# GENETIC DIVERSITY AND STRUCTURE OF *CYMODOCEA NODOSA* MEADOWS IN THE AEGEAN SEA, EASTERN MEDITERRANEAN

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**Abstract.** Genetic diversity and structure were investigated among seven meadows of *Cymodocea nodosa* in the Aegean Sea, eastern Mediterranean basin. Analysis of three genetic loci, corresponding to the nuclear rDNA operon, revealed 12 distinct multilocus RFLP genotypes. The observed $F_{ST}$ pairwise values revealed, in most cases, a previously unidentified genetic diversity in Aegean populations. A relative sub-structuring was revealed within Pagassitikos gulf, suggesting a fragmentation possibly due to the significant factor of the previously recorded cyclonic system within the studied gulf. Moreover, individual-based landscape approach analysis supports the latter observation, suggesting the presence of three different sub-populations overall in the studied area. The observed barriers maybe related to angiosperms re-colonization from warmer parts of the eastern Mediterranean, after the last glacial maximum. AMOVA indicated the existence of hierarchical significant genetic variation between meadows within gulfs and among samples within meadows, rather than between the two gulfs, as a potential consideration for unidentified limited dispersal and demographic habits of the species in question. On the other hand, sexual reproduction events may contribute to the maintenance of genetic diversity of *C. nodosa* structuring in the Aegean Sea, where the species is also exhibiting extensive morphological plasticity.
**Keywords**: *seagrasses, barrier to gene flow, genetic structure, sexual reproduction*

## Introduction

Seagrass meadows are important coastal marine ecosystems (i.e. bio-indicators) (Constanza et al., 1997; Duarte et al., 2005; Larkum et al., 2006; Directive 2000/60/EC), which are experiencing a worldwide decline, probably due to the increased anthropogenic disturbance and related global climate change (Schramm and Nienhuis, 1996; Orth et al., 2006; Hughes et al., 2009; Waycott et al., 2009). Marine angiosperms are capable of both sexual reproduction through the production of seeds and clonal reproduction *via* rhizome elongation through vegetative production of ramets (Hemminga and Duarte, 2000). The extent of asexual reproduction could influence both the ecological and evolutionary processes in seagrass meadows (Becheler et al., 2010), leading to lower contribution of sexual reproduction in marginal edge habitats (Billingham et al., 2003; Olsen et al., 2004) and, consequently to inter-population genetic divergence (Procaccini et al., 2002; Coyer et al., 2004).
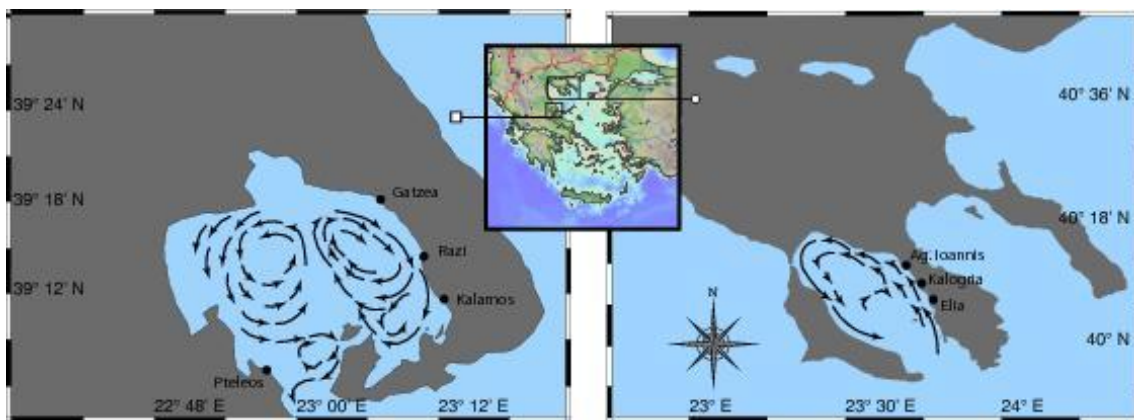
Various genetic analyses implying high-resolution molecular markers have revealed a wide range of genotypic richness across such meadows (Arnaud-Haond et al., 2005; Alberto et al., 2006; Arnaud-Haond et al., 2007a). The distribution patterns of genotypic richness for marine partially clonal organisms was addressed (Olsen et al., 2004; Coyer et al., 2004; Arnaud-Haond et al., 2007b; Alberto et al., 2008). In general, limited dispersal was reported (e.g. Alberto et al., 2005); however, long-distance dispersal (van Dijk et al., 2009) may be a result of rare jump-dispersal events (Ruggiero et al., 2005; Kendrick et al., 2012).

