Genetic identification and population analyses of mussel *Mytilus galloprovincialis* along the eastern Adriatic coast: heterozygote/hybrid characterization

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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 obscures, 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*.

METHODS

- **Mussel sampling (Fig. 2)**
- **Morphometric analysis (Fig. 3)**
- **Total genomic DNA extraction**
- **PCR analyses using Me15/16, 5S rDNA and COI mtDNA primers**
- **Sequencing of 5S clones and PCR COI products**

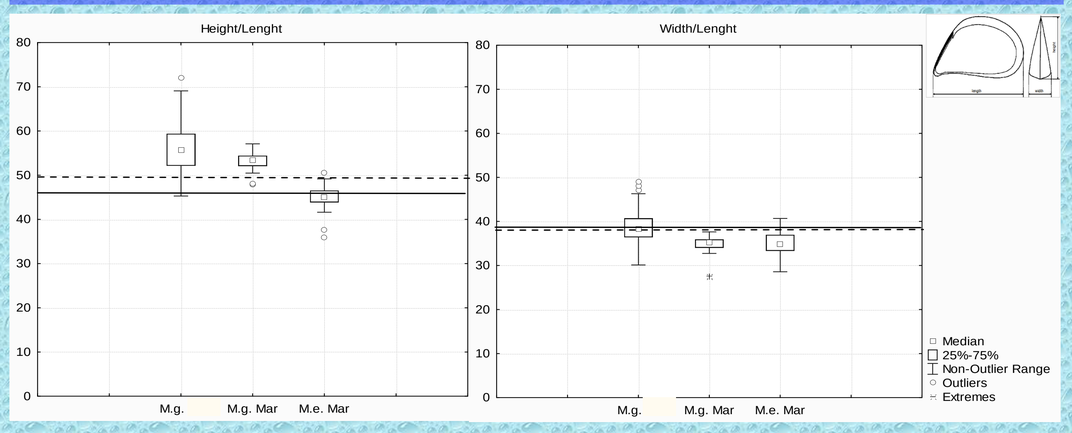

Fig. 3. Comparison of width/length and height/length ratios of Mediterranean mussel (M.g.) collected along Eastern Adriatic coast, commercial blue mussel (M.e. Mar) and commercial Mediterranean mussel (M.g. Mar). Heterozygous mussel GE (solid line): h/l 46.90%, w/l 38.45% and GT (dashed line): h/l 48.91 %, w/l 39.11%.

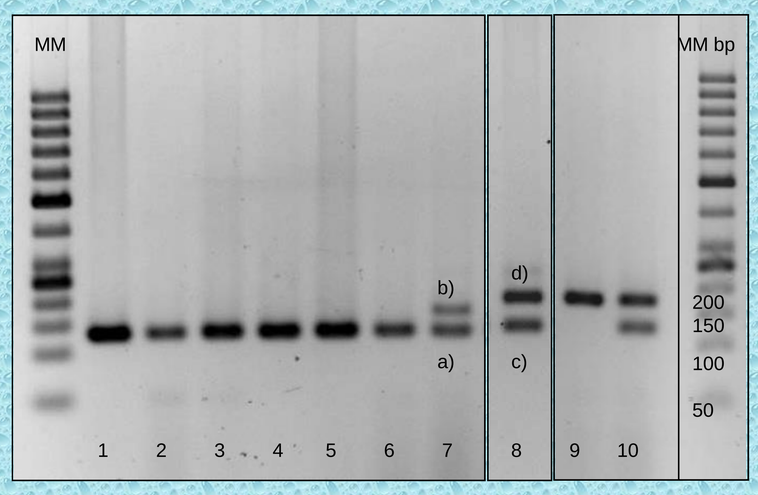

Fig. 4. Agarose gel with Me 15/16 PCR products: 1-6) *M. galloprovincialis*, 7) heterozygous mussel with *M. galloprovincialis* and *M. trossulus*-type alleles, 8) heterozygote mussel *M. galloprovincialis* and *M. edulis*-type alleles, 9) control *M. edulis* and 10) composite sample of *M. edulis* and *M. galloprovincialis* with Mw markers (MM). Mw of the bands: Lines 1- 6) GG 126 bp, 7a) G 126 bp, 7b) T 168 bp, 8c) G 126 bp, 8d) E 180 bp, 9) EE 180 bp.

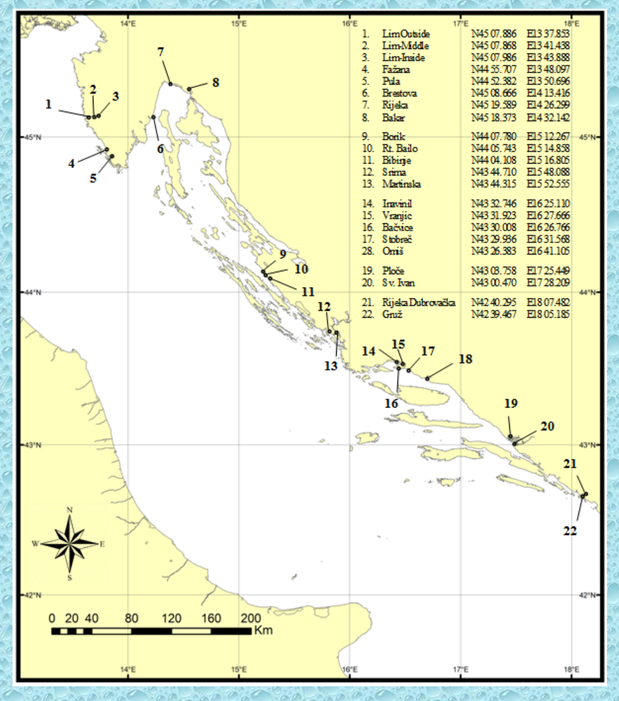

Fig. 2. Sampling locations along the Croatian coastal area of the Adriatic Sea.

