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Mitochondrial DNA Variation as a Tool for Systematic Status Clarification of Commercial Species – The Case of Two High Commercial *Flexopecten* Forms in the Aegean Sea

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1. Introduction

The determination and the identification of species constitute some of the first basic steps for biodiversity monitoring and conservation (Dayrat, 2005). Species identification is usually carried out by taxonomists, but in many cases it is restricted by the lack of available morphological characters. This is the case for two *Flexopecten* taxa: *Flexopecten glaber* L. and *F. proteus* Dillwyn ex Solander ms. The smooth scallop *F. glaber* is an edible bivalve, with a maximum length of 8.66 cm (Pisor & Poppe, 2008). It is an epibenthic species of soft and/or hard substrate and its habitat is characterized as muddy and sandy, with organic detritus. The species has a fast growth rate. Its reproduction takes place mainly during late summer, while the gonad activity can be observed all the year. It inhabits between 5 and 900 m depth or more (even 1600 m), in Mediterranean and Black Seas (Poppe & Goto, 1993) and its distribution appears in Figure 1.

The expansion of *F. proteus* is controversial, since some authors reported restricted distribution of the species to Adriatic Sea (Pountiers, 1987). However, this form has been reported as a commercial species in the area of Thessaloniki Gulf (North Western Aegean Sea), where it coexists with *F. glaber* (Zenetos, 1996; Galinou-Mitsoudi & Sinis, 2000). According to these authors, the occurrence ratio of *F. glaber* to *F. proteus* in three random fisheries samples of 99, 130 and 120 individuals in total was 0.74, 1.28 and 6.50, respectively. Besides Thessaloniki Gulf, *F. proteus* is found in Ionian Sea (Amvrakikos and Korinthiakos Gulfs) and also in Aegean Sea (Pagasitikos Gulf , N. Evoikos Gulf , Saronikos Gulf , Lesvos Island and Lemnos Island) (Koutsoubas et al., 2007; Zenetos, 1996) (Fig. 1).

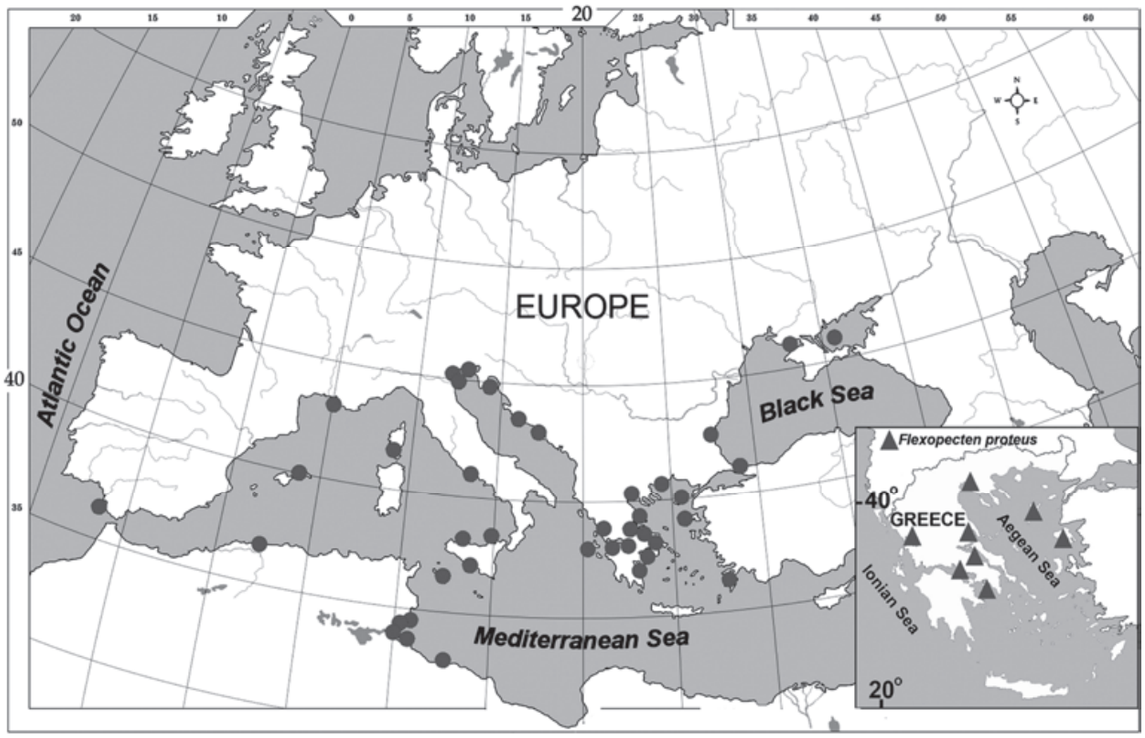

Fig. 1. *F. glaber* distribution in Mediterranean and Black Sea. In inlaid map of Greece the *F. proteus* distribution is denoted, according to Zenetos (1996).

These commercial scallops are major target species due to their high demand in the national and European market. Nevertheless, the species seems to be overexploited, as the annual Hellenic production from over 20 t between mid 1970s and late 1980s, was reduced to 5 t from the late 1980s to 1990s (Koutsoubas et al., 2007). However, *Flexopecten* scallops fishing is forbidden in the Thessaloniki Gulf since 2002, due to the presence of high concentrations of heavy metals (Cd), while the stocks in the central Aegean Sea have rather collapsed in 2003 (Koutsoubas et al., 2007).

The two forms of *F. glaber* and *F. proteus* are also exploitable in Adriatic Sea (Pujolar et al., 2010). Besides the fishing, *Flexopecten* scallops having high growth rate, high demand and good price/kg, are also potential species for aquaculture.

