

Possibility of Natural Hybridization between Red Seabream (*Pagrus major*) and Blackhead Seabream (*Acanthopagrus schlegeli*)

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During the storage of these two species in a large conservation tank, fertilized eggs were collected and the offspring were raised. During culturing of the offspring, individuals with mixed characteristics of these two species were observed, and 96 individuals were randomly tested using microsatellite markers applicable to both species. Among the 96 individuals, 15 individuals with mixed morphological characteristics were confirmed to be hybrids showing both of genotypes red seabream and blackhead seabream. Additionally, based on sequence analysis of mitochondrial cytochrome oxidase subunit 1 (mtDNA CO1), 81 showed 99% nucleotide sequence identity to that of black sea bream, and the remaining 15 individuals showed over 99% sequence identity to that of red seabream. So, hybrids were produced by female red seabream and male blackhead seabream. These results suggest that hybrids may form in nature between these two species if their habitats overlap due to the influence of humans or global climate change.

Key words : Habitats overlap, hybrid, microsatellite, seabream

Introduction

In undisturbed ecosystems, reproductive isolation is primarily maintained by spatial and temporal barriers [10]. However, such barriers to interbreeding may be lost because of habitat disturbance and introduction of non-native species, which can be exacerbated by habitat modification, translocation of fish species, and global climate change [1]. In addition to hybridization in natural ecosystems (usually between native and nonnative taxa), artificially induced hybridization has been used to improve aquaculture productivity through heterosis in fish [2, 6, 12, 15]. Hybridization is more common in fish than in any other vertebrate because of their external fertilization and similar mating behaviors [9]. Long-term hybridization, especially in cases leading to genomic introgression, may result in speciation, and thereby contribute to diversity within taxa [4]. However,

hybridization can also be destructive, because it can lead to sterile or nonviable progenies, loss of unique genetic diversity, breakdown of adaptive multi-gene complexes, and the collapse of such substructures [4]. Since the late 1960s, there have been increasing reports of hybridization between diverse animals, including fish species [5]. Although the majority of hybridization is due to the introduction of non-native species, hybridization between sympatric species has also been reported [3, 13, 16]. In contrast to hybridization between sympatric freshwater fish species, less is known regarding the hybridization between sympatric marine fish species [14]. In this study, we analyzed offspring in a stocking tank containing a mixture of male and female red seabream (*Pagrus major*) and blackhead seabream (*Acanthopagrus schlegeli*). These two species inhabit Korean and Japanese waters to the South China Sea, and are very important aquatic species for recreational fishing (*A. schlegeli*) and commercial aquaculture (*P. major*). Hybrids for higher productivity have been developed and cultured using artificial fertilization of eggs from *P. major* and sperm from *A. schlegeli* [7]. However, hybridization between these two species has not been performed using artificial fertilization, and to the best of our knowledge, this is the first evidence of natural hybridization between these two species.

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Materials and Methods

Sample collection and DNA extraction

The red (n=23) and black (n=12) seabream broodstock were collected from the wild, and maintained for stock preservation in a tank of 100 metric tons with approximately 100 other fish of about 10 species at the Jeju Hatchery Center, National Fisheries Research & Development Institute in Korea. The floating eggs fertilized by natural mating were collected using a net of nylon mesh during April, which is the middle of the seabream spawning season. The hatched larvae were cultured using running water culturing methods before analysis. A total of 96 progeny (6 months of age and average weight of 8.5 g) and all individuals of the two parental species were analyzed for genetic inheritance. Small pieces of fin tissue were cut from individuals and preserved in 100% ethanol. Total DNA was isolated from each sample using a MagExtractor MFx-6100 automated DNA extraction system (Toyobo, Japan). The extracted genomic DNA was quantified using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and stored at -20°C until microsatellite genotyping analysis.

PCR amplification and genotyping

The 20 microsatellite markers developed for blackhead seabream by Kim *et al.* [8] were first tested for cross species transferability to red seabream, and 9 of them showing transferability were used for analysis. For multiplex PCR, the 5' -end of each forward primer was labeled with one of three fluorescent dyes; namely, 6-FAM and HEX (Applied Biosystems, Foster City, CA, USA). PCR for amplification of microsatellite loci was performed in a 10 µl reaction volume containing 1× ExTaq buffer, 10-50 ng template DNA, 0.2 mM dNTPs, 0.5 µM of each primer, and 0.25 U *Taq*DNA polymerase (Takara, Shiga, Japan) using an RTC 200 thermocycler

(MJ Research, Waltham, MA, USA). The PCR conditions were as follows: initial denaturation at 95°C for 11 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at optimal temperature for each primer set for 1 min, extension at 72°C for 1 min, and a final extension at 72°C for 5 min. A 1 µl aliquot of PCR product was mixed with a genotyping reaction mixture containing formamide and a size standard using GeneScan400HD ROX (Applied Biosystems), and was electrophoresed with an ABI3130 DNA sequencer (Applied Biosystems). The fragment lengths of the PCR products were determined using GeneMapper software ver. 4.0 (Applied Biosystems).

