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Assigning morphological variants of *Fucus* (Fucales, Phaeophyceae) in Canadian waters to recognized species using DNA barcoding

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Abstract

The intertidal brown algal genus *Fucus* (Phaeophyceae) consists of individuals with a generally dichotomously branched habit. Morphological variability within species, combined with morphological similarity between species, renders field identification difficult. In light of recent taxonomic revisions, which reduced ten taxa traditionally recognized in Canada to four species, we tested the utility of the DNA barcode (mitochondrial cytochrome oxidase 1, 5') for assigning individuals to these species. We sequenced the DNA barcode for 125 specimens representing all morphologies recognized. We confirmed our results by sequencing the internal transcribed spacer region for 69 specimens. This is the first study to establish that the DNA barcode successfully assigns different morphologies of brown algae to known species as well as other single-gene molecular markers currently used. Furthermore, the results uncovered substantial phenotypic plasticity in Pacific *Fucus distichus*, from moss-like fragments embedded in estuarine mud, strap-like morphs on exposed rocky coasts, to 'spiralis'-like morphs in the upper intertidal whereas phenotypic expression for this species was more restricted in the Atlantic.

Key Words: *Fucus*, DNA barcoding, internal transcribed spacer, species identification, brown algae, cytochrome c oxidase subunit I

Introduction

The brown algal genus *Fucus* is a prominent component of the intertidal seaweed flora along the rocky shorelines of the cold temperate waters of the northern hemisphere. *Fucus* species inhabit a variety of exposure habitats from exposed rocky shores to quiet bays and estuarine areas. Thalli are flattened, more or less dichotomously branched, and terminate in tips that swell to become reproductive receptacles when fertile (Graham and Wilcox 2000).

Traditionally, ten taxa of *Fucus* were recognized in Canada. Reported from the Atlantic were: *Fucus vesiculosus* Linneaus; *Fucus vesiculosus* var. *spiralis* (Linneaus) C. Agardh; *Fucus cottonii*-like morphologies (as *F. vesiculosus* var. *muscoides* Cotton); *F. distichus* ssp. *anceps* (Harvey & Ward ex Carruthers) H.T. Powell; *F. distichus* ssp. *distichus* H.T. Powell; *F. distichus* ssp. *edentatus* H.T. Powell; *F. distichus* ssp. *evanescens* C. Agardh; and *F. serratus* Linneaus. Reported from the Pacific: *F. gardneri* P.C. Silva; and *F. cottonii*-like morphologies of unknown taxonomic designation. *Fucus spiralis* Linneaus is reported from both Atlantic and Pacific coasts (Sears 1998; Gabrielson et al. 2000). However, many *Fucus* individuals in Canadian waters are difficult to assign to distinct taxa in the field. Individuals of the species *Fucus serratus*, with serrated margins, and *F. vesiculosus*, with paired vesicles on the thallus and a dioecious mating system, are relatively easily recognized; however, there are a number of difficulties in resolving the other *Fucus* 'species' and 'subspecies' morphologically. Distinguishing features traditionally used include: habitat; shape of the thallus; receptacle shape; and reproductive characteristics such as monoecy versus dioecy (Sears 1998). Individuals commonly display morphologies that are intermediate in one or more of the distinguishing characters. Members of the various subspecies of *Fucus distichus* are particularly difficult to identify. While *F. distichus* ssp. *distichus* is distinctive in that it occupies upper tide pools and has a small, tough, terete thallus shape, *Fucus distichus* ssp. *edentatus* and *F. distichus* ssp. *evanescens* are difficult to tell

apart; the former with thin blades, uniform in width (1-2cm), with thin, long receptacles and the latter with broad blades (2-4cm) and broad receptacles (Sears 1998). However, in the field we do not observe a distinct division between individuals with narrow and wide blades and specimens of intermediate width are frequently encountered.

Using a mitochondrial marker, Coyer et al. (2006a) suggested that all of the subspecies of *F. distichus,* as well as *F. gardneri,* should be collapsed into a single species, *Fucus distichus*. Coyer et al. (2006a) also recognized *F. spiralis* in the Pacific, and *F. spiralis, F. vesiculosus* and *F. serratus* in the Atlantic, as the only other species in Canada. Coyer et al. (2006a) continued to recognize *F. spiralis* and *F. vesiculosus* as separate species despite the fact that their data could not distinguish them. In the molecular studies completed to date, this closely related species pair is distinguishable only by microsatellite analyses, which also often failed to provide a 'correct' identification (Engel et al. 2003; Wallace et al. 2004; Billard et al. 2005a). Even with this radical reduction to only four taxa, problems still persist in the assignment of samples to species. For example, in the northeast Pacific, *F. spiralis* and *F. distichus* are distinguished partially based on distribution - the former reportedly positioned highest in the intertidal, whereas the latter is found lower. In between these upper and lower limits where individuals in the field appear morphologically distinct, there is a zone of *Fucus* variously exhibiting morphologies of both species. This intermediate zone may be the result of phenotypic plasticity of one or both species, hybridization between the two species, or some combination of these events. The taxonomic identity of the *F. cottonii*-like morphologies is unclear; particularly in the northeast Pacific where their identity/source relative to the two known species in the flora is uncertain. In the Atlantic, the *F. cottonii*-like morphologies have been described as *F. vesiculosus* var. *muscoides* Cotton (Mathieson and Dawes 2001; Wallace et al. 2004) and are found as tiny, dichotomously branched

thalli embedded in the mud in estuarine areas. These Atlantic morphologies have been attributed to hybrids of *F. vesiculosus* and *F. spiralis* (Wallace et al. 2004).

