
457	Site 6	*F. vesiculosus* (large attached)	126	144	142	142	137	137	363	363	165	165
458	Site 6	*F. vesiculosus* (large attached)	144	144	142	142	137	137	384	384	165	165
459	Site 6	*F. vesiculosus* (large attached)	126	146	142	146	139	139	369	384	165	188
460	Site 6	*F. vesiculosus* (large attached)	144	144	142	142	139	139	384	384	165	165
461	Site 6	*F. vesiculosus* (large attached)	126	126	146	151	228	228	384	384	188	188
462	Site 6	*F. vesiculosus* (large attached)	126	142	142	142	133	133	384	384	165	165
463	Site 6	*F. vesiculosus* (large attached)	126	144	142	142	137	137	384	384	165	165
464	Site 6	*F. vesiculosus* (large attached)	126	144	146	151	137	226	369	380	188	188
465	Site 6	*F. vesiculosus* (large attached)	144	144	142	142	137	228	384	384	165	165
466	Site 6	*F. vesiculosus* (large attached)	144	144	142	142	137	137	384	384	165	165
467	Site 6	*F. vesiculosus* (large attached)	126	126	142	146	135	228	369	380	188	188
468	Site 6	*F. vesiculosus* var. *spiralis* or *F. spiralis*	126	126	142	159	226	226	361	371	168	168
469	Site 6	*F. vesiculosus* var. *spiralis* or *F. spiralis*	126	140	142	159	226	226	361	371	176	176
476	Site 6	*F. spiralis* (vegetative)	142	144	142	142	133	133	378	378	165	165
477	Site 6	*F. spiralis* (vegetative)	144	142	142	142	137	228	384	384	165	165
478	Site 6	*F. spiralis* (vegetative)	144	144	142	142	?	?	384	384	165	165
479	Site 6	*F. spiralis* (vegetative)	144	144	142	142	137	137	384	384	165	165
480	Site 6	*F. spiralis* (vegetative)	144	144	142	142	137	137	?	?	165	165
481	Site 6	*F. spiralis* (vegetative)	144	144	142	142	135	228	382	382	174	174
482	Site 6	*F. spiralis* (vegetative)	144	144	142	142	137	224	382	382	165	165
483	Site 6	*F. spiralis* (vegetative)	144	144	142	142	137	137	384	384	174	174
484	Site 6	*F. spiralis* (vegetative)	144	144	142	142	137	137	384	384	165	165
485	Site 6	*F. spiralis* (vegetative)	144	144	142	142	137	137	380	384	165	165
486	Site 6	*F. spiralis* (vegetative)	144	144	142	142	137	137	378	378	188	188

Gulf of Maine Table continued.

Number	Site	Morphological ID	F227		F90		F300		F26II		L94	
487	Site 6	*F. spiralis* (vegetative)	144	144	142	142	137	137	378	384	165	165
488	Site 6	muscoides-like *Fucus*	126	144	142	146	137	228	369	384	165	188
489	Site 6	muscoides-like *Fucus*	126	144	142	146	137	228	369	384	174	196
490	Site 6	muscoides-like *Fucus*	126	144	142	146	135	228	369	384	165	188
491	Site 6	muscoides-like *Fucus*	126	144	142	146	137	228	369	378	174	196
492	Site 6	muscoides-like *Fucus*	126	144	142	146	137	228	369	384	165	188
493	Site 6	muscoides-like *Fucus*	126	144	142	146	137	228	369	384	174	196
494	Site 6	muscoides-like *Fucus*	126	144	142	146	137	228	369	384	165	188
495	Site 6	muscoides-like *Fucus*	126	144	142	146	137	228	386	386	?	?
496	Site 6	muscoides-like *Fucus*	126	144	142	146	137	226	369	384	165	188
497	Site 6	muscoides-like *Fucus*	126	144	142	146	137	228	369	384	165	188
498	Site 6	muscoides-like *Fucus*	126	144	142	146	137	228	369	384	165	188
499	Site 6	muscoides-like *Fucus*	126	144	142	146	135	228	369	384	165	188
500	Site 1	*F. vesiculosus* ecad *volubilis*	126	148	142	146	228	234	369	369	168	168
501	Site 1	*F. vesiculosus* ecad *volubilis*	126	144	146	146	135	228	361	361	170	188
502	Site 1	*F. vesiculosus* ecad *volubilis*	126	144	146	146	137	228	361	361	146	188
503	Site 1	*F. vesiculosus* ecad *volubilis*	126	144	146	146	137	228	361	361	163	188
504	Site 1	*F. vesiculosus* ecad *volubilis*	126	144	146	146	137	228	361	361	188	188
505	Site 1	*F. vesiculosus* ecad *volubilis*	126	144	146	146	135	228	361	361	146	188
506	Site 1	*F. vesiculosus* ecad *volubilis*	126	144	146	146	139	228	361	361	146	188
507	Site 1	*F. vesiculosus* ecad *volubilis*	126	144	146	146	135	228	361	361	146	188
508	Site 1	*F. vesiculosus* ecad *volubilis*	126	144	142	146	137	228	361	361	188	188
509	Site 1	*F. vesiculosus* ecad *volubilis*	126	144	142	146	135	228	361	361	188	188
510	Site 1	*F. vesiculosus* ecad *volubilis*	126	144	146	146	135	228	361	361	146	188
511	Site 1	*F. vesiculosus* ecad *volubilis*	126	144	142	146	?	?	369	369	165	188
512	Site 1	*A. nodosum* ecad *scorpiodes*	126	138	142	142	?	?	?	?	188	188
513	Site 1	*A. nodosum* ecad *scorpiodes*	126	138	142	142	?	?	?	?	154	154

Gulf of Maine Table continued.

Number	Site	Morphological ID	F227		F90		F300		F26II		L94	
514	Site 1	*A. nodosum* ecad *scorpiodes*	126	126	142	142	?	?	?	?	154	154
515	Site 1	*F. vesiculosus* var. *spiralis*?	126	144	142	142	137	137	363	363	142	165
516	Site 1	*F. vesiculosus* var. *spiralis*?	126	144	142	142	137	137	345	363	142	165
517	Site 1	*F. vesiculosus* var. *spiralis*?	126	144	142	142	137	137	361	361	140	165
518	Site 1	*F. vesiculosus* var. *spiralis*?	126	144	142	142	137	137	363	363	165	165
519	Site 1	*F. vesiculosus* var. *spiralis*?	126	144	142	142	137	137	363	363	165	165
520	Site 1	*F. vesiculosus* var. *spiralis*?	126	144	142	142	137	220	363	363	142	165
521	Site 1	*F. vesiculosus* var. *spiralis*?	126	144	142	142	137	137	363	363	142	165
522	Site 1	*F. vesiculosus* var. *spiralis*?	126	144	142	142	137	137	363	363	142	165
523	Site 1	*F. vesiculosus* var. *spiralis*?	126	144	142	142	137	137	363	363	165	165
524	Site 1	*F. vesiculosus* var. *spiralis*?	126	144	142	142	137	137	363	363	165	165
525	Site 1	*F. vesiculosus* var. *spiralis*?	126	144	142	142	137	137	363	363	142	165
526	Site 1	*F. vesiculosus* var. *spiralis*?	126	144	142	142	137	137	363	363	142	165
527	Site 1	*F. vesiculosus* many vessicles	126	126	142	146	135	228	380	380	168	188
528	Site 1	*F. vesiculosus* many vessicles	126	142	142	142	125	228	369	369	188	188
529	Site 1	*F. vesiculosus* many vessicles	126	138	142	146	228	228	?	?	188	188
530	Site 1	*F. vesiculosus* many vessicles	138	138	146	146	139	236	369	389	168	188
531	Site 1	*F. vesiculosus* many vessicles	126	126	146	146	228	228	369	369	168	168
532	Site 1	*F. vesiculosus* many vessicles	126	150	142	142	125	232	369	380	168	188
533	Site 1	*F. vesiculosus* many vessicles	126	126	146	146	127	230	365	365	188	188
534	Site 1	*F. vesiculosus* many vessicles	126	144	146	146	125	228	369	380	188	188
535	Site 1	*F. vesiculosus* many vessicles	126	142	146	146	230	230	369	380	168	168
536	Site 1	*F. vesiculosus* var. *spiralis*	126	126	142	142	127	228	369	369	168	174
537	Site 1	*F. vesiculosus* var. *spiralis*	126	126	142	142	125	228	369	369	168	174
538	Site 1	*F. vesiculosus* var. *spiralis*	126	126	142	142	127	228	369	369	242	251
539	Site 1	*F. spiralis* ecad *lutarius*	126	144	142	146	137	228	369	369	165	165
540	Site 1	*F. spiralis* ecad *lutarius*	126	142	142	146	137	228	369	369	165	188

Gulf of Maine Table continued.

Number	Site	Morphological ID	F227		F90		F300		F26II		L94	
541	Site 1	*F. spiralis* ecad *lutarius*	126	144	146	146	228	228	361	361	188	188
542	Site 1	*F. spiralis* ecad *lutarius*	126	144	142	146	137	228	365	369	165	188
543	Site 1	*F. spiralis* ecad *lutarius*	126	144	142	146	137	228	365	369	165	188
544	Site 1	muscoides-like *Fucus*	126	144	142	146	137	228	365	369	165	188
545	Site 1	muscoides-like *Fucus*	126	144	142	146	135	135	369	369	165	188
546	Site 1	muscoides-like *Fucus*	126	144	142	146	137	228	363	369	165	188
547	Site 1	muscoides-like *Fucus*	126	144	142	146	137	228	365	369	165	188
548	Site 1	muscoides-like *Fucus*	126	144	142	146	137	228	365	369	165	188
549	Site 1	muscoides-like *Fucus*	126	144	142	146	137	228	363	369	165	188
550	Site 1	muscoides-like *Fucus*	126	144	142	146	137	228	363	369	165	188
551	Site 1	*F. vesiculosus* no vessicles	144	144	142	142	137	137	365	365	165	165
552	Site 4	*F. vesiculosus* no vessicles	126	126	142	151	226	230	371	371	182	182
553	Site 4	*F. vesiculosus* no vessicles	144	144	142	142	137	137	363	363	188	188
554	Site 4	*F. vesiculosus* no vessicles	144	144	142	142	137	226	365	365	165	165
555	Site 4	*F. vesiculosus* no vessicles	144	144	142	142	137	137	365	365	165	165
556	Site 4	*F. vesiculosus* no vessicles	126	146	142	146	139	139	365	367	165	165
557	Site 4	*F. vesiculosus* no vessicles	126	126	142	151	125	228	363	380	201	201
558	Site 4	*F. vesiculosus* no vessicles	144	144	142	142	137	137	363	363	165	165
559	Site 4	*F. vesiculosus* (material poor)	126	140	142	146	228	228	380	380	168	174
560	Site 4	*F. vesiculosus* (material poor)	126	142	144	144	133	133	369	369	?	?
561	Site 4	*F. vesiculosus* var. *spiralis* or *F. spiralis*	126	126	151	159	228	228	361	365	188	188
562	Site 4	*F. vesiculosus* no vessicles	126	144	142	142	137	137	365	365	165	165
566	Site 2	*F. vesiculosus* var. *spiralis*	144	144	142	142	137	137	384	384	165	165
567	Site 2	*F. vesiculosus* var. *spiralis*	144	144	142	142	137	137	384	384	165	165
568	Site 2	*F. vesiculosus* var. *spiralis*	144	144	142	142	137	137	384	384	165	165
569	Site 2	*F. vesiculosus* var. *spiralis*	144	144	142	142	137	137	384	384	165	165
570	Site 2	*F. vesiculosus* var. *spiralis*	126	144	142	142	125	230	365	384	174	188

Gulf of Maine Table continued.

Number	Site	Morphological ID	F227		F90		F300		F26II		L94	
571	Site 2	*F. vesiculosus* var. *spiralis*	140	140	142	142	135	135	365	365	165	165
572	Site 2	*F. vesiculosus* var. *spiralis*	126	126	151	151	127	232	369	369	168	168
573	Site 2	*F. vesiculosus* var. *spiralis*	144	144	142	142	137	137	363	363	168	168
574	Site 2	*F. vesiculosus* var. *spiralis*	144	144	?	?	137	137	363	363	165	165
575	Site 2	*F. vesiculosus* var. *spiralis*	144	144	142	142	137	137	384	384	168	168
576	Site 2	*F. vesiculosus* var. *spiralis*	144	144	142	142	137	137	363	363	168	168
577	Site 2	*F. vesiculosus* var. *spiralis*	144	144	142	142	135	135	384	384	165	165
578	Site 2	ecad *lutarius*? (larger muscoides)	126	144	142	146	137	228	380	389	165	188
579	Site 2	ecad *lutarius*? (larger muscoides)	126	144	142	146	137	228	380	380	165	188
580	Site 2	ecad *lutarius*? (larger muscoides)	126	142	142	146	137	228	380	389	165	188
581	Site 2	ecad *lutarius*? (larger muscoides)	126	144	142	146	137	228	380	389	165	188
582	Site 2	ecad *lutarius*? (larger muscoides)	126	144	142	146	137	228	380	389	165	188
583	Site 2	ecad *lutarius*? (larger muscoides)	126	144	142	142	137	228	380	389	165	188
585	Site 2	muscoides-like *Fucus*	126	144	142	146	137	228	380	389	165	188
586	Site 2	muscoides-like *Fucus*	126	144	142	146	137	228	380	389	165	188
587	Site 2	muscoides-like *Fucus*	126	144	164	164	137	228	380	389	165	188
588	Site 2	muscoides-like *Fucus*	126	144	142	146	137	228	380	389	165	188
589	Site 2	muscoides-like *Fucus*	126	144	142	146	137	228	380	389	165	188
590	Site 2	muscoides-like *Fucus*	126	144	142	146	137	228	380	397	165	188
591	Site 2	muscoides-like *Fucus*	?	?	142	142	?	?	?	?	?	?
592	Site 2	muscoides-like *Fucus*	126	144	142	146	137	228	380	389	165	188
593	Site 2	muscoides-like *Fucus*	126	144	142	146	137	228	380	389	165	188
594	Site 2	muscoides-like *Fucus*	126	144	142	146	137	137	380	389	165	188
595	Site 2	muscoides-like *Fucus*	126	144	142	146	137	228	380	389	165	188
596	Site 2	*F. spiralis* (vegetative)	144	144	142	142	137	137	384	384	165	165
597	Site 2	*F. spiralis* (vegetative)	144	144	142	142	137	137	384	384	165	165
598	Site 2	*F. spiralis* (vegetative)	144	144	142	142	137	137	384	384	165	165

Gulf of Maine Table continued.

