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# Karyological study reveals a putatively distinctive population of the European flat oyster (*Ostrea edulis*) in Mali Ston Bay, Croatia

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*The European flat* oyster (Ostrea edulis) *was described in Mali Ston Bay (Eastern Adriatic coast, Croatia). Metaphase spreads were made from the flat oyster larvae and studied using conventional karyotyping methods. The study revealed the presence of 5 metacentric and 4 submetacentric pairs of chromosomes as well as a single pair of subtelocentric ones with an arm ratio of 3.72 ± 0.42 and a centromeric index of 21.42 ± 1.87 which has not been described previously. This suggests that the oyster population cultured in Mali Ston Bay in Croatia represents a genetically different lineage of the species.*

**Key words:** oyster, *Ostrea edulis*, chromosome, cytogenetics

## INTRODUCTION

European flat oyster, *Ostrea edulis* is a sessile bivalve mollusk with a distribution range from Norway to Morocco in the Atlantic Ocean, whole Mediterranean Sea and even extending into the Black Sea. In the past, this species had the significant economic importance along the European coasts. Massive declines in abundance were observed in the late 1800s and early 1900s, probably due to overexploitation, habitat deterioration and unidentified diseases. Recently, a further drastic decline occurred due to two parasitic diseases, marteiliosis and bonamiosis that caused heavy mortalities in Western Europe (LAPÈGUE *et al*., 2007; LALLIAS *et al*., 2010).

The Eastern Adriatic populations were isolated from these events, mainly due to ban on import of spat from Western Europe. The population of Mali Ston Bay in Croatia has some specificity in comparison with other Adriatic populations: it has been cultured during the last four centuries, with a major production growth to 150 t/year in the last 40 years; most of the population's reproduction potentials lie in cultured animals; the wild population is usually wiped out by illegal harvest; the culture practice

is to sell oyster when they reach the size of 6-7 cm and this means that reproduction potentials rely mainly on fast growing +1 and +2 age oysters before marketing while slow growers are unsold and cultured for 3-years (MARČELJA *et al*., 2004).

A genetic study of the European flat oyster (*Ostrea edulis*) showed that the population from Mali Ston Bay is specific and distant from the others (LAUNEY *et al*., 2002). Enzymes analysis (SAAVEDRA *et al*., 1995) together with microsatellite and mitochondrial markers (SOBOLEWSKA & BEAUMONT, 2005; LAUNEY *et al*., 2002; DIAZ-ALMELA *et al*., 2004) revealed moderate differentiations between Atlantic and Mediterranean populations. In this study, results of karyological investigations of the flat oyster population from Mali Ston Bay, Eastern Adriatic coast, Croatia are presented.

## MATERIAL AND METHODS

*O. edulis* individuals were collected at a commercial farm in Mali Ston Bay, Croatia. Individuals were taken into the laboratory of the University of Dubrovnik and their upper shells were removed. In individuals containing veliger larvae (n = 5), the larvae were washed out of the shells using filtered sea water. Larvae (approximately 50 000) were incubated in 0.1% colchicine solution prepared in filtered sea water for 2 hours. Following colchicine treatment they were incubated in a hypotonic 0.9% sodium citrate solution for 40 minutes. Finally, the larvae were fixed in a 1:3 mixture of acetic acid and methanol, which was replaced three times with 20-minute intervals (MISKOLCZI *et al*., 2005).

Before the preparation of slides, larvae were suspended in a 50% solution of acetic acid on a watch glass. The suspensions were spread on slides and stained in 4% Giemsa (Riedel-de Haën, Germany, ref.number: 32884) for 20 min. Chromosome counting and evaluation was done at 1000x magnification on well spread metaphases using a Nikon Eclipse E600 microscope (Nikon, Japan). Photomicrographs of metaphase spreads were taken by a computer-controlled CCD camera (Micro Publisher 3.3).