Populations at the margins of their geographic distribution are such useful systems to unlock mechanisms of colonization but also interpret the population response to modern environmental changes (Petit et al., 2003, 2004; Alberto et al., 2008). The Mediterranean Sea is a challenging area for testing such hypotheses since major geological events, like continental drift and paleoclimatic changes, may have created dispersal barriers and split continuous distributions in the past, resulting in vicariance (Lüning, 1990; Orfanidis and Breeman, 1999; Arnaud-Haond et al., 2007b). Within the Mediterranean Sea many areas were exposed to thermohaline changing (Myers et al., 1998) during the last glacial maximum (20,000 - 18,000 years BP). Seagrasses distribution and population structure studies are proned to trace locations of former refugia regarding historical demographic fluctuations (e.g. Waycott et al., 1997, 2009; Van Dijk et al., 2009). Consequently, angiosperm species exhibiting distinct and/or local population structure are subject to post-glacial recolonization and adaptation events (Ruggiero et al., 2002).

*Cymodocea nodosa* (Ucria) Ascherson is a dioecious seagrass widely distributed in the Mediterranean Sea and adjacent saline lagoons (den Hartog, 1970; Sfriso and Ghetti, 1998; Agostini et al., 2003; Nicolaidou et al., 2005; Pasqualini et al., 2006). The known present southern limit is Senegal (den Hartog, 1970). The species is also found in the Atlantic from Mauritania and the Canary Isles up to central Portugal (Reyes et al., 1995; Barberá et al., 2005; Espino et al., 2008). Its vegetative growth renders an extensive morphological plasticity (Barberá et al., 2005; Orfanidis et al., 2010). In the Aegean Sea it forms extensive patchy meadows, often mixed with *Zostera noltii*, being one of the main benthic primary producers of the area (Orfanidis et al., 2005). Genetic heterogeneity in inter-meadow scale has been comprehensively explored in a series of marine angiosperms including *C. nodosa* (Alberto et al., 2008; van Dijk et al., 2009; Becheler et al., 2010). Thus, records of genetically differentiated meadows of seagrasses correspond either to particular distinct genotypes as soon as you move beyond the patch scale, with a few exceptions where very large clones have been found, or even to ecotypes which should be clarified in order to understand any structure profile across the Aegean Sea.

The eastern Mediterranean basin is consisted of a divergent topography due to the relief-forming geotectonic processes during the late glacial period (Lyberis, 1984). Thus, the Aegean Sea is composed of shallow platforms and relatively wide gulfs (Mascle and Martin, 1990). As a result of the differentiated morphology, the climate can be seasonally distinguished with transition periods between them (Theocharis et al., 2002; Karageorgis and Anagnostou, 2003). Although the circulation patterns are usually transient, in the case of Pagassitikos gulf there is an almost stable dipole, an anticyclone in the east and a cyclone in the central-western part, accompanied by smaller jets and eddies (Petihakis et al., 2002) (*Figure 1*). On the other hand, Toronaios gulf forms a continuous cyclonic system (Hyder et al., 2002), implying a steady-state condition

(Savvidis et al., 2005). Eastern Mediterranean basin represents a hot spot of *C. nodosa* genetic diversity; with Cyprus and Aegean populations being rather closely related (Alberto et al., 2008). Taking into account the recent sea surface temperature regime throughout the species distribution range, water temperatures during the last glacial maximum might have been too low for growth, or even for survival of a species adapted to such warm temperate conditions like *C. nodosa* (Procaccini et al., 2007; Orfanidis et al., 2010). Therefore, populations of this species in relatively colder coasts, like those in the Aegean Sea, may have resulted by re-colonization from warmer parts of the eastern Mediterranean. Such a hypothesis was demonstrated to be valid in the case of *Posidonia oceanica* (Ruggerio et al., 2002) and also applies for *C. nodosa* in the Mediterranean Sea (Procaccini et al., 2007). Moreover, *C. nodosa* seems to have survived the last sapropelic (oxygen starvation) events which occurred in the eastern Mediterranean and Aegean basins within the last 10,000 years (Kotthoff et al., 2008).