Number	**Site**	**Morphological ID**	**F227**		**F90**		**F300**		**F26II**		**L94**	
599	Site 2	*F. spiralis* (vegetative)	144	144	142	142	137	137	384	384	165	165
600	Site 2	*F. spiralis* (vegetative)	144	144	142	142	137	137	384	384	165	165
601	Site 2	*F. spiralis* (vegetative)	144	144	142	142	137	137	384	384	165	165
602	Site 2	*F. spiralis* (vegetative)	144	144	142	142	137	224	384	384	165	165
603	Site 2	*F. spiralis* (vegetative)	140	140	142	142	121	121	384	384	165	165
604	Site 2	*F. spiralis* (vegetative)	142	142	142	142	137	228	384	384	165	165
605	Site 2	*F. spiralis* (vegetative)	140	140	142	142	137	224	363	363	165	165
606	Site 2	*F. spiralis* (vegetative)	144	144	142	142	137	137	384	384	165	165
607	Site 2	*F. spiralis* (vegetative)	140	140	142	142	135	135	363	363	153	153
611	Site 5	*F. vesiculosus* var. *spiralis*	126	126	146	146	215	230	365	365	168	168
612	Site 5	*F. vesiculosus* var. *spiralis*	126	144	146	151	226	226	365	365	168	168
613	Site 5	*F. vesiculosus* var. *spiralis*	126	126	146	155	215	228	365	369	188	188
614	Site 5	*F. vesiculosus* var. *spiralis*	126	126	146	155	228	238	365	369	188	188
615	Site 5	*F. vesiculosus* var. *spiralis*	126	126	146	151	228	228	365	365	168	188
616	Site 5	*F. vesiculosus* var. *spiralis*	126	126	142	146	228	228	363	380	168	188
617	Site 5	*F. vesiculosus* var. *spiralis*	126	144	146	146	228	228	365	365	188	188
618	Site 5	*F. vesiculosus* var. *spiralis*	128	128	151	151	228	228	365	369	165	186
619	Site 5	*F. vesiculosus* var. *spiralis*	126	126	146	146	232	232	365	365	188	188
620	Site 5	*F. vesiculosus* var. *spiralis*	126	126	146	151	228	228	365	361	134	188
621	Site 5	*F. vesiculosus* var. *spiralis*	126	126	146	151	226	226	361	361	134	153
622	Site 5	*F. vesiculosus* var. *spiralis*	126	126	146	151	228	228	365	369	188	188
626	Site 5	*F. v* var *sp.* or *F. sp.* High shoreline[1]	126	126	146	151	228	228	365	380	188	188
627	Site 5	*F. v* var *sp.* or *F. sp.* High shoreline	126	126	146	151	228	228	365	369	188	188
628	Site 5	*F. v* var *sp.* or *F. sp.* High shoreline	126	126	151	155	228	228	363	369	165	165
629	Site 5	*F. v* var *sp.* or *F. sp.* High shoreline	126	126	142	151	228	228	363	380	165	165
630	Site 5	muscoides-like *Fucus*	126	144	142	151	137	137	380	386	165	188
631	Site 5	muscoides-like *Fucus*	126	126	142	151	137	137	380	384	165	188

Gulf of Maine Table continued.

Number	Site	Morphological ID	F227		F90		F300		F26II		L94	
632	Site 5	muscoides-like *Fucus* (*Ascophyllum*?)	126	144	?	?	137	137	378	386	165	188
633	Site 5	muscoides-like *Fucus*	126	144	142	151	137	137	380	384	165	188
634	Site 5	muscoides-like *Fucus*	126	144	142	151	137	137	380	384	165	188
635	Site 5	muscoides-like *Fucus*	126	144	142	151	137	137	380	384	165	188
636	Site 5	muscoides-like *Fucus*	126	126	142	151	137	137	380	384	165	188
637	Site 5	muscoides-like *Fucus* (*Ascophyllum*?)	136	140	?	?	137	137	380	384	165	188
638	Site 5	muscoides-like *Fucus* (*Ascophyllum*?)	126	150	?	?	127	228	365	381	188	188
639	Site 5	muscoides-like *Fucus*	126	144	146	153	137	137	380	386	165	188
640	Site 5	muscoides-like *Fucus*	126	144	142	151	137	137	380	384	165	188
641	Site 5	muscoides-like *Fucus*	126	126	146	151	137	137	380	384	165	188
642	Site 5	muscoides-like *Fucus*	126	138	142	153	137	137	380	384	165	188
643	Site 5	muscoides-like *Fucus*	126	144	146	146	137	137	380	384	165	188
644	Site 5	muscoides-like *Fucus*	126	144	142	151	137	137	382	384	165	188
645	Site 5	*F. spiralis*	126	144	146	146	228	228	355	365	188	188
646	Site 3	*F. vesiculosus* var. *spiralis* probably	126	126	142	151	137	228	384	384	165	188
647	Site 3	*F vesiculosus* var. *spiralis* probably	126	126	142	142	137	228	384	384	165	188
648	Site 3	*F. vesiculosus* var. *spiralis* probably	126	144	142	149	137	228	386	386	165	188
649	Site 3	*F vesiculosus* var. *spiralis* probably	126	126	144	151	137	228	384	384	165	188
650	Site 3	*F. vesiculosus* var. *spiralis* probably	?	?	142	151	?	?	?	?	?	?
651	Site 3	*F vesiculosus* var. *spiralis* probably	126	144	142	151	137	228	386	386	165	188
652	Site 3	*F. vesiculosus* var. *spiralis* probably	126	144	142	151	137	228	384	384	165	188
653	Site 3	*F vesiculosus* var. *spiralis* probably	126	144	142	151	137	228	384	384	165	188
654	Site 3	*F. vesiculosus* var. *spiralis* probably	126	132	146	151	137	228	384	384	165	188
655	Site 3	*F vesiculosus* var. *spiralis* probably	126	142	142	151	137	228	384	384	165	188
656	Site 3	*F. vesiculosus* var. *spiralis* probably	126	126	142	151	137	228	384	384	165	188
657	Site 3	*F vesiculosus* var. *spiralis* probably	126	144	146	146	137	228	384	384	165	188
658	Site 3	*A. nodosum* ecad *scorpiodes*	142	142	?	?	?	?	?	?	153	153

Gulf of Maine Table continued.

Number	Site	Morphological ID	F227		F90		F300		F26II		L94	
659	Site 3	*A. nodosum* ecad *scorpiodes*	?	?	?	?	?	?	?	?	153	153
660	Site 3	*A. nodosum* ecad *scorpiodes*	126	?	?	?	?	?	?	?	153	153
661	Site 3	Strange *lutarius*/muscoides	126	144	142	151	137	228	378	384	165	188
662	Site 3	Strange *lutarius*/muscoides	126	126	142	146	137	228	384	384	165	188
663	Site 3	Strange *lutarius*/muscoides	128	140	142	151	137	228	384	384	165	188
664	Site 10	ecad *volubilis* or spiraled *lutarius*	126	126	151	151	228	228	369	386	165	188
665	Site 10	ecad *volubilis* or spiraled *lutarius*	126	126	151	151	228	228	369	386	165	188
666	Site 10	ecad *volubilis* or spiraled *lutarius*	126	126	151	151	228	228	369	386	165	188
667	Site 10	ecad *volubilis* or spiraled *lutarius*	126	126	155	155	226	226	380	380	168	188
668	Site 10	ecad *volubilis* or spiraled *lutarius*	126	126	151	151	137	228	369	386	188	188
669	Site 10	ecad *volubilis* or spiraled *lutarius*	126	126	151	151	228	228	365	365	188	188
670	Site 10	ecad *volubilis* or spiraled *lutarius*	126	126	151	151	228	228	386	386	165	188
671	Site 10	ecad *volubilis* or spiraled *lutarius*	126	126	151	151	228	228	369	384	165	188
672	Site 10	ecad *volubilis* or spiraled *lutarius*	126	126	151	155	228	228	380	380	168	188
673	Site 10	ecad *volubilis* or spiraled *lutarius*	126	126	151	151	228	228	369	386	165	188
674	Site 10	ecad *volubilis* or spiraled *lutarius*	126	126	151	151	135	228	369	386	165	188
675	Site 10	ecad *volubilis* or spiraled *lutarius*	126	126	151	151	135	228	369	378	165	188
679	Site 10	*F. spiralis*	144	144	142	142	137	137	384	384	165	165
680	Site 10	*F. spiralis*	126	126	146	146	228	228	365	391	174	188
681	Site 10	*F. spiralis*	144	144	142	142	137	137	384	384	165	165
682	Site 10	*F. spiralis*	126	144	146	151	135	228	384	384	168	188
683	Site 10	*F. spiralis*	126	142	142	142	?	?	369	369	165	188
684	Site 10	*F. spiralis*	144	126	142	142	137	137	382	382	165	165
685	Site 10	*F. spiralis*	126	126	142	142	228	228	380	380	174	188
686	Site 10	*F. spiralis*	126	126	142	151	228	228	380	394	174	188
687	Site 10	*F. spiralis*	126	126	142	146	137	228	369	369	188	188
688	Site 10	*F. spiralis*	126	126	146	146	228	228	380	380	165	174

Gulf of Maine Table continued.

Number	Site	Morphological ID	F227		F90		F300		F26II		L94	
689	Site 10	*F. spiralis*	144	144	142	142	137	137	384	384	165	165
690	Site 10	*F. spiralis*	126	126	142	151	228	228	369	391	188	188
691	Site 10	*F. spiralis* ecad *lutarius* (large)	126	126	142	146	137	224	380	389	165	188
692	Site 10	*F. spiralis* ecad *lutarius* (large)	126	144	142	146	137	224	380	389	165	188
693	Site 10	*F. spiralis* ecad *lutarius* (large)	126	144	142	146	137	224	380	389	165	188
694	Site 10	*F. spiralis* ecad *lutarius* (large)	126	144	142	146	137	224	380	389	165	188
695	Site 10	*F. spiralis* ecad *lutarius* (large)	126	144	142	146	137	224	380	389	165	188
696	Site 10	*F. spiralis* ecad *lutarius* (large)	126	144	142	142	137	224	380	389	165	188
697	Site 10	*F. spiralis* ecad *lutarius* (large)	126	144	142	146	137	224	380	389	165	188
698	Site 10	*F. spiralis* ecad *lutarius* (large)	126	144	142	146	137	224	380	389	165	188
699	Site 10	*F. spiralis* ecad *lutarius* (large)	126	144	138	146	137	224	380	389	165	188
700	Site 10	*F. spiralis* ecad *lutarius* (large)	126	144	142	146	137	224	380	389	165	188
701	Site 10	*F. spiralis* ecad *lutarius* (large)	126	144	142	146	137	224	380	389	165	188
702	Site 10	*F. spiralis* ecad *lutarius* (large)	126	144	142	146	137	224	380	389	165	188
703	Site 10	*F. vesiculosus* var. *spiralis*	126	126	151	151	137	228	380	380	168	188
704	Site 10	*F. vesiculosus* var. *spiralis*	126	126	151	151	226	226	365	389	188	188
705	Site 10	*F. vesiculosus* var. *spiralis*	126	126	151	151	228	228	380	380	168	188
706	Site 10	*F. vesiculosus* var. *spiralis*	126	126	151	155	228	228	380	380	168	188
707	Site 10	*F. vesiculosus* var. *spiralis*	126	150	142	151	137	228	386	386	165	188
708	Site 10	*F. vesiculosus* var. *spiralis*	126	126	151	151	228	228	365	386	188	188
709	Site 10	*F. vesiculosus* var. *spiralis*	126	150	142	151	137	228	386	386	165	188
710	Site 10	*F. spiralis*	126	126	142	151	228	228	359	363	188	188
711	Site 10	*F. spiralis*	126	126	146	151	135	228	382	382	165	188
712	Site 10	*F. spiralis*	144	144	142	142	139	139	384	384	165	165
713	Site 10	*F. spiralis*	126	140	142	142	226	226	389	389	188	188
714	Site 10	*F. spiralis*	126	126	146	146	228	228	365	394	188	188
715	Site 10	*F. spiralis*	144	144	142	142	137	137	384	384	165	165

Gulf of Maine Table continued.