Results and Discussion

Offspring production

Based on sequence analysis of mitochondrial cytochrome oxidase subunit 1 (mtDNA CO1), 81 out of 96 randomly selected individuals showed 99% nucleotide sequence identity to that of black sea bream (HQ846832.1), and the remaining 15 individuals showed over 99% sequence identity to that of red seabream (JF952802.1). In addition, these 15 individuals showed specific morphological characteristics of both species; they had more round heads with bulging on the forehead, which is a characteristic of the red seabream. The dorsal fin of the red seabream is composed of 12 spines, with the posterior formed of 10 soft rays in contrast to the blackhead seabream that has 11 spines and soft rays. The suspected hybrid had dorsal pins with 12 spines and 10 soft rays. However, these individuals showed faint residual vertical lines that can be seen on blackhead seabream (Fig. 1). Hybrids have been produced using artificial insemination between female red seabream and male blackhead seabream [7]. Therefore, these 15 individuals were assumed to be natural hybrids between red seabream dam and blackhead seab-

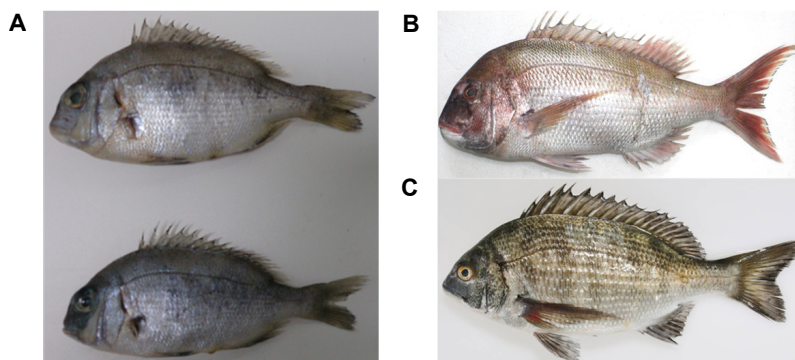


Fig. 1. Morphological characteristics of parental seabream species and their hybrid offspring. The hybrid offspring (A) have the forehead, the lateral line, and dorsal pins with 12 spines that resemble the maternal red seabream (B), but have faint vertical lines and 'V' form tail fin shape similar to the paternal blackhead seabream (C).

ream sire, and further genotyping was performed.

Molecular identification of hybrids

Among the nine markers that showed amplification both in blackhead seabream and red seabream [8], microsatellite marker AS392 (GenBank accession number GU121420) showed clear differences in the size of amplified products used for hybrid identification. This marker has a sequence composition of (AC)₁₃AT(AC)₄ and produced polymorphic PCR products ranging from 109-135 base pairs (bp) in blackhead seabream. The cross species transferability of this marker to red seabream was confirmed later, and produced polymorphic PCR products of 90 bp in red seabream (unpublished data). The typical amplification patterns of the parent and offspring are shown in Fig. 2. The parental red seabream and blackhead seabream showed a peak of 90 and

130 bp, respectively. The 81 individuals identified as blackhead seabream showed a peak of 130 bp corresponding to their parent. However, the suspected hybrid showed peaks at both positions. Therefore, the morphological and molecular characterization confirmed that these 15 individuals are hybrids between female red seabream and male blackhead seabream.

Hybrids between red and blackhead seabream have been generated to incorporate the high growth rates of red seabream and resistance to environmental stress of blackhead seabream, and the resulting hybrid showed mixed characteristics [7]. However, these hybridizations have been conducted using artificial insemination of eggs from red seabream and sperm of blackhead seabream; this is the first report on the identification of these two species using a method other than artificial insemination. Although hybrids have