*Figure 1. Sampling sites of Cymodocea nodosa analyzed meadows [Pteleos (N = 30), 39.01 °N 22.57 °E / Gatzea (N = 30), 39.18 °N 23.06 °E / Kalamos (N = 30), 39.13 °N 23.11 °E / Razi (N = 30), 39.16 °N 23.09 °E / Ag. Ioannis (N = 30), 40.11 °N 23.40 °E / Elia (N = 30), 40.08 °N 23.45 °E / Kalogria (N = 30), 40.10 °N 23.43 °E]. Water surface circulation system of the two studied areas (Pagassitikos and Toronaios gulfs, to the left and the right of the figure, respectively). Arrows represent sea current patterns.*

In the present study the genetic diversity, using PCR-RFLP profiles, in seven meadows of *C. nodosa* in two geographically distinct areas of the Aegean Sea (Pagassitikos and Toronaios gulfs, eastern Mediterranean) was assessed. Thus, effort was focused mainly on addressing the following critical questions: (a) to what extent these contemporary meadow populations are differentiated with respect to the documented different cyclonic systems? (b) to assess connectivity and barriers to gene flow between areas within the frame of geographical limit of dispersion.

## Material and methods

### Study sites and sampling design

*Cymodocea nodosa* samples were collected from seven coastal, perennial, meadows in two main geographically distinct gulfs (Pagassitikos and Toronaios) of the Aegean Sea, eastern Mediterranean. In Pagassitikos gulf four putative populations (Gatzea,

Pteleos, Kalamos and Razi) were sampled where in Toronaios gulf three putative populations were sampled (Ag. Ioannis, Elia and Kalogria) (*Figure 1*). All meadows, at least in most cases, were fairly continuous at an average depth of 3 - 4 m, covering an area of about 1,500 m² each. The geographical distance between meadow pairs ranged from 3 to 140 km.

Thirty samples (N = 30) were collected from each meadow by SCUBA diving within an area of 30 × 30 m (perpendicular to shore) by using a grid with square meshes (minimal 5 m distance). Samples were obtained during species' spring - summer vegetation growth period. After collection, sheath tissue was removed, thoroughly cleaned from epiphytes and preserved in silica gel.

### DNA extraction, PCR amplification and restriction enzyme analysis

Approximately one milligram of dried tissue from each sample was grounded using liquid nitrogen. Following CTAB-based DNA extraction (Doyle and Doyle, 1987), three universal primer pairs were used to amplify polymorphic nuclear ribosomal regions. The primer pairs ITSB-F (5'-AACTTAAAGGAATTGACGGAAG-3') / ITSB-R (5'-GGTCCGTGTTTCAAGACGGG-3') and ITSA-F (5'-TTTCCGTAGGTGAACCTGC-3') / ITSA-R (5'-ATATGCTTAAGTTCAGCGGGT-3') were used to amplify the nuclear rDNA operon (ITS1 - 5.8S - ITS2) (Provan et al., 2005). In order to isolate nuclear 18S rDNA region, the forward primer AT18F01 (5'-YACCTGGTTGATCCTGCCAGTAG-3') and the reverse primer AT18R01 (5'-TGATCCTTCYGCAGGTTCACC-3') were used (Ki and Han, 2007). These amplified regions were abbreviated as ITSB, ITSA and 18S, identically corresponding to the respective fragments targeted. Amplification reaction mixtures consisted of 100 ng DNA template, 0.2 mM of each deoxyribonucleotide triphosphate (dNTPs), 0.2 μM of each primer, 5 μl of 10× reaction buffer, 1 U of DyNAzyme II DNA polymerase (Finnzymes, Espoo, Finland), with sterilized water added to make up a final volume of 50 μl. PCR amplifications were performed under the following conditions: initial denaturation at 94 °C for 5 min, followed by 35 thermal cycles of denaturation at 94 °C for 45 s, annealing at 50 - 53 °C (51°C for ITSB, 50 °C for ITSA and 53 °C for 18S), for 1 min, and extension at 72 °C for 1 min 30 s. The final extension was performed at 72 °C for 10 min. All PCR products were separated by electrophoresis on 1.2% agarose gels buffered with 1× Tris-Borate-EDTA (TBE), stained with ethidium bromide and visualized under UV light.

Amplified PCR products were purified by precipitation by adding 0.1 volume of 3 M sodium acetate and 2.5 volumes of absolute ethanol to remove excess of dNTPs and primers, and then resuspended in sterile distilled water. Genetic variation was analyzed by restriction fragment length polymorphism (RFLP) performed on the previous PCR amplified products. All the amplified fragments corresponding to three loci were screened with the following four restriction endonucleases: *Alu*I, *Rsa*I, *Hae*III and *Eco*R1 (New England Biolabs, Beverly, MA) according to the manufacturer's instructions. For each sample, 10 μl of the PCR product was fully and separately digested at 37 °C for 1 h with the aforementioned restriction enzymes. A deactivation temperature was used for 2 min for each reaction (65 °C for *Alu*I, *Rsa*I and *Eco*R1 and 80 °C for *Hae*III). Electrophoresis of the restriction fragments was carried out in 1.5% agarose or 3.5% (wt/vol) metaphor gel (FMC Bioproducts, Rockland, ME) in 1× TBE buffer. The molecular size of each fragment was estimated precisely using the original

UVIDoc Mw package (St. John's Innovation Center, Cambridge, UK) software by comparisons with standard DNA molecular weight markers (Cambrex, Rockland, ME). The obtained distinct restriction patterns were coded with capital letters, generating composite genotypes consisting of 12 capital letters, one of each restriction enzyme.