Mitochondrial DNA (mtDNA) has been shown to be useful for analyzing relationships among bivalve populations (Hare & Avise, 1996; Rawson & Hilbish, 1998; Katsares et al., 2008), due to its maternal inheritance, its haploid nature and the fact that its effective population size is the one fourth of that of the nuclear DNA. The invertebrate mitochondrial 16S rDNA gene has been largely used for studying the population structure and the levels of genetic variation in many Pectinidae taxa (Kong et al., 2003; Mahidol et al., 2007; Yuan et al., 2009; Pujolar et al., 2010). As the Aegean populations of *F. glaber* and *F. proteus* are the main commercial stocks of these bivalves in Greece, the main objective of our study was to conduct a survey on the genetic resources of the two taxa using mitochondrial 16S rDNA nucleotide sequences.

Chlamys proteus or *F. proteus* Dillwyn has been considered since long, as a species of the bivalve family Pectinidae (Cossignani et al., 1992; Delamotte & Vardala–Theodorou, 1994; Doneddu & Trainito, 2005; Repetto et al., 2005; Zenetos et al., 2005). The majority of these sources mention that *F. proteus* is another similar species to *F. glaber*. The main systematic characteristic for the recognition of the two pectinids, is the difference in the number of major and dominated ribs which are 9-12 in *F. glaber*, but only five in *F. proteus* (Fig. 2). On the other hand, according to Poppe & Goto (1993), CLEMAM & ERMS, *F. proteus* is accepted as a subspecies or a form of *F. glaber*. Also, Raines & Poppe (2006) underline that *F. proteus* "is treated only as a synonyme" of *F. glaber.*

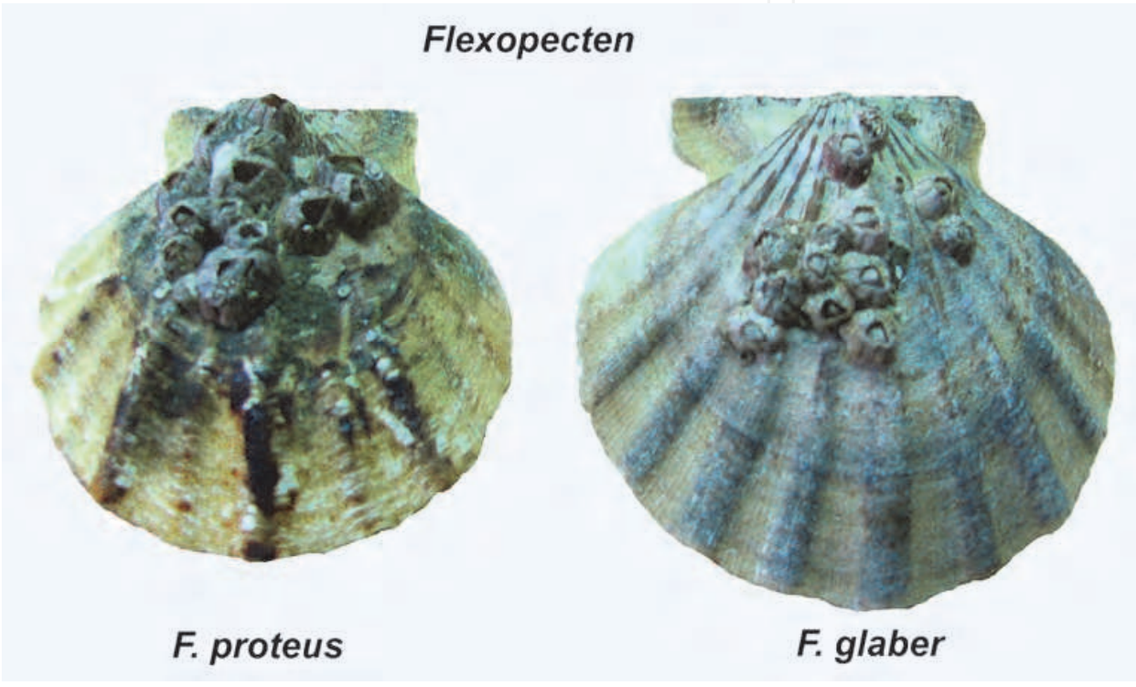

Fig. 2. Fished scallops in the study area of the two *Flexopecten* forms *proteus* and *glaber,* with epibiots on their left valves

Many biologists have argued that the future of descriptive taxonomy will depend on successfully embracing new techniques. Many ideas have been proposed and much progress has been achieved by using molecular data that provide a complementary approach to discriminate species separated by subtle morphological characters (Knowlton, 1993; Avise, 1994; Chan & Chu, 1996; Sarver et al., 1998; Mathews et al., 2002; Goetze, 2003). The invertebrate mtDNA 16S rDNA gene has been relatively well studied due to availability of universal primers (Kocher et al., 1989; Palumbi, 1996) and was used to resolve taxonomic problems in the family Mytilidae (Rawson & Hilbish, 1995), Veneridae (Canapa et al., 1996) and Pectinidae (Canapa et al., 2000a; Saavedra & Pena, 2004; Pujolar et al., 2010).

Taking into account all the existed considerations and the uncertain systematic status of these two common *Flexopecten* "forms", another aim of our work was an attempt to solve the identification problem of the two Aegean taxa, using the sequence analysis of the mtDNA

16S rDNA gene. A parallel and similar study (Pujolar et al., 2010), was also made for the Adriatic populations of the *Flexopecten* complex.