An emerging molecular tool for species identification, the DNA barcode (5'-COI), has been successfully used to distinguish among species, and identify new species (Hebert et al. 2003a; Hebert et al. 2003b). DNA barcoding has the advantage of being an objective species identification tool in cases where identification is ambiguous and has been used in the red algae to delimit species that are morphologically similar (Saunders 2005; Robba et al. 2006). In the only study to date to assess DNA barcoding as a species identification tool in brown algae, Lane et al. (2007) were able to resolve distinct mitotypes, but these were not associated with recognized species owing to rampant introgression, hence the usefulness of DNA barcoding remained equivocal. Given that we now have a broad understanding of the diversity among *Fucus* species (Coyer et al. 2006a), we can test the utility of the DNA barcode for discriminating among and assigning individuals to the four accepted species in Canada, particularly those that are difficult to identify in the field providing an indication of the ability of this marker to discriminate more generally among species of brown algae.

In cases where species are known to undergo hybridization, or if introgression is suspected, it is beneficial to confirm mitochondrial DNA results (such as DNA barcoding) with a nuclear marker (Funk and Omland 2003). As hybridization has been extensively documented among *Fucus* species (Coyer et al. 2002b; Wallace et al. 2004; Bergstrom et al. 2005; Billard et al. 2005a; Engel et al. 2005), and mitochondrial are inherited maternally in *Fucus* (Coyer et al. 2002b), we confirmed our DNA barcode results with the internal transcribed spacer of the ribosomal cistron (ITS). The ITS has been used extensively to assess algal species limits (Coyer et al. 2001; Ross et al. 2003), identify cases of hybridization in algae (Liptack and Druehl 2000; Kooistra et al. 2001; Druehl et al. 2005), and, in *Fucus,* to investigate species relationships

(Leclerc et al. 1998; Serrao et al. 1999a). The last-mentioned were largely unsuccessful, in part due to recent divergence of *Fucus* species, but possibly aggravated by the sequencing approach used in the studies (see discussion). Here we use the ITS marker to confirm genetic species groupings identified with the DNA barcode and to look directly for evidence of hybridization between *F. spiralis* and *F. distichus* in the Pacific.

Materials and methods

Collections

We collected multiple individuals for each of the species and subspecies listed in the taxonomic identification keys (Sears 1998; Gabrielson et al. 2000), as well as specimens with morphologies that did not match the various descriptions. Individuals were collected by harvesting the entire specimen, pressing it on herbarium paper as a voucher, and preserving a piece in silica gel for DNA work. Field identifications, collection information and sequence accessions of each individual are listed in Appendix 1.

Sequence acquisition and analysis

The dried material was ground in liquid nitrogen and an organelle extraction followed by a phenol/chloroform DNA extraction was conducted as outlined in Lane et al. (2006). Gene regions were amplified by polymerase chain reaction (PCR) with the primers GazF2 (5'CCAACCAYAAAGATATWGGTAC3') and GazR2 (5'GGATGACCAAARAACCAAAA3') for the DNA barcode (Lane et al. 2007). The fragment length was 654bp excluding the primers regions. The forward and reverse primers used in PCR of the ITS were P1 and G4, respectively (Tai et al. 2001). The PCR profiles were as follows: for DNA barcode - an initial denaturation at 94°C for 4 minutes, 38 cycles of 94°C denaturation for 1

minute, 50°C for annealing for 30 seconds, 72°C elongation for 1 minute and a final extension at 72°C for 7 minutes; and for ITS - an initial denaturation at 94°C for 3 minutes, 38 cycles of 94°C denaturation for 30 seconds, 45°C for annealing for 45 seconds, 72°C elongation for 2 minutes and a final extension at 72°C for 10 minutes. PCR products were then cooled to 4°C and maintained at that temperature. Amplified DNA fragments were purified by gel electrophoresis (Saunders 1993). Following purification, fragments were sequenced using the same primers as for PCR and for the ITS we additionally used K1R1 (reverse to P1) and KP5 (forward to G4) (Lane et al. 2006). Sequencing was done using PE Applied Biosystems Big Dye (Foster City, CA, USA) cycle sequencing kit and an ABI 3100 genetic analyzer. Sequences were edited using the computer software package Sequencher v.4.2 (Gene Codes Corporation, Ann Arbor, MI, USA). A multiple sequence alignment was constructed by eye using MacClade v4.08 (Maddison and Maddison 2005), and clustering was performed with the neighhbour-joining algorithm in PAUP* v4.0b10 (Swofford 2002) on uncorrected dissimilarities (p).

Anatomical observations

Following genetic analysis, we examined reproductive structures of nine collections of *F. spiralis* (5 Pacific, 4 Atlantic) and nine collections of *F. distichus* (6 Pacific, 3 Atlantic), to identify useful characteristics for distinguishing these two species in the Pacific. We chose to focus on antheridia because a cursory overview of other structures failed to reveal differences between the species. We examined approximately four or five antheridia from one conceptacle in each of the 9 collections for a total of 48 *F. spiralis* antheridia and 44 *F. distichus* antheridia. We measured antheridial length and width using an optical micrometer on a Leica DM 5000 B (Wetzlar, Germany) compound microscope. We averaged the measurements of the antheridia for each

collection and compared the mean antheridial lengths and widths for the two species using an unpaired, two-tailed Student's T-test.

