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Identification of partial MHC class II B exon 2 sequences in two closely related snake species: *Natrix tessellata* and *Natrix natrix*

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Abstract. MHC genes are well characterized in mammalian and bird species however few data are available from reptile species, especially from snakes. Herein we report the identification of a 113 bps long sequence of the MHC class II B gene exon 2 in two Natricine species, *Natrix tessellata* (dice snake) and *Natrix natrix* (grass snake). Being too short our sequences cannot be used for population structure or phylogeographic analysis, however they can be used for the design of specific primers and they are a stepping stone for the identification of the whole MHC class II genes in these snake species.

Key words: MHC, *Natrix natrix*, *Natrix tessellata*.

INTRODUCTION

The MHC (major histocompatibility complex) is a large genomic region than can be found in all jawed vertebrates. Most of the genes in this region play a key role in the immune system. The role of the MHC class II proteins is to present the extracellular antigen to the helper T cells and to elicit this way the immune response. The peptide binding grove of this protein is encoded by the exon 2 of the B chain coding gene. The exon 2 of the B gene is the most polymorphic sequence

from a vertebrate genome (Kelley et al. 2005). There are more theories that predict the high variability of this site: heterozygote advantage, frequency dependent selection, disassortative mating preferences (Hedrick 1999, Penn & Potts 1999). Due to its high variability it could be used as an effective marker to predict population structure. There were also successful trials to use MHC genes for phylogeography (Breggren 2005).

Although in most of the mammalian and bird species the MHC genes are well characterized we have few data from reptiles and especially snakes. The sequences identified by us are too short only 113 bps long which is not enough for a precise population structure analysis, but they can serve as stepping stone for further primer design and identification of the whole MHC class II B genes in these snake species.

METHODS

Two individuals of *Natrix tessellata* (N. tesselata1 lat. 43º05′, long. 22º30′, and N. tesselata2 lat. 46º58′, long. 22º31′) and one individual of *Natrix natrix* (N. natrix lat. 46º58′, long. 22º31′) were used in our study. Although the tow species are closely related they can be easily distinguished by morphological traits such as colour pattern. To demonstrate that our sequences are indeed MHC sequences and construction of phylogenetic tree, we used the following homologue MHC sequences from the NCBI: FL589895.1 (*Micrurus corallinus*), FL590235.1 (*Micrurus corallinus*), HO056457.1 (*Bungarus multicinctus*), AF256651.1 (*Caiman crocodilus*), AY937204.1 (Mauremys *reevesii*), FJ886736.1 (*Crocodylus niloticus*) AY491421.1 (*Alligator sinensis*), AY772946.1 (*Eumeces chinensis*), EF210744.1 (*Bombina vaiegata*), EF210760.1 (*Rana temporaria*), FJ448004.1 (*Triturus cristatus*), EU512174.1 (*Mesotriton alpestris*), AB302187.2 (*Edyptula minor*), AY694406.1 (*Gallinago media*), FJ853407.1 (*Anser cygnoides*), HQ230724.1 *(Hyaena hyaena*), L77103.1 (*Galago garnetti*), GU825757.1 (*Tupaia belangeri*), AB490486.1 (*Melursus ursinus*), AJ555156.1 (*Homo sapiens*).

The DNA was extracted from a 3 mm long tail clip of the individuals, by the MACHEREY NAGEL Nucleospin®Tissue Kit standard protocol for DNA extraction.

For primer design we used the two *M. corallinus* sequences (FL589895.1 FL590235.1) since alignments with the other reptile sequences yielded few conserved regions. The designed degenerate primers SnakeMHC F: AGC GGG TGC GGT TCC TSS and SnakeMHC R: GTC CRC ATC CGS CTC CCC yielded a 113 bps long product at the

annealing temperature of 59ºC. The PCR product was extracted from gel, using the MACHEREY NAGEL Nucleospin® Extract Kit and subcloned into pTZ57R/T (InsTAclone™ PCR Cloning Kit Fermentas). XL1 blue *Escherichia coli* was used for transformation. Ten white colonies of *E. coli* for each snake individual were selected for DNA extraction. Plasmid DNA was extracted by GeneJET™ Plasmid Miniprep Kit (Fermetas). The 10 clones for each snake indiviudal were sequenced at Macrogen Inc., Korea, using the universal M13 F primer. For the sequence editing we used the VectorNTI 10 and for sequence alignment the MEGA5. Phylogenetic trees were constructed with MEGA5 (maximum-likelihood method with 1000 bootstraps). Although our sequence was 113 bps long we only used 112 bps long sequences in our alignment so that our sequences would totally overlap with the homolog sequences from the database.