2. Methods and tools

2.1 Sampling

Specimens were collected from three different regions of the Gulf of Thessaloniki, northern Aegean Sea (Fig. 3). In total, 78 adult individuals of *F. glaber* and 57 adult individuals of *F. proteus* from the three different locations, were collected (Table 1). Specimens were classified according to the number of dominant ribs.

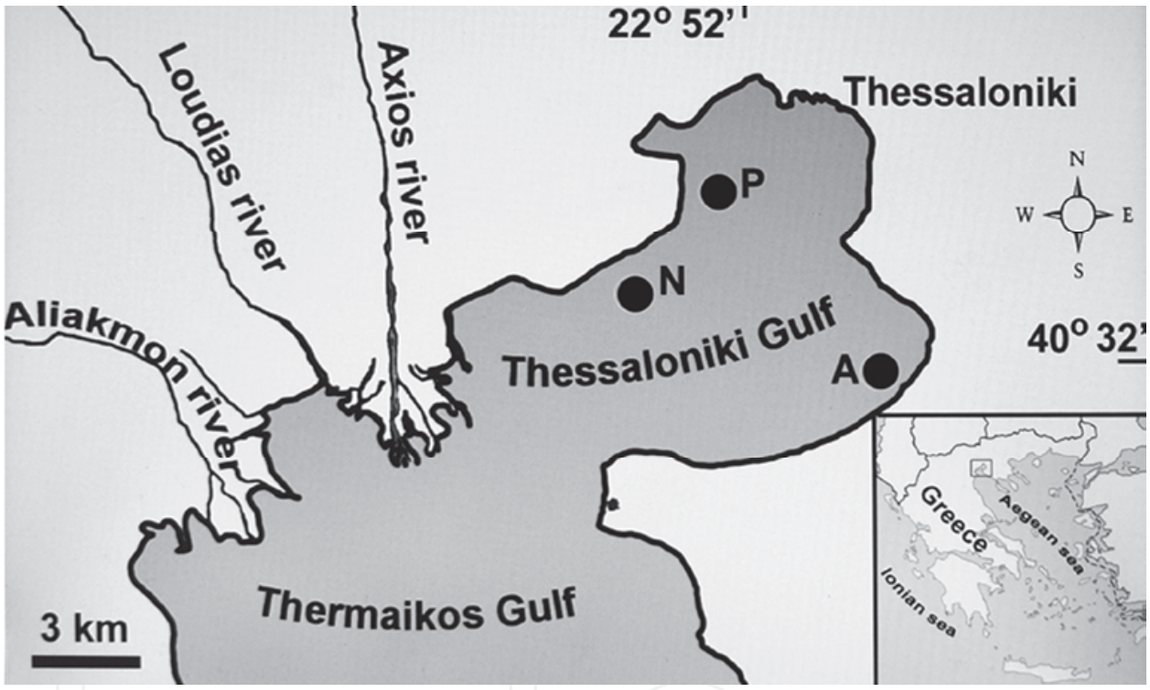

Fig. 3. Sampling sites of the analyzed population for both *F. glaber* and *F. proteus*. A: Airport; P: Paliomana; N: Naziki.

2.2 DNA extraction and PCR amplification

Total DNA was extracted from the anterior adductor muscle according to Hillis et al. (1996). A universal primer set (Palumbi, 1996) was used for the amplification of the 16S rDNA gene in both *F. glaber* and *F. proteus*. The reaction mixture contained template DNA (approximately 100 ng), 1X PCR buffer, 2.2 mM $MgCl_2$, 20 pmol of each primer, 0.25 mM of each dNTP and 0.5 U of Promega polymerase. Amplification was started at 94°C for 3 min, followed by 31 cycles at 94°C for 50 s, 50°C for 50 s, 72°C for 50 s and a final extension at 72°C for 5 min.

Electrophoresis of 3 μl of the PCR product was performed in 1XTBE buffer for 1 h at 150 V, in 1.5% agarose gel containing 0.5 μg/ml ethidium bromide. The size of the PCR products

was checked against a 100 bp DNA ladder and was approximately 500 bp for both taxa. The resulting DNA fragments were visualized by UV transilumination and photographed.

2.3 DNA sequencing

A sequencing analysis on a 3730Xl DNA Analyzer (Applied Biosystems) was followed using both forward and reverse primers for crosschecking. DNA sequences were deposited to GenBank (accession numbers GU320272 – GU320288; HM 627014 – HM627051).

2.4 Data analysis

The nucleotide sequences of all individuals were aligned using the Clustal X software (Thompson et al., 1997) and the BioEdit software (Hall, 1999), set to default parameters and corrected by eye. Mt DNA 16S rDNA diversity was estimated by the number of haplotypes, haplotype frequency, haplotype diversity (h) and nucleotide diversity (π), via Arlequin version 3.5 (Excoffier & Lischer, 2010). Pairwise genetic distances among haplotypes were computed based on the 2-parameter Kimura distance model (Kimura, 1980) using MEGA software (Tamura et al., 2007; Kumar et al., 2008). The obtained pairwise genetic distances were used to construct a Neighbor-Joining tree with the same software. The species *Aequipecten opercularis* (GenBank: AM494412) was used as outgroup and two relevant ingroups (*Argopecten irradians*: GU119971; *Chlamys multistriata*: FN667665) were also included in the analysis. Pairwise sample differentiation was assessed using the exact test described by Raymond & Rousset (1995), included in the Arlequin package. Analysis of molecular variance was conducted also with the same package (Arlequin version 3.5; Excoffier & Lischer, 2010), to determine the genetic differentiation of populations.