Results

A total of 125 specimens were sequenced for the DNA barcode, which resolved as three genetic species groups (for an explanation of species-level divergence see Discussion): *F. serratus* a *F. vesiculosus*/*F. spiralis* complex, and *Fucus distichus* (Fig. 1). The amount of within species variation (0-2 differences; 0-0.3%) was typical of what is seen among many other organisms, including red algae; however, the level of between-species variation (5-23 differences; 0.7-3%; Fig. 1) was lower than that typically observed between red algal species (usually >30 differences (0.05%); Saunders 2005). There were 69 sequences obtained for the ITS marker, which also resolved three genetic species groups, identical in composition to the groupings of DNA barcode marker (Fig. 2). The ITS data showed levels of within species group variation slightly higher than the DNA barcode (Fig. 2). The original field identifications and the subsequent molecular identifications by DNA barcode for each collection are summarized in Appendix 1.

F. serratus *species group*

With both the DNA barcode and ITS markers, *F. serratus* was resolved as a monophyletic genetic species that consisted only of specimens that were correctly field-identified (Fig. 3a). There were 5-21 (0.7-3%) nucleotide differences between individuals of *F. serratus* and the two other species groups resolved with the DNA barcode, and 8-22 (0.7-2%) differences in the ITS.

F. vesiculosus *and* F. spiralis *species group*

In the DNA barcode analysis, *Fucus vesiculosus* and *Fucus spiralis* formed a single monophyletic group containing 39 collections, with an overall variation of 0-2 (0-0.3%) differences (Fig. 1). The morphological and geographic variability of the *F. vesiculosus*/*spiralis* genetic species group included all 11 collections of typical *F. vesiculosus* (Fig. 3b) (Atlantic), all six collections of *F. vesiculosus* var. *spiralis* (Fig. 3c) (Atlantic), 11 Atlantic *F. spiralis* specimens (Fig. 3d), six specimens of *F. spiralis* from the Pacific, and all five collections of *F. cottonii*-like morphologies (Fig. 3e) from the Atlantic. The *Fucus vesiculosus* collections (all Atlantic) were all resolved within a single subcluster within this species group, however, *F. spiralis* were not.

In the ITS analysis, the monophyletic *F. vesiculosus/spiralis* genetic species group contained 19 specimens with 0-2 (0-0.3%) differences between them (Fig. 2). There were several individuals for which the majority of the ITS1 (840bp) sequence could not be obtained. These individuals exhibited within-individual heterogeneity at two positions in the sequence. Within-individual heterogeneity can result from recent hybridization and/or incomplete lineage sorting (Coen et al. 1982; Doyle 1997; Okuyama et al. 2005). In this case, within-individual sequence heterogeneity was exhibited as varying length repeats at regions of multiple thymine residues, such that the sequence following the repeats is in two or more reading frames and difficult to read accurately. Unfortunately, these areas were found both near the 5' and 3' ends of the ITS1 region and, therefore, affected both the forward and reverse sequences (P1 primer and K1R1 primer) used to acquire the ITS1 data. The shorter (345 bp) ITS2 region showed no differences between *F. vesiculosus* and *F. spiralis*.

F. distichus *species group*

The DNA barcode assigned 78 individuals to the *F. distichus* genetic species group with genetic variation of 0-2 (0-0.3%) nucleotide differences (Fig.1). The group included all *F. distichus* subspecies from the Atlantic, all collections of *F. gardneri* from the Pacific and several Pacific specimens that were field-identified as *F. spiralis*. Pacific collections of this species group exhibited a wide range of morphologies: the typical *F. gardneri* morphology (long pointed receptacles; flat, strap-like thalli; caecostomata present; Fig. 3f); specimens of a rigid, strictly dichotomously branched morphology (Fig. 3g,h; *Fucus gardneri* rigid morph); mud-embedded *F. cottonii*-like morphologies (Fig. 3i,j); several specimens field-identified as *F. spiralis* found in the upper intertidal and intermixed with true *F. spiralis*; all morphological intermediates between *F. gardneri* and *F. spiralis*; and several morphs recorded as "*F. spiralis* undulate morph" found high in estuarine areas attached to small stones or wood and having undulated fronds (Fig. 3k,l). The ITS results (Fig. 2) also resolve *F. distichus* and *F. gardneri* as a single genetic species group, with 0-5 (0-0.7%) nucleotide differences among members. Subclusters within the *F. distichus* group did not correspond to any single morphology or geographic pattern, and though one DNA barcode sub-cluster consisted of all Pacific samples, Pacific collections were also found within the other sub-clusters in the group (Fig. 1). Atlantic collections exhibited more limited morphological variation and consisted of: *F. distichus* ssp. *distichus* (Fig. 3m), *F. distichus* ssp. *evanescens* (Fig. 3n), *F. distichus* ssp. *edentatus* (Fig. 3o), *F. distichus* ssp. *anceps* (Fig. 3p), and one specimen field-identified as *F. spiralis*.

To evaluate further the possibility of hybridization between the Pacific populations of *F. spiralis* and *F. distichus* as a putative explanation for individuals of intermediate morphology, we sequenced a short, single strand region of the ITS1 (final aligned length = 398 nucleotides; amplified with primers P1 and G4, and sequenced with K1R1; see Supplementary Data), which contained nine fixed differences between *F. spiralis* and *F. distichus* for 62 additional specimens

representing both morphologies (field identified *F. spiralis* n=24, *F. distichus* n=9) and morphological intermediates (n=29). We visually examined the chromatograms with the expectation that if hybridization was occurring, we would observe double peaks at the variable sites, due to the bi-parental inheritance of the ITS marker (also see Lane et al. 2007). In all cases, the variable sites contained no ambiguities and clearly assigned each specimen to either *F. distichus* or *F. spiralis*, thus no evidence of hybridization was uncovered.