## Data analysis

Linkage disequilibrium (LD - using Fisher's exact tests) was tested through the software ARLEQUIN v.3.5 (Excoffier and Lischer, 2010). Allelic frequencies were estimated according to the "round robin fashion" mode as described by Parks and Werth (1993) and clonal assignment probability was calculated for sampling units sharing the same genotype as a previously encountered unit to be derived from a distinct sexual reproductive event (as opposed to being part of the same genetic individuals). The likelihood of a given repeated genotype to have resulted from different recombination events $P_{sex}$ (Parks and Werth, 1993; Arnaud-Haond et al., 2007a) was assessed using GenClone v.2.0 software (Arnaud-Haond and Belkhir, 2007). Genotypic richness, R (G/N ratio) was calculated, where G is the number of different observed genotypes and N is the number of samples (ramets) analyzed.

To estimate the extent of genetic differentiation between meadows, the fixation index $F_{ST}$ (Wright, 1951) was used, as implemented in ARLEQUIN v.3.5 software (Excoffier and Lischer, 2010). The latter software was also used to test deviation from mutation drift equilibrium, the Tajima's D index (Tajima, 1989). To evaluate patterns of spatial genetic structure, a hierarchical analysis of molecular variance (AMOVA) (Excoffier and Lischer 2010) was performed to partition variance components attributable to (1) variance between gulfs; (2) variance between meadows within gulfs; and (3) variance among samples within meadows. The significance of the resulting F-indices and variance components were permutated 10,000 times using a Bonferroni correction (Rice, 1989).

Population structure was assessed using the software STRUCTURE v.2.3 (Pritchard et al., 2000). The Correlated Allele Frequency Model (Falush et al., 2003) records the allele frequencies in a hypothetical 'ancestral' population without specifying geographic area as a prior. To test the convergence of the priors and the appropriateness of the chosen burn-in length and simulation length, three independent repeats were run for each value of K; the number of clusters ($1 \leq K \leq 10$). Burn-in length and length of simulation were set at 500,000 and 1,000,000 repetitions, respectively. The online software HARVESTER was used to obtain the likelihood value of the different K values and for detection of the best value that fits to the data in hand (Earl and von Holdt, 2012).

The software BARRIER v.2.2 (Manni et al., 2004) was utilized in order to identify locations and the directions of barriers to gene flow using a computational geometry approach. The Monmonier (1973) maximum difference algorithm provided a more realistic representation of the barriers in a genetic landscape and a significance test was implemented by means of bootstrap matrix analysis. In order to obtain a geometric satisfactory map from a list of geographic x, y coordinates, a Voronoï tessellation (Voronoï, 1908) calculator was used. Out of this tessellation a Delaunay triangulation (Brassel and Reif, 1979) was obtained.

## Results

ITSB, ITSA and 18S fragments were consistently amplified; at least one recognition site per each of the amplified fragments was identified. A total of 40 allelic restriction sites were revealed after LD test; 12 different composite genotypes were revealed for the four surveyed restriction enzymes for all loci among the 210 samples overall. Composite genotypes with their frequencies in each sampling site, along with $P_{sex}$ significances, used to discriminate among the seven sample units, are presented in *Table 1*. Tajima's D index was non-significant. It is worth mentioning that $h_{12}$ was recorded as Kalogria meadow-specific genotype in Toronaios gulf. $P_{sex}$ clonal assignment was significant (P < 0.001, after Bonferroni correction) only for Kalogria sample unit in Toronaios gulf, indicating that repeated genotypes were likely the result of clonal reproductive events in this particular sample site. Furthermore, an extra analysis was performed, assuming all the sampling sites as one population; only Kalogria meadow was still consistent for clonal reproductive events. Thus, the latter sample site was omitted from further analysis. Consequently, the remaining analyzed distinct sample units were not unlikely to be the result of distinct reproduction events. Genotypic richness (R) ranged from 0.03 in meadows of Toronaios Gulf to 0.16 in Gatzea meadow in Pagassitikos gulf.

