

## STATUS OF *MYTILUS GALLOPROVINCIALIS* LAMARCK, 1819, IN THE SOUTHEASTERN ADRIATIC CONFIRMED BY GENETIC MARKERS

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### Abstract

The species of the *Mytilus* complex are difficult to differentiate morphologically because of their high degree of similarity and the impact of the environment on the shell shape. A nuclear DNA marker Me 15 and 16 (adhesive protein gene), which is diagnostic for *Mytilus galloprovincialis*, *M. edulis* and *M. trossulus* was examined for 262 individuals (wild and cultured) sampled along the south-eastern Adriatic coast. All individuals proved to be *M. galloprovincialis*.

**Keywords :** *Adriatic Sea, Biodiversity, Bivalves, Genetics.*

*Mytilus* spp. are considered key species of the coastal ecosystem in the Adriatic sea and are commercially valuable. Genetic characterization of their populations may serve to monitor changes in diversity, and possibly for understanding the biodiversity changes in the coastal ecosystems in the Adriatic sea. Changes in current directions, temperature and salinity, related to possible global climate changes, higher number of commercial vessels may impact the distribution of mussels [3]. Around Mali Ston *Mytilus* cultivation has long and successful tradition, which has been mass-cultured since the second half of the 20th century. The origins of *Mytilus* spp. in Mali Ston culture facilities, as well as in other parts of eastern Adriatic coast, are unknown. During 2005 and 2006, as part of comprehensive project concerning *Mytilus* species complex in the Mediterranean and parts of the eastern Atlantic, we conducted series of samplings along the eastern Adriatic from Boka Kotorska bay to the Limski channel. The results of these investigations are presented in this paper.

Me 15 CCA GTA TAC AAA CCT GTG AAG A  
Me 16 TGT TGT CTT AAT AGG TTT GTA AGA

Fig. 1. Sequences of Me15 and 16 PCR primers.

Species within the *Mytilus* complex are difficult to differentiate morphologically because of their high degree of similarity and due the impact of the environment on the shell shape [3]. However some differences in certain "variable region" of a sequence in the nonrepetitive domain of the foot protein 1 are correlated with the taxonomic rank of these species. The length of nonrepetitive region fragments amplified with Me15 and 16 [2] is specific to each species and differs interspecifically, enabling the use of these primers as diagnostic markers for determination and confirmation of the species.

We sampled 262 individuals from 10 locations: 1. Boka Kotorska bay (cultured); 2. Boka Kotorska bay (wild); 3. Konavle Rocks (wild); 4. Rijeka Dubrovacka (wild); 5. Elafiti islands (wild) 6. Vrnjak (wild); 7. Bistrina bay (cultured); 8. Brijesta (wild); 9. Brijesta (cultured); 10. Ploce harbour (wild). Following DNA extraction (Quiagen, Dneasy Tissue kit), and PCR amplification using genetic markers, electrophoresis revealed one single and uniform band for all of investigated individuals on the position characteristic for *M. galloprovincialis*. Expected size is 126 bp because the sequence of *M. galloprovincialis* contains deletion of 18 amino acids. For the final verification we obtained the standard confirmed total DNA of *M. galloprovincialis*, *M. edulis* and *M. trossulus* and upon comparison of these bands on electrophoreses - all of our individuals equaled the band position as that of standard *M. galloprovincialis* (Fig. 2). We suggest that *M. galloprovincialis* is an invasive species by its nature [1] and that trait may be the reason for preserving its purity.

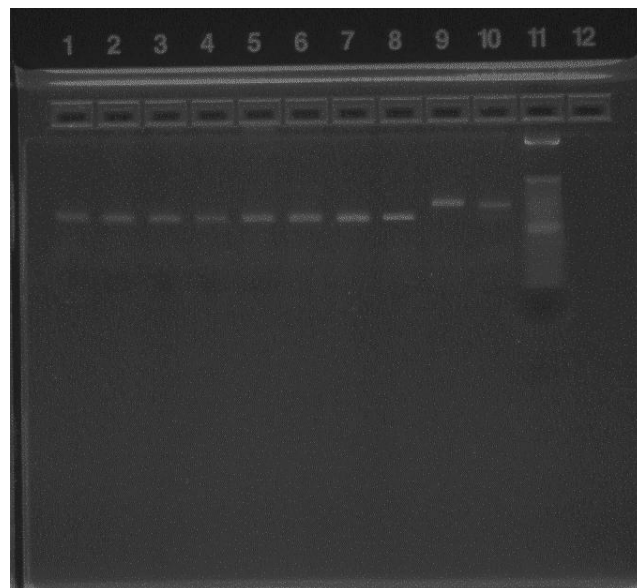


Fig. 2. Photo of electrophoresis gel. Lane 1 to 7: mussels sampled during this investigation; lane 8: standard DNA of *M. galloprovincialis*; lane 9: standard DNA of *M. edulis*; lane 10: standard DNA of *M. trossulus*; lane 11: DNA ladder.

### References

- 1 - Geller JB, Carlton JT, power DA (1994) PCR-based detection of mt DNA haplotypes of native and invading mussels on the northern pacific coast: latitudinal pattern of invasion. *Marine Biology* 119:243-249.
- 2 - Inoue K, Waite JH, Matsuoka M, Odo S, Harayama S (1995) Interspecific variations in adhesive protein gene sequence of *Mytilus edulis*, *M. galloprovincialis* and *M. trossulus*. *Biol Bull (Woods Hole)* 198: 370-375.
- 3 - Smietanka b, Zbawicka M, Wolowicz M, Wenne R (2004) Mitochondrial DNA lineages in the European populations of mussels (*Mytilus* spp.). *Marine Biology* 146: 79-92.