Anatomical observations

We found a statistically significant difference (p=0.0006) between the mean lengths of antheridia of *F. spiralis* (n=9) and *F. distichus* (n=9). There was no significant difference between the widths of antheridia (14±2.0 µm, p=0.63). The mean antheridial length for *F. spiralis* was 39±3.8 µm with less than 5 of the measured antheridia reaching lengths of 50-52 µm. The mean antheridial length for *F. distichus* was 48±5.1 µm with only a few (<5) with lengths of 32-38 µm.

Discussion

The only published study to asses the DNA barcode for species identification among brown algae failed to resolve the utility of this marker owing to introgression (Lane et al. 2007). In the current study we establish that the DNA barcode can resolve brown algal species as well as all other single-locus markers currently used for this purpose (e.g., Coyer et al. 2006a) including the ITS marker, as confirmed here. We noted that COI divergence values within genetic species groups ranged from 0-0.3%, which are typical values among animal lineages (Hebert et al. 2003a) and red algae (Saunders 2005, 2008). Although interspecific divergence values (typically 3%) were slightly lower than those presented by Saunders (2005, 2008; lower limit ca. 3.5%),

our values were generally an order of magnitude greater than intraspecific divergences (3% vs. 0.3%), which is consistent with the lower threshold generally used for the characterization of species in other barcoding studies (Hebert et al. 2004; Barrett and Hebert 2005; Cywinska et al. 2006; Borisenko et al. 2007; Clare et al. 2007). Saunders (2005, 2008) noted a few exceptional cases for which divergence between closely related species (based on additional molecular, morphological, anatomical and ecological data) was as low as 0.8-1.2%, which is equivalent to our results for *Fucus serratus* relative to *Fucus distichus* (0.7%). As with the red algal genera studied by Saunders (2005, 2008), ample evidence supports species level distinction of these two taxa (data here; Leclerc et al. 1998; Serrao et al. 1999b; Coyer et al. 2002a; Coyer et al. 2006a). Such exceptional cases of low interspecific divergence values are also reported in other DNA barcoding studies (e.g. Burns et al. 2007) and serve to illustrate the effectiveness of DNA barcoding for distinguishing among even closely related species. The relatively low between species group variation in both the DNA barcode and the ITS among the four species currently recognized in Canada is consistent with previously published work suggesting that the genus *Fucus* has undergone a recent radiation (Leclerc et al. 1998; Serrao et al. 1999a; Coyer et al. 2006a). Our study is the first to demonstrate that the DNA barcode is an effective tool to distinguish among species of brown algae.

It is necessary to reiterate that DNA barcoding is a tool for assigning biological specimens to species (Hebert et al. 2003a; Hebert et al. 2003b; Schander and Willassen 2005; Borisenko et al. 2007; Clare et al. 2007). Thus, simple clustering algorithms as implemented here are sufficient to provide a visual representation of these assignments (e.g. Fig. 1). It is critical to emphasize, however, that these phenograms should not be interpreted in a phylogenetic context owing to the simple models of sequence evolution that were applied. Nonetheless, the short region of COI used for DNA barcoding does have phylogenetic signal at the appropriate

taxonomic level and thus can be embedded in more robust phylogenetic analysis with additional data to resolve evolutionary relationships among species (e.g.Lissovsky et al. 2007; Saunders 2008).

Despite a low level of variation, DNA barcoding distinguishes recognized species of *Fucus* in Canada in all but one case (and in this case, other single gene locus molecular markers also fail). We were able to use the DNA barcode to assign species designations to all specimens that could not be identified using traditional morphological characteristics or that had been identified incorrectly except for *F. spiralis* versus *F. vesiculosus*.

Congruent with the findings of Coyer et al. (2006a), *Fucus serratus* forms a distinct monophyletic grouping closely related to the *F. distichus* species group. Our results, along with a wealth of published work, show that *F. serratus* is genetically distinct from other species of *Fucus* in Canada. This species has been introduced to Canada and has a relatively limited range in Atlantic Canada (Edelstein et al. 1973).

Previously published work has indicated that *F. spiralis* and *F. vesiculosus* are a recently diverged species pair (Serrao et al. 1999a; Engel et al. 2003; Wallace et al. 2004; Billard et al. 2005a; Billard et al. 2005b; Engel et al. 2005; Coyer et al. 2006a). Consistent with this, we were unable to distinguish these two species using either the DNA barcode or the ITS marker. Genetic isolation between *F. spiralis* and *F. vesiculosus* has only been established at the microsatellite level by Billard et al. (2005a). Because both Billard et al. (2005a) and Coyer et al. (2006a) suggest maintaining these as separate species, we follow their recommendation, while recognizing that the DNA barcode cannot distinguish between these species. The DNA barcode marker was also not variable enough to detect if there is a genetic basis for the *F. vesiculosus* var. *spiralis* morphology. The Atlantic collections of *F. cottonii*-like morphs fell within the *F. vesiculosus/spiralis* group, in agreement with the work of Wallace et al. (2004) and Coyer et al.

