Genetic population structure of scallops going through a rapid population decline in the Eastern Mediterranean

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Abstract
Genetic structure of the endemic *Pecten jacobaeus* of Aegean inferred from mitochondrial 16s DNA sequence analysis was performed. This genetic marker is a powerful tool for measuring genetic variation and gene flow among populations. Valuable scallop stocks were abundant in the past in Euboikos gulf and Aegean but now are severely depleted due to overfishing, pollution and ocean warming. To restock and conserve this bivalve, a better understanding of its genetic variability is essential. DNA was isolated using the nucleospin tissue kit after homogenization of ligament and PCR using genus specific primers. The PCR products were analyzed by sequencing. The genomic comparison was performed by Blast analysis. We studied two hypotheses: **1**. Scallops (*P. maximus & P. jacobeus*) which can be easily distinguished by the shell morphology belong to the same species without a significant genetic variation or not **2**. The possibility to extract DNA from dead shells, a good practice for protected or endangered species. We showed that it is possible to extract DNA from ligaments of dead shells exposed for longtime on the seabed. A high similarity (>99%) between our results for *P. jacobeus* (16s rRNA genome) and *P. Jacobeus* or *P. maximus* nucleotide collections was revealed
Keywords: Pecten, sequencing, genetic differentiation

1. Introduction

In Europe the most valuable species are the Atlantic (*Pecten maximus*) which enters in Mediterranean up to Almeria Oran front and further in the E. Mediterranean basin it is replaced by *P. jacobaeus*. These two scallops have been considered for decades as two different species with distinct morphological characteristics (phenotype). The superfamily Pectinoidea possess an alivincular ligament system having a central, internal resilium with a medial core of ligamental material (Waller, 2006). Valuable scallop stocks were abundant in Greek waters since antiquity but now are severely depleted due to aquaculture, overfishing, pollution, and ocean warming. Apart from natural processes, the genetic variability of wild populations can be affected by anthropic pressures (Telahigue et al, 2018) that affect the scallop population dynamics in Euboikos bay. Understanding the connectivity among the two scallops populations and the scale of their interaction is crucial for the study of their population dynamics and sustainable fisheries management. DNA is a hydrophilic molecule and both elution and degradation may contribute to the quickly reduced suitability of archived/dead shells. Previous studies demonstrated that exposure time of dead shells to habitat result in a fast natural degradation of the size and quantity of DNA. The highest yield of DNA obtained from fresh shell decreased to 0% for dead ones being exposed to stream water for more than 3 months (Geist et al, 2008). The inevitable degradation of DNA by endogenous enzymes & microbes after death presents problems in genetics. However, degradation of DNA molecules is slower if the DNA is built-into a mineral matrix like shell (Doherty et al, 2007).

2. Materials and methods

We focused our study on the mitochondrial gene (16S rRNA) because it has proved to be very useful (Canapa et al, 2000) for systematics and phylogenetic relationships of bivalves. The evolutionary rate of the mitochondrial DNA of bivalves is highly variable, and Pecteniidae seems to be in the low-rate side of the distribution (Saavedra & Pena, 2004). Sampling of alive specimens in declining and endangered marine species such as *P. jacobeus* is not optional because their populations is almost extinct but empty or dead shells are still abundant on the sea bottom of bays providing an easy and nondestructive sampling alternative. In order to extent the study of *P. jacobeus* further to the East, 4 samplings of dead scallops around S. Euboikos Bay by divers took place in 2018; at locations where in the past robust populations lived. Also, an archived specimen fished in 1986 in Korinthiakos bay was examined (in pool s.2). The time exposure of our shells into habitat varied from some hours (one shell was undamaged as fresh) to many years. The biometry of 25 shells measured (L= 42-104 mm) and their ligament (24 scallops) or adductor muscle of 1 scallop (muscle s.2) was collected with clean forceps. Ligaments of 11 dead shells were pooled (pool s.2). Afterwards, ligaments were pulverized on a metal mortar and stored in clean tubes for DNA extraction, amplification and sequencing (partial sequences of 16S r RNA gene) according to (Saavedra & Pena, 2005) for a better comparison with historical data.

