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- SHORT COMMUNICATION -

Microsatellite loci for Black Sea turbot *Scophthalmus maeoticus*

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Abstract

Several microsatellite loci which were developed and reported for turbot *Scophthalmus maximus* were examined for *Scophthalmus maeoticus*. Only five of these microsatellite loci were successfully amplified with new developed PCR conditions and can be used for routine analysis of stock identification of *S. maeoticus*.

Keywords:
Black Sea turbot, *Scophthalmus maeoticus*, microsatellite marker

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Introduction

Black Sea Turbot *Scophthalmus maeoticus* (Pallas, 1814) is an economically important flatfish species belong to the family of Scopthalmidae, and there is a remarkable relevance in this species with respect to fisheries and aquaculture. *S. maeoticus* is one of the most valuable species in the Black Sea Basin (Pradonov et al., 1997; Mikhailov & Prodanov, 2003). Long-term former catch data of the countries sharing the stocks in the Black Sea show that majority

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of the turbot (72%) had been caught by Turkey and followed by the Commonwealth of Independent States (19%), Bulgaria (7%) and Romania (3%) (Pradanov et al., 1997).

There are two more species of the genus Scopthalmus as *Scopthalmus maximus* and *Scopthalmus rhombus* for European fisheries and aquaculture. These three species are closely related congeneric species (Pardo et al., 2005; Azevedo et al., 2008; Turan, 2007) which show a similar distributional range (Blanquer et al., 1992; Pardo et al., 2001). Molecular genetics techniques utilize to identify stock structure of marine species. In recent years, microsatellite markers have increasingly been used to identify genetics differences between stocks (Shaw et al., 1999; Liu & Cordes, 2004). There are various genetic studies about microsatellite analysis of *Scophthalmus maximus* that revealed heterogeneity between stocks.

Florin & Höglund (2007) detected significant genetic differentiation among turbot (*Psetta maxima*) in the Baltic Sea by using 8 polymorphic microsatellite markers. Nielsen et al. (2004) investigated *S. maximus* populations in the Northeast Atlantic with 8 highly polymorphic microsatellite loci and found significant genetic differentiation among samples. Iyengar et al. (2000) developed highly polymorphic microsatellite markers for turbot *S. maximus* with potential for use other several flatfish species and reported characterization of an entire suite of novel polymorphic microsatellite markers.

Until this time, as we know, no specialized microsatellite markers currently exist for the Black Sea turbot *S. maeoticus*.. In the present study, various microsatellite loci were tried for *S. maeoticus* by using other microsatellite primers which were characterized or developed for *S. maximus* in order to figure out whether they are suitable on usage for *S. maeoticus*. Only five microsatellite loci were successfully amplified and used for routine analysis of *S. maeoticus* stock identification.

Material and Method

Genomic DNA was extracted using Standard phenol-chloroform isoamyl alcohol technique (Sambrook et al., 1989), and total 50 individuals were extracted and total DNA was visualized by gel electrophoresis (1.5%) and quantified by spectrophotometric assay. In order to specify and avoid amplifying non-specific sequences touchdown PCR method has been used.

After screening of the primers developed for *S. maximus* with different PCR conditions, only five microsatellite loci were found to be appropriate for routine analysis. The annealing temperature for each primer set was arranged according to the melting temperature of the primer sets (Table 1).

Polymerase chain reaction (PCR) was carried out using a reaction volume of 15 µl containing 1 U Taq polymerase (Thermo scientific), 2 µM of each primer, 200 mM dNTPs, 25 mM $MgCl_2$, 10 mM Tris-HCI pH 8.8, 50 mM KCI and 1 µl template DNA (≈10-25 ng). PCR temperature profile consisted of two stages. In first stage; pre denaturation at 95°C for 1 min followed by 5 cycles of denaturation at 94°C for 20 s, annealing at primer-specific temperature for 55 s, extension at 72°C for 2 s. Second stage followed by 25 cycles of denaturation at 94°C for 20 s and final extension at 72°C for 20 s.

The forward primers were fluorescently labeled with FAM. The quality of the PCR amplifications was checked by subjecting the PCR products to electrophoresis in 1.5 % agarose gels. Allele size was determined in an ABI-PRISM 3100 sequencer (Applied Biosystems, Foster City CA, U.S.A.). Raw data from the sequencer were processed with the PEAK SCANNER1.0 software for peak identification and fragment sizing (Applied Biosystems).

Result and Discussion

The primer sets *B12-I GT14* (Iyengar et al., 2000), *3/9CA15* (Iyengar et al., 2000), *Sma1-125INRA* (Estoup et al., 1998), *Sma-02* (Bouza et al., 2002) and *Sma3-12INRA* (Estoup et al., 1998) have been chosen for a successful amplifying process. New PCR conditions were developed for each primer.

Table.1 Five microsatellite loci for Black Sea turbot (*Scophthalmus maeoticus*)

Locus	GenBank No	Primer sequences	Repeat motif	Size range (bp)	T_m°C
B12-I GT14	AF182086.1	F: GTGATGGAAGATTGTACCAG R: CACAATAAAGGATAGACCAG	$(GT)_{14}$	175-185	56-54
3/9CA15	AF182091.1	F: AGAGTGAAGAACGTACCTGC R: CAATGGAGAGGCAGTATCGG	$(CA)_{15}$	165-197	58-60
Sma1-125INRA	No data	F: CACACCTGACAAAGCTCAAC R:GCTGAACATTTTCATGTTGATAG	$(TAGA)_{11}$-$(TG)_4$	112-152	58
Sma-02	No data	F: GGAGGATGTATTGAAAGTGT R: AGAGCAGGTCATTATACAGC	$(TG)_{16}$	93-141	54-56
Sma3-12INRA	No data	F: CACAATTGAATCACGAGATG R: GCCACCACTGCGTAACAC	$(TG)_{21}$	88-110	54-58

This study preliminary display reliable usage of microsatellite loci for Black Sea turbot *S. maeoticus*. Consequently, the chosen microsatellite loci which was previously developed and characterized in other studies for *S. maximus* (Iyengar et al., 2000; Estoup et al., 1998; Bouza et al., 2002) can be used for *S. maeoticus* with the PCR conditions described here.

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