Number	Site	Morphological ID	F227		F90		F300		F26II		L94	
716	Site 10	*F. spiralis*	126	144	142	146	139	230	369	384	165	188
717	Site 10	*F. spiralis*	126	144	142	146	137	230	384	384	165	188
718	Site 10	*F. spiralis*	126	144	142	146	137	228	380	384	165	188
719	Site 10	*F. spiralis*	126	126	142	151	228	228	382	382	165	188
720	Site 10	*F. spiralis*	126	144	142	151	137	230	384	384	165	188
721	Site 10	*F. spiralis*	144	144	142	142	135	135	384	384	165	165
722	Site 10	*F. spiralis* ecad *lutarius*	126	126	142	146	137	228	384	384	165	174
723	Site 10	*F. spiralis* ecad *lutarius*	144	144	142	146	137	228	384	384	165	174
724	Site 10	*F. spiralis* ecad *lutarius*	144	144	142	146	137	226	386	386	165	174
725	Site 10	*F. spiralis* ecad *lutarius*	126	144	142	146	137	228	384	384	165	174
726	Site 10	*F. spiralis* ecad *lutarius*	126	144	142	146	137	228	386	386	165	174
727	Site 10	*F. spiralis* ecad *lutarius*	144	144	142	146	137	228	384	384	165	174
728	Site 10	*F. spiralis* ecad *lutarius*	144	144	142	146	137	226	384	384	165	174
729	Site 10	*F. spiralis* ecad *lutarius*	144	144	142	146	137	228	386	386	165	174
730	Site 10	*F. spiralis* ecad *lutarius*	144	144	142	146	137	226	384	384	165	174
731	Site 10	*F. spiralis* ecad *lutarius*	144	144	142	146	137	228	384	384	165	174
732	Site 10	*F. spiralis* ecad *lutarius*	144	144	142	146	137	228	384	384	165	174
733	Site 10	*F. spiralis* ecad *lutarius*	144	144	142	146	137	228	384	384	165	174
734	Site 8	*F. vesiculosus*	126	126	146	146	228	228	363	369	174	188
735	Site 8	*F. vesiculosus*	126	126	146	151	228	228	365	391	188	188
736	Site 8	*F. vesiculosus*	126	126	146	146	228	232	369	380	174	188
737	Site 8	*F. vesiculosus*	126	126	146	151	228	228	369	380	188	188
738	Site 8	*F. vesiculosus*	126	126	146	153	228	228	369	369	188	188
739	Site 8	*F. vesiculosus*	126	126	142	151	137	228	365	380	188	188
740	Site 14	*F. spiralis* germling	126	126	146	151	228	228	380	380	188	188
741	Site 14	*F. spiralis* germling	126	126	142	151	228	228	380	386	188	188
742	Site 14	*F. spiralis* germling	126	126	142	151	228	228	369	380	188	188

Gulf of Maine Table continued.

Number	Site	Morphological ID	F227		F90		F300		F26II		L94	
743	Site 14	*F. spiralis* germling	126	126	146	155	228	228	380	380	168	188
744	Site 14	*F. spiralis* germling	126	126	146	155	230	230	380	380	165	168
745	Site 14	*F. spiralis* germling	126	126	151	151	228	228	369	391	188	188
746	Site 14	*F. spiralis* germling	126	126	146	155	228	228	367	380	188	188
747	Site 14	*F. spiralis* germling	126	126	142	151	226	226	380	394	174	188
748	Site 14	*F. spiralis* germling	126	126	146	146	228	228	369	380	174	188
749	Site 14	*F. spiralis* germling	126	126	142	153	226	226	380	389	168	188
750	Site 14	*F. vesiculosus*	126	126	142	151	228	228	369	380	188	188
751	Site 14	*F. vesiculosus*	126	126	146	151	228	234	369	380	188	188
752	Site 14	*F. vesiculosus*	126	126	148	153	228	228	365	380	188	188
753	Site 14	*F. vesiculosus*	126	126	142	142	230	230	369	369	188	188
754	Site 14	*F. vesiculosus*	126	126	151	151	210	228	380	380	168	188
755	Site 14	*F. vesiculosus*	126	126	142	142	228	228	380	380	165	188
756	Site 14	*F. vesiculosus*	126	126	151	151	228	228	369	376	188	188
757	Site 14	*F. vesiculosus*	126	138	146	151	228	228	369	380	188	188
758	Site 14	*F. vesiculosus*	126	126	151	151	228	228	365	380	168	188
759	Site 14	*F. vesiculosus*	126	126	142	151	228	228	380	380	174	188
760	Site 14	*F. vesiculosus*	126	126	142	151	228	228	363	369	165	188
761	Site 14	*F. vesiculosus*	126	126	146	151	228	228	367	380	165	188
762	Site 14	*F. vesiculosus*	126	126	151	151	228	234	365	380	188	188
763	Site 14	*F. vesiculosus*	142	142	146	151	228	228	380	380	188	188
764	Site 14	*F. vesiculosus*	126	126	151	151	228	228	380	394	188	188
765	Site 14	*F. vesiculosus* forma *gracillumus*	126	126	142	146	228	228	380	380	165	188
766	Site 14	*F. vesiculosus* forma *gracillumus*	126	126	146	146	137	228	380	391	188	188
767	Site 14	*F. vesiculosus* forma *gracillumus*	126	126	146	146	228	228	380	391	188	188
768	Site 14	*F. vesiculosus* forma *gracillumus*	126	126	146	146	131	228	380	391	188	188
769	Site 14	*F. vesiculosus* forma *gracillumus*	126	126	146	146	133	228	380	391	188	188

Gulf of Maine Table continued.

Number	Site	Morphological ID	F227		F90		F300		F26II		L94	
770	Site 14	*F. vesiculosus* forma *gracillumus*	126	126	146	146	127	228	380	391	188	188
771	Site 14	*F. vesiculosus* forma *gracillumus*	126	138	142	151	131	230	?	?	?	?
772	Site 14	*F. vesiculosus* forma *gracillumus*	126	140	146	151	228	228	380	380	165	188
773	Site 14	*F. vesiculosus* forma *gracillumus*	126	126	142	151	125	228	380	380	165	168
774	Site 14	*F. vesiculosus* forma *gracillumus*	126	126	146	153	228	228	380	380	188	188
775	Site 14	*F. vesiculosus* forma *gracillumus*	126	126	146	146	125	228	380	391	188	188
776	Site 14	*F. vesiculosus* forma *gracillumus*	126	126	151	151	133	228	380	391	165	188
777	Site 14	*F. vesiculosus* forma *gracillumus*	126	126	146	146	137	228	380	391	188	188
778	Site 14	*F. vesiculosus* forma *gracillumus*	126	126	146	146	135	228	380	391	188	188
779	Site 14	*F. vesiculosus* forma *gracillumus*	126	136	146	146	228	228	380	391	188	188
780	Site 8	*F. vesiculosus*	126	126	146	151	228	228	386	386	174	188
781	Site 8	*F. vesiculosus*	126	126	153	153	135	228	363	363	174	188
782	Site 8	*F. vesiculosus*	126	150	151	151	226	226	369	386	174	188
783	Site 8	*F. vesiculosus*	126	136	?	?	228	228	?	?	?	?
784	Site 8	*F. vesiculosus*	126	126	142	146	228	228	369	369	174	188
785	Site 8	Attached *Fucus*	126	126	142	151	228	228	391	391	188	188
786	Site 8	Attached *Fucus*	126	126	151	151	160	228	386	386	168	188
787	Site 8	Attached *Fucus*	126	126	151	151	123	228	380	380	168	174
788	Site 8	Attached *Fucus*	126	126	146	146	228	228	363	389	188	188
789	Site 8	Attached *Fucus*	126	150	146	151	228	228	363	369	188	188
790	Site 8	Small ecad *volubilis* or *lutarius*	126	150	142	153	118	137	380	384	165	188
791	Site 8	Small ecad *volubilis* or *lutarius*	126	144	142	159	137	228	369	384	165	168
792	Site 8	Small ecad *volubilis* or *lutarius*	126	144	142	159	228	228	369	384	165	168
793	Site 8	Small ecad *volubilis* or *lutarius*	126	126	142	151	228	228	380	386	165	188
794	Site 8	Small ecad *volubilis* or *lutarius*	126	150	142	153	137	137	380	384	165	188
795	Site 8	muscoides-like *Fucus*	126	126	?	?	?	?	?	?	?	?
796	Site 8	muscoides-like *Fucus*	126	144	142	151	137	230	365	384	165	188

Gulf of Maine Table continued.

Number	Site	Morphological ID	F227		F90		F300		F26II		L94	
797	Site 8	muscoides-like *Fucus*	126	144	142	151	137	230	365	384	165	188
798	Site 8	muscoides-like *Fucus*	126	144	142	151	137	230	365	384	165	188
799	Site 8	muscoides-like *Fucus*	126	144	142	151	137	230	365	384	165	188
800	Site 8	muscoides-like *Fucus*	126	144	142	151	137	224	380	384	165	188
801	Site 8	muscoides-like *Fucus*	126	144	142	151	137	228	365	384	165	188
802	Site 8	muscoides-like *Fucus*	126	144	142	151	137	228	365	384	165	188
803	Site 8	muscoides-like *Fucus*	126	144	142	151	135	228	365	384	165	188
804	Site 8	muscoides-like *Fucus*	126	144	142	151	137	230	365	384	165	188
805	Site 8	muscoides-like *Fucus*	126	144	142	151	137	230	365	384	165	188
806	Site 8	muscoides-like *Fucus*	126	144	142	151	137	228	365	384	165	188
807	Site 8	muscoides-like *Fucus*	126	144	142	142	131	228	365	384	165	165
808	Site 8	muscoides-like *Fucus*	126	144	142	151	137	230	365	384	165	188
809	Site 8	muscoides-like *Fucus*	126	144	142	151	137	230	365	384	165	188
810	Site 13	*F. spiralis*	144	144	142	142	135	226	382	382	165	165
811	Site 13	*F. spiralis*	144	144	142	142	137	236	382	382	165	165
812	Site 13	*F. spiralis*	144	144	142	142	137	137	382	382	165	165
813	Site 13	*F. spiralis*	144	144	142	142	137	137	382	382	165	165
814	Site 13	*F. spiralis*	126	144	142	148	135	226	382	382	165	165
815	Site 13	*F. spiralis*	144	144	142	142	137	137	382	382	165	165
816	Site 13	*F. spiralis*	144	144	142	142	137	226	382	382	165	165
817	Site 13	*F. spiralis*	144	144	142	142	137	137	382	382	165	165
818	Site 13	*F. spiralis*	144	144	142	142	137	226	382	382	165	165
819	Site 13	*F. spiralis*	144	144	142	142	137	230	382	382	165	165
820	Site 13	*F. spiralis*	144	144	142	142	137	226	382	382	165	165
821	Site 13	*F. spiralis*	126	144	142	146	135	226	382	382	165	165
822	Site 13	*F. spiralis*	126	144	142	142	137	220	367	367	165	165
823	Site 13	*F. spiralis*	144	144	142	142	137	137	382	382	165	165

Gulf of Maine Table continued.

Number	Site	Morphological ID	F227		F90		F300		F26II		L94	
824	Site 13	*F. spiralis*	144	144	142	142	139	230	384	384	165	165
825	Site 13	*F. vesiculosus* ecad *volubilis*	126	126	142	151	228	228	367	367	188	188
826	Site 13	*F. vesiculosus* ecad *volubilis*	126	126	146	159	228	228	363	363	168	188
827	Site 13	*F. vesiculosus* ecad *volubilis*	126	126	144	151	228	228	363	367	188	188
828	Site 13	*F. vesiculosus* ecad *volubilis*	126	126	151	151	228	228	363	363	188	188
829	Site 13	*F. vesiculosus* ecad *volubilis*	126	126	142	151	228	228	363	367	188	188
830	Site 13	*F. vesiculosus* ecad *volubilis*	126	126	146	146	137	228	363	369	168	188
831	Site 13	*F. vesiculosus* ecad *volubilis*	126	126	151	151	228	228	363	363	188	188
832	Site 13	*F. vesiculosus* ecad *volubilis*	126	126	151	151	228	228	?	?	188	188
833	Site 13	*F. vesiculosus* ecad *volubilis*	126	126	151	151	228	228	363	363	188	188
834	Site 13	*F. vesiculosus* ecad *volubilis*	126	126	144	144	228	228	363	367	188	188
835	Site 13	*F. vesiculosus* ecad *volubilis*	126	126	142	151	228	228	363	367	188	188
836	Site 13	*F. vesiculosus* ecad *volubilis*	126	126	151	151	228	228	363	363	188	188
837	Site 13	*F. vesiculosus* ecad *volubilis*	126	126	146	159	228	228	363	369	168	188
838	Site 13	*F. vesiculosus* ecad *volubilis*	126	126	151	151	228	228	363	367	188	188
839	Site 13	*F. vesiculosus* ecad *volubilis*	126	126	151	151	228	228	363	367	188	188
840	Site 7	*F. vesiculosus*	126	150	142	151	228	228	369	369	188	188
841	Site 7	*F. vesiculosus*	126	150	142	151	228	228	369	369	188	188
842	Site 7	*F. vesiculosus*	126	126	146	153	228	228	369	380	168	168
843	Site 7	*F. vesiculosus*	126	126	146	153	228	228	369	382	168	168
844	Site 7	*F. vesiculosus*	126	126	142	151	228	228	369	369	188	188
845	Site 7	*F. vesiculosus*	126	126	146	151	228	228	363	363	168	188
846	Site 7	*F. vesiculosus*	126	126	146	151	228	228	380	389	168	174
847	Site 7	*F. vesiculosus*	126	144	142	146	137	228	369	389	168	168
848	Site 7	*F. vesiculosus*	126	150	142	151	228	228	369	369	188	188
849	Site 7	*F. vesiculosus*	126	126	146	153	228	228	369	382	168	168
850	Site 7	*F. vesiculosus*	126	126	146	153	228	228	369	382	168	168

Gulf of Maine Table continued.