(2006b) in which these morphologies were reported to be hybrids between *F. spiralis* and *F. vesiculosus* or polyploids of *F. vesiculosus*. Owing to morphological and transplant observations and overlap in genetic signal at several mitochondrial loci with both *F. spiralis* and *F. vesiculosus,* it has been hypothesized that *F. cottonii*-like morphs may also be derived from pure *F. spiralis* and *F. vesiculosus* parental forms having undergone habitat selection for embedded growth habit (Mathieson et al. 2006); however, Mathieson et al. (2006) also state that their results would support a hybrid origin for *F. cottonii*-like forms. Wallace et al. (2004) hypothesize that the hybrid *F. cottonii*-like morphologies in the Atlantic may be a vector for gene flow between *F. vesiculosus* and *F. spiralis*, however, whether hybrids exist as attached individuals has not yet been directly tested and unattached *F. cottonii*-like populations have not been observed to reproduce sexually. Here, we observed the presence of within-individual heterogeneities of ITS sequence in representatives of all of the different morphologies within the *F. vesiculosus* and *F. spiralis* cluster, which is a possible signature of hybridization and introgression, but could simply represent within lineage heterogeneity, as is commonly encountered in the ITS. Previous studies employing the ITS marker for *Fucus* (Leclerc et al. 1998; Serrao et al. 1999a) have similarly not been able to resolve relationships between *F. vesiculosus* and *F. spiralis*. While the DNA barcode will consistently assign both *F. vesiculosus* and *F. spiralis* collections within Atlantic Canada to the *F. vesiculosus/F. spiralis* group, we would recommend using microsatellites (Billard et al. 2005a) in combination with morphological and anatomical features (*F. vesiculosus* having paired vesicles and separate sexes and *F. spiralis* lacking vesicles and having combined sexes) to distinguish between the species, although this also, at times, results in misidentifications (Billard et al. 2005b).

Using the ITS marker, Serrao et al. (1999) reported within species variability of 0-5.6%, overall in *Fucus,* whereas our data show lower levels (0-0.3%). One possible explanation for this

discrepancy may be differences in the method used for sequencing between our study and both Serrao et al. (1999) and Leclerc et al. (1998). In the previous studies, ITS sequence was obtained by cloning PCR product and sequencing from a single clone, whereas our study employed direct sequencing of PCR product. During PCR, errors in nucleotide sequence may be introduced in many of the thousands of strands generated during amplification due to imperfect fidelity of the polymerase enzyme. By selecting an individual PCR product by cloning, these errors are amplified, whereas in direct sequencing individual errors in the various strands are overwhelmed by copies of correct sequence (Strachan and Read 1999).

Our results are in concordance with the work of Coyer et al. (2006a) in showing that all of the subspecies of *Fucus distichus,* as well as *Fucus gardneri,* should be subsumed into this species, as we found little to no nucleotide divergence among them in both the DNA barcode and ITS (see results, Figs. 1 and 2). Of particular interest are our DNA barcoding results from the northeast Pacific. Several individuals that were identified in the field as *F. spiralis* (Fig. 1), were identified by both the DNA barcode and ITS data as belonging to the *F. distichus* genetic species group. These specimens often had classic *F. spiralis*-like morphologies (absence of caecostomata, presence of cryptostomata and sterile wings around the receptacles). The misidentified specimens also included individuals with undulate thalli, which, though not a traditional taxonomic character, is often associated with *F. spiralis*. Several of these latter collections were made high in the intertidal in a quiet, estuarine bay, attached to wood or small stones (*F. spiralis* undulate morphs; Fig. 3k,l). Other misidentified specimens were found in the upper intertidal, attached to rocks and intermixed with true *F. spiralis* (Appendix 1). To the contrary, in no case was a Pacific *F. distichus* misidentified as *F. spiralis.* Our finding that the antheridia of *F. distichus* are significantly longer than those of *F. spiralis* may be of use in distinguishing these species in the Pacific. In our observations, there was some overlap in size,

but measuring a number of antheridia from one plant and taking the average is a good estimator of species identity when molecular techniques are not available. Our preliminary observations suggest that there may be other anatomical features that differentiate these two species and this is worthy of future investigation.

In the Pacific where only two species of *Fucus* coexist and true *F. spiralis* appears to be rare in occurrence (our results; Luning 1990), *F. distichus* tends to take morphologies and inhabit niches that in the Atlantic are characteristic of *F. spiralis, F. vesiculosus* and their hybrids (as *F. cottonii*-like morphs). Recently, it has been suggested that there is some aspect of the *F. vesiculosus* genome required for inhabiting salt marshes (Coyer et al. 2006b), however, our results indicate that this is not the case, since Pacific *F. distichus* is also capable of mud-embedded estuarine existence.

Fucus spiralis is considered a recent introduction to the Pacific (Luning 1990; Coyer et al. 2006a); perhaps in the absence of competition (niche exclusion), *F. distichus* has filled the niches that are occupied by members of the *F. vesiculosus/spiralis* species group in the Atlantic. Displacement of phenotypically plastic characters has been shown to occur in response to competition and phenotypically similar individuals in the same habitat experience the greatest level of competition (Pfennig 1992). In the absence of competition, it is conceivable that species capable of phenotypic plasticity will display all or most of the range of their phenotypes, should appropriate habitat be available. In the presence of competition, species may displace into habitats for which they are either specialized—in the case of non-plastic species, or into available habitat—in the case of plastic species. We hypothesize that competition between *F. distichus* and *F. spiralis* in the upper intertidal and estuarine areas in the Atlantic has been in place for long enough to restrict *F. distichus* to tide pool habitats in the upper intertidal (as *F. distichus* ssp. *distichus* morphs), and to mid and lower intertidal areas. In the Pacific where *F. spiralis* may be a

recent introduction (or re-introduction), competitive interactions are still being established, and *F. distichus* fills many of the niches from which it is excluded in the Atlantic. It is also possible that environmental factors, perhaps subtle, are sufficiently different between the intertidal zones of the Canadian Atlantic and Pacific coasts that *F. distichus* can out compete *F. spiralis* over a wider variety of habitats in the Pacific.