Chromosome measurements were performed on photomicrographs using the ImageJ open source software. Chromosomes were separated and aligned horizontally for measurements. Lengths of short and long arms were measured using the software. Five measurements were made for each arm. Values of the five measurements were averaged and relative length (total length of the chromosome multiplied by 100 and divided by the length of the haploid genome),

*Table 1. Relative length (proportions of the length of individual chromosomes to the length of the entire haploid compartment), arm ratios (proportions of long arms to short arms) as well as centromeric indices (proportions of the short arms to the total length of the chromosome) in the ten pairs of chromosomes of the European flat oyster (Ostrea edulis). Classification nomenclature is as follows: m: metacentric, sm: submetacentric, st: subtelocentric. Data are expressed as mean ± SD (n = 9)*

| Pairs | Relative length | | Arm ratio | | Centromeric index | | Classification |
|---|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD | |
| 1 | 12.76 | 0.75 | 1.36 | 0.51 | 43.72 | 6.35 | m |
| 2 | 11.89 | 0.84 | 1.25 | 0.23 | 44.82 | 3.83 | m |
| 3 | 11.02 | 0.61 | 2.03 | 0.16 | 33.18 | 1.75 | sm |
| 4 | 10.91 | 0.33 | 1.2 | 0.14 | 45.71 | 2.82 | m |
| 5 | 10.39 | 0.36 | 1.28 | 0.16 | 44.38 | 3.05 | m |
| 6 | 9.74 | 0.55 | 1.3 | 0.18 | 43.85 | 3.39 | m |
| 7 | 9.53 | 0.52 | 1.94 | 0.42 | 34.73 | 4.88 | sm |
| 8 | 8.85 | 0.60 | 2.45 | 0.51 | 29.76 | 3.91 | sm |
| 9 | 8.44 | 1.13 | 3.72 | 0.42 | 21.42 | 1.87 | st |
| 10 | 6.2 | 0.73 | 2.36 | 0.19 | 30.04 | 1.58 | sm |

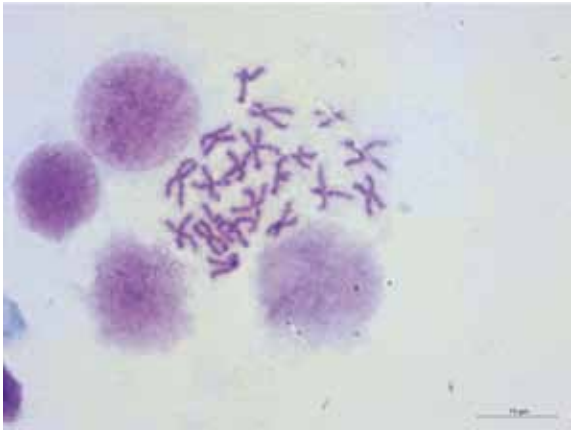

*Fig.1. Metaphase plate of a larva of the European flat oyster (Ostrea edulis) from Mali Ston Bay, Eastern Adriatic coast, Croatia*

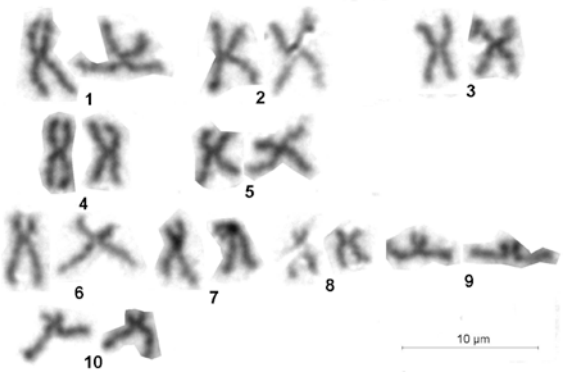


*Fig. 2. Karyogram of a larva of the European flat oyster (Ostrea edulis). Chromosome pairs 1-5 are metacentric, 6-9 are submetancentric and pair 10 is subtelocentric*

arm ratio (length of the long arm divided by the length of the short arm) as well as the centromeric index (length of the short arm multiplied by 100 and divided by the total length of the chromosome – the sum of the short and long arms) were calculated. Calculations of relative length, arm ratio and centromeric index, then classification of chromosomes as metacentric, submetacentric and subtelocentric were carried out according to LEVAN *et al*. (1964).