Number	Site	Morphological ID	F227		F90		F300		F26II		L94	
851	Site 7	*F. vesiculosus*	126	144	142	146	135	228	369	389	168	168
852	Site 7	*F. vesiculosus*	150	150	146	151	228	228	369	389	174	188
853	Site 7	*F. vesiculosus*	126	126	146	153	228	228	369	380	168	168
854	Site 7	*F. vesiculosus*	126	126	146	153	228	228	369	380	168	168
855	Site 7	*F. vesiculosus* var. *spiralis*	126	150	142	146	228	228	365	369	168	168
856	Site 7	*F. vesiculosus* var. *spiralis*	126	150	142	146	228	228	365	369	168	168
857	Site 7	*F. vesiculosus* var. *spiralis*	126	150	142	146	228	228	365	369	168	168
858	Site 7	*F. vesiculosus* var. *spiralis*	126	146	142	146	228	228	365	369	168	168
859	Site 7	*F. vesiculosus* var. *spiralis*	126	144	151	151	139	228	365	369	168	168
860	Site 7	*F. vesiculosus* var. *spiralis*	126	144	151	151	139	228	365	369	168	168
861	Site 7	*F. vesiculosus* var. *spiralis*	126	150	142	146	230	230	365	369	168	168
862	Site 7	*F. vesiculosus* var. *spiralis*	126	150	142	146	228	228	365	369	168	168
863	Site 7	*F. vesiculosus* var. *spiralis*	126	146	153	153	137	228	365	369	168	168
864	Site 7	*F. vesiculosus* var. *spiralis*	126	150	146	146	228	228	365	369	168	168
865	Site 7	*F. vesiculosus* var. *spiralis*	126	144	151	151	139	228	365	369	168	168
866	Site 7	*F. vesiculosus* var. *spiralis*	126	144	151	151	137	228	365	369	168	168
867	Site 7	*F. vesiculosus* var. *spiralis*	126	144	151	151	137	228	365	369	168	168
868	Site 7	*F. vesiculosus* ecad *volubilis*	126	126	151	155	228	228	380	380	168	188
869	Site 7	*F. vesiculosus* ecad *volubilis*	?	?	144	151	?	?	?	?	?	?
870	Site 7	*F. vesiculosus* ecad *volubilis*	126	126	146	153	246	246	369	369	165	168
871	Site 7	*F. vesiculosus* ecad *volubilis*	126	126	155	155	246	246	382	382	168	188
872	Site 7	*F. vesiculosus* ecad *volubilis*	126	126	151	155	246	246	380	380	168	188
873	Site 7	*F. vesiculosus* ecad *volubilis*	126	126	151	155	246	246	380	380	168	188
874	Site 7	*F. vesiculosus* ecad *volubilis*	126	126	146	153	246	246	369	369	165	168
875	Site 7	*F. vesiculosus* ecad *volubilis*	126	126	155	155	246	246	380	380	168	188
876	Site 7	*F. vesiculosus* ecad *volubilis*	126	126	151	155	246	246	380	380	168	188
877	Site 7	*F. vesiculosus* ecad *volubilis*	126	126	146	153	?	?	369	369	165	168

Gulf of Maine Table continued.

Number	Site	Morphological ID	F227		F90		F300		F26II		L94	
878	Site 7	*F. vesiculosus* ecad *volubilis*	126	126	146	153	228	228	369	369	165	168
879	Site 7	*F. vesiculosus* ecad *volubilis*	126	126	151	155	246	246	380	380	168	188
880	Site 7	*F. vesiculosus* ecad *volubilis*	126	144	151	151	246	246	380	380	188	188
881	Site 7	*F. vesiculosus* ecad *volubilis*	126	126	146	153	246	246	369	369	165	168
882	Site 9	*F. vesiculosus* ecad *volubilis*	126	126	142	151	246	246	380	380	168	188
883	Site 9	*F. vesiculosus* ecad *volubilis*	126	126	151	155	246	246	380	380	168	188
884	Site 9	*F. vesiculosus* ecad *volubilis*	126	126	151	151	246	246	371	394	188	188
885	Site 9	*F. vesiculosus* ecad *volubilis*	126	126	151	155	246	246	380	380	168	188
886	Site 9	*F. vesiculosus* ecad *volubilis*	126	144	142	155	137	246	380	386	165	168
887	Site 9	*F. vesiculosus* ecad *volubilis*	126	126	151	155	246	246	380	380	168	188
888	Site 9	*F. vesiculosus* ecad *volubilis*	126	144	142	155	137	246	380	386	165	168
889	Site 9	*F. vesiculosus* ecad *volubilis*	126	126	151	151	246	246	371	394	188	188
890	Site 9	*F. vesiculosus* ecad *volubilis*	?	?	?	?	246	246	?	?	?	?
891	Site 9	*F. vesiculosus* ecad *volubilis*	126	126	151	155	246	246	380	380	168	188
892	Site 9	*F. vesiculosus* ecad *volubilis*	126	126	151	155	135	135	380	380	168	168
893	Site 9	*F. vesiculosus* ecad *volubilis*	126	126	142	151	228	228	369	384	165	168
894	Site 9	*F. vesiculosus* ecad *volubilis*	126	144	142	159	135	246	361	384	165	188
895	Site 9	*F. vesiculosus* ecad *volubilis*	144	144	?	?	?	?	?	?	188	188
896	Site 9	*F. vesiculosus* ecad *volubilis*	126	126	155	155	246	246	380	380	168	188
897	Site 9	*F. vesiculosus* ecad *volubilis*	126	126	151	151	246	246	380	380	168	168
898	Site 9	*F. vesiculosus* ecad *volubilis*	126	126	142	159	137	246	365	386	165	168
899	Site 9	*F. vesiculosus* ecad *volubilis*	126	126	151	151	246	246	371	394	188	188
900	Site 9	*F. vesiculosus* ecad *volubilis*	126	126	151	151	246	246	380	380	168	188
901	Site 9	*F. vesiculosus* ecad *volubilis*	126	126	151	155	246	246	380	380	168	188
902	Site 9	*F. spiralis*	126	144	142	148	137	137	386	386	165	165
903	Site 9	*F. spiralis*	144	144	142	142	137	137	384	384	165	165
904	Site 9	*F. spiralis*	144	144	142	151	137	137	384	384	165	165

Gulf of Maine Table continued.

Number	Site	Morphological ID	F227		F90		F300		F26II		L94	
905	Site 9	*F. spiralis*	144	144	140	140	135	135	384	384	165	165
906	Site 9	*F. spiralis*	144	144	142	142	137	137	384	384	165	165
907	Site 9	*F. spiralis*	144	144	142	142	137	226	384	384	165	165
908	Site 9	*F. spiralis*	144	144	142	148	137	137	384	384	165	165
909	Site 9	*F. spiralis*	126	144	142	142	137	137	?	?	165	165
910	Site 9	*F. spiralis*	126	144	142	142	137	137	?	?	165	165
911	Site 9	*F. spiralis*	126	144	142	142	137	137	386	386	165	165
912	Site 9	*F. spiralis*	144	144	142	142	137	137	384	384	165	165
913	Site 9	*F. spiralis*	126	144	142	142	137	137	?	?	165	165
914	Site 9	*F. spiralis*	144	144	140	140	137	137	386	386	165	165
915	Site 9	*F. spiralis*	144	144	142	142	137	137	386	386	165	165
916	Site 9	*F. spiralis*	144	144	142	142	137	137	384	384	165	165
917	Site 9	*F. spiralis*	144	144	142	142	137	137	386	386	165	165
918	Site 9	*F. spiralis*	144	144	142	151	137	137	?	?	165	165
919	Site 9	*F. spiralis*	144	144	142	151	137	137	384	384	165	165
920	Site 9	*F. spiralis*	144	144	142	142	137	137	384	384	165	165
921	Site 9	*F. spiralis*	144	144	142	142	137	137	386	386	165	165
922	Site 9	muscoides-like *Fucus*	126	144	142	146	137	226	369	384	165	188
923	Site 9	muscoides-like *Fucus*	126	144	142	151	137	137	340	380	165	188
924	Site 9	muscoides-like *Fucus*	126	144	142	151	137	137	340	380	165	188
925	Site 9	muscoides-like *Fucus*	126	144	142	151	137	137	380	384	165	188
926	Site 9	muscoides-like *Fucus*	126	144	142	151	137	137	380	382	165	188
927	Site 9	muscoides-like *Fucus*	126	144	142	151	137	137	380	384	165	188
928	Site 9	muscoides-like *Fucus*	126	144	142	151	137	137	340	380	165	188
929	Site 9	muscoides-like *Fucus*	126	144	142	146	137	137	369	384	165	188
930	Site 9	muscoides-like *Fucus*	126	144	142	151	137	137	340	380	165	188
931	Site 9	muscoides-like *Fucus*	126	144	142	151	137	137	380	384	165	188

Gulf of Maine Table continued.

Number	Site	Morphological ID	F227		F90		F300		F26II		L94	
932	Site 9	muscoides-like *Fucus*	126	144	142	151	137	137	340	380	165	188
933	Site 9	muscoides-like *Fucus*	126	144	142	151	137	137	380	384	165	188
934	Site 9	muscoides-like *Fucus*	126	144	142	151	137	137	380	384	165	188
935	Site 9	muscoides-like *Fucus*	126	144	142	146	137	137	384	384	165	188
936	Site 9	muscoides-like *Fucus*	126	144	142	151	137	137	340	380	165	188
937	Site 9	muscoides-like *Fucus*	126	144	142	146	137	137	369	384	165	188
938	Site 9	muscoides-like *Fucus*	126	144	142	151	137	137	340	380	165	188
939	Site 9	muscoides-like *Fucus*	126	144	142	146	137	137	369	384	165	188
940	Site 9	muscoides-like *Fucus*	126	144	142	151	137	137	340	380	165	188
941	Site 9	muscoides-like *Fucus*	126	144	146	146	137	224	369	384	165	188
942	Site 9	*F. vesiculosus* ecad *volubilis*	126	126	151	155	133	228	380	380	165	188
943	Site 9	*F. vesiculosus* ecad *volubilis*	126	126	151	151	228	228	371	371	188	188
944	Site 9	*F. vesiculosus* ecad *volubilis*	126	126	151	151	228	228	371	394	188	188
945	Site 9	*F. vesiculosus* ecad *volubilis*	126	144	142	159	137	228	361	384	165	188
946	Site 9	*F. vesiculosus* ecad *volubilis*	126	126	151	155	228	228	380	380	168	188
947	Site 9	*F. vesiculosus* ecad *volubilis*	126	144	142	159	137	228	361	384	165	188
948	Site 9	*F. vesiculosus* ecad *volubilis*	126	144	142	151	137	228	361	384	165	188
949	Site 9	*F. vesiculosus* ecad *volubilis*	126	126	151	155	133	228	380	380	168	188
950	Site 9	*F. vesiculosus* ecad *volubilis*	126	144	142	159	137	228	361	384	165	188
951	Site 9	*F. vesiculosus* ecad *volubilis*	126	144	142	159	137	228	361	384	165	188
952	Site 9	*F. vesiculosus* ecad *volubilis*	126	126	151	155	228	228	380	380	168	188
953	Site 9	*F. vesiculosus* ecad *volubilis*	126	144	142	159	137	228	361	384	165	188
954	Site 9	*F. vesiculosus* ecad *volubilis*	126	126	151	155	228	228	380	380	168	188
955	Site 9	*F. vesiculosus* ecad *volubilis*	126	126	151	155	137	228	380	380	168	188
956	Site 9	*F. vesiculosus* ecad *volubilis*	126	126	151	159	228	228	361	365	168	188
957	Site 9	*F. vesiculosus* ecad *volubilis*	126	126	151	155	228	228	380	380	168	188
958	Site 9	*F. vesiculosus* ecad *volubilis*	126	144	142	159	137	228	361	384	165	188

Gulf of Maine Table continued.

Number	Site	Morphological ID	F227		F90		F300		F26II		L94	
959	Site 9	*F. vesiculosus* ecad *volubilis*	126	126	151	155	228	228	380	380	168	188
960	Site 9	*F. vesiculosus* ecad *volubilis*	126	126	151	151	133	228	371	394	188	188
961	Site 9	*F. vesiculosus* ecad *volubilis*	126	126	151	151	228	228	371	394	188	188
962	Site 9	*F. spiralis*	144	144	142	142	137	137	384	384	165	165
963	Site 9	*F. spiralis*	126	126	142	142	?	?	384	384	165	165
964	Site 9	*F. spiralis*	126	144	142	142	137	137	384	384	165	165
965	Site 9	*F. spiralis*	144	144	142	142	142	230	384	384	165	165
966	Site 9	*F. spiralis*	126	144	142	142	142	142	384	384	165	165
967	Site 9	*F. spiralis*	144	144	142	151	142	142	384	384	165	165
968	Site 9	*F. spiralis*	126	144	142	142	?	?	384	384	165	165
969	Site 9	*F. spiralis*	126	144	142	142	?	?	384	384	165	165
970	Site 9	*F. spiralis*	126	144	142	151	?	?	384	384	165	165
971	Site 9	*F. spiralis*	?	?	?	?	?	?	?	?	?	?
972	Site 9	*F. spiralis*	144	144	142	142	?	?	384	384	165	165
973	Site 9	*F. spiralis*	126	144	142	142	?	?	384	384	165	165
974	Site 9	*F. spiralis*	144	144	142	142	?	?	384	384	165	165
975	Site 9	*F. spiralis*	144	144	142	142	?	?	384	384	165	165
976	Site 9	*F. spiralis*	144	144	142	142	142	142	384	384	165	165
977	Site 9	*F. spiralis*	144	144	142	142	137	137	386	386	165	165
978	Site 9	*F. spiralis*	144	144	142	142	137	226	384	384	165	165
979	Site 9	*F. spiralis*	126	144	142	142	137	137	384	384	165	165
980	Site 9	*F. spiralis*	144	144	142	142	142	142	384	384	165	165
982	Site 9	muscoides-like *Fucus*	125	144	142	151	137	137	340	380	165	188
983	Site 9	muscoides-like *Fucus*	144	144	142	146	139	228	369	386	165	188
984	Site 9	muscoides-like *Fucus*	126	144	142	151	137	137	340	380	165	188
985	Site 9	muscoides-like *Fucus*	126	144	142	151	137	137	340	380	165	188
986	Site 9	muscoides-like *Fucus*	126	144	142	142	137	137	369	384	165	188

Gulf of Maine Table continued.

