SHORT COMMUNICATION

SARSIA

LOW LEVELS OF INTERSPECIFIC DNA SEQUENCE VARIATION OF THE MITOCHONDRIAL 16S rRNA IN NORTH ATLANTIC REDFISH SEBASTES (PISCES, SCORPAENIDAE)

ROLF C. SUNDT & TORILD JOHANSEN

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During an attempt to develop a molecular method for identification of species and morphs of Atlantic *Sebastes*, extremely low levels of interspecific sequence variation in a mitochondrial large subunit (16S) ribosomal RNA were found. Nucleotide sequencing of a 480 base pair portion of the gene from representatives of six phenotypes of four species of *Sebastes* was executed. No variation was found among the individuals of 'regular' *S. marinus*, 'giant' *S. marinus*, 'oceanic' *S. mentella*, and 'deep-sea' *S. mentella* analyzed. Sequences from *S. fasciatis* and *S. viviparus* differed with only one base each from the *marinus/mentella* haplotype. The low level of genetic variation at this gene makes it unsuitable as a target for methods to identify North Atlantic *Sebastes*. Level of variation may indicate recent evolutionary divergence between the species.

Rolf C. Sundt, Institute of Marine Research, P.O. Box 1870 Nordnes, N-5024 Bergen, Norway. E-mail: rolf sundt@imr.no – Torild Johansen, University of Bergen, Department of Fisheries and Marine Biology, Bergen High-Technology Center, N-5020 Bergen, Norway. E-mail: torild.johansen@ifm.uib.no

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INTRODUCTION

There are four common species of *Sebastes* in the North Atlantic: The commercially exploited Acadian redfish *S. fasciatus* (STORER, 1854) is found in the western North Atlantic and the unexploited Norway redfish *S. viviparus* (KRÖYER) in the eastern North Atlantic. The two other species, deep-sea redfish *S. mentella* (TRAVIN, 1951) and the golden redfish *S. marinus* (LINNEUS, 1758) are found in demersal and pelagic habitats in the North Atlantic. Each of these species exhibits a morphological polymorphism with two morphs. *S. mentella* is separated into a 'deep-sea' and an 'oceanic' type (MAGNUSSON & MAGNUSSON 1995) and *S. marinus* consist of a 'regular' and a 'giant' type. The two morphs of both these species are exploited commercially.

Due to problems with morphologically based identification, data on catches has to be pooled by management authorities (MCGLADE & al. 1983). Methods for unambiguous identification of *Sebastes* species are needed to avoid the disadvantages this causes. Where morphological or physiological evidence is unclear, genetic characters may provide accurate and unambiguous indicators for species separation (WILSON & al. 1985). Although genetic methods have given considerable new knowledge about the relation among redfish species and the structure within the species (NEDREAAS & NÆVDAL 1989; JOHANSEN & al. 1997; MCGLADE & al. 1983), there is still a need for more potent methods to be used in addition to the ones in use.

Mitochondrial ribosomal genes have been used for species and family-level studies (e.g. GELLER & al. 1993; MILINKOVITCH & al. 1993). In the present study a 480 base pair portion of 16S ribosomal RNA from individuals with known species/morph affiliations was sequenced. The initial objective was to clarify the molecular relationship among species and morphs of *Sebastes*. We also wanted to evaluate the potential for a routine molecular method for identification of morphs and species based on amplified DNA from the 16S gene.

METHODS

Sampling information is given in Table 1 and Fig. 1. Species were identified based on morphological characters according to BARSUKOV & al. (1984); NEDREAAS & NÆVDAL (1989); MAGNUSSON & MAGNUSSON (1995). The 16S RNA gene was PCR amplified from phenol/chloroform extracted DNA using the primers 16SAR (5' - CGCCTGTTTAACAAAAACAT - 3')