Pairwise $F_{ST}$ analysis revealed that the highest heterogeneity was accounted between Pagassitikos gulf western meadows (Gatzea and Pteleos) and Toronaios gulf meadows (P < 0.001; *Table 2*). A high $F_{ST}$ value implies a considerable degree of differentiation among these meadows, indicating relative sub-structuring.

**Table 1.** *Twelve composite haplotypes (haplotypes-denoted with capital letters) based on RFLP digests of four restriction endonucleases (AluI, RsaI, HaeIII and EcoR1) in ITSB, ITSA and 18S fragments of r-DNA operon and their relative frequencies per population along with $P_{sex}$ significances (ns = non-significant, \*\*\*: P < 0.001); genotypic Richness (R) and Tajima's D index along with its significance (ns = non-significant, N/A = not applicable).*

| Haplotype | Composite genotype | Pagasitikos gulf | | | | Toronaios gulf | | |
|:---:|:---:|:---:|:---:|:---:|:---:|:---:|:---:|:---:|
| | | Gatzea | Pteleos | Kalamos | Razi | Elia | Ag. Ioannis | Kalogria |
| **h1** | ABCE ADEF ABAD | 0.5 (ns) | 0.5 (ns) | | | | | |
| **h2** | ABCF ADEF ABAD | | 0.1 (ns) | | | | | |
| **h3** | ABCD ADEF ABAD | | | 0.6 (ns) | 0.4 (ns) | | | |
| **h4** | ABCE ADEF ACAD | 0.1 (ns) | | | | | | |
| **h5** | ABCF ADEF ACAD | 0.1 (ns) | | | | | | |
| **h6** | ABDE ADEF ABAD | | | 0.1 (ns) | 0.5 (ns) | 1 (ns) | 1 (ns) | |
| **h7** | ABCE BEEE ABAD | | | 0.2 (ns) | | | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **h8** | ABCF BEEF ABAD | $0.2\,(^{ns})$ | $0.1\,(^{ns})$ | | | | |
| **h9** | ABCE BEEF ABAD | | | | $0.1\,(^{ns})$ | | |
| **h10** | ABDE BEEF ACAD | $0.1\,(^{ns})$ | $0.3\,(^{ns})$ | | | | |
| **h11** | ABCD BEEF ABAD | | | $0.1\,(^{ns})$ | | | |
| **h12** | ABDD CEEF ABAD | | | | | | $1^{***}$ |
| **R=G/N** | | 0.16 | 0.13 | 0.13 | 0.1 | 0.03 | 0.03 | 0.03 |
| **Tajima's D** | | $0.536^{\,ns}$ | $0.747^{\,ns}$ | $0.483^{\,ns}$ | $0.721^{\,ns}$ | N/A | N/A | N/A |

**Table 2.** $F_{ST}$ *values between sampled units of Cymodocea nodosa (\*\*\*: P < 0.001, after Bonferroni correction).*
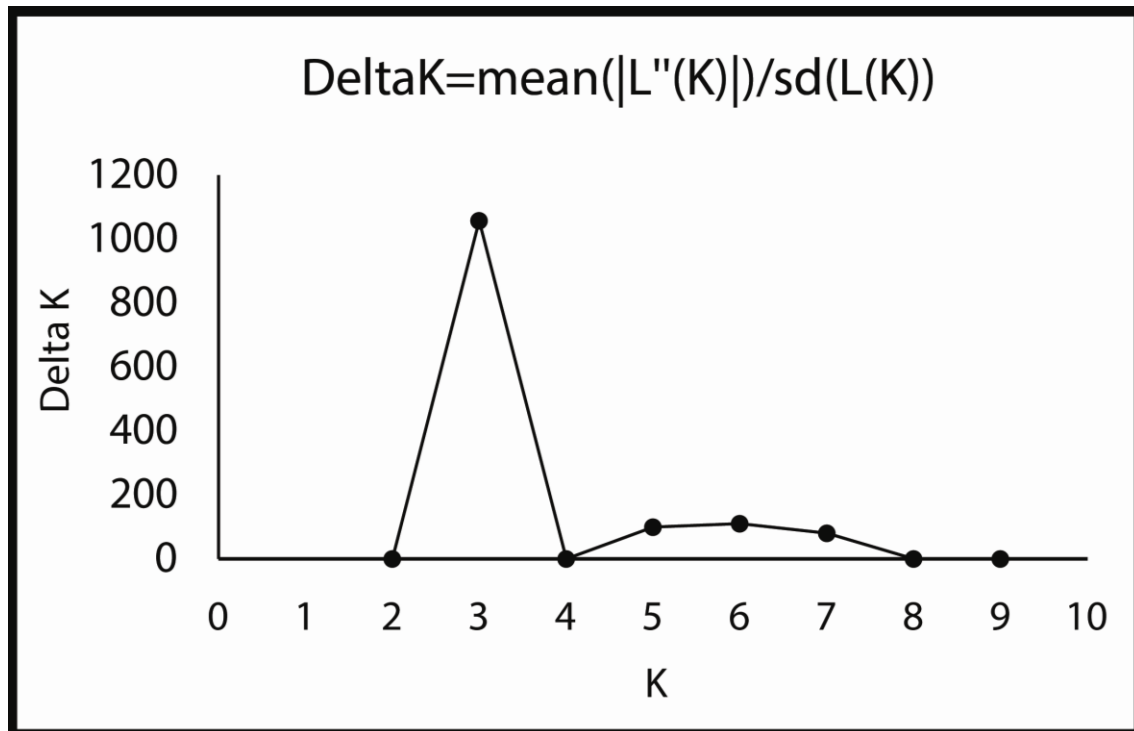
| | Gatzea | Pteleos | Kalamos | Razi | Elia | Ag. Ioannis |
|---|---|---|---|---|---|---|
| **Gatzea** | - | | | | | |
| **Pteleos** | 0.014 | - | | | | |
| **Kalamos** | $0.769^{***}$ | $0.764^{***}$ | - | | | |
| **Razi** | $0.808^{***}$ | $0.805^{***}$ | 0.077 | - | | |
| **Elia** | $0.875^{***}$ | $0.872^{***}$ | $0.400^{***}$ | $0.317^{***}$ | - | |
| **Ag. Ioannis** | $0.875^{***}$ | $0.872^{***}$ | $0.400^{***}$ | $0.317^{***}$ | 0 | - |