Number	Site	Morphological ID	F227		F90		F300		F26II		L94	
987	Site 9	muscoides-like *Fucus*	126	144	142	146	137	137	369	384	165	188
988	Site 9	muscoides-like *Fucus*	126	144	142	151	139	228	340	380	165	188
989	Site 9	muscoides-like *Fucus*	126	144	142	146	137	137	369	384	165	188
990	Site 9	muscoides-like *Fucus*	126	144	142	151	137	137	340	380	165	188
991	Site 9	muscoides-like *Fucus*	126	144	142	146	137	137	369	384	165	188
992	Site 9	muscoides-like *Fucus*	126	144	142	151	137	224	340	380	165	188
993	Site 9	muscoides-like *Fucus*	126	144	142	151	137	137	340	380	165	188
994	Site 9	muscoides-like *Fucus*	126	144	142	151	139	139	340	380	165	188
995	Site 9	muscoides-like *Fucus*	126	144	142	142	137	137	369	384	165	188
996	Site 9	muscoides-like *Fucus*	126	144	146	146	137	137	369	384	165	188
997	Site 9	muscoides-like *Fucus*	126	144	142	151	137	137	340	380	165	188
998	Site 9	muscoides-like *Fucus*	126	144	142	151	137	137	340	380	165	188
999	Site 9	muscoides-like *Fucus*	126	144	142	151	137	137	340	380	165	188
1000	Site 9	muscoides-like *Fucus*	126	144	142	151	139	139	369	384	165	188
1001	Site 9	muscoides-like *Fucus*	126	144	142	146	137	137	340	380	165	188
1002	Site 11	*F. spiralis*	126	126	151	151	228	228	365	391	188	188
1003	Site 11	*F. spiralis* or *F. vesiculosus*	126	126	142	153	228	228	361	365	188	188
1004	Site 11	*F. spiralis* or *F. vesiculosus*	126	126	148	151	226	226	363	363	188	188
1005	Site 11	*F. spiralis* or *F. vesiculosus*	144	144	142	142	139	139	384	384	165	165
1006	Site 11	*F. spiralis* or *F. vesiculosus*	126	126	146	151	228	228	361	397	188	188
1007	Site 11	*F. spiralis* or *F. vesiculosus*	126	126	151	151	228	228	365	391	188	188
1008	Site 11	*F. spiralis* or *F. vesiculosus*	126	126	148	151	228	228	365	365	188	188
1009	Site 11	*F. spiralis* or *F. vesiculosus*	126	126	148	148	228	228	361	361	188	188
1010	Site 11	*F. spiralis* or *F. vesiculosus*	124	126	148	157	228	228	361	397	165	188
1011	Site 11	*F. spiralis* or *F. vesiculosus*	126	126	148	151	228	228	361	380	188	188
1012	Site 11	*F. spiralis* or *F. vesiculosus*	126	126	148	148	228	228	378	397	188	188
1013	Site 11	*F. spiralis* or *F. vesiculosus*	126	126	146	151	228	228	361	361	188	188

Gulf of Maine Table continued.

Number	Site	Morphological ID	F227		F90		F300		F26II		L94	
1014	Site 11	*F. spiralis* or *F. vesiculosus*	124	124	146	146	228	228	361	361	188	188
1015	Site 11	*F. spiralis* or *F. vesiculosus*	124	124	146	151	228	228	365	365	188	188
1016	Site 11	*F. spiralis* or *F. vesiculosus*	124	126	148	148	228	228	361	369	188	188
1017	Site 11	*F. spiralis* or *F. vesiculosus*	124	126	148	153	228	228	361	380	188	188
1018	Site 11	*F. spiralis* or *F. vesiculosus*	126	126	148	151	228	228	363	363	188	188
1019	Site 11	*F. spiralis* or *F. vesiculosus*	124	126	151	151	228	228	361	361	188	188
1020	Site 11	*F. spiralis* or *F. vesiculosus*	124	126	148	151	228	228	361	361	188	188
1021	Site 11	*F. spiralis* or *F. vesiculosus*	126	144	142	148	228	228	361	367	188	188
1022	Site 11	*F. vesiculosus* ecad *volubilis*?	126	126	151	151	228	228	378	384	188	188
1023	Site 11	*F. vesiculosus* ecad *volubilis*?	126	126	142	153	228	228	365	365	188	188
1024	Site 11	*F. vesiculosus* ecad *volubilis*?	126	126	142	151	228	228	365	365	188	188
1025	Site 11	*F. vesiculosus* ecad *volubilis*?	126	126	142	153	228	228	361	365	188	188
1026	Site 11	*F. vesiculosus* ecad *volubilis*?	124	126	148	148	228	228	378	380	188	188
1027	Site 11	*F. vesiculosus* ecad *volubilis*?	126	126	142	153	139	228	369	380	168	188
1028	Site 11	*F. vesiculosus* ecad *volubilis*?	126	126	142	153	139	228	369	380	168	188
1029	Site 11	*F. vesiculosus* ecad *volubilis*?	126	126	142	153	139	228	369	380	168	188
1030	Site 11	*F. vesiculosus* ecad *volubilis*?	126	126	142	151	228	228	365	365	188	188
1031	Site 11	*F. vesiculosus* ecad *volubilis*?	126	126	142	151	228	228	365	365	188	188
1032	Site 11	*F. vesiculosus* ecad *volubilis*?	126	126	142	151	228	228	365	365	188	188
1033	Site 11	*F. vesiculosus* ecad *volubilis*?	126	126	142	151	228	228	365	365	188	188
1034	Site 11	*F. vesiculosus* ecad *volubilis*?	126	126	142	153	139	228	369	380	168	188
1035	Site 11	*F. vesiculosus* ecad *volubilis*?	126	126	142	153	139	228	369	380	168	188
1036	Site 11	*F. vesiculosus* ecad *volubilis*?	126	126	142	151	228	228	365	365	188	188
1037	Site 11	*F. vesiculosus* ecad *volubilis*?	126	126	142	151	228	228	365	365	188	188
1040	Site 11	*F. vesiculosus* ecad *volubilis*?	126	126	?	?	139	228	369	380	168	188
1041	Site 11	*F. vesiculosus* ecad *volubilis*?	126	126	?	?	228	228	365	365	188	188
1042	Site 12	*F. spiralis* or *F. vesiculosus*	144	144	151	151	228	228	363	363	188	188

Gulf of Maine Table continued.

Number	Site	Morphological ID	F227		F90		F300		F26II		L94	
1043	Site 12	*F. spiralis* or *F. vesiculosus*	126	126	153	153	228	228	369	369	188	188
1044	Site 12	*F. spiralis* or *F. vesiculosus*	124	124	142	151	228	228	365	365	188	188
1045	Site 12	*F. spiralis* or *F. vesiculosus*	124	126	151	155	228	228	369	389	188	188
1046	Site 12	*F. spiralis* or *F. vesiculosus*	124	126	151	151	228	228	363	363	188	188
1047	Site 12	*F. spiralis* or *F. vesiculosus*	124	126	142	151	228	228	380	384	165	188
1048	Site 12	*F. spiralis* or *F. vesiculosus*	124	126	153	153	123	228	359	389	188	188
1049	Site 12	*F. spiralis* or *F. vesiculosus*	126	126	151	151	228	228	369	391	168	188
1050	Site 12	*F. spiralis* or *F. vesiculosus*	126	126	142	142	228	228	363	389	188	188
1051	Site 12	*F. spiralis* or *F. vesiculosus*	124	126	151	151	228	228	357	357	188	188
1052	Site 12	*F. spiralis* or *F. vesiculosus*	126	126	142	142	228	228	369	369	168	188
1053	Site 12	*F. spiralis* or *F. vesiculosus*	126	126	151	151	228	228	363	394	168	188
1054	Site 12	*F. spiralis* or *F. vesiculosus*	126	126	146	151	135	228	386	389	188	188
1055	Site 12	*F. spiralis* or *F. vesiculosus*	126	126	151	151	137	228	394	394	188	188
1056	Site 12	*F. spiralis* or *F. vesiculosus*	124	126	142	151	137	228	357	384	188	188
1057	Site 12	*F. spiralis* or *F. vesiculosus*	126	126	146	146	127	228	355	365	188	188
1058	Site 12	*F. spiralis* or *F. vesiculosus*	124	126	151	155	228	228	367	367	188	188
1059	Site 12	*F. spiralis* or *F. vesiculosus*	126	126	142	155	228	228	378	394	188	188
1060	Site 12	*F. spiralis* or *F. vesiculosus*	126	144	142	155	137	226	394	394	188	188
1061	Site 12	*F. spiralis* or *F. vesiculosus*	126	126	146	151	137	228	367	384	188	188
1062	Site 12	muscoides-like *Fucus*	126	144	142	151	137	230	365	382	165	188
1063	Site 12	muscoides-like *Fucus*	126	144	151	151	137	230	365	384	165	188
1064	Site 12	muscoides-like *Fucus*	126	144	142	151	137	228	363	384	165	188
1065	Site 12	muscoides-like *Fucus*	126	144	142	151	137	228	363	384	165	188
1066	Site 12	muscoides-like *Fucus*	126	144	142	151	137	228	363	384	165	188
1067	Site 12	muscoides-like *Fucus*	126	144	?	?	137	137	?	?	165	165
1068	Site 12	muscoides-like *Fucus*	126	126	?	?	?	?	?	?	?	?
1069	Site 12	muscoides-like *Fucus*	126	144	142	155	137	228	363	382	165	165

Gulf of Maine Table continued.

Number	**Site**	**Morphological ID**	**F227**		**F90**		**F300**		**F26II**		**L94**	
1070	Site 12	muscoides-like *Fucus*	126	144	142	151	137	228	363	384	165	188
1071	Site 12	muscoides-like *Fucus*	126	144	?	?	?	?	?	?	?	?
1072	Site 12	*F. vesiculosus* ecad *volubilis*?	126	126	142	151	228	228	365	365	188	188
1073	Site 12	*F. vesiculosus* ecad *volubilis*?	126	126	142	151	228	228	365	365	188	188
1074	Site 12	*F. vesiculosus* ecad *volubilis*?	124	144	151	151	228	228	363	394	188	188
1075	Site 12	*F. vesiculosus* ecad *volubilis*?	126	126	142	151	228	228	365	365	188	188
1076	Site 12	*F. vesiculosus* ecad *volubilis*?	126	126	142	151	228	228	365	365	188	188
1077	Site 12	*F. vesiculosus* ecad *volubilis*?	126	126	142	151	228	228	365	365	188	188
1078	Site 12	*F. vesiculosus* ecad *volubilis*?	126	126	142	151	228	228	365	365	188	188
1079	Site 12	*F. vesiculosus* ecad *volubilis*?	126	126	142	151	228	228	365	365	188	188
1080	Site 12	*F. vesiculosus* ecad *volubilis*?	126	126	146	151	228	228	365	365	188	188
1081	Site 12	*F. vesiculosus* ecad *volubilis*?	126	126	142	151	228	228	365	365	188	188
1082	Site 12	*F. vesiculosus* ecad *volubilis*?	126	126	142	151	228	228	365	365	188	188
1083	Site 12	*F. vesiculosus* ecad *volubilis*?	126	126	142	151	137	228	365	365	188	188
1084	Site 12	*F. vesiculosus* ecad *volubilis*?	126	126	142	142	?	?	365	365	?	?
1085	Site 12	*F. vesiculosus* ecad *volubilis*?	126	126	142	151	228	228	365	365	188	188
1086	Site 12	*F. vesiculosus* ecad *volubilis*?	126	126	142	153	139	228	369	380	168	188
1087	Site 12	*F. vesiculosus* ecad *volubilis*?	126	126	142	153	139	228	369	380	168	188
1088	Site 12	*F. vesiculosus* ecad *volubilis*?	126	126	142	153	139	228	369	380	168	188
1089	Site 12	*F. spiralis* ecad *lutarius*	126	126	142	153	139	228	369	380	168	188
1090	Site 12	*F. spiralis* ecad *lutarius*	126	126	142	153	139	228	369	380	168	188
1091	Site 12	*F. spiralis* ecad *lutarius*	126	144	142	155	135	228	363	382	165	165
1092	Site 12	*F. spiralis* ecad *lutarius*	126	126	142	153	139	228	369	380	168	188
1093	Site 12	*F. spiralis* ecad *lutarius*	126	126	142	151	139	228	369	380	168	188
1094	Site 12	*F. spiralis* ecad *lutarius*	126	142	142	153	137	228	369	380	168	188
1095	Site 12	*F. spiralis* ecad *lutarius*	126	126	142	151	139	228	369	380	168	188
1096	Site 12	*F. spiralis* ecad *lutarius*	126	126	142	153	139	228	369	380	168	188

Gulf of Maine Table continued.

Number	Site	Morphological ID	F227		F90		F300		F26II		L94	
1097	Site 12	*F. spiralis* ecad *lutarius*	126	126	142	153	139	228	369	380	168	188
1098	Site 12	*F. spiralis* ecad *lutarius*	126	144	142	153	137	228	363	386	165	188
1099	Site 12	*F. spiralis* ecad *lutarius*	126	126	142	151	139	228	369	380	168	188
1100	Site 12	*F. spiralis* ecad *lutarius*	126	144	142	153	137	228	363	384	165	188
1101	Site 12	*F. spiralis* ecad *lutarius*	126	144	142	153	137	228	363	382	165	165
1102	Site 12	*F. spiralis* ecad *lutarius*	126	126	142	153	139	228	369	380	168	188
1103	Site 12	*F. spiralis* ecad *lutarius*	126	126	142	153	139	228	369	380	168	188
1104	Site 12	*F. spiralis* ecad *lutarius*	126	126	142	153	139	228	367	378	168	188
1105	Site 12	*F. spiralis* ecad *lutarius*	126	126	142	153	139	228	369	380	168	188
1106	Site 12	*F. spiralis* ecad *lutarius*	126	144	142	155	137	228	363	382	165	165
1107	Site 12	*F. spiralis* ecad *lutarius*	126	144	142	151	137	228	363	384	165	188
1108	Site 12	*F. spiralis* ecad *lutarius*	124	144	142	155	137	228	363	384	165	165

Collection Data and Genotype Information for All Samples at Rosmuc, Ireland (Chapter IV, Figure 11)

Number	Morphological Identification	Date	F90		F26II		L94		L58	
1109	*Fucus cottonii*	5/20/04	141	141	381	381	165	165	129	129
1110	*Fucus cottonii*	5/20/04	129	129	369	373	169	177	138	138
1111	*Fucus cottonii*	5/20/04	141	141	340	356	165	173	125	125
1112	*Fucus cottonii*	5/20/04	143	143	354	354	173	179	129	129
1113	*Fucus cottonii*	5/20/04	149	149	354	354	173	179	127	127
1114	*Fucus cottonii*	5/20/04	141	141	375	391	165	173	125	129
1115	*Fucus cottonii*	5/20/04	137	141	352	352	173	173	129	129
1116	*Fucus cottonii*	5/20/04	139	141	365	365	188	188	?	?
1117	*Fucus cottonii*	5/20/04	141	141	375	375	173	173	125	129

Rosmuc Table continued.