In summary, our results show that on both western and eastern coasts *F. distichus* exhibits a high level of phenotypic plasticity and can inhabit a range of habitats. These include: upper most tide pools as small, strictly dichotomously branched *F. distichus* ssp. *distichus* morphs; mid intertidal wave exposed areas as strap-like *F. gardneri* (Pacific), *F. distichus* ssp. *anceps* and F. *distichus* ssp. *edentatus* (Atlantic) morphs; as well as sheltered bays as wide *F. distichus* ssp. *evanescens* (Atlantic) and *F. spiralis* (Pacific) undulate morphologies; and, mud-embedded upper intertidal estuarine areas as the tiny *F. cottonii*-like morphs (Pacific); and finally Pacific upper intertidal areas as morphologies indistinguishable from *F. spiralis*. On the other hand, *F. spiralis* in the Pacific is relatively rare (though widely distributed) and restricted to the upper intertidal, growing attached to the rocky substrate, and displays a restricted level of phenotypic plasticity relative to Atlantic populations which, in association with *F. vesiculosus,* occupy a wider niche and morphological range.

We establish the ability of the DNA barcode marker to assign individuals that are difficult or impossible to identify in the field to one of three genetic species groups: *F. serratus, F. vesiculosus/spiralis* and *F. distichus*. With this level of discrimination, we were able to assign individuals from a number of morphologically unusual populations to distinct species and explore the distribution and level of phenotypic plasticity characteristic of each species. This finding is significant because as DNA barcode technology and the associated database grow and progress,

our data will allow for the standardized COI marker to be applied to *Fucus* in future ecological and biogeographical studies.

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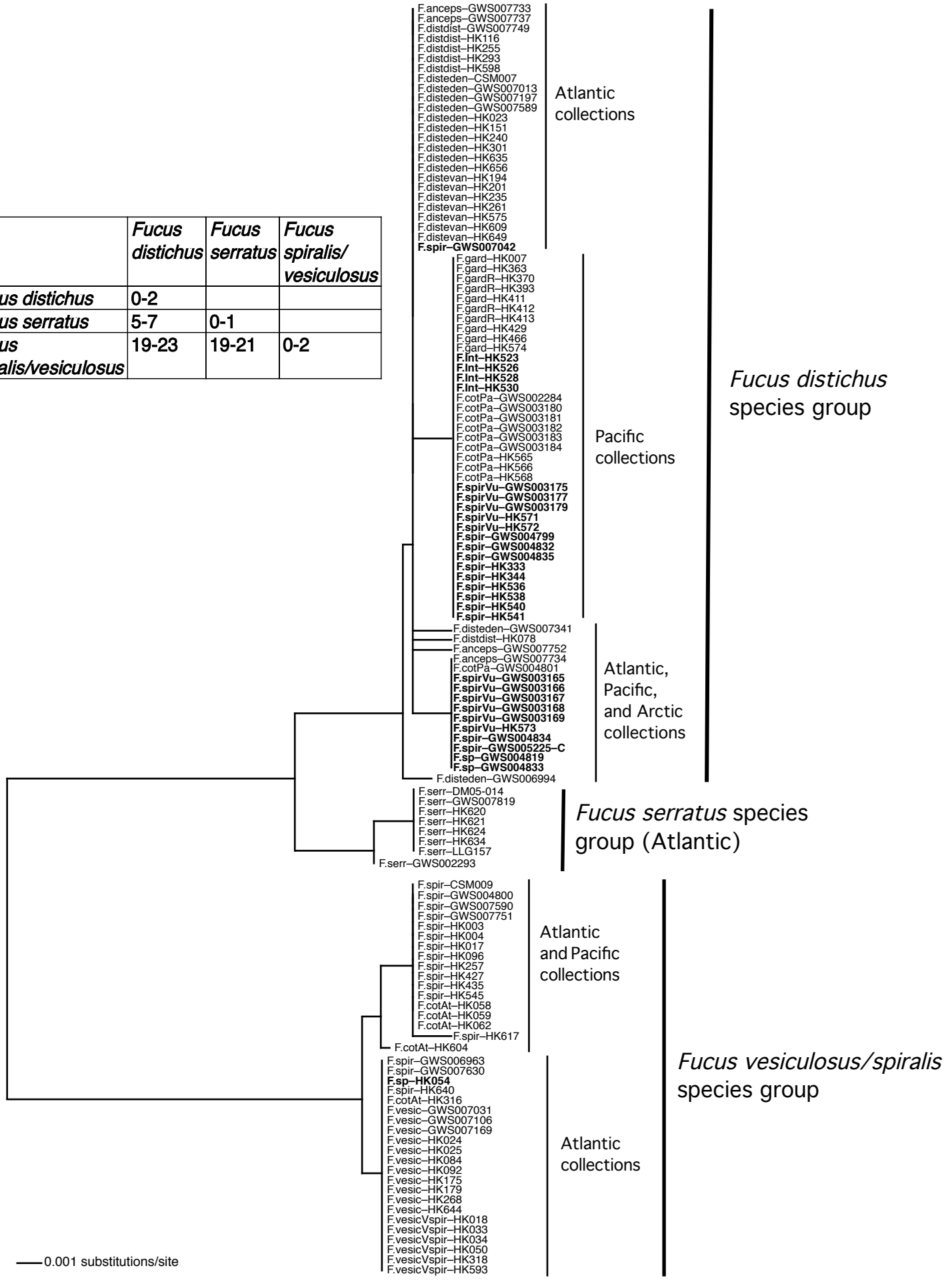
Figure 1. Neighbor-joining analysis of DNA barcode data, and a matrix of actual distances between sequences. Specimens that were mis-identified or unidentified in the field are shown in bold. Geographic locals for specimen subclusters are shown, for detailed collection information for each sample refer to Appendix 1. For each taxon, abbreviations and reference representative photographs are as follows: F.serr, *Fucus serratus* (Fig. 3a); F.vesic, *Fucus vesiculosus* (Fig. 3b); F. vesicVspir, *Fucus vesiculosus* var. *spiralis* (Fig. 3c); F.spir*, Fucus spiralis* (the single collection from Churchill MN is followed by a "C") (Fig. d); F.cotAt, *Fucus cottonii*-like morphs from the Atlantic (Fig. 3e); F.gard*, Fucus gardneri* (Fig. 3f); F. gardR, *Fucus gardneri* rigid morph (Fig. 3g,h); F.cotPa, *Fucus cottonii*-like morphs from the Pacific (Fig. 3i,j); F.spirVu, *Fucus spiralis* undulate morph (Pacific) (Fig. 3k,l); F.Int, morphs intermediate in morphology between *F. spiralis* and *F. gardneri*; F.distdist, *Fucus distichus* ssp. *distichus* (Fig. 3m); F.distevan*, Fucus distichus* ssp. *evanescens* (Fig. 3n); F.disteden, *Fucus distichus* ssp. *edentatus* (Fig.3o); F.anceps, *Fucus distichus* ssp. *anceps* (Fig. 3p); F. sp, unidentified *Fucus.*