Moreover, when applying a hierarchical analysis of molecular variance (AMOVA) (*Table 3*) for meadows of *Cymodocea nodosa*, 61.67% of the overall genetic variation was attributed rather to between meadows within gulfs than among samples within meadows, or between the two distinct gulfs (22.18% and 16.15%, respectively). Significant F-statistics were observed at the following hierarchical levels: between meadows within gulfs ($F_{SC}$) and among samples within meadows ($F_{ST}$). $F_{CT}$ value (0.161) was non-significant (P = 0.142), indicating that a relative amount of variation was not partitioned to biogeographical boundaries. STRUCTURE revealed three putative populations (K = 3; Ln (PD) = -152.8; *Figure 2*). The latter was also supported by BARRIER; a significant barrier to gene flow was generated among the two Pagassitikos gulf western meadows (Gatzea and Pteleos) and the rest sampled meadows (*Figure 3*). Frequency distribution of composite genotypes showed that Pagassitikos gulf eastern meadows (Razi and Kalamos) shared similar sub-populations with Toronaios gulf meadows, implying that latitude may have a greater influence on differentiation due to allele frequencies shifting.
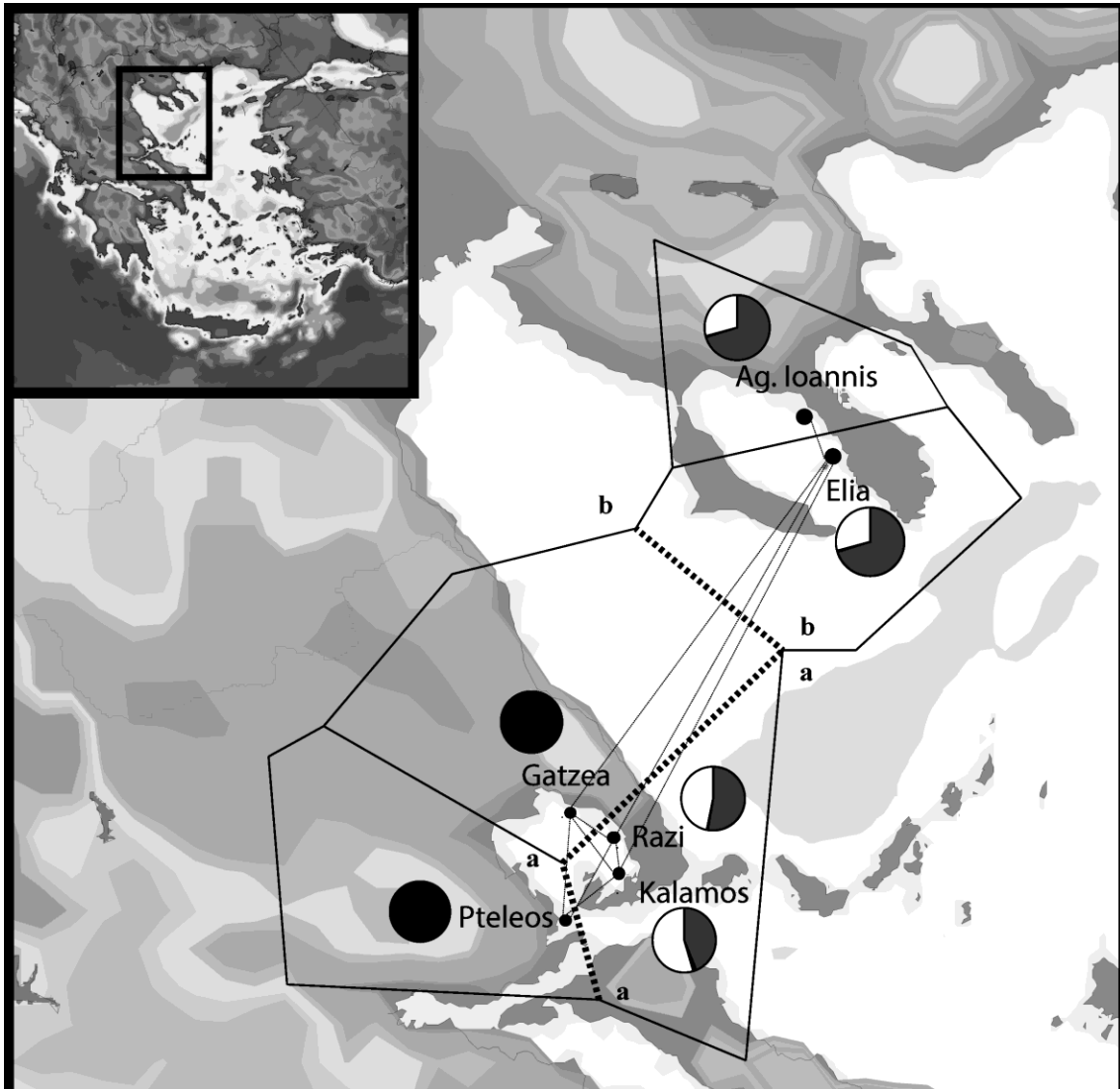
***Table 3.*** *Analysis of molecular variance (AMOVA) of Cymodocea nodosa grouped in two areas; Pagassitikos and Toronaios gulfs. $F_{CT}$ = variation between gulfs divided by total variation, $F_{SC}$ = variation between sub-areas within gulfs divided by the sum of variation between sub-areas within gulfs and variation within sub-areas, $F_{ST}$ = the sum of variation between gulfs and variation between sub-areas within gulfs divided by total variation (ns = non-significant, \*\*\*: P < 0.001).*