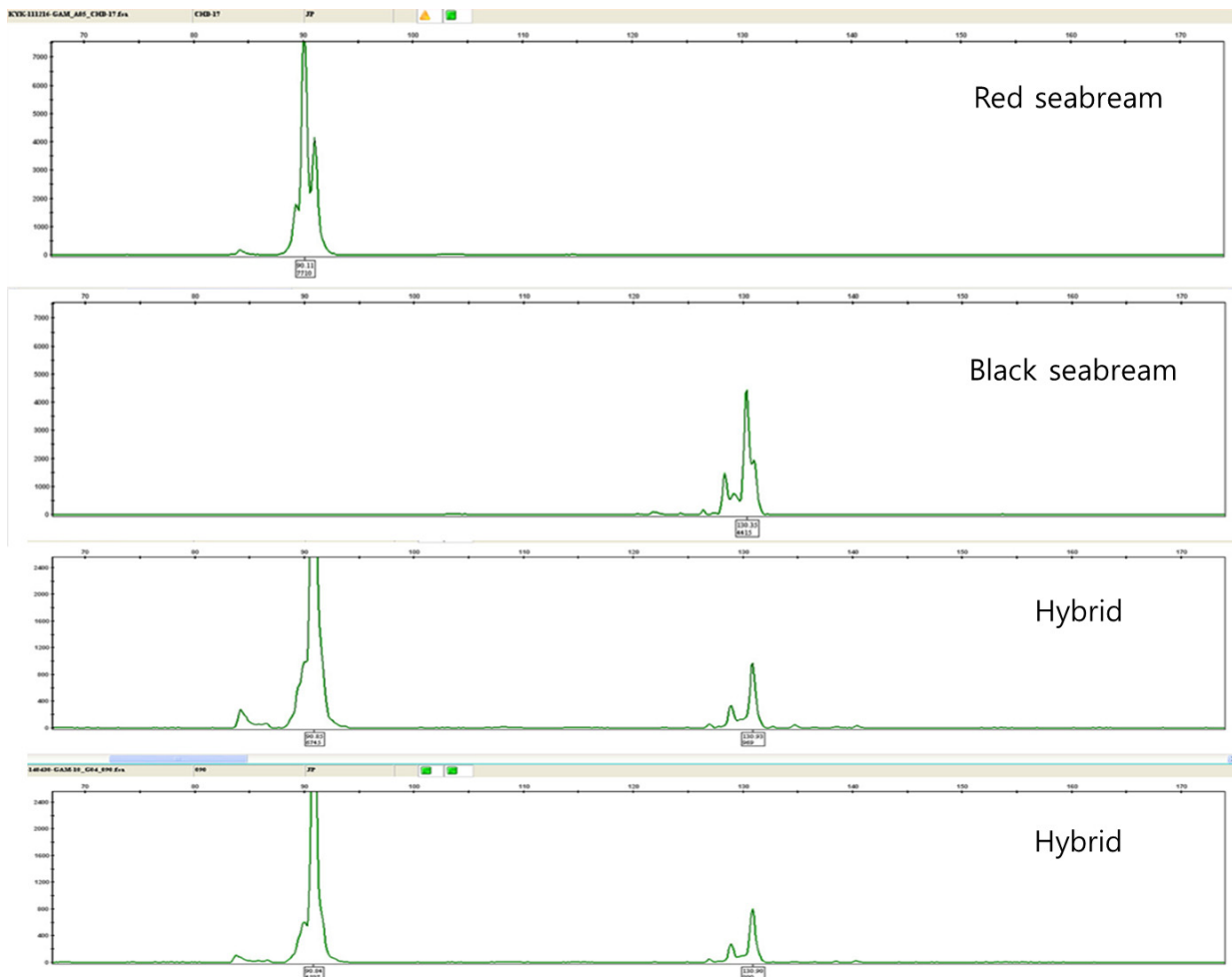


Fig. 2. Genotyping of parental seabream species and their hybrid offspring using microsatellite marker AS392. The paternal red seabream and the maternal blackhead seabream shows a peak of 90 bp and 130 bp, respectively and their hybrid shows the peaks of both parents.