## RESULTS

Metaphase spreads were successfully prepared from the larvae of the European flat oyster. The diploid chromosome number of 2n = 20 was found for the investigated larvae (Fig. 1 and 2). The chromosome set of this larvae population contained 5 pairs of metacentric chromosomes, 4 pairs of submetacentric and 1 pair of subtelocentric chromosomes (Table 1). The 9$^{th}$ pair had an arm ratio of 3.72 ± 0.42 and centromeric index of 21.42 ± 1.87 which clearly classifies it as subtelocentric.

## DISCUSSION

This is the first reported attempt to prepare metaphase spreads from the larvae of *O. edulis*. Previous reports on using oyster larvae for cytogenetic studies were only reported in the Pacific oyster (*Crassostrea gigas*) where they were used for genotoxicity assessment (CHEUNG *et al*., 2006). Larvae offer a convenient and quicker alternative to chromosome preparation from adult individuals, as larvae are typically available in large numbers during the reproductive season, they contain a relatively high number of mitotic divisions and they require shorter colchicine treatment which considerably reduces preparation time.

The presence of a sub-telocentric pair in karyotype of *O. edulis* has not been reported before. According to cytogenetic studies carried out on Atlantic populations, this species has 5 metacentric and 5 submetacentric pairs of chromosomes (THIRIOT-QUIÉVREUX, 1984; LEITÃO *et al*., 2002). Subtelocentric chromosomes were present in some Atlantic populations of this species according to THIRIOT-QUIÉVREUX (1984), however their status was uncertain due to the high variation in the centromeric index, thus they have always been characterized as submetacentric – subtelocentric. Later studies (LEITÃO *et al*., 2002) have not confirmed the presence of a subtelocentric pair in the Atlantic stocks of *O. edulis*. Our results suggest that the oyster population of Mali Ston Bay represents a genetically distinctive lineage of this species. This also suggests that previously detected differences between Mediterranean and Atlantic populations of the species by using enzymatic and DNA markers (LAUNEY *et al*., 2002; SOBOLEWSKA & BEAUMONT, 2005; DIAZ-ALMELA *et al*., 2004) are also apparent

using a classical cytogenetic approach. This is a potential tool for the identification of populations, although further studies are needed on other Mediterranean oyster populations to elucidate whether the presence of a subtelocentric chromosome pair is characteristic for only one population or it is more common. In Mali Ston Bay, a high degree of inbreeding due to the isolation of this stock and dependence on cultured individuals could have also contributed to these differences.

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# Genetička osobitost Europske plosnate kamenice (*Ostrea edulis*) iz Malostonskoga zaljeva potvrđena kariološkom analizom

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## SAŽETAK

U radu je opisana genetička osobitost populacije europske plosnate kamenice (*Ostrea edulis*) iz Malostonskog zaljeva. U istraživanju je korištena uobičajena metoda za dobivanje metafaznih kromosoma, kojom je utvrđena prisutnost 5 metacentričnih i 4 submetacentrična para kromosoma te jednog para subtelocentričnih kromosoma, koji do sada nije bio opisan kod europske plosnate kamenice. Omjer krakova subtelocentričnog para je 3,72 ± 0,42, a centromerni indeks 21,42 ± 1,87. Ovi rezultati ukazuju da populacija europske plosnate kamenice koja se uzgaja u Malostonskom zaljevu predstavlja potencijalno različitu genetičku liniju ove vrste.

**Ključne riječi:** europska plosnata kamenica, *Ostrea edulis*, kromosom, citogenetika