| | df | Sum of squares | Variance components | Percentage of variation | Fixation indices |
|---|---|---|---|---|---|
| between gulfs | 1 | 52.375 | 0.26727Va | 16.15 | $F_{CT} = 0.161^{ns}$ |
| between meadows within gulfs | 4 | 123.975 | 1.02088Vb | 61.67 | $F_{SC} = 0.735^{***}$ |
| among samples within meadows | 174 | 63.900 | 0.36724Vc | 22.18 | $F_{ST} = 0.778^{***}$ |
| Total | 179 | 240.250 | 1.65539 | | |



***Figure 2.*** *Determination of the number of clusters (K) including all three repetitions for each K without geographical area as a prior. The highest peak denotes the most likely number of clusters (K = 3) according to the Pritchard Bayes Formula.*

***Figure 3.*** *Pies denote the different sub-populations (black, gray, white colour) after STRUCTURE analysis. Voronoi tessellation (in solid line) of the points (sampling units) according to geographical locations (black spots) and the corresponding Delaunay triangulation (in light gray colour). The dotted black line indicates the resulted barriers to gene flow.*

## Discussion

Analysis of genetic diversity of *Cymodocea nodosa* meadows in the Aegean Sea was performed at all geographical hierarchical levels and a significant degree of variation among samples within meadows and between meadows within distinct geographic areas (gulfs) was detected. The relative pattern of genetic variability between studied meadows of the two gulfs (> 100 km scale) may suggest that they are a result of post-glacial colonization from eastern Mediterranean Pleistocene glacial refugia at the edges of the species' distribution range (Ruggiero et al., 2002, 2004). The eastern Mediterranean is the most parsimonious location for an ancestral center of distribution of this species (Alberto et al., 2008). This might be by itself an interesting and novel

aspect of this study in what concerns the signature of post Pleistocene latitudinal recolonization patterns.