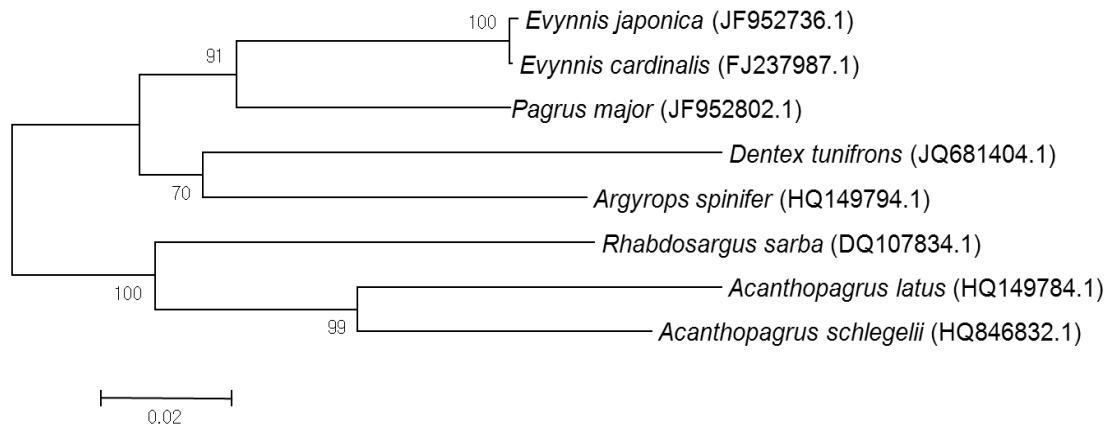


Fig. 3. Neighbor-joining tree of the Sparidae inhabiting the Korean Peninsula based on the *CO1* gene sequence. The numbers near the nodes represent bootstrap support.

not been identified under completely natural conditions, there was sufficient space and interaction with other fish species in this report.

Despite our observations, the possibility of hybridization is very low for several reasons. First, the habitats of these two species are different. Red seabream inhabit rocky deep sea areas ranging from 10-200 m, while blackhead seabream lives in sandy areas rich in seaweeds near the shore of 5-50 m. Red seabream tends to be a shoal fish, although the size of the shoals decreases with age. In contrast, mature blackhead seabream lives in solitary. Therefore, the chance for these two fish to inhabit a common area is low. However, local changes in the habitats by destruction or global climate change can result in the co-inhabitation of these two species in a common area. Firstly, write result and then discussion.

The other reason for the low chance of hybridization is their genetic distance. As shown in Fig. 3, these two species are genetically separated. Seabream with a red color including *P. major*, *Dentex tumifrons*, and *Evynnis japonica* have 12 spines in their dorsal fin. In contrast, black-colored seabream such as *A. schelegeli* and *Rhabdosargus sarba* have 11 spines. One interesting feature in our analysis is that we did not observe any red seabream offspring. There can be several reasons for this observation. In the analysis, the ratio of hybrid was very low (16%) and the majority of offspring were blackhead seabream. This indicated that only a few female red seabream were involved in spawning. The spawning of red seabream is affected by water temperature and salinity, and the optimal conditions were a water temperature of 15°C to 22°C and salinities from 18% to 36% [11]. The optimal temperature for blackhead seabream spawning is 20°C, but details of other conditions are not

available. This sentence needed more detail expression. However, it is suspected that the water conditions were conducive to the spawning of blackhead seabream, and most of the eggs originated from blackhead seabream. The other reason for the absence of red seabream could be the mating style and characteristics of the fish species. Red seabream is monogamous, and blackhead seabream is more aggressive and compete for the female of interest. If only a few red seabreams were involved in the spawning, less aggressive red seabream males could be excluded from the fertilization. This could be examined using parental analysis of all offspring; however, this is complicated because of the complex mating partnerships that occur.

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초록 : 참돔(*Pagrus major*)과 감성돔(*Acanthopagrus schlegeli*) 종간 자연 잡종 가능성

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참돔과 감성돔은 우리나라 주변에 서식하는 고유 어종으로, 참돔의 암컷과 감성돔의 수컷을 이용한 인공수정에 의하여 잡종은 생산되었으나, 자연 상태에서는 이들 간의 잡종이 보고된 바 없다. 이들 두 어종의 암수 및 타 어종을 섞어서 대형 수조에서 사육하는 과정에서 생산된 수정란을 회수하여 부화시켜 육성하는 과정에서 이들 두 종간의 잡종의 형태를 보이는 개체들이 관찰되었다. 임의로 96개체를 선택하여 두 종에 모두 적용할 수 있는 microsatellite marker를 이용하여 유전학적 분석을 실시한 결과 96개체 중 두 종의 혼합된 형태적 특징을 보이는 15개체는 참돔 암컷과 감성돔 수컷 간의 잡종으로 판명되었으며, 나머지 81개체는 감성돔 치어로 확인되었다. 사육 수조의 크기가 매우 컸으며 다른 어류들도 함께 들어 있었다는 점과, 이와 같이 유전적으로 구분되는 두 종간의 잡종이 자연상태와 유사한 환경에서 생산되었다는 점을 고려할 때 본 연구의 결과는 자연 상태에서도 인위적인 영향이나 기후 변화에 의하여 이들의 서식지가 중복될 경우 두 종간의 잡종이 생산될 가능성이 있다는 것을 시사한다.