To test whether the populations underwent recent demographic population growth we calculated the mismatch distribution in Arlequin package, which assumes an infinite sites model of selectively neutral nucleotide substitutions and assesses significance via coalescent simulations of a large, neutrally evolving population of constant size (Slatkin & Hudson, 1991). Finally, Tajima's *D* (Tajima, 1989) was used to examine the selective neutrality of mitochondrial fragment and it was also used in mismatch distribution, because significant negative *D*-values are what the hypothesis of population growth predicts (Bertorelle & Slatkin, 1995).

3. Results

A total of 436 base pairs of the 16S rRNA gene fragment were successfully sequenced for 135 individuals from both taxa, and 50 polymorphic sites were identified (Appendix 1). The transition/transversion rate ratios were 2.312 for purines and 6.283 for pyrimidines while the overall transition/transversion bias was 1.7. No species specific positions were detected that could discriminate the two species. Fifty one haplotypes were detected among all samples (37 for *F. glaber* and 32 for *F. proteus*) and their frequencies are given in Appendix 1. Haplotype 5 (20% in total) was shared among all samples of both taxa. Haplotype 1 revealed a higher percentage of appearance among samples (25.2%) and it was dominant in all samples apart from Paliomana population of *F. proteus* (Appendix 1). Haplotypes 3, 10 and 15 were present in three samples in total of the same and different taxa, whereas haplotypes 6, 19 and 21 were shared between two samples of different taxa. Haplotypes 8, 45 and 50 were observed twice in the same population and all the other haplotypes were unique.

Haplotype diversity, nucleotide diversity, number of polymorphic sites and number of haplotypes are given also in Appendix 1. The highest values of haplotype and nucleotide diversity were revealed in the population of *F. proteus* from Paliomana (0.9744 and 0.0078 respectively) while the lowest values of these indices were observed in the sample of *F. glaber* from Airport (0.7793 and 0.0027). Mean haplotype and nucleotide diversity for *F. glaber* populations were h=0.854 and π=0.004 respectively, whereas for *F. proteus* were h=0.892 and π=0.005.

Values of genetic distances between pairs of populations are given in Table 1 and ranged from 0.003 (between pA and gA) to 0.008 (between pP and pN). Genetic distance between the two taxa was estimated in a value of D=0.005. Pairwise genetic distances among haplotypes were used to construct a neighbour joining tree. As it can be seen by Figure 4, no clustering of haplotypes corresponded to specific taxon and/or sampling site was detected. All internal nodes are supported by relatively low bootstrap values (≤67%).

	gA	**gP**	**gN**	**pA**	**pP**	**pN**
gA		[0.001]	[0.002]	[0.001]	[0.002]	[0.001]
gP	0.005		[0.002]	[0.001]	[0.002]	[0.002]
gN	0.005	0.006		[0.002]	[0.002]	[0.002]
pA	0.003	0.005	0.005		[0.002]	[0.001]
pP	0.006	0.008	0.006	0.007		[0.002]
pN	0.004	0.006	0.007	0.004	0.008	

Table 1. Genetic distances among the studied population samples, based on 16S rDNA sequences and the 2-parameter Kimura model. SE values are shown in brackets. gA: *F. glaber* from Airport; gP: *F. glaber* from Paliomana; gN: *F. glaber* from Naziki; pA: *F. proteus* from Airport; pP: *F. proteus* from Paliomana; pN: *F. proteus* from Naziki.

The analysis of the partitioning of the haplotype diversity indicated that the majority of the genetic variation (96.98%) was distributed within populations (Table 2) and only a percentage of 4.53% could be attributed to variation among populations within groups. AMOVA with two groups (i.e. *F. glaber* versus *F. proteus*) revealed a low F_{ST} value of 0.0295 and showed that only a 1.52% of the genetic variation occurred among groups. Pairwise exact test (Raymond & Rousset, 1995) for samples of both taxa showed no population differentiation with all *P* values>0.05 (*P*=1.000 for all the estimates). When pooling together

Source of variation	**Df**	**Variance components**	**Percentage of variation**
Among groups	1	-0.006	-1.52
Among populations within groups	4	0.020	4.53
Within populations	129	0.430	96.98
Total	134	0.444	100

Table 2. Analysis of molecular variance, in populations of both taxa. Df: degrees of freedom. The fixation indices are: F_{CT}=- 0.01518; F_{SC}=0.03017; and F_{ST}=0.04467.