Although the limited power of RFLPs, a relatively high local genetic differentiation in Pagassitikos gulf meadows was observed and this could be a result of limited dispersal ability in the marine environment, as has been earlier hypothesized elsewhere (Vekeman and Hardy, 2004; Alberto et al., 2005; Becheler et al., 2010). $F_{ST}$ pairwise values were significant among the western and eastern part of the gulf, illustrating a potential small-scale local population structure for the species in question. To this extent, the geometric map analysis revealed barriers to gene flow between the eastern and western parts of the gulf, suggesting a structuring subdivision of the species. The Baeysian individual assignment approach proposes the presence of three distinct sub-populations. The latter differentiated pattern may propose an exhibiting spatial difference with respect to the cyclonic system taking place in the area (Petihakis et al., 2002). This relatively small degree of differentiation may be attributed to the differential dispersion of the seeds through the current patterns between the two gulfs.

A possible scenario for such differentiations between meadows within gulfs could be the effect of a non-recent colonization which allowed sufficient evolutionary time to divergence. In order to test this scenario, Tajima's D (Tajima, 1989) values were non-significantly positive in the present study. Thus, the detected differentiation among meadows of *C. nodosa* was not attributed to a more recent than the post-glacial colonization, and probably reflects a prolonged and stable demographic equilibrium among them. However, it is very important that the above test used is consistent, given any sample size, and powerful enough to distinguish between different possible evolutionary processes (Ferretti et al., 2010).

Despite the observed genetic pattern, the limited power of RFLPs used in clonal plant species is arisen, since several population features cannot be assessed (levels of clonality and its consequences on population dynamics). Since one could struggle to discriminate clones, the results could be potentially biased by this very rate of clonality. When such markers are chosen, the consequent analyses and their interpretations should take into account the properties of such markers. It is worth mentioning that, the level of differentiation may be influenced by plethora of factors, such as effective population size, genetic drift, mutation rate and it cannot be ruled out that molecular markers may vary on the matter. Thus, it is challenging to fully estimate the degree of geographic differentiation between the power of each of the selected markers. According to O'Brien (1991), RFLP markers are type I markers; type I are markers associated with known genes. Since genetic variation may be subject to selection, due to the fact of the importance of mutation in natural populations (Liu and Cordes, 2004), the apparent geographical genetic pattern of the species in question, using RFLPs, may be due to selective forces, reflecting adaptive differentiation (see Cai et al., 2004).

During sampling in the western part of Pagassitikos gulf, *Posidonia oceanica* meadows were scarce or even absent compared to the eastern part. *P. oceanica* degraded beds are prone to invasion by one or more potential substitutes, such as *C. nodosa* (Bianchi and Pierano, 1995; Montefalcone et al., 2006). The apparent successful invasion of *C. nodosa* in the western Pagassitikos gulf may be dependent on the species capacity to adapt to a spatially varying environment regarding physicochemical features that could act as evolutionary pressure mechanisms. Finally, it could be a result of secondary contact among previously extended geographically isolated populations,

which is likely to have occurred mainly because of anthropogenic dispersal capabilities in these sampling sites (Amoutzopoulou-Schina, 2007).

The samples collected mainly from Elia and Ag. Ioannis meadows corresponded either to patchy meadows, or to shallow borders of continuous seagrass beds, possibly fragmented by wave fluctuations caused by southern winds that deposit organic material close to the coast (Lazaridou et al., 1997). In some cases, such disturbances directly affect the uniformity of seagrass meadows and may promote recovery mechanisms through vegetative propagation (Olsen et al., 2002). Although the highly significant factor "between meadows within gulfs" represents a relatively higher degree of the total variance component, *C. nodosa* may actually have a somewhat dispersal potential in Pteleos, Gatzea meadows through young developing seedlings and may address the also highly significant factor "among samples within meadows" to be the key one. It is likely that a potential colonization in Pagassitikos gulf may have resulted through rare jump dispersal events in consistence with that observed at the Canary Islands (Alberto et al., 2006).

To conclude, the local genetic differentiation in Pagassitikos gulf, also evident by the ability of the species to grow in different habitats due to its morphological plasticity (Cancemi et al., 2002; Cunha and Duarte, 2005) and to different oceanographic features related to the recorded cyclonic pattern (Petihakis et al., 2002), could be partially attributed to the previously presumed (e.g. Alberto et al., 2008) limited dispersal ability. The low genetic differentiation between the eastern meadows of Pagasitikos gulf and Toronaios gulf ones may be referred as an area which retains the most ancestral gene pool. Alternatively, *C. nodosa* meadows in western Pagasitikos gulf could have evolved from a less broadly distributed progenitor and its seed dispersal is evolutionary preferred and better suited to this particular habitat.

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