Genomic DNA was extracted from 30-50 mg of tissue using the NucleoSpin Tissue kit (Macherey-Nagel) and DNA was purified by means of the Nucleospin Extract II kit (Machery-Nagel) following the supplier's protocol. The identification achieved by comparing the sequences/ alignments found in our samples with 24 reference databases published in GenBank for a wide distribution range of Pectinidae by NCBI BLAST software (Table 1).

3. Results & Conclusion

Table 1. BLAST output in hit table format. E-values is 0 for each alignment pair in this search. Queries are our sequences against 24 reference sequence databases. Only the identities **id.%** ≥ 99 % are shown. (**a.l**. is alignment length, **s.b**. is bit score)

Query	*Subject:* Ref. databases	id.%	a.l.	s.b.
s.14F	*JQ611455.1 P.jacobaeus & JX624722.1 P.maximus*	100	520	961
s.14F	*AY650057.1 P.maximus & MF183948.1 P.jacobaeus*	100	510	942
	JQ611455.1 P.jacobaeus & JX624722.1 P.maximus	100	464	857
	AY650057.1 P.maximus & MF183948.1 P.jacobaeus	100	483	893
s.14F	*FN667668.1 P._maximus*	99.8	540	992
s.14F	*MF183949.1 P.jacobaeus*	99.8	509	935
muscle S.2	*FN667668.1 P.maximus*	99.8	484	889
muscle S.2	*AY650060.1 P.maximus & MF183949.1 P.jacobaeus &*	99.8	483	887
s.14F	*AJ972436.1 P.maximus*	99.8	478	878
s.14F	*AJ245394.1 P.jacobaeus*	99.6	520	948
s.13F	*JQ611455.1 P.jacobaeus & JX624722.1 P.maximus*	99.2	519	944
s.13F	*AY650057.1 P._maximus*	99.2	510	928
s.13F	*MF183948.1 P.jacobaeus*	99.2	509	926
pool s.2	*KF982791.1 P. maximus*	98.6	492	876
pool s.2	*JQ611455.1 P.jacobaeus & JX624722.1 P.maximus*	98.9	464	835
pool s.2	*MF183948.1 P.jacobaeus*	99	483	870

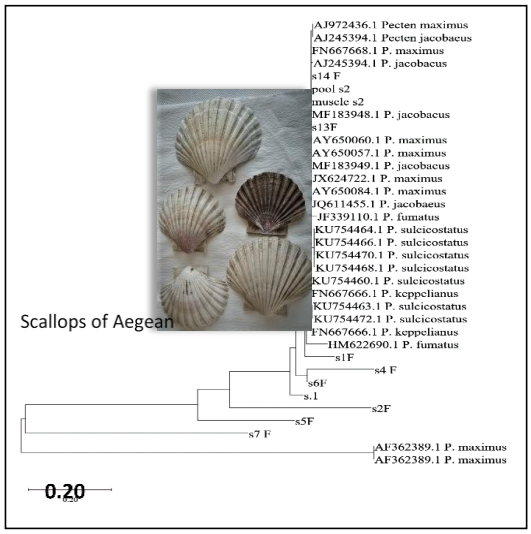

Figure 1. The evolutionary history was inferred by using the Maximum Likelihood method and Tamura-Nei model by MEGA X. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site, next to the branches. This analysis involved 35 nucleotide sequences, with a total of 1405 positions in the final dataset.

Conclusions. Our results show that the ligament taken from dead scallops lying on the seabed for a long time period can be a good source of DNA especially if pool samples are used. Separate ligament analysis (s. 1, 2, 4, 5, 6, 7) showed more mismatches and substitutions per site. It was shown no genetic differentiation between European scallops, supporting the hypothesis that they could be races or conspecies. An interesting output is also the low genetic differentiation between *P.maximus & P.jacobaeus* and *P.sulcicostatus P.fumatus & P. keppelianus*. Particularly, samples s.13F, s.14F & s.2 showed **identities** >97% with **bit scores** (>830) with those 3 species abovementioned. Genetic differentation in the Euboikos bay however, can also be heavily influenced by various anthropogenic activities (illegal fisheries, aquaculture, pollution, etc). Further study using more genes are necessary to understand better the evolutionary relationship among all scallop species albeit the genetic similarity between Mediterranean scallops is very high.

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