Figure 2. Neighbor-joining analysis of internal transcribed spacer data, and a matrix of actual distances between sequences for each genetic species group. Specimens that were mis-identified or unidentified in the field are shown in bold. Geographic locals for specimen subclusters are shown, for detailed collection information for each sample, refer to Appendix 1. Abbreviations and photographic references are as for Figure 1.

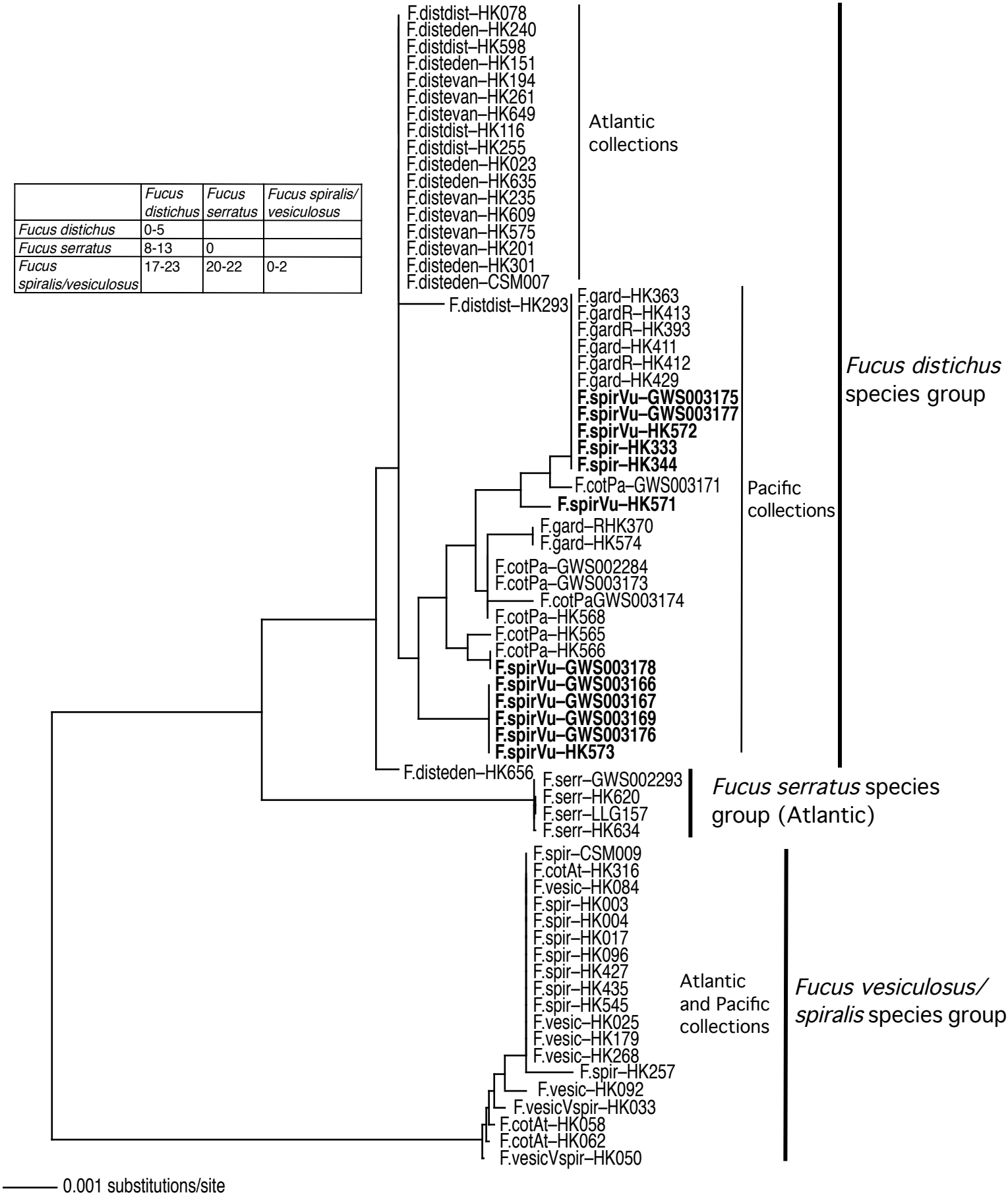
Figure 3. Morphological variation in *Fucus;* scale provided as a scale bar or with a centimeter ruler. a) *Fucus serratus,* b) *Fucus vesiculosus*, c) *Fucus vesiculosus* var. *spiralis,* d) *Fucus spiralis* typical morph*,* e) *Fucus cottonii*-like morphology from the Atlantic, scale bar = 0.3cm, f) *Fucus gardneri* typical morphology, g,h) *Fucus gardneri* rigid morphology, scale bar for h = 2 cm, i,j) *Fucus cottonii*-like morphologies from the

