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Research Article

Pacific false kelpfish, *Sebastiscus marmoratus* (Cuvier, 1829) (Scorpaeniformes, Sebastidae) found in Norwegian waters

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Abstract

During an angling competition in the Oslofjord, Southern Norway, a fish species previously unknown to the anglers was caught. Subsequent morphological studies and DNA barcoding identified it as a false kelpfish, *Sebastiscus marmoratus* (Cuvier, 1829), a species native to the Western Pacific from southern Hokkaido, Japan to the Philippines. The specimen was a female with a length of 29.2 cm and weighing 453 g. Stomach contents revealed fish remains, as well as the brachyuran *Xantho pilipes* A. Milne-Edwards, 1867 and remains of anomuran decapods. Parasitological examination revealed infections with the locally common generalist parasites *Derogenes varicus* (Digenea) and *Hysterothylacium aduncum* (Nematoda) that likely have been acquired through prey fish. A literature study of the parasites of *S. marmoratus* was carried out, listing at least 31 species. To the best of our knowledge, this is the first report of this fish species in the Atlantic. The introduction route is unknown, but the most likely possibility is via a ship's ballast water as a larva or fry, which again would imply that the specimen has been in temperate waters for several years. This, in addition to the fact that the fish was caught on a bait and thus actively foraging, indicates it was thriving and is a strong indication that this fish species could survive well in the conditions present in Norwegian waters. It was also clear from this study that available cytochrome oxidase 1 (CO1) sequences cannot differentiate between *S. marmoratus* and *S. tertius* and thus, cannot be used alone for barcoding and discriminating these two species.

Key words: marble rockfish, parasite fauna, DNA barcoding, introduced species, ballast water

Introduction

The life cycle of many marine organisms, including fish, contains a planktonic stage that drift or swim in the water column. The consequence is that even large species such as fish can be transferred to new areas via ballast water due to transfer of small larvae. Accidental transfer of species via ballast water is common for fish and e.g. Wonham et al. (2000) identified 32 fish species that had been transported and introduced to new areas via ballast water, of which as many as 24 established viable populations.

The false kelpfish, *Sebastiscus marmoratus* (Cuvier, 1829) (Scorpaeniformes, Sebastidae), is a species native to the Western Pacific from southern Hokkaido, Japan to the Philippines (see www.fishbase.org, accessed 20th September, 2017) and has never been found outside its native range. In this paper we present the first finding of false kelpfish in the Atlantic Ocean.

Methods

A specimen of a fish species unknown to the anglers was caught on hook and bait during an angling competition in the Oslofjord, Southern Norway (59 15.933′N; 10 37.332′E) on the 21st August 2016. To obtain a species identification of the fish specimen

the fish was frozen and shipped to the Norwegian Veterinary Institute for further analyses. At the NVI the fish was weighed, measured and photographed. A full parasitological examination and an examination of the stomach and rectum content was performed to see whether the fish specimen had been infected by parasites and/or had been foraging in Norwegian waters. Based on photographs of the specimen it was morphologically determined by Prof. Hiroyuki Motomura, The Kagoshima University Museum, to be *Sebastiscus marmoratus*. To verify this species determination, the morphological characteristics of the specimen were compared to the literature and in addition, samples of muscle and gill tissue were taken for genetic species identification by DNA barcoding of the mitochondrial cytochrome oxidase 1 gene.

DNA from muscle and gill tissue from the fish and from some selected parasites and other stomach content was extracted with the DNeasy kit on a QiaCube extraction machine (Qiagen®) according to the instructions from the manufacturer. The PCR reaction was carried out with puRe Taq Ready-to-Go PCR beads (Amersham Biosciences) in a GeneAmp PCR System 9700 (Applied Biosystems). For fish barcoding the primers FishF1/FishR1 (Ward et al. 2005) were used to amplify approximately 650 base pairs of the mitochondrial cytochrome oxidase 1 (CO1). For identification of nematodes recovered from the stomach we used the primers NC5/NC2 to amplify approximately 900 base pairs of the ribosomal (r)DNA region (Newton et al. 1998), and for identification of the crab we used the universal primers LCO1490/HCO2198 to amplify approximately 700 base pairs of CO1 (Folmer et al. 1994). The following protocol was used for PCR: 4 min at 95 °C, followed by 35 cycles of 1 min at 95 °C, 1 min at 55 °C (50 °C for FishF1/FishR1) and 2 min at 72 °C. The PCR products were sequenced on an ABI 3700 XL (Applied Biosystems®) and proofread in Vector NTI ver 11.5 (Invitrogen). After proofreading, the sequences were subjected to a BlastN search in Genbank. To assess possible genetic variation between published records of *S. marmoratus*, CO1 sequences in GenBank representing *Sebastiscus* were downloaded and aligned in MEGA (7.0) software (Kumar et al. 2016). Phylogenetic relationships were inferred by neighbor-joining (NJ), where genetic distances were calculated according to the Kimura 2-parameter method (K2) and bootstrap support was estimated by running 1000 replicates. During initial analysis it was seen that some of the downloaded sequences were erroneous and some grouped with other species than *Sebastiscus*. Therefore only the most closely related sequences were used for the alignment and phylogenetic analyses.

Results and discussion

The specimen caught in the Oslofjord was a female measuring 292 mm in total length and weighing 453 g (425 g after bleeding and freezing). The morphological characteristics of the specimen were as follows: pectoral fins: 18; pelvic fins: I+5, dorsal fin: XII+13; Anal fin: III+I+6; caudal fin: 15. The gonad weight was 8.4 g and the liver weight was 5.3 g. Colour was brown (Figure 1). The whole specimen and a tissue sample is included in the fish collection and the DNA bank at the Natural History Museum, Oslo, Norway under accession numbers NHMO J 7177 and NHMO-DFH-771, respectively.

The sequencing of the PCR product (obtained by using the primers FishF1/FishR1) yielded a 671 bp sequence of the CO1 gene and the BlastN search (as of 22.09.2017) showed the highest sequence similarity to sequences from individuals identified as both *S. marmoratus* and *S. tertius* (e.g. KM366112). The CO1 sequence representing the specimen from Norway is deposited under GenBank accession number MG030723 and BOLD:AAC5047.

The subsequent phylogenetic analysis of 40 sequences is based on 499 base pairs in order to achieve a global alignment without missing information. The NJ-tree (Figure 2) show that the sequence from the Norwegian specimen group with sequences representing *S. marmoratus* from southern China and Japan, but this group also contain sequences representing *S. tertius* from Taiwan and Korea. Two sequences of *S. marmoratus*, KM366121 (unpublished) and KT189678 (Shin et al. 2016), were divergent from sequences in this first group (0.046 K2-distance between KM366121 and DQ678413 (*S. marmoratus*) (see Figure 2), and grouped, although with low support with sequences of *Sebastiscus albofasciatus* and might represent misidentifications. The K2-distance between KM366121 and KU892820 (*S. albofasciatus*) was 0.036, which might indicate separate species status of the former. Another four sequences of *S. marmoratus* from the South-China Sea (JQ738542-5) were highly divergent from other sequences of *S. marmoratus* and based on the current analyses they appear to be *Sebastes pachycephalus* Temminck and Schlegel, 1843 (KF836442) (see Figure 2). Cuvier *in* Valenciennes and Cuvier (1829) originally described *S. marmoratus* from Japan, and thus we consider the genotypes from that area to represent *S. marmoratus* (i.e. Katoh and Tokimura 2001; Hyde and Vetter 2007).

It is clear that the CO1 sequences from *S. marmoratus* and *S. tertius* that are available in GenBank cannot differentiate between the two species.

Figure 1. Photographs of *Sebastiscus marmoratus* (A to D); (A) whole specimen (with , (B) dorsal view of head, (C) left pectoral fin and (D) swim bladder/gonads. (E) stomach content from *S. marmoratus* showing 1) crab, *Xantho pilipes* and 2) part of vertebra from consumed fish. All photos by the authors.

This poses a question on the correctness of the species identifications or the separate species status of these two species, which again have implications for barcoding of these fishes. The three available *S. tertius* sequences (KF965464, KM366112, KU943360) are from fishes caught in Taiwan (Chang et al. 2017) and Korea (unpublished). However, according to Katoh and Tokimura (2001), Ishii (1997) found substitution differences in CO1 between the species, although the unbiased genetic distance was low (sequences not available). According to Katoh and Tokimura (2001) these species can be separated by morphology and allozymes, but ambiguous specimens may occur. Hence the specific status of *S. tertius* is still unclear.

For the morphological separation of these two similar species, the number of rays on the pectoral fin is important (Barsukov and Chen 1978). This character alone, the presence of 18 rays in the present specimen, appears to classify our specimen as *S. marmoratus* with some 85% certainty (Katoh and Tokimura 2001). In addition, the brownish pigmentation of *S. marmoratus* correctly identifies most specimens (98%) among the other *Sebastiscus* spp. according to Katoh and Tokimura (2001). *Sebastiscus tertius* on the other hand, tend to be pink to red. Also, branched rays in the pectoral (Figure 1) is a characteristic of *S. marmoratus* when compared to *S. tertius*. Therefore, based on morphology, we identify the specimen as *S. marmoratus* and the current CO1 sequence is thus deposited in GenBank and BOLD under the name *S. marmoratus*.

Only a few parasite specimens were found during the parasitological examination. Altogether 7 specimens of the nematode *Hysterothylacium aduncum* were found in the intestinal lumen (6) or stomach (1) and 2 specimens of the trematode *Derogenes varicus* were found in the stomach. The nematodes were adults (up to 24 mm long) or stage 4 preadults. The species identity of the *H. aduncum* specimens were confirmed by molecular methods while *D. varicus* was identified based on morphology only.

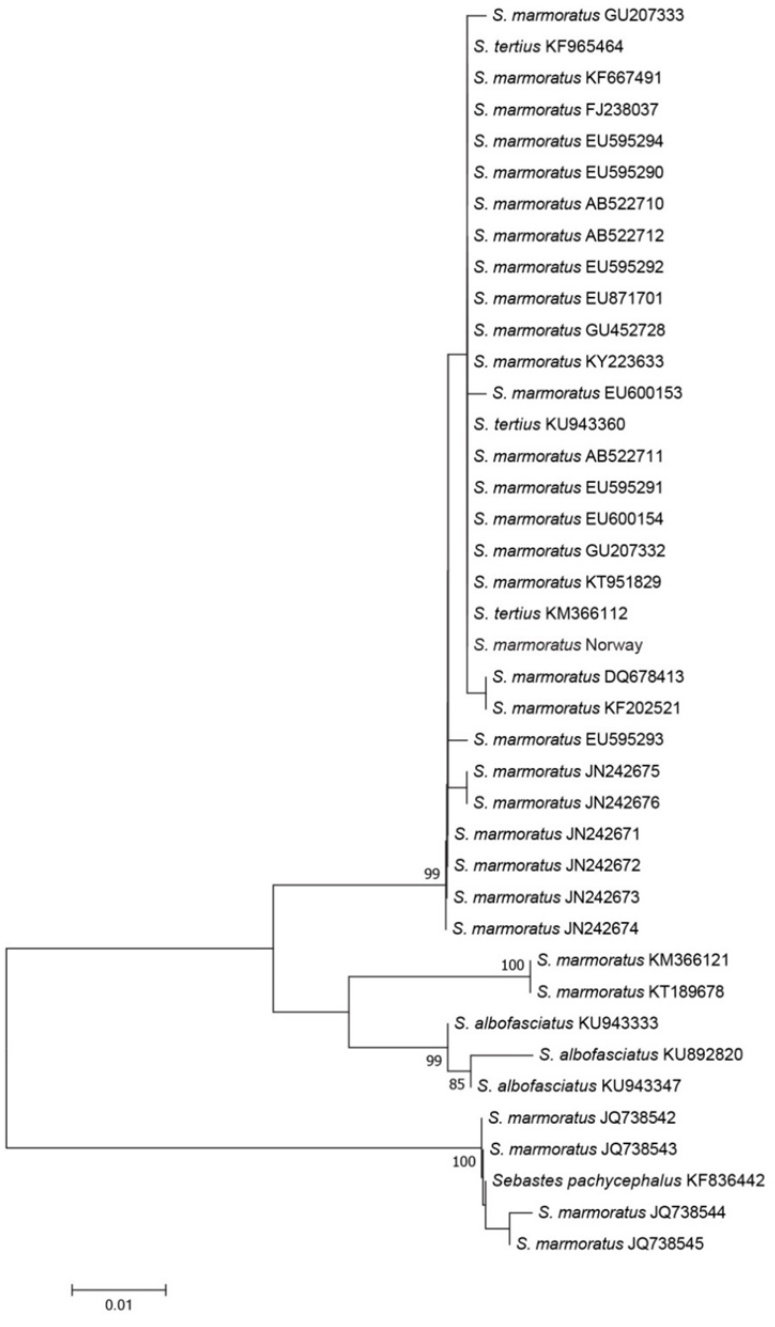

Figure 2. Neighbor-joining (NJ) tree of 499 bp cytochrome oxidase I sequences of *Sebastiscus marmoratus* and related species. Bootstrap support is indicated as percentages of 1000 replicates; only bootstrap values > 80 are given. The evolutionary distances were computed using the Kimura 2-parameter method and are in the units of the number of base substitutions per site. The rate variation among sites was modeled with a gamma distribution (shape parameter = 1). Details of sequences are given in Supplementary information Table S2.

At least 31 species of parasites have been recorded from *S. marmoratus* in its native range (see Supplementary information Table S1). Both *D. varicus* and *H. aduncum* occur in Japanese waters, but have not been recorded from *S. marmoratus*. However, *D. varicus* has been found in the related *S. albofasciatus* from the Japan Sea (Yamaguti 1938; Kamegai and Ichihara 1972). Both parasite species are common in the Oslofjord where the fish was caught, and could have been acquired from infected prey.

The stomach and rectum content analyses revealed that the fish had been eating in Norwegian waters as both the crab species *Xantho pilipes*, fish vertebra and remains of a decapod (probably *Galathea* sp.) were found (Figure 1). *Xantho pilipes* is only found in the north and south Atlantic oceans (Udekem d'Acoz 1999). The crab species was confirmed by DNA bar-

coding (99% similarity to JQ306052). A molecular identification of the fish vertebra found in the stomach proved difficult and the fish species was not identified. These types of prey, i.e. crabs and fish, are very typical for this fish in its native range (see e.g. Lee et al. 2012). This together with the fact that the false kelpfish was actually foraging when it was caught on bait indicates that it was thriving and that the species could survive well in Norwegian waters, especially with increasing water temperatures due to climate change. The large size of the specimens would suggest that it was some 7 or more years old, if caught in Japan (Yokogawa et al. 1992). However, we did not collect the otoliths in order to examine this, since we did not want to destroy the specimen before its inclusion in a museum collection. The occurrence of this West-Pacific species in a Norwegian fjord is remarkable and the only likely vector for its introduction is ballast water (Seebens et al. 2013). The large size of the specimen then suggests that the introduction event happened several years ago, when the specimen was a larva. The occurrence of native parasites and food items (*X. pilipes*) also fits such a scenario, although introduction of exotic infectious agents and parasites is possible also through fish larvae. However, we cannot rule out the possibility that the specimen in the current study was the result of a more recent introduction as Wonham et al. (2000) refers to at least one incident were fish larger than the specimen in the current study were found in ballast tanks. We conclude that both the morphological (see Figure 1) and molecular analyses show that the specimen caught in the Oslofjord represent the first record of false kelpfish, *S. marmoratus* in Norway.

Comment on some erroneous sequences in GenBank not included in the phylogenetic analyses

The sequences said to represent *S. marmoratus* with the accession numbers HM180870, HM180871, and HM180872 from Kim et al. (2012) were found to be highly divergent from other sequences from *S. marmoratus* in GenBank. As the closest hits when submitting the sequences to a separate BlastN search were *Pseudomonas* spp. (Bacteria) we conclude that they were erroneous (likely contamination) and they were therefore disregarded.

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Supplementary material

The following supplementary material is available for this article:

Table S1. Parasites recorded from *Sebastiscus marmoratus* in its native range.

Table S2. List of mitochondrial cytochrome oxidase 1 sequences used in the phylogenetic analyses.

Appendix 1. List of references for Table S1.

This material is available as part of online article from:
http://www.reabic.net/journals/bir/2018/Supplements/BIR_2018_Hansen_Karlsbakk_SupplementaryTables.xlsx