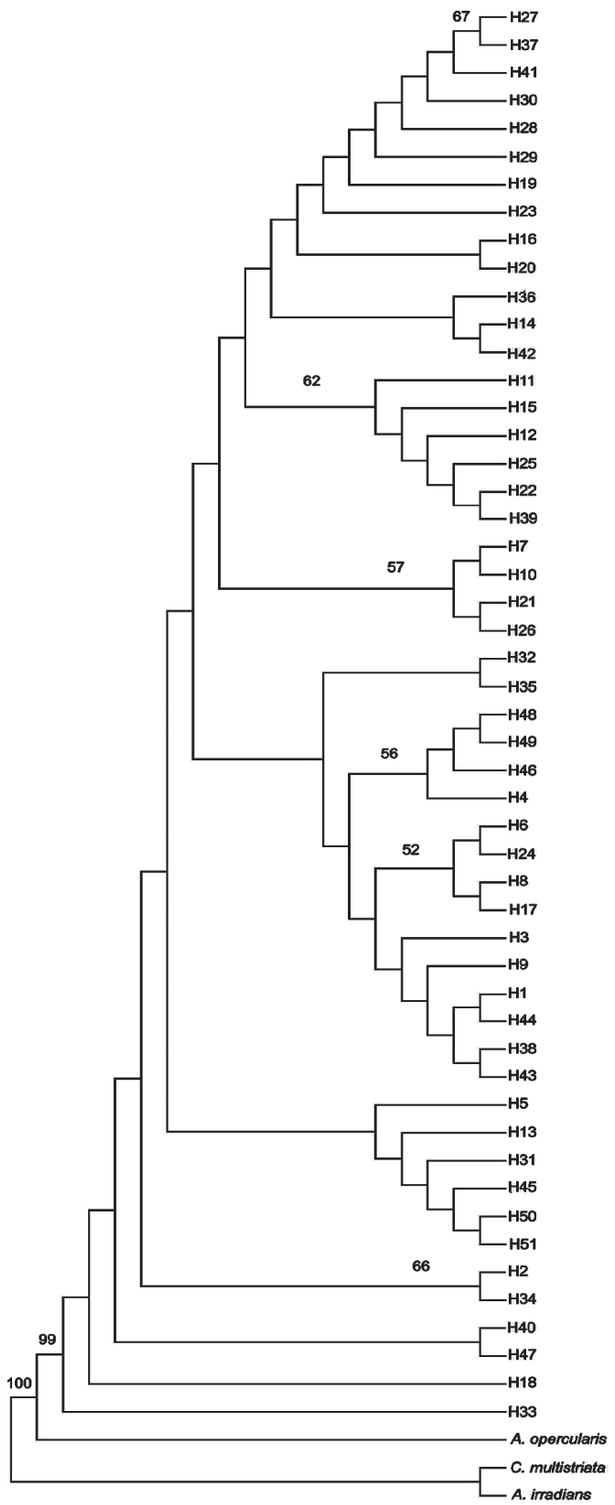

Fig. 4. Phylogenetic tree of the 51 haplotypes of *F. glaber* and *F. proteus* taxa recovered from 16S rRNA sequences, estimated by the Neighbor-joining method. Only bootstrap values based on 100 replications higher than 50% are displayed.

the three sampling sites of *F. glaber* and *F. proteus* respectively, the exact test of population differentiation was still not statistically significant (*P* values=0.20).

Mismatch distribution was calculated for all studied populations of both taxa (Fig. 5). All populations followed unimodal distribution (i.e a bell-shaped distribution) that it is assumed to be the signature of population expansion occurred probably after a bottleneck event. However, Tajima's *D*-values were not significantly negative for any of the studied populations (p=0.05–0.1), thus rejecting the hypothesis for population growth.

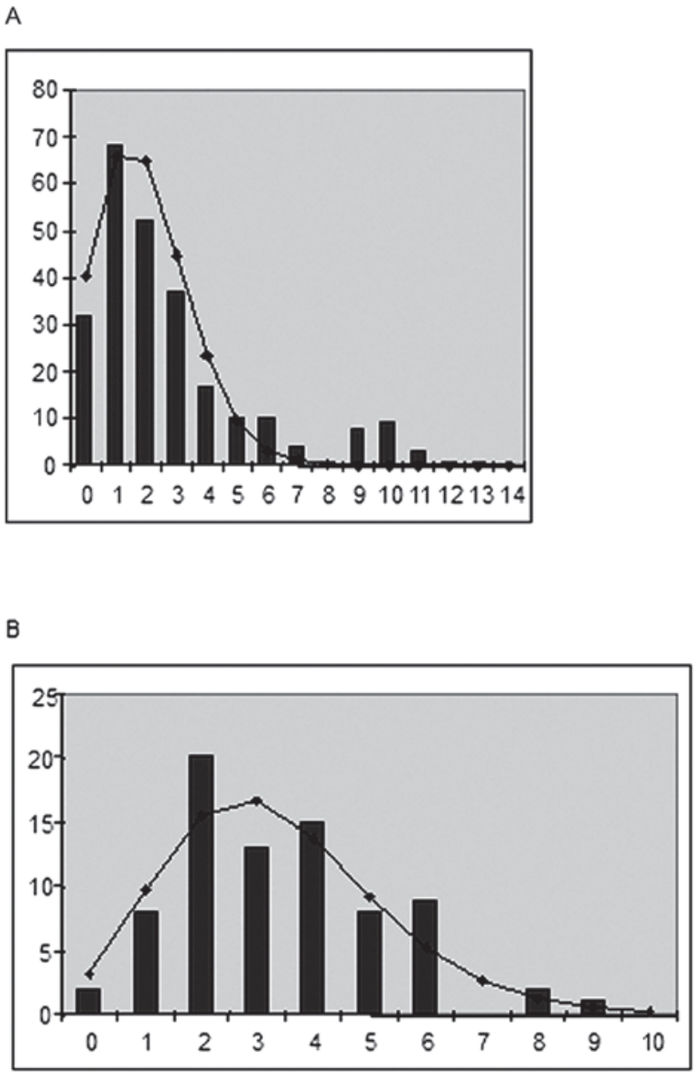

Fig. 5. Mismatch distributions for A) *F. glaber* and B) *F. proteus*. All studied samples followed the same unimodal distribution and thus graphs depict frequencies of pairwise differences for one of the populations of each taxon. The observed frequency of pairwise nucleotide differences among sequences is represented by black bars, and expected frequencies under a model of sudden population expansion are represented by continuous line.