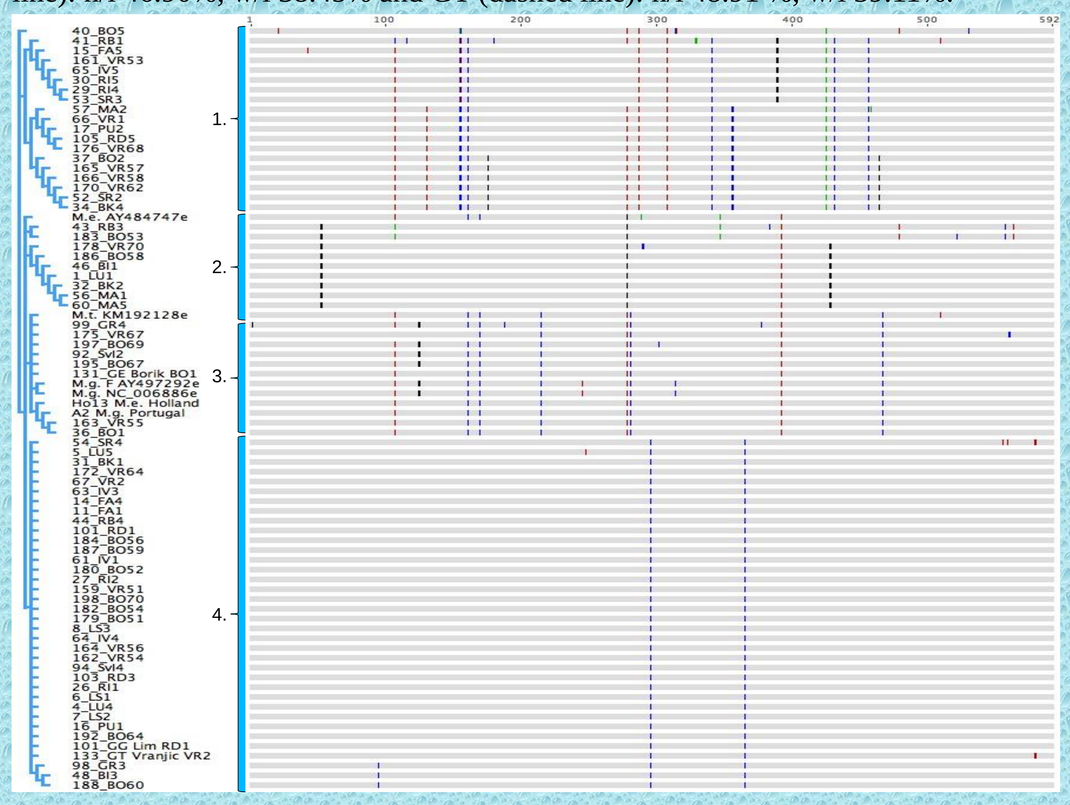

Fig. 5. Alignment of COI sequences with N-J tree: Heterozygous mussel 133_GT Vranjic and 131_GE Borik; *M. edulis* Ho13 M.e. Holland, *M. galloprovincialis* A2_M.g. Portugal; Mitochondiron NCBI COI sequences M.g. NC_006886, M.e. AY484747, M.t. KM192128.

RESULTS

Morphometric characteristics of mussel shells

Morphometric characterization was done for collected *Mytilus galoprovincialis* (M.g). on the basis of 110 specimens, commercial *M. edulis* (M.e. Mar) and *M. galloprovincialis* (M.g. Mar) 20 specimens each. Morphometric measures were within normal values for *M. galloprovincials*: height/length 55.89 ± 5.08 % and width/length 38.76 ± 3.42 % (Fig. 3).

PCR sample analyses using Me 15/16 primers

Mytilus galloprovincialis (G) alleles were identified in all mussel samples/specimens 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 mitochondiron COI sequences: *M. galloprovincialis* NC_006886, *M. edulis* AY484747 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 mitochondrion COI sequences). Interesting only our heterozygote GE mussel match *M. edulis* COI haplotype.

Sequence analyses of 5S 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 *M. edulis* (AJ312082) and *M. galloprovincialis* (AJ312076), indicating recent hybridization or presence of an ancient *Mytilus* stock.

CONCLUSION

- **Morphometric measures were within normal values for *M. galloprovincials*.**
- **Dominant presence of Mediterranean mussel *M. galloprovincialis* along eastern Adriatic coast at all 22 sites (GG, Me 15/16 locus).**
- **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.t. 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 relict 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*.**

References

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