Number	Morphological ID	Date	F90		F26II		L94		L58	
1118	*Fucus cottonii*	5/20/04	141	141	352	352	173	173	129	129
1119	*Fucus cottonii*	5/20/04	141	141	352	352	173	173	127	129
1120	*Fucus cottonii*	5/20/04	141	141	373	373	173	173	129	129
1121	*Fucus cottonii*	5/20/04	?	?	352	352	179	179	127	127
1122	*Fucus cottonii*	5/20/04	141	145	352	352	169	173	125	129
1123	*Fucus cottonii*	5/20/04	141	141	352	352	173	173	129	129
1124	*Fucus cottonii*	5/20/04	141	141	373	373	173	173	129	129
1125	*Fucus cottonii*	5/20/04	141	141	354	354	?	?	127	127
1126	*Fucus cottonii*	5/20/04	133	133	373	373	173	179	125	129
1127	*Fucus cottonii*	5/20/04	141	141	354	354	173	173	127	129
1128	*Fucus cottonii*	5/20/04	133	133	352	352	173	173	129	129
1129	*Fucus cottonii*	5/20/04	141	141	373	373	173	173	129	129
1130	*Fucus cottonii*	5/20/04	141	141	354	354	165	173	125	129
1131	*Fucus cottonii*	5/20/04	141	161	373	373	165	173	125	125
1132	*Fucus cottonii*	5/20/04	141	141	352	352	173	173	129	129
1133	*Fucus cottonii*	5/20/04	141	141	373	373	173	173	129	129
1134	*Fucus cottonii*	5/20/04	141	141	352	352	173	179	129	129
1135	*Fucus cottonii*	5/20/04	141	141	354	373	173	173	129	129
1136	*Fucus cottonii*	5/20/04	133	133	354	354	165	179	127	129
1137	*Fucus cottonii*	5/20/04	131	131	354	373	173	173	127	129
1138	*Fucus cottonii*	5/20/04	141	141	352	352	173	173	129	129
1139	*Fucus cottonii*	5/20/04	141	141	352	352	173	173	129	129
1140	*Fucus cottonii*	5/20/04	141	141	344	344	165	173	133	133
1141	Putative Hybrid[2]	5/20/04	139	139	344	344	167	167	125	129
1142	Putative Hybrid	5/20/04	139	139	344	344	173	179	125	129

Rosmuc Table continued.

Number	Morphological ID	Date	F90		F26II		L94		L58	
1143	Putative Hybrid	5/20/04	149	149	352	356	167	173	125	127
1144	Putative Hybrid	5/20/04	151	151	356	365	173	179	125	129
1145	Putative Hybrid	5/20/04	141	165	356	360	173	179	125	129
1146	Putative Hybrid	5/20/04	165	165	352	352	173	173	125	127
1147	Putative Hybrid	5/20/04	163	163	385	391	173	173	125	125
1148	Putative Hybrid	5/20/04	163	163	344	344	173	173	125	125
1149	Putative Hybrid	5/20/04	137	137	356	395	173	173	127	127
1150	Putative Hybrid	5/20/04	155	155	352	358	173	179	125	125
1151	Putative Hybrid	5/20/04	141	145	360	371	173	188	125	125
1152	Putative Hybrid	5/20/04	141	145	350	352	173	173	125	125
1153	Putative Hybrid	5/20/04	141	141	?	?	173	179	125	125
1154	Putative Hybrid	5/20/04	141	141	350	391	173	173	127	127
1155	Putative Hybrid	5/20/04	141	141	356	356	173	185	125	125
1156	Putative Hybrid	5/20/04	153	153	356	360	173	173	125	125
1157	Putative Hybrid	5/20/04	151	151	352	362	173	173	129	133
1158	Putative Hybrid	5/20/04	145	145	352	352	173	173	125	129
1159	Putative Hybrid	5/20/04	145	145	356	356	173	173	125	125
1160	Putative Hybrid	5/20/04	155	155	356	360	173	173	125	125
1161	Putative Hybrid	5/20/04	149	153	352	360	175	175	125	125
1162	Putative Hybrid	5/20/04	141	145	352	360	173	173	125	129
1163	Putative Hybrid	5/20/04	141	145	352	362	173	173	129	129
1164	Putative Hybrid	5/20/04	143	143	352	352	173	173	127	127
1165	Putative Hybrid	5/20/04	151	151	362	362	165	173	125	125
1166	Putative Hybrid	5/20/04	145	145	360	360	173	173	127	127
1167	Putative Hybrid	5/20/04	153	155	348	348	179	179	125	129

Rosmuc Table continued.

Number	Morphological ID	Date	F90		F26II		L94		L58	
1168	Putative Hybrid	5/20/04	?	?	352	352	173	179	125	129
1169	Putative Hybrid	5/20/04	131	145	352	352	173	179	125	125
1170	Putative Hybrid	5/20/04	139	139	352	352	173	173	125	129
1171	Putative Hybrid	5/20/04	143	143	356	356	173	173	125	125
1172	Putative Hybrid	5/20/04	153	153	352	391	173	173	125	129
1173	Putative Hybrid	5/20/04	159	163	360	367	173	173	125	129
1174	Putative Hybrid	5/20/04	137	163	356	356	171	173	125	125
1175	Putative Hybrid	5/20/04	153	153	356	356	173	173	125	129
1176	Putative Hybrid	5/20/04	149	149	356	365	173	173	125	129
1177	Putative Hybrid	5/20/04	147	147	352	358	173	173	125	129
1178	Putative Hybrid	5/20/04	153	153	352	358	173	173	125	125
1179	Putative Hybrid	5/20/04	147	147	365	365	173	179	125	125
1180	Putative Hybrid	5/20/04	147	147	360	360	173	173	129	133
1181	Putative Hybrid	5/20/04	141	141	360	360	173	179	125	129
1182	Putative Hybrid	5/20/04	153	157	352	352	173	173	125	125
1183	Putative Hybrid	5/20/04	159	163	385	391	173	173	125	129
1184	Putative Hybrid	5/20/04	135	135	354	356	167	179	125	129
1185	Putative Hybrid	5/20/04	145	145	358	365	173	185	125	127
1186	Putative Hybrid	5/20/04	151	151	352	365	179	179	125	125
1187	Putative Hybrid	5/20/04	153	153	395	395	173	173	125	125
1188	Putative Hybrid	5/20/04	147	147	352	371	173	173	125	125
1189	Putative Hybrid	5/20/04	141	147	356	391	173	173	125	129
1190	Putative Hybrid	5/20/04	139	139	356	365	173	173	125	129
1191	Putative Hybrid	5/20/04	141	165	352	380	173	179	127	127
1192	Putative Hybrid	5/20/04	141	141	391	391	173	183	125	125

Rosmuc Table continued.

Number	Morphological ID	Date	F90		F26II		L94		L58	
1193	Putative Hybrid	5/20/04	139	139	344	385	173	173	125	125
1194	*Fucus spiralis*	5/20/04	141	141	336	336	151	159	125	125
1195	*Fucus spiralis*	5/20/04	141	141	350	350	165	165	125	125
1196	*Fucus spiralis*	5/20/04	141	141	350	350	165	165	125	125
1197	*Fucus spiralis*	5/20/04	141	141	383	383	165	165	125	125
1198	*Fucus spiralis*	5/20/04	139	139	336	336	165	165	125	125
1199	*Fucus spiralis*	5/20/04	141	141	350	365	165	165	125	125
1200	*Fucus spiralis*	5/20/04	141	141	350	350	165	165	125	125
1201	*Fucus spiralis*	5/20/04	141	141	365	365	165	165	125	125
1202	*Fucus spiralis*	5/20/04	141	141	383	383	161	165	125	125
1203	*Fucus spiralis*	5/20/04	139	139	336	336	165	165	135	135
1204	*Fucus spiralis*	5/20/04	141	141	328	328	165	165	?	?
1205	*Fucus spiralis*	5/20/04	139	141	350	350	165	165	125	125
1206	*Fucus spiralis*	5/20/04	139	139	362	362	165	165	125	125
1207	*Fucus spiralis*	5/20/04	151	151	373	373	167	167	?	?
1208	*Fucus spiralis*	5/20/04	141	141	350	350	165	165	125	125
1209	*Fucus spiralis*	5/20/04	141	141	336	336	165	165	125	125
1210	*Fucus spiralis*	5/20/04	141	141	336	336	165	165	125	125
1211	*Fucus spiralis*	5/20/04	141	141	336	365	165	165	125	125
1212	*Fucus spiralis*	5/20/04	139	139	350	350	161	165	125	125
1213	*Fucus spiralis*	5/20/04	139	141	350	350	165	165	125	125
1214	*Fucus spiralis*	5/20/04	141	141	365	365	165	165	125	125
1215	*Fucus spiralis*	5/20/04	141	141	336	350	165	165	125	125
1216	*Fucus spiralis*	5/20/04	141	141	336	336	165	165	125	125
1217	*Fucus spiralis*	5/20/04	141	141	350	350	165	165	125	125

Rosmuc Table continued.

Number	Morphological ID	Date	F90		F26II		L94		L58	
1218	*Fucus spiralis*	5/20/04	129	141	385	385	165	165	125	125
1219	*Fucus spiralis*	5/20/04	141	141	350	365	165	165	125	125
1220	*Fucus spiralis*	5/20/04	141	141	350	350	163	163	125	125
1221	*Fucus spiralis*	5/20/04	139	141	336	336	165	165	125	125
1222	*Fucus spiralis*	5/20/04	141	141	336	336	165	165	125	125
1223	*Fucus spiralis*	5/20/04	139	141	350	350	165	165	125	125
1224	*Fucus spiralis*	5/20/04	141	141	365	365	165	165	125	125
1225	*Fucus spiralis*	5/20/04	141	141	350	350	165	165	125	125
1226	*Fucus spiralis*	5/20/04	141	141	350	350	165	165	125	125
1227	*Fucus spiralis*	5/20/04	141	141	350	350	163	163	125	125
1228	*Fucus spiralis*	5/20/04	141	141	336	350	169	169	125	125
1229	*Fucus spiralis*	5/20/04	141	141	350	350	165	165	125	125
1230	*Fucus spiralis*	5/20/04	141	141	336	365	165	165	125	125
1231	*Fucus spiralis*	5/20/04	141	141	350	350	165	165	125	125
1232	*Fucus spiralis*	5/20/04	141	141	350	365	165	173	125	125
1233	*Fucus spiralis*	5/20/04	151	151	358	373	165	165	125	125
1234	*Fucus spiralis*	5/20/04	139	141	348	350	165	165	125	125
1235	*Fucus spiralis*	5/20/04	151	151	?	?	167	167	125	125
1236	*Fucus spiralis*	5/20/04	129	129	328	328	165	165	125	125
1237	*Fucus spiralis*	5/20/04	141	141	336	336	165	165	125	125
1238	*Fucus spiralis*	5/20/04	129	141	365	365	165	165	125	125
1239	*Fucus spiralis*	5/20/04	151	151	350	350	165	165	125	125
1240	*Fucus spiralis*	5/20/04	141	141	348	348	165	165	125	125
1241	*Fucus spiralis*	5/20/04	141	151	383	383	165	175	125	125
1242	*Fucus spiralis*	5/20/04	141	141	365	365	165	165	125	125

Rosmuc Table continued.

Number	Morphological ID	Date	F90		F26II		L94		L58	
1243	*Fucus spiralis*	5/20/04	137	141	336	350	165	173	125	125
1244	*Fucus vesiculosus*	5/20/04	145	145	356	369	173	179	125	125
1245	*Fucus vesiculosus*	5/20/04	155	155	352	362	173	185	125	125
1246	*Fucus vesiculosus*	5/20/04	141	145	344	356	173	173	125	129
1247	*Fucus vesiculosus*	5/20/04	161	161	356	356	173	179	125	125
1248	*Fucus vesiculosus*	5/20/04	141	141	356	356	167	173	125	125
1249	*Fucus vesiculosus*	5/20/04	139	139	344	356	173	173	125	129
1250	*Fucus vesiculosus*	5/20/04	135	137	323	323	173	173	125	125
1251	*Fucus vesiculosus*	5/20/04	141	141	365	369	173	179	125	129
1252	*Fucus vesiculosus*	5/20/04	141	153	352	356	173	179	125	125
1253	*Fucus vesiculosus*	5/20/04	147	153	358	358	173	173	125	129
1254	*Fucus vesiculosus*	5/20/04	167	167	365	365	173	185	125	125
1255	*Fucus vesiculosus*	5/20/04	157	157	360	365	173	173	125	129
1256	*Fucus vesiculosus*	5/20/04	147	157	352	381	173	173	127	129
1257	*Fucus vesiculosus*	5/20/04	167	167	356	365	173	173	125	125
1258	*Fucus vesiculosus*	5/20/04	141	141	352	356	167	188	125	125
1259	*Fucus vesiculosus*	5/20/04	141	141	391	391	173	173	125	125
1260	*Fucus vesiculosus*	5/20/04	141	141	391	391	173	173	125	131
1261	*Fucus vesiculosus*	5/20/04	139	141	344	344	173	173	125	129
1262	*Fucus vesiculosus*	5/20/04	129	143	362	373	173	173	129	129
1263	*Fucus vesiculosus*	5/20/04	153	153	352	358	173	179	125	129
1264	*Fucus vesiculosus*	5/20/04	153	153	352	358	179	179	125	129
1265	*Fucus vesiculosus*	5/20/04	?	?	354	360	?	?	?	?
1266	*Fucus vesiculosus*	5/20/04	155	155	350	350	173	188	127	129
1267	*Fucus vesiculosus*	5/20/04	141	141	362	365	173	185	125	127

Rosmuc Table continued.