RESULTS AND DISCUSSION

In case of the 20 *N. tessellata* clones from the 2 individuals we only found 2 alleles (N. tessellata 1 and N. tessellata 2). In case of the single *N. natrix* individual we found 4 alleles (clone1-4). The phylogenetic tree indicates that our MHC sequences cannot be used to distinguish between species. The N. natrix clone1 and clone3 clusters together with the N. tessellata 1, while the N. natrix clone2 and clone4 cluster together with N. tessellata 2, and the M. corallinus sequences. It is important to note that the snake MHC sequences cluster together with the mammalian MHC sequences instead of the rest of reptile species, which seem to be closer to the bird MHC sequences (Fig. 1).

The alignment of our sequences with the snakes sequences from the NCBI database, FL589895.1 (*Micrurus corallinus*), FL590235.1 (*Micrurus corallinus*), and HO056457.1 (*Bungarus multicinctus*) proves the high variability of these gene sequences. Regardless of the primer binding sites, on a short sequence of 77 bps we could find 28 variable sites between the 4 snake species (Fig. 2).

As far as we know these MHC II B sequences identified in the two *Natrix* species are the 3^{d} and 4th available snake sequences for this gene. Although they are too short for a precise population analysis they can be used for the design of primers targeting the whole MHC II B gene sequence.

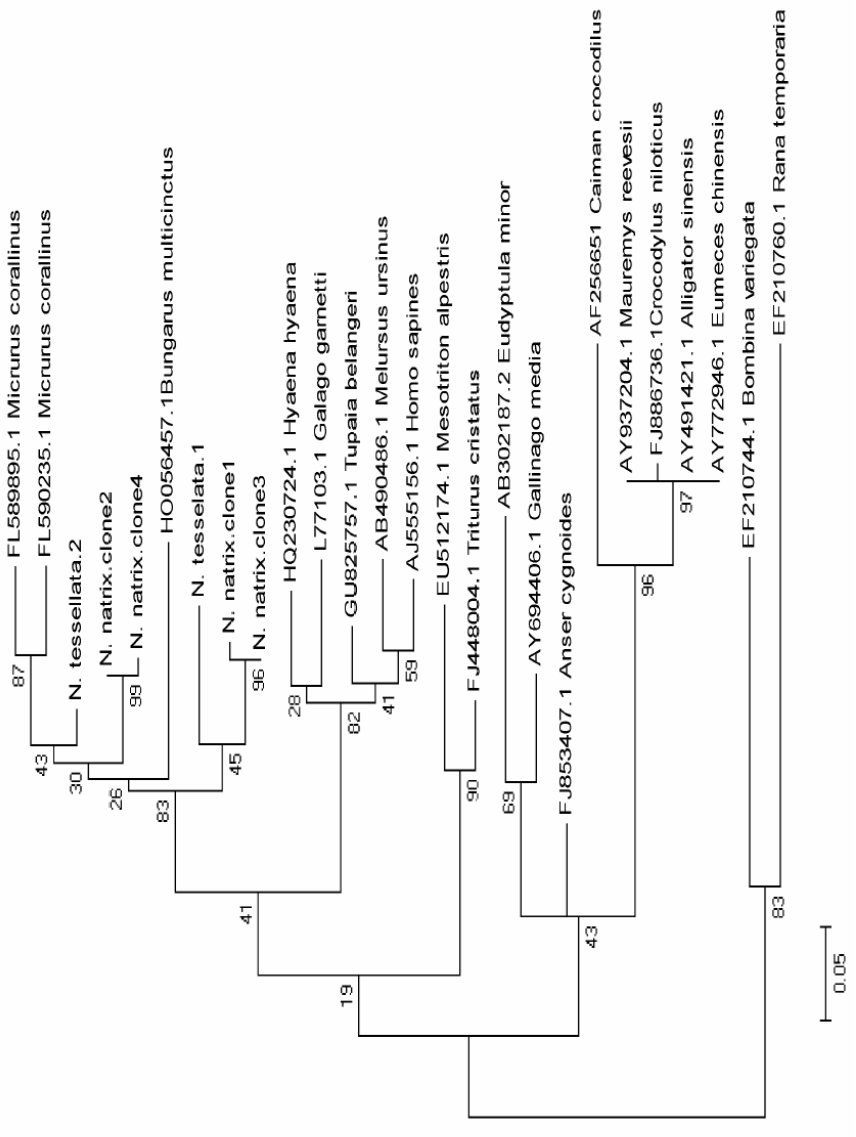

Figure 1: Maximum likelihood phylogenetic tree of the partial MHC class II B exon 2 sequences from the two Natrix species together with the homologue sequences from other species. The Natrix sequences (N. tessellata 1 and N. tessellata 2, N. natrix clone1-4) cluster together with the other snake sequences (*Micrurus corallinus* and *Bungarus multicinctus*) but they are closer to the mammalian sequences than the other reptile sequences. Bootstrap values are shown in the left nodes.

