INTRODUCTION

The mussels of *Mytilus* genus belong to a group of key species in marine, coastal ecosystems. Species of the "*Mytilus edulis* complex": *M. edulis* Linnaeus, 1758; *M. galloprovincialis* Lamarck, 1819 and *M. trossulus* Gould, 1850 look very similar to each other, and hybrids are also indistinguishable from the pure species [1,2] (Fig. 1). In Europe *M. galloprovincialis* is found in the Black Sea, the Mediterranean Sea and the Iberian Atlantic coasts, *M. edulis* predominates in central and northern Europe, and *M. trossulus* occurs in the Baltic Sea and presumably in some other areas in northern Europe [3,4]. Identification based on shell shape and morphometric parameters is usually uncertain because of the extreme shape plasticity exhibited by mussels under environmental variation [2,4,5]. Molecular markers now allow for positive identification of species although introgression and hybridisation sometimes obscure, if not biases when dismissed, the interpretation [5,6].

**Fig. 1.** Mussel species of "*Mytilus edulis complex": (M.g.) *M. galloprovincialis*, (M.e.) *M. edulis* and (M.t.) *M. trossulus*.

CONCLUSION

- Morphometric measures were within normal values for *M. galloprovincialis*.
- Dominant presence of Mediterranean mussel *M. galloprovincialis* along eastern Adriatic coast at all 22 sites (GG, Me 15/16 homes).
- Different alleles are reported in Croatian Adriatic Sea populations (E - *M. edulis* and T – *M. trossulus*). The presence of the E allele at low frequency in *M. galloprovincialis* mussels can simply be explained by introgression, as *M. edulis* and *M. galloprovincialis* are known to hybridise and exchange genes. Faraway from *M. trossulus* distribution area, the presence of the T allele is very surprising.
- COI sequences analysis identified typical Adriatic haplotypes (group 1. and 2.) with presence of *M. edulis* complex (M.g., M.L and M.e.) Atlantic/Baltic haplotypes. The unexpected haplotypes detected can be explained by *Mytilus* maternally and paternally inherited mitochondrial genomes, recent hybridization and/or relic of an ancient stock introgression [6].
- Further detailed morphometric and genetic analyses (different nuclear and mitochondrial loci) on larger numbers of specimens are needed to clarify the processes underlying origin and evolution of Mediterranean mussels *M. galloprovincialis*.

RESULTS

Morphometric characteristics of mussel shells

Morphometric characterization was done for collected *Mytilus galloprovincialis* (M.g.), on the basis of 110 specimens, commercial *M. edulis* (M.e.) and *M. galloprovincialis* (M.g.) Mar 20 specimens each. Morphometric measures were within normal values for *M. galloprovincialis*:

Extremes

<table>
<thead>
<tr>
<th>Metric</th>
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<th>25%-75%</th>
<th>Non-Outlier Range</th>
<th>Outliers</th>
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<tr>
<td>Height/Lenght</td>
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<tr>
<td>M.g. PJ</td>
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<td>M.g. Mar</td>
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<tr>
<td>M.e. Mar</td>
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<td></td>
<td>50</td>
<td>60</td>
<td>20-70</td>
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PCR sample analyses using Me 15/16 primers

*Mytilus galloprovincialis* (G) alleles were identified in all mussel specimens/samples at all 22 investigated sites. The presence of mussel heterozygote genotype were detected: *M. galloprovincialis - M. edulis* (GE-type alleles, site 9, Borik) and *M. galloprovincialis - M. trossulus* (GT-type alleles, site 15, Vranjic) (Fig. 4). Further there was a tendency for the height/length values of heterozygote specimens to be closer to *M. edulis* (GE: h/l 46.90 %; w/l 38.45 %, GT: h/l 48.91 %, w/l 39.11 %) (Fig. 3).

Sequence analyses of PCR COI products

Purified bands were directly sequenced using both primers and contig of obtained sequences were afterwards aligned with mitochondrial COI sequences: *M. galloprovincialis* NC_006886, *M. edulis* ATY484747 and *M. trossulus* KM192128 (Fig. 5). Neighbor-Joining consensus tree nucleotide alignment grouped sequences into four haplotype group: Most of our Adriatic mussels were in 1, 19 and 4, group (36 seq.), 2 was *M. edulis* haplotype group (9) and 3 was group sharing Atlantic and Baltic haplotype (M.g. and M.t. reference mitochondrial COI sequences). Interesting only our heterozygote Gussel match *M. edulis* COI haplotype.

Sequence analyses of SS ribosomal DNA

The 5S rDNA of heterozygous mussel GE, GT and representative GG (Lim bay) was amplified by PCR using contiguous primers. Two main products of 250 (alpha) and 760 bp (beta) were cloned and sequenced, revealing two classes of 5S rDNA units. Obtained GG, GE and GT alpha sequences were most similar to (beta) were cloned and sequenced, revealing two classes of 5S rDNA units. Obtained GG, GE and GT alpha sequences were most similar to

References