Number	**Morphological ID**	**Date**	**F90**		**F26II**		**L94**		**L58**	
1268	*Fucus vesiculosus*	5/20/04	143	143	360	360	165	165	125	127
1269	*Fucus vesiculosus*	5/20/04	153	153	352	381	173	183	127	129
1270	*Fucus vesiculosus*	5/20/04	147	147	356	391	173	185	131	131
1271	*Fucus vesiculosus*	5/20/04	167	167	344	360	173	183	125	127
1272	*Fucus vesiculosus*	5/20/04	139	141	377	377	173	185	125	125
1273	*Fucus vesiculosus*	5/20/04	131	143	365	383	173	173	129	129
1274	*Fucus vesiculosus*	5/20/04	165	165	334	395	179	188	125	129
1275	*Fucus vesiculosus*	5/20/04	143	143	344	397	173	179	125	125
1276	*Fucus vesiculosus*	5/20/04	141	141	360	360	173	179	125	125
1277	*Fucus vesiculosus*	5/20/04	153	153	344	356	173	188	125	125
1278	*Fucus vesiculosus*	5/20/04	155	155	356	365	173	173	127	129
1279	*Fucus vesiculosus*	5/20/04	141	141	365	373	165	188	125	129
1280	*Fucus vesiculosus*	5/20/04	143	143	352	352	173	183	125	125
1281	*Fucus vesiculosus*	5/20/04	141	147	340	356	173	173	125	125
1282	*Fucus vesiculosus*	5/20/04	141	141	354	354	173	183	125	125
1283	*Fucus vesiculosus*	5/20/04	141	147	340	356	177	177	125	125
1284	*Fucus vesiculosus*	5/20/04	141	141	395	395	173	173	125	131
1285	*Fucus vesiculosus*	5/20/04	151	151	356	365	173	179	125	125
1286	*Fucus vesiculosus*	5/20/04	149	149	352	381	183	183	127	129
1382	*Fucus vesiculosus*	5/20/04	141	141	381	381	?	?	125	129
1383	*Fucus vesiculosus*	5/20/04	141	141	365	365	165	165	125	125

[1] *F. v* var *sp.* or *F. sp.* High shoreline = *F. vesiculosus* var. *spiralis* or *F. spiralis* High Shoreline.

[2] The Putative Hybrid was identified by Dr. Robert Wilkes.

APPENDIX III

MICROSATELLITE DEVELOPMENT RESULTS

Summary of the Results of Microsatellite Development (Chapter II)

All plasmids are stored at –80°C in Rudman Hall Room 333

Plasmid #	Insert (Y/N)	Sequencing Primer	Microsatellite Motif	Sequence Quality	Comments
1	Y	T7	Bad Sequence	Bad	Not Usable
2	Y	T7	No	Bad	Not Usable
3	Y	T7	No	Great	N/A
4	N				
5	Y	T7	Multiple (CA)>5	Good	Not Usable
6	Y	T7	Multiple (CA)>5	Great	Not Usable
7	Y	T7	Multiple (CA)>5	Great	Not Usable
8	Y	T7-SP6	Multiple (CA)>5	Good	Not Usable
9	Y	T7	A-(CA)9-C-(CA)3-T	Good	Not Usable
10	Y	T7	T-(CA)13.5-G; T-(CA)20.1-C	Fair	Sequence further
11	Y	T7	Multiple (CA)>5	Poor	Not Usable
12	?				
13	Y	T7	(GT)>5	Bad	Not Usable
14	Y	T7-SP6	G-(CA)6-T-(CA)20-G	Fair	Sequence further
15	Y	T7	No	Great	N/A
16	Y	T7-SP6	T-(CA)20.5-C	Poor	Sequence further
17	Y	T7	Bad Sequence	Bad	Not Usable
18	Y	T7-SP6	T-(GA)13.5-A-(GA)8.5-T	Good	Amplification problems present
19	Y	T7	Multiple (CA)>5	One good, one bad	Not Usable
20	Y	T7	Multiple (CA)>5	Good	Not Usable
21	Y	T7	No	One bad, one poor	Not Usable
22	N				
23	Y	T7	A-(GT)11-T	Good	Not Usable
24	Y	T7	Bad Sequence	Bad	Not Usable
25	Y	T7	Multiple (CA)	Good	Not Usable
26	**Y**	**T7**	**(AC)25**	**Good**	**GenBank Accession: AY484942**
27	Y	T7	No	Poor	Not Usable

Summary of the Results of Microsatellite Development (Chapter II)

All plasmids are stored at –80°C in Rudman Hall Room 333

Plasmid	Insert	Sequencing	Microsatellite Motif	Sequence	Comments
28	Y	T7	Multiple (CA)>5	Bad	Not Usable
29	Y	SP6	T-(GT)11.5-A-(GT)16-G	Good	Sequence further
30	Y	T7-SP6	T-(CA)13.5-G; T-(CA)21-C	Good	Potentially Develop
31	Y	T7	A-(CA)12-GCTT-(CA)9	Fair	Not Usable
32	Y	T7	C-(GA)3-G-(GA)10-A	Good	Not Usable
33	Y	T7	Multiple (GT)>5	Good	Not Usable
34	Y	T7	No	Bad	Not Usable
35	Y	T7	T-(GT)6.5-A-(GT)2.5-A also C-(GT)13-G	Good	Not Usable
36	Y	T7	No	Good	Not Usable
37	Y	T7	No	Good	Not Usable
38	Y	T7	Bad Sequence	Bad	Not Usable
39	N				
40	Y	T7-M13	T-(CA)12.5-G	one good, one bad	Not Usable
41	Y	T7	No	Bad	Not Usable
42	Y	T7	A-(CA)1.5-T-(CA)10.5-T-(CA)4-G-(CA)4.5-G	Good	Not Usable, Same as 209
43	Y	T7	A-(GT)14.5-T	Fair	Not Usable
44	Y	T7	T-(CA)3-G-(CA)14.5-T	Good	Not Usable
45	N				
46	Y	T7-SP6	C-(GT)16-T	Good	GenBank Accession: AY993956
47	Y	T7	Possible CA repeat	Poor	Not Usable
48	N				
49	Y	T7	Large CA interrupted	Fair	Not Usable
50	Y	T7	G-(CAA)9-T; G-(CA)10.5-A	Fair	Not Usable
51	Y	T7	G-(CA)10.5-T	Fair	Not Usable
52	Y	M13, T7	Multiple GT>5	M13-Good, T7-Bad	Not Usable
53	Y	T7	Bad Sequence	Bad	Not Usable
54	Y	T7	Multiple (CA)>5	Good	Not Usable
55	Y	T7	G-(GT)9-T	Fair	Not Usable
56	Y	T7-SP6	A-(GT)26.5-A	Fair	Potentially Develop

Summary of the Results of Microsatellite Development (Chapter II)

All plasmids are stored at –80°C in Rudman Hall Room 333

Plasmid	Insert	Sequencing	Microsatellite Motif	Sequence	Comments
57	Y	T7	No	Good	Not Usable
58	Y	T7	T-(CA)14.5-T	Good	Not Usable
59	N				
60	N				
61	Y	T7	Multiple (CA)>5	Good	Not Usable
62	Y	T7	Bad Sequence	Bad	Not Usable
63	Y	T7	T-(CA)4.5-T-(CA)18-T	Poor	Not Usable
64	Y	T7-SP6	T-(CA)33.5-A	Fair	Test Further
65	Y	T7	Bad Sequence	Bad	Not Usable
66	Y	T7	Bad Sequence	Bad	Not Usable
67	N				
68	Y	T7	T-(CA)16.5-CC-(G)10-A	Good	Potentially Develop
69	Y	M13	(AC)26	Poor	GenBank Accession: AY993957
70	N				
71	Y	T7	Bad Sequence	Bad	Not Usable
72	Y	T7	Bad Sequence	Bad	Not Usable
73	Y	T7	C-(GT)12.5-T	Good	Not Usable
74	Y	T7, M13, SP6	T-(GT)11.5-A; A-(GT)19.5-C	Fair	Sequence further
75	Y	T7	G-(GT)-CTA-(GT)1.5-A-(GT)14.5-T	Good	Not Usable
76	Y	T7	A-(GT)2.5-C-(GT)20.5-A	Good	Sequence further
77	Y	T7	Several large CA	Good	Not Usable
78	Y	T7	No	Good	Not Usable
79	Y	T7	T-(CA)4.5-G-(CA)-10.5-G	Good	Not Usable, same insert as 80
80	Y	T7	T-(CA)4.5-G-(CA)10-G	Fair	same insert as 79
81	Y	T7	A-(GT)15.5-T	Fair	Sequence further
82	Y	T7	A-(GT)7.5-T, small CA repeats	Good	Not Usable
83	Y	T7	No	Poor	Not Usable
84	Y	T7	Bad Sequence	Bad	Not Usable

Summary of the Results of Microsatellite Development (Chapter II)

All plasmids are stored at –80°C in Rudman Hall Room 333

Plasmid	Insert	Sequencing	Microsatellite Motif	Sequence	Comments
85	Y	T7	C-(GT)10-G-(GT)2-A	Good	Not Usable
86	Y	T7	Huge CA interrupted	Fair	Test Further
87	Y	T7-M13	T-(CA)23.5(AC)3.5-A	Good	Potentially Develop
88	N				
89	N/A				
90	Y	T7-SP6	A-(CA)21-A	Good	**GenBank Accession: AY484941**
91	**Y**	**T7, SP6, M13**	**A-(CA)23.5-T**	**Good**	Polymorphic – Same as insert 90
92	Y	T7	Bad Sequence	Bad	Not Usable
93	Y	T7	A-(GT)11.5-A-(GT)1.5-(GT)13.5-T	Good	Not Usable
94	Y	?	(GT)>50		
95	Y	T7, SP6, M13	(GT)33.5	Good	GenBank Accession: AY993958
96	Y	T7	T-(CA)6-C-(CA)8.5-T	Good	Not Usable
97	Y	T7	Bad Sequence	Bad	Not Usable
98	Y	T7	A-(CA)13.5-G	Good	Not Usable
99	Y	T7	A-(CA)9-C-(CA)3-T	Good	Not Usable
100	Y	?			
101	Y	T7	Multiple (CA)>5	Fair	Not Usable
102	Y	T7	Multiple (CA)>5	Good	
103	Y	T7	Bad Sequence	Bad	Not Usable
104	Y	T7	Bad Sequence	Bad	Not Usable
105	Y	T7	A-(CA)12.5-T	Good	Not Usable
106	Y	T7	G-(CT)10-G;A-(GT)14.5-C-(GT)8-A	Good	Not Usable
107	N				
108	Y	T7	Bad Sequence	Bad	Not Usable
109	Y	T7	No	Fair	Not Usable
110	Y	T7	Bad Sequence	Bad	Not Usable
111	Y	T7	Bad Sequence	Bad	Not Usable
112	Y	T7	Bad Sequence	Bad	Not Usable

Summary of the Results of Microsatellite Development (Chapter II)

All plasmids are stored at –80°C in Rudman Hall Room 333

Plasmid	Insert	Sequencing	Microsatellite Motif	Sequence	Comments
113	Y	T7	Bad Sequence	Bad	Not Usable
114	Y	T7	No	Bad	Not Usable
115	Y	T7-SP6	Disparity	Fair	Sequence further
116	Y	T7	No	Poor	Not Usable
117	Y	T7	No	Poor	Not Usable
118	Y	T7	No	Poor	Not Usable
119	Y	T7	GT>5	Poor	Not Usable
120	Y	T7	Bad Sequence	Bad	Not Usable
121	Y	T7	Multiple (CA)>5	Poor	Not Usable
122	Y	T7	Multiple GT>5	Poor	Not Usable
123	Y	T7	GT>5	Bad	Not Usable
124	N				
125	Y	T7	Bad Sequence	Bad	Not Usable
126	Y	T7	T-(CA)9-C	Fair	Not Usable
127	Y	T7	C-(GT)14.5-A	Good	Not Usable
128	N				
129	Y	T7	C-(GT)17-N	Bad	Sequence further
130	N				
131	Y	T7	G-(CA)14-T	Poor	
132	Y	T7-SP6	A-(CA)24.5-A-(CA)23.5-G	Fair	Developed, N/A
133	Y	T7	No	Good	Not Usable
134	Y	M13, SP6, T7	G-(CA)20.5-T	Good	Developed, N/A
135	Y	M13, SP6, T7	T-(CA)10.5-T-(CA)4-G-(CA)4.5-G	Fair	Not Usable
136	Y	M13	G-(CA)20.5-T	Good	Not Usable
137	N				
138	Y	M13	Bad Sequence	Bad	Not Usable
139	Y	T7	Bad Sequence	Bad	Not Usable
140	Y	T7-SP6	(GT)>5	Bad	Not Usable

Summary of the Results of Microsatellite Development (Chapter II)

All plasmids are stored at –80°C in Rudman Hall Room 333

Plasmid	Insert	Sequencing	Microsatellite Motif	Sequence	Comments
141	Y	T7	(GT)>50	Fair	Not Usable
142	N				
143	Y	T7	(GT)>60	Fair	Not Usable
144	N				
145	Y	T7	No	Good	Not Usable
146	Y	T7	Bad Sequence	Bad	Not Usable
147	Y	T7-SP6	Large CA interrupted	Fair	Test Furthur
148	N				
149	Y	T7	Multiple (CA)>5	Fair	Not Usable
150	N				
151	Y	T7	Multiple (CA), (GT)>5	Fair	Not Usable
152	Y	T7	Large CA interrupted	Fair	Sequence further
153	Y	T7	T-(CA)28-C; A-(CA)14-A	Fair	Sequence further
154	Y	T7	No	Fair	Not Usable
155	Y	T7	No	Fair	Not Usable
156	Y	T7	A-(GT)11.5-T	Fair	Sequence further
157	Y	T7	A-(CA)2-T-(CA)12	Good	Not Usable
158	Y	T7	A-(GT)15-T	Good	Sequence further
159	Y	T7-SP6	T-(CA)28-C	Fair	Potentially Develop
160	N				
161	Y	T7	A-(GT)12.5-T	Fair	Not Usable
162	Y	T7	Large CA interrupted	Fair	Not Usable
163	Y	T7	A-(CA)12.5-G	Fair	Not Usable
164	Y	T7-SP6	T-(CA)5.5-T-(CA)19.5-T	Good	Test Further
165	Y	T7	A-(GT)3.5-AT-(GT)12.5-T-(GT)4.5-A	Fair	Not Usable
166	Y	T7	T-(CA)21-C	Good	Potentially Develop
167	Y	T7	No	Good	Not Usable
168	Y	T7	A-(CA)17.5-G	Fair	Potentially Develop
169	N				