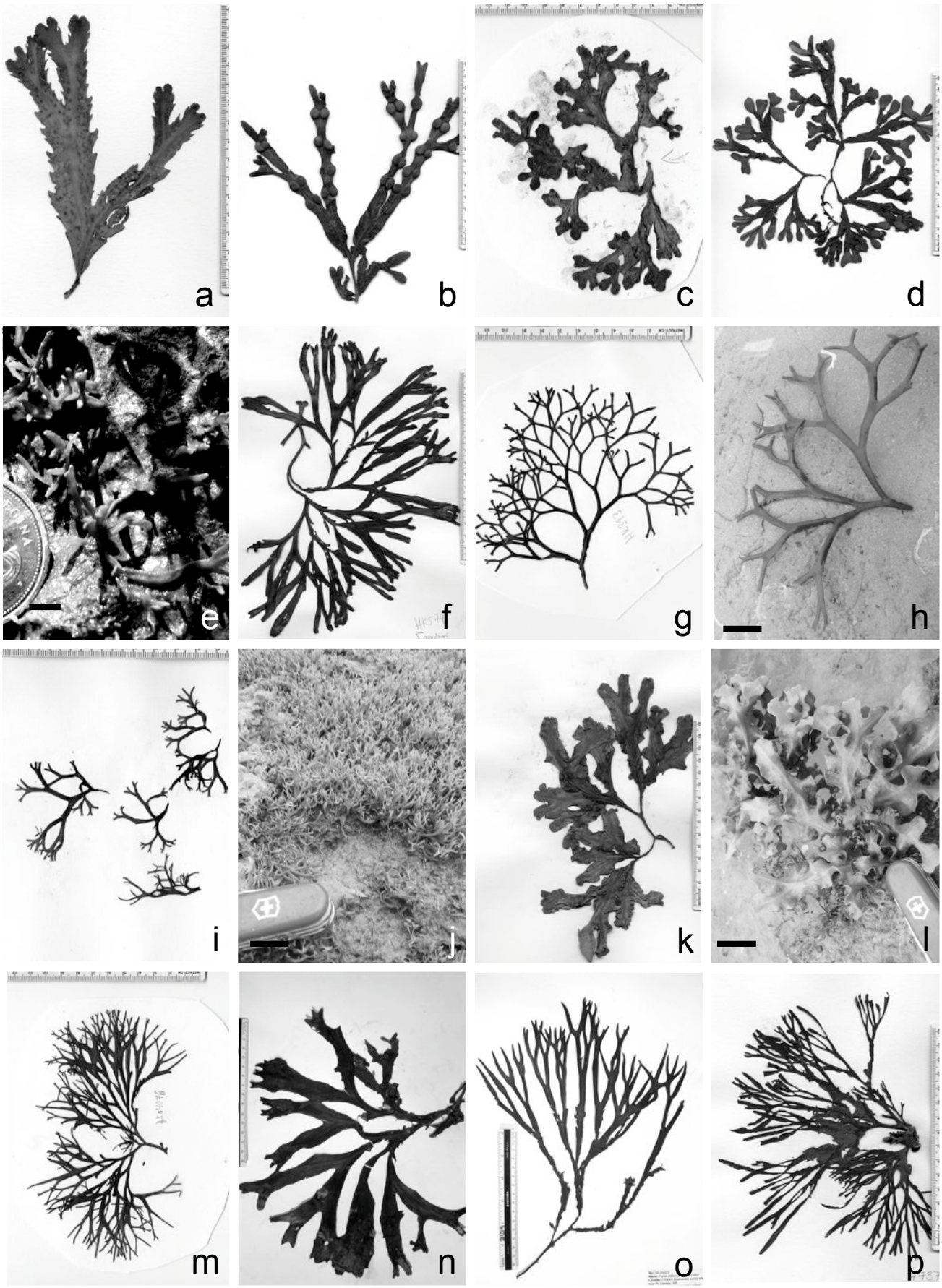
Pacific, scale bar for j = 2cm, k,l) *Fucus spiralis* undulate morphology, scale bar for l = 3 cm, m) *Fucus distichus* ssp. *distichus*, n) *Fucus distichus* ssp. *evanescens,* o) *Fucus distichus* ssp. *edentatus*, p) *Fucus distichus* ssp. *anceps.*

	Fucus distichus	*Fucus serratus*	*Fucus spiralis/ vesiculosus*
Fucus distichus	0-2		
Fucus serratus	5-7	0-1	
Fucus spiralis/vesiculosus	19-23	19-21	0-2



	Fucus distichus	*Fucus serratus*	*Fucus spiralis/ vesiculosus*
Fucus distichus	0-5		
Fucus serratus	8-13	0	
Fucus spiralis/vesiculosus	17-23	20-22	0-2

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2008

Assigning morphological variants of Fucus (Fucales, Phaeophyceae) in Canadian waters to recognized species using DNA barcoding

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