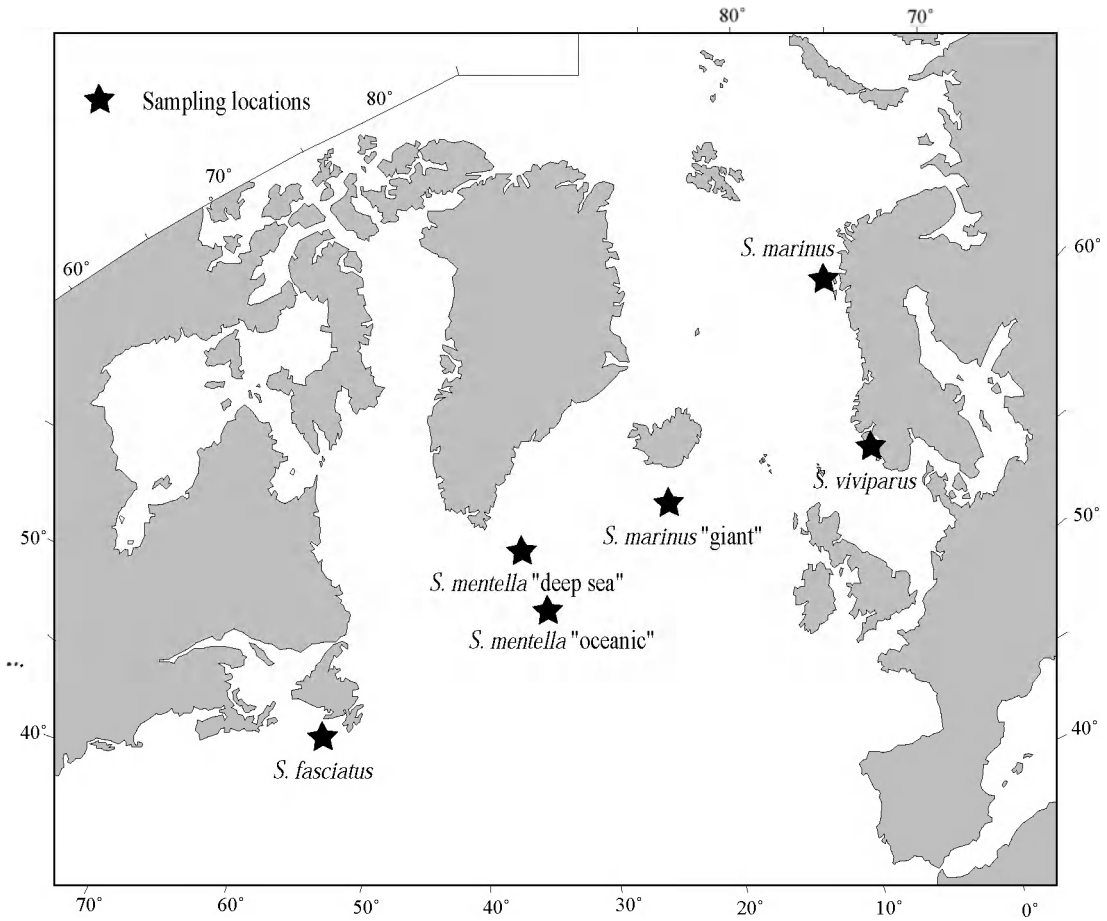

Fig. 1. Sampling locations for the different redfish species and morphs.

and 16SBR (5' - CCGGTTTGAACTCAGATCACGT - 3', Palumbi & al. 1991). Running conditions were: 94 °C for 2 min and (1 min at 94 °C, 2 min at 37 °C, 3 min at 72 °C) for 40 cycles. Negative controls (no DNA added) were run with each amplification. Products were run in 2 % Metaphore™, cut out from the gel and purified using Sephaglas™ BandPrep Kit (Pharmacia Biotech). Templates from one individual of each phenotype were sequenced in both directions using the 16SAR/16SBR primers and unincorporated primers and dNTPs were removed using Autoseq™ G-50 columns (Pharmacia Biotech). Nucleotide sequencing was carried out in an Applied Biosystems, Inc., Automated DNA Sequencer, Model 377.

RESULTS AND DISCUSSION

No variation was found among the individuals of 'regular' *S. marinus*, 'giant' *S. marinus*, 'oceanic' *S. mentella*, and 'deep-sea' *S. mentella* analyzed. Sequences from *S. fasciatus* and *S. viviparus* differed from the *marinus/*

Table 1. Sampling information for *Sebastes* samples.