Summary of the Results of Microsatellite Development (Chapter II)

All plasmids are stored at –80°C in Rudman Hall Room 333

Plasmid	Insert	Sequencing	Microsatellite Motif	Sequence	Comments
170	Y	T7	GT>5	Fair	Not Usable
171	Y	T7	Multiple (GT)>5	Fair	Not Usable
172	Y	T7	Multiple (CA)>5	Poor	Not Usable
173	Y	T7	G-(GT)34-A	Fair	Sequence further
174	Y	T7	(GT)>5	Poor	Not Usable
175	Y	T7	C-(CT)5-(CA)10-A	Fair	Not Usable
176	Y	T7	No	Poor	Not Usable
177	Y	T7	A-(CA)14.5-G, T-(CA)8.5-T	Good	Sequence further
178	Y	T7	(CA)>5	Fair	Not Usable
179	N				
180	Y	T7	Bad Sequence	Bad	Not Usable
181	Y	T7	No	Good	Not Usable
182	Y	T7	Multiple (CA)>5	Good	Not Usable
183	Y	T7-SP6	Multiple (CA)>5	Fair	Sequence further
184	Y	T7	No	Good	Not Usable
185	N				
186	Y	T7	No	Poor	Not Usable
187	Y	T7	C(GT)8.5-(GA)15	Good	Sequence further
188	Y	T7	No	Fair	Not Usable
189	Y	T7	(CA)>5	Bad	Not Usable
190	Y	T7	T-(CA)16-C-(CA)4.5-C	Fair	Sequence further
191	Y	T7	No	Good	Not Usable
192	N				
193	Y	T7	G-(CA)9-T	Good	Not Usable
194	Y	T7	T-(CA)12.5-G	Good	Not Usable
195	N				
196	Y	T7	No	Good	Not Usable
197	Y	T7-SP6	G-(CA)20.5	Fair	Test Furthur
198	Y	T7	Bad Sequence	Bad	Not Usable

Summary of the Results of Microsatellite Development (Chapter II)

All plasmids are stored at –80°C in Rudman Hall Room 333

Plasmid	Insert	Sequencing	Microsatellite Motif	Sequence	Comments
199	Y	T7	Bad Sequence	Bad	Not Usable
200	Y	T7	Bad Sequence	Bad	Not Usable
201	Y	T7	T-(CA)2.5-T-(CA)11.5-G	Good	Not Usable
202	Y	T7	No	Poor	Not Usable
203	Y	T7	Bad Sequence	Bad	Not Usable
204	Y	T7	No	Fair	Not Usable
205	Y	T7	T-(CA)14-T	Bad	Not Usable
206	N/A				
207	Y	T7-SP6	T-(GT)17-G; G-(GA)7.5-A	Fair	Sequence Further
208	Y	T7	GT>5	Fair	Not Usable
209	Y	T7	A-(CA)1.5-T-(CA)10.5-T-(CA)4-G-(CA)4.5-G	Fair	Same insert as 42
210	Y	T7-SP6	GT>5	Fair	Not usable
211	Y	T7-SP7	Large GT>5	Good	Sequence Further
212	Y	M13F +R	C-(GA)19.5-T	M13F-Bad/ R-Good	Ordered
213	Y	M13F +R	T-(CA)18.5-A	M13F-Bad/R-Fair	Looked At, N/A same insert as 223-4
214	Y	M13F	No	Great	N/A
215	N				
216	N				
217	Y	M13F	T-(GA)18.5-G	Poor	Sequence M13R
218	Y	M13F +R	No	M13F-Good/R-Bad	N/A
219	N				
220	Y	M13R	G-(CA)23-T	Fair	Sequence further
221	Y	M13R	No	Great	
222	Y	M13R	A-(CA)47.5?-N	Poor	Sequence M13F
223	Y	M13R	T-(CA)37.5-T	Fair	same insert as 213
224	Y	M13R	Long CA MSAT	Poor	same insert as 213
225	Y	M13F	C-(GA)13.5-A-(GA)4.5-A	Great	Not Usable
226	Y	M13F	C-(GA)22.5-T	Good	GenBank Accession: AY993959
227	**Y**	**M13F(2X)+**	**G-(CT)24.5-C**	**Good**	**GenBank Accession: AY484939**

Summary of the Results of Microsatellite Development (Chapter II)

All plasmids are stored at –80°C in Rudman Hall Room 333

Plasmid	Insert	Sequencing R	Microsatellite Motif	Sequence	Comments
228	Y	M13F+R	C-(GA)22.5-T	Fair	Sequence further - same as 232
229	Y	M13F	No	Good	Not Usable
230	Y	M13F	No	Bad	Not Usable
231	Y	M13F	No	Good	Not Usable
232	Y	M13F(2X) +R	C-(GA)22.5-T	Good	same insert as 228
233	Y	M13F	No	Poor	Not Usable
234	N				
235	Y	M13F	No	Fair	Not Usable
236	N				
237	Y	M13F	Long GA	Bad	Sequence M13R
238	Y	M13F	T-(ATAC)5-ATACACG-(TACA)16.5-A	Good	Sequence M13R -Sequence further
239	Y	M13F	GA>5	Good	Not Usable
240	Y	M13F	Bad Sequence	Bad	Not Usable
241	Y	M13F	Bad Sequence	Bad	Not Usable
242	Y	M13F	Bad Sequence	Bad	Not Usable
243	Y	M13F	Bad Sequence	Bad	Not Usable
244	Y	M13F	Bad Sequence	Bad	Not Usable
245	N				
246	N				
247	Y	M13F	No	Poor-Bad	Not Usable, M13F side of insert same as 251
248	Y	M13F	T-(GA)26.5-A	Good	Sequence further
249	Y	M13F	T-(GA)14.5-A	Fair	Not Usable
250	Y	M13F	No	Great	N/A
251	Y	M13F	T-(GA)26.5-A	Good	Sequence M13R, same as 248
252	Y	M13F	(TGTA)>22.5 interrupted	Fair	Not Usable
253	Y	M13F	C-(GA)23.5-T	Good	Potentially develop
254	Y	No sequence data			

Summary of the Results of Microsatellite Development (Chapter II)

All plasmids are stored at –80°C in Rudman Hall Room 333

Plasmid	Insert	Sequencing	Microsatellite Motif	Sequence	Comments
255	N				
256	N				
257	N				
258	Y	No sequence data			
259	N				
260	Nothing				
261	Nothing				
262	Y	No sequence data			
263	N				
264	N				
265	N				
266	N				
267	N				
268	Y	M13F	No	Great	N/A
269	N				
270	Y	M13F	A-(TACA)5-CATACG-(TACA)16.5-AA-(TACA)2.75	Good	Sequence further
271	Y	M13F	No	Great	N/A
272	Y	M13F	Bad Sequence	Bad	Not Usable
273	Y	M13F	No	Great	N/A
274	Y	M13F	No	Great	N/A
275	N				
276	N				
277	Y	M13F	No	Poor	Not Usable
278	N				
279	N				
280	Y	M13F	Bad Sequence	Bad	Not Usable
281	N				
282	Y	M13F	Bad Sequence	Bad	Not Usable
283	N				

Summary of the Results of Microsatellite Development (Chapter II)

All plasmids are stored at –80°C in Rudman Hall Room 333

Plasmid	Insert	Sequencing	Microsatellite Motif	Sequence	Comments
284	Y	M13F	No	Great	N/A
285	Y	M13F	Bad Sequence	Bad	Not Usable
286	N				
287	N				
288	N				
289	N				
290	Y	M13R	No	Good	N/A
291	Y	M13R	G-(CA)23-T	Good	Sequence further
292	N				
293	N				
294	Y	M13F	Multiple CA >5	Fair	Not Usable
295	N				
296	N				
297	Y	M13F	Multiple CA >5	Poor	Not Usable
298	N				
299	Y	M13F	Bad sequence	Poor	Not Usable
300	**Y**	**M13F(2X)**	**T-(GA)34-T**	**Good**	**GenBank Accession: AY484940**
301	Sequence further	M13R	Bad Sequence	Bad	Sequence M13F
302	Sequence further	M13R	Bad Sequence	Bad	Sequence M13F
303	Y	M13F +R	Many (CA)(GA)MSATS>5	M13F-Poor/R-Fair	Not Usable
304	Y	M13F + R	T-(GA)33.5-T	Good	Same as #299
305	Y	M13R	No	Fair	Not Usable
306	Y	M13R	No	Great	N/A
307	Y	M13R	C-(GA)24.5-T	Good	Sequence further - same as 308
308	Y	M13R	C-(GA)24.5-T	Good	same insert as 307
309	Y	M13R	Bad Sequence	Bad	Not Usable
310	Y	M13R	C-(CT)14.5-C-(CT)7.5-C	Good	Not Usable
311	N				
312	Y	M13R	No	Good	N/A

Summary of the Results of Microsatellite Development (Chapter II)

All plasmids are stored at –80°C in Rudman Hall Room 333

Plasmid	Insert	Sequencing	Microsatellite Motif	Sequence	Comments
313	Y	M13R	Bad sequence	Bad	Not Usable
314	Y	M13R	No	Good	N/A
315	Y	M13R	Bad sequence	Bad	Not Usable
316	Y	M13R	Bad sequence	Bad	Not Usable
317	N				
318	Y	M13R	No	Good	N/A - same insert as 319
319	Y	M13R	No	Good	N/A - same insert as 318
320	Y	M13R	Yes-Needs Sequencing	Good	SEQM13F
321	Y	M13R	Yes-Needs Sequencing	Good	SEQM13F
322	Y	M13R	No	Great	N/A
323	Y	M13R	Bad sequence	Bad	Not Usable
324	N				
325	Y	M13R	T-(CA)19.5(GA)10.5-G-(GA)17.5-G	Good	Sequence further
326	N				
327	Y	M13R	No	Good	N/A
328	Y	M13R	T-(CA)13.5(GA)27-A	Good	Sequence further
329	N				
330	Y	M13R	Bad sequence	Bad	Not Usable
331	Y	M13R	No	Good	N/A
332	Y	M13R	Bad sequence	Bad	Not Usable
333	N				
334	N				
335	N				
336	Y	M13F	Bad sequence	Bad	Not Usable
337	Y	M13F	T-(GA)33.5?-A	Poor	Sequence M13F+R
338	Y	M13F	Bad sequence	Bad	Not Usable
339	Y	M13F	C-(CT)28(GT)19-A	Good	Sequence further
340	Y	M13F	A-(GA)17.5-C-(GA)13-A	Fair	Sequence M13R -same insert as 342
341	N				

Summary of the Results of Microsatellite Development (Chapter II)

All plasmids are stored at –80°C in Rudman Hall Room 333

Plasmid	Insert	Sequencing	Microsatellite Motif	Sequence	Comments
342	Y	M13F	A-(GA)17.5-C-(GA)13-A	Good	same insert as 340
343	Y	M13F	T-(GA)32.5-A	Good	same insert as 344
344	Y	M13F	T-(GA)32.5-A	Good	same insert as 343
345	Y	M13F+R	No	Good	N/A
346	Y	M13F	No	Fair	N/A
347	Y	M13F	A-(CA)2(GA)6.5-A-(GA)20.5-C	Fair	Sequence M13F+R
348	N				
349	Y	M13F	(GA)n-Bad Sequence	Bad	Sequence M13R
350	Y	M13F	Huge (GA)(CA)MSATS	Fair	Not Usable -similar to 351
351	Y	M13F	Huge (GA)(CA)MSATS	Fair	Not Usable- similar to 350
352	Y	M13F	C-(GA)19.5-T	Good	Unfinished
353	Y	No sequence data			
354	Y	M13F	Bad sequence	Bad	Not Usable
355	Y	M13F	T-(CT)16-C-(CT)5(GA)33-T	Fair	Sequence M13R - same as 357
356					
357	Y	M13F	T-(CT)16-C-(CT)5(GA)33-T	Fair	Sequence M13R - same as 355
358	N				
359	N				
360	Y	M13F	No	Good	N/A
361	Y	M13F	Multiple GA,CA>5	Good	Not Usable
361B	Y	M13F	A-(CA)23-C	Good	Don't Use
362	Y	M13F	No	Poor	N/A
363	Y	M13F	(ATGT)interrupted	Good	N/A
364	N				
365	Y	M13F	Bad sequence	Bad	Not Usable
366	N				
367	Sequence further				
368	Y	M13F	Bad sequence	Bad	Not Usable
369	Y	Not Sequenced			

Summary of the Results of Microsatellite Development (Chapter II)

All plasmids are stored at –80°C in Rudman Hall Room 333

Plasmid	Insert	Sequencing	Microsatellite Motif	Sequence	Comments
370	N	Not Sequenced			
371	Y	Not Sequenced			
372	N	Not Sequenced			
373	Y	Not Sequenced			
374	Y	Not Sequenced			
375	Y	Not Sequenced			
376	Y	Not Sequenced			
377	Y	Not Sequenced			
378	Y	Not Sequenced			
379	N	Not Sequenced			
380	Y	Not Sequenced			
381	Y	Not Sequenced			
382	Y	Not Sequenced			
383	Y	Not Sequenced			
384	Y	Not Sequenced			
385	Y	Not Sequenced			

In Appendix III, all plasmids are listed. Of these, four (plasmids 26, 90, 227, and 300) were subsequently found to contain inserts that corresponded to polymorphic microsatellite loci, and four plasmids (F46, F69, F95, and F226) contained monomorphic loci. GenBank accession numbers are provided for these eight sequences. In addition, the “Comments” column contains observations regarding which plasmids contain inserts that may repay further examination. Plasmids listed as “Potentially develop” are promising candidates for future primer design, while those listed as “Sequence further” show interesting motifs, but require further sequencing prior to developing primer pairs.