4. Discussion

4.1 Levels of population variation

Low levels of intraspecific variation reported by many authors (Palumbi, 1996; Stepien et al., 1999; Saavedra & Pena, 2004; Pujolar et al., 2010) indicate that the mt 16S rDNA gene is highly conserved in many taxa, apparently due its functional role in protein assembly. However, it was not the case in the present study. Values of nucleotide diversity found for *F. glaber* (π=0.004) and *F. proteus* (π=0.005) are higher than the values reported for Adriatic populations of the same taxa (π=0.001 and π=0.000 respectively) (Pujolar et al., 2010). Also our values are higher than those estimated for other Pectinidae taxa as *Chlamys nobilis* (π =0.0012; Yuan et al., 2009), *C. farreri* (π=0.0035; Kong et al., 2003), *Pecten jacobaeus* (π=0.0015), *P. novaezelandiae* (π=0.0020), *P. fumatus* (π=0.000) and *P. maximus* (π=0.0039) (Saavedra & Pena, 2004).

The levels of intrapopulation variation for *F. glaber* and *F. proteus*, revealed by sequences of the mt DNA 16S rDNA region in the present study are also relatively high. Values of haplotype diversity ranged from 0.7793 to 0.9744 for populations of both taxa, are much higher than the values of 0.000 and 0.400 reported for the Adriatic populations (Pujolar et al., 2010) and higher than the values of 0.5356 and 0.7328 reported for two wild populations of *C. nobilis*, using sequences of the same region (Yuan et al., 2009). Also the highest value of π revealed in our study (0.0078) exceeds much the value of π=0.0012 observed in *C. nobilis* populations (Yuan et al., 2009). Kong et al. (2003) reported lower to similar values of π (0.00175 to 0.00591) and similar values of h (0.750 to 1.000) for different populations of Zhikong scallop *C. farreri*, based on the sequencing analysis of 16S ribosomal gene. Also, values of nucleotide and haplotype diversity observed in our samples are considerably higher than the values observed for different populations of the Asian moon scallop *Amusium pleuronectes* (0.000 to 0.0017 for π; 0.000 to 0.0511 for h) (Mahidol et al., 2007b).

The demographic analysis revealed that all the studied samples of both taxa were probably stable over time. Pujolar et al. (2010) who have studied the demographic history of the Adriatic *Flexopecten* complex using three mitochondrial and one nuclear gene proposed that the lack of large demogarphic expansion since the last glaciations, suggests that colonization must have been conducted by a substantial number of individuals that occupied the new habitat relatively fast. Demographic expansion does not appear either as a correct explanation of the observed genetic variation in our samples. A possible explanation for high values of intrapopulation indices for *F. glaber* and *F. proteus* could be the large effective population sizes as genetic variability is abundant. It is common knowledge that large *Ne* results to large amounts of neutral variability in populations.

An alternative hypothesis to explain this finding is that there is more than one species in the data set, but the character used to identify it is not valid. In other words, there may be more than one evolutionary entity, so we are artificially lumping more than one species in the analysis. One way to sort this out was to include other taxa in the analysis and make a phylogenetic analysis of the haplotypes, to see if they form unique phylogenetic species. There may simply be cryptic species to be considered or perhaps the morphotype doesn't correspond to the phylotype, which would certainly not be the first time in molluscs (Knowlton, 2000). The phylogenetic analysis of haplotypes revealed no clustering corresponding to each putative species (*F. glaber* and *F. proteus*). Also, the taxa *A. irradians* and *C. multistriata* formed a separate group in the Neighbour – Joining tree.

The decreased intrapopopulation genetic diversity can have serious consequences on the survival and reproduction of the species (Soule, 1980). Nevertheless, in the marine invertebrates reduction of genetic variation is expressed mainly with the loss of rare haplotypes despite with the total reduction of heterozygosity in a population (Gosling, 2003). Our samples are characterized from high DNA variability and the existence of some rare haplotypes, a result which indicates the good population status for both taxa. As the fishing of the Aegean *F. glaber* and *F. proteus* stocks is forbidden since 2002 because of heavy metals (Cd) presence in high concentrations (Koutsoubas et al., 2007), our data are fully justified.

Despite the high degree of genetic variation found within populations, the results of the statistical analysis of the 16S rDNA haplotypes indicated that little geographic structure was present among populations. The genetics of marine species with pelagic larval development has often been characterized by low genetic variation among populations, a pattern driven by high dispersal capabilities and large scale oceanic mixing (Reichow & Smith 2001; Rivera et al., 2004). After spawning and external fertilization, developing larvae spend a variable period of time as part of the plankton, which can be passively drifted by water currents (Seed, 1969; Cho et al., 2007). Similarly, gene flow among the populations of the present study seems not to be significantly blocked in this relatively small area of the Gulf of Thessaloniki. Consequently, our results support the general observation that marine invertebrates possessing a planktonic larval stage have a high genetic relatedness due to increased potential for larval dispersal (Crisp, 1978).

4.2 Systematic status of the two taxa

The presence of *F. proteus* in the Aegean Sea does not support the restricted gene flow or the recent origin of this form, reported by Pulojar et al. (2010). It seems that the high dispersal of the larval stage has provoked the expansion of this morph outside of the Adriatic Sea or *F. proteus* has appeared for the first time in other Mediterranean region, apart from Adriatic Sea.

The phylogenetic position of *Chlamys glabra* (now *F. glaber*) is under discussion. Previous phylogenetic data (Canappa et al., 2000a, 2000b; Barucca et al., 2004; Liu et al., 2007) support the idea that *C. glabra* seems to belong to a completely distinct genus from *Chlamys*, in agreement with some malacologists who have included it in the genus *Flexopecten* (Vaught, 1989; Rombouts, 1991). According to these studies *C. glabra* is clustered together with *Aequipecten opercularis* and not with other species of the genus *Chlamys* i. e. *C. islandica*, *C. varia* and *C. farreri*.

Later on Saavedra & Pena (2006) used the updated latin name *F. glaber* instead of *C. glabra* in a phylogenetic analysis for American scallops, and they also found that taxa *F. glaber* and *A. opercularis* clustered together. There has been one effort for identification of *C. glabra* larvae from other bivalve species larvae, with a method based on PCR - SSCP combined with sequencing of partial 18S rDNA region, but it was not successful (Livi et al., 2006). In the latest study of the two Adriatic *Flexopecten* forms using three mitochondrial and one nuclear gene, Pujolar et al. (2010) suggest that *F. glaber* and *F. proteus* are the same species as evidenced by both putative species appearing mixed in all genetic trees, with no clustering according to species.

The present study tried to resolve the two Aegean taxa relationship using a mitochondrial gene. Sequencing analysis of 16S rDNA revealed no taxon specific positions that could discriminate between different species. Additionally, two highly frequent and six less frequent haplotypes are shared between the two taxa, while no clustering in different groups was detected. This is an evidence of interbreeding between *F. glaber* and *F. proteus*, suggesting that they are conspecific taxa. Similarly, Wilding et al. (1999) found no high frequency private haplotypes within *P. maximus* and *P. jacobaeus* after a mtDNA PCR - RFLP analysis, and this fact together with the low genetic divergence demonstrated that the two species are also capable of hybridization. Later on, Saavedra & Pena (2004), in a phylogenetic analysis of *P. maximus* and *P. jacobaeus* noticed that the two most common haplotypes appeared in both taxa and this supports the held view of conspecificity between the two European taxa.

Another evidence of conspecific taxa was the levels of genetic distance. The genetic distance found between the two taxa (D=0.005) does not suggest genetic differences between two species, and shown to be of a similar magnitude to intraspecific than interspecific values. This value is considerably lower than expected for congeneric species (Liu et al., 1998; Stepien et al., 1999; Saavedra & Pena 2004; Yu et al., 2004; Mahidol et al., 2007a). Our data cannot even justify the classification of *F. proteus* as a subspecies of *F. glaber*. According to Therriault et al. (2004), the previous classification of *Dreissena bugensis* as a subspecies of *D. rostriformis* should be revised because genetic distance between them was relatively low (0.003–0.004; a value similar to our results), suggesting that these two taxa constitute a single species.

The existence of a single species in our study is also reinforced by the exact test of population differentiation. When pooling together the three sampling sites of *F. glaber* and *F. proteus* respectively, no statistically significant population differentiation was detected. So, our findings support the aspect that *F. proteus* is only a form or simply a synonyme of *F. glaber*.

In Bivalve, differences in shell morphology are often the result of phenotypic plasticity, so that many past descriptions of species were unjustified (reviewed by Knowlton, 2000). Consequently, the number of genetic studies that have resulted in the synonimization of species is probably higher for bivalves than most other marine invertebrate phyla. Our study is one example of numerous cases where distinctions between sympatric taxa based on shell morphology were not supported by genetic data (Wilding et al., 1999; reviewed by Knowlton, 2000). Present data reinforce also the validity of the mtDNA markers for clarifying uncertain systematic relationships among taxa.

A global DNA-based barcode identification system that is applicable to all animal species provides a universal tool for the identification of different species. The barcode system is based on sequence diversity in a single gene region (a section of the mitochondrial DNA cytochrome c oxidase I gene, COI). When the reference sequence library is in place, new specimens can be identified by comparing their DNA barcode sequences against this barcode reference library. Herbert et al. (2004a, 2004b) have demonstrated that the COI region is appropriate for discriminating between closely related species and these results have prompted international efforts to accelerate the process of cryptic species identification (Herbert et al., 2003). A similar survey based on the barcode identification system, could be done in the future for the two *Flexopecten* forms, in order to confirm the existence of a single species.

5. Appendix

	Variable nucleotide sites					Haplotype frequency							
	1	1111112222	2222222223	3333333333	3344444444								
	555556790	1559990111	2335666781	2223566889	9900013333								
	8567890628	7244785678	9450035343	2699836693	7912603456	gA(30)		gP(23)	gN(25)	pA(18)	pP(13)	pN(26)	Total
H1	TGTCTTTTCT	TGCAAATGGG	GGTTGAATCT	TTTTGTCTTT	GCGTTTATTG	12	7	3	7	–	5	34	
H2	....	....G.....	....	....	.T........	1	–	–	–	–	–	1	
H3	....	....	....	....	........A.	1	1	–	–	–	2	4	
H4	....	....	....	.......C..	....	1	–	–	–	–	–	1	
H5	....	....	....	....	.T........	8	5	1	4	2	8	28	
H6	C	....	....	....	....	1	–	–	–	–	1	2	
H7	....	....	....	C.........	.A........	1	–	–	–	–	–	1	
H8	....	....	.....G....	....	....	2	–	–	–	–	–	2	
H9	....	......C...	....	....	....	1	–	–	–	–	–	1	
H10	....	....	....	C.........	.T........	2	2	–	1	–	–	5	
H11	....	...G......	....	.......C..	.T........	–	1	–	–	–	–	1	
H12	C.........	...G......	....	....	.T......A.	–	1	–	–	–	–	1	
H13	....	....	....	....	.T......A.	–	1	–	–	–	–	1	
H14	..CA......	...G......	C	....	.T..A.....	–	1	–	–	–	–	1	
H15	....	...G......	....	....	.T........	–	1	3	–	1	–	5	
H16	....	...G...TTC	....C.....	.CA.A.....	..C.......	–	1	–	–	–	–	1	
H17	C	....	.....G....	....	....	–	1	–	–	–	–	1	
H18	....	....	......C...	....	.T......G.	–	1	–	–	–	–	1	
H19	....	...G......	....	....	....	–	–	7	–	2	–	9	
H20	....	...G......	....	....	.TC.......	–	–	1	–	–	–	1	
H21	....	...G......	....	C.........	.T........	–	–	1	–	1	–	2	
H22	....	...G......	....	...A......	.T.....G..	–	–	1	–	–	–	1	
H23	....	...G......	.A........	....	....	–	–	1	–	–	–	1	
H24	C	...G......	C.........	....	....	–	–	1	–	–	–	1	
H25	....	...G......	........T.	....	.T........	–	–	1	–	–	–	1	
H26	....	C..G......	....	C.........	....	–	–	1	–	–	–	1	
H27	.C........	...G......	....	....	....	–	–	1	–	–	–	1	
H28	....	...G......	.......C..	....	....	–	–	1	–	–	–	1	
H29	....	...G......	....	....	.......G..	–	–	1	–	–	–	1	
H30	....	...G......	....	......T...	....	–	–	1	–	–	–	1	
H31	....	....	....	....	.T.......T	–	–	–	1	–	–	1	
H32	....	....	...C......	....	.T........	–	–	–	1	–	–	1	
H33	....	....	......C...	....	.T........	–	–	–	1	–	–	1	
H34	....	....G.....	....	....	.T......G.	–	–	–	1	–	–	1	
H35	....	....	...C......	....	....	–	–	–	1	–	–	1	

H36	..C....... ...G......	–	–	–	1	–	–	1
H37	.T........ ...G...... ..C.......	–	–	–	–	1	–	1
H38	.....A..T.	–	–	–	–	1	–	1
H39	....A..... ...G...... .... .T........	–	–	–	–	1	–	1
H40	.... .AT....... .... .T........	–	–	–	–	1	–	1
H41	....G...... ....G.... ....G..	–	–	–	–	1	–	1
H42	..CT..A... ...G...... ...C...... .... .T.....G..	–	–	–	–	1	–	1
H43	....G.	–	–	–	–	1	–	1
H44	....G....	–	–	–	–	–	1	1
H45	.... .T....T.G.	–	–	–	–	–	2	2
H46	.......A.. ....A....G	–	–	–	–	–	1	1
H47	....T....... .... .T........	–	–	–	–	–	1	1
H48	.......A.. ....A..AC. A..CC.....	–	–	–	–	–	1	1
H49	.......A.. ....C...... ....CC.....	–	–	–	–	–	1	1
H50	.... .T......G.	–	–	–	–	–	2	2
H51	...T...... .... .T........	–	–	–	–	–	1	1
No. polymorphic sites		9	21	15	8	16	15	
No. haplotypes		10	12	15	9	11	12	
Haplotype diversity (h)		0.7793	0.8735	0.9100	0.8235	0.9744	0.8769	
Nucleotide diversity (π)		0.0027	0.0065	0.0042	0.0032	0.0078	0.0053	

Appendix 1. Polymorphic sites, haplotype frequencies and genetic diversity indices, in all three studied populations of both taxa. The nucleotides in each position are given in comparison to haplotype H1. For all haplotypes variable sites are indicated, while identity is given by dots. In brackets it is the sample size. gA: *F. glaber* from Airport; gP: *F. glaber* from Paliomana; gN: *F. glaber* from Naziki; pA: *F. proteus* from Airport; pP: *F. proteus* from Paliomana; pN: *F. proteus* from Naziki.

6. Conclusion

We developed a DNA methodology based on PCR amplification and sequencing analysis of the mitochondrial 16S rDNA gene, in order to discriminate two different taxa of the family Pectinidae - *Flexopecten glaber* and *Flexopecten proteus* - and for a population study of both taxa. Fifty polymorphic nucleotide sites and fifty one different haplotypes were revealed in total. The majority of the genetic variation (96.98%) was distributed within populations. The levels of haplotype and nucleotide diversity were relatively high for both taxa, probably due to the large effective population sizes and the good population status. No species specific position was found and the two highly frequent haplotypes are shared between taxa, suggesting possible hybridization between them. Genetic distance between *F. glaber* and *F. proteus* was D = 0.005 and no genetic differentiation was revealed among taxa, when pooling together samples from different localities. Our results show that *F. glaber* and *F. proteus* are conspecific, which is in agreement with the latest classification.

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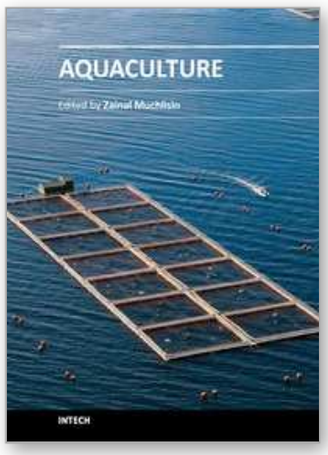

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This book provides an understanding on a large variety of aquaculture related topics. The book is organized in four sections. The first section discusses fish nutrition second section is considers the application of genetic in aquaculture; section three takes a look at current techniques for controlling lipid oxidation and melanosis in Aquaculture products. The last section is focused on culture techniques and management, ,which is the larger part of the book. The book chapters are written by leading experts in their respective areas. Therefore, I am quite confident that this book will be equally useful for students and professionals in aquaculture and biotechnology.

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