	Species	Type (length in cm/sex)	Time	Location	Depth	Gear
1	*S. marinus*	'regular' (37/M)	autumn 95	Norwegian Sea	300 m	trawl
2	*S. marinus*	'giant' (77/F)	spring 96	Reykjanes ridge	800 m	longline
3	*S. mentella*	'oceanic' (38/F)	autumn 95	Irminger Sea	300 m	trawl
4	*S. mentella*	'deep-sea' (31/F)	summer 95	Irminger Sea	500 m	trawl
5	*S. fasciatus*	Canadian (16/M)	autumn 96	Gulf of St. Lawrence	255 m	trawl
6	*S. viviparus*	Masfjorden (23/M)	spring 92	Norwegian coast	20 m	gillnet

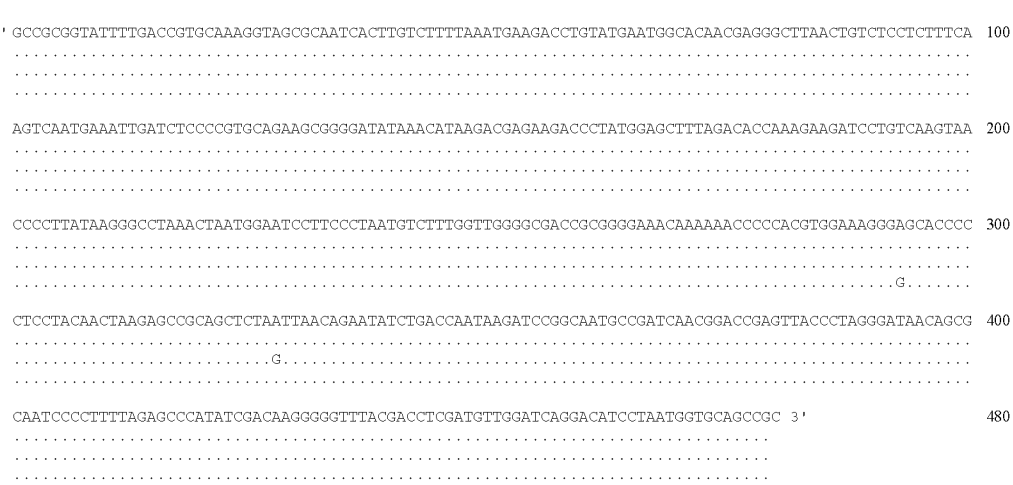

Fig. 2. Sequence data for a 480 base pair region of the mitochondrial 16s rRNA for *Sebastes* spp. *S. marinus* sequence represents both 'regular' and 'giant' morphs, *S. mentella* sequence represents 'deep-sea' and 'oceanic' morphs. (· = base same as that of the reference sequence)

mentella haplotype with only one transition (one purine base has been substituted for the other) each (Fig. 2). Since only one individual of each morph/species was analyzed, this study gives no information about possible intraspecific variation in the 16S gene. Difference in *S. fasciatus* sequence is within the cut site for the restriction enzyme TspE1. Provided within-species homogeneity at the site, restriction cutting of the PCR products could be employed to separate individuals of *S. fasciatus* from the other species studied.

16S sequences have been used to infer phylogeny for a number aquatic organisms, e.g. marine trematoid fishes (Ritchie & al. 1996), cyprinodontid killifishes (Parker & Kornfield 1995), Gonostomatidae (Miya & Nishida 1996), cephalopods (Bonnaud & al. 1994), marine copepods (Bucklin & al. 1995) and freshwater mussels (Lydeard & al. 1996). Mitochondrial ribosomal genes generally seem to show relatively low levels of variation within a genus, e.g. a study of the 12S/16S on piranhas, revealed 0-5.8 % sequence variation (Orti & al. 1996). The next step will be to check if the DNA sequence variation level in North Atlantic *Sebastes* is as low in nuclear genes that evolve faster and in other parts of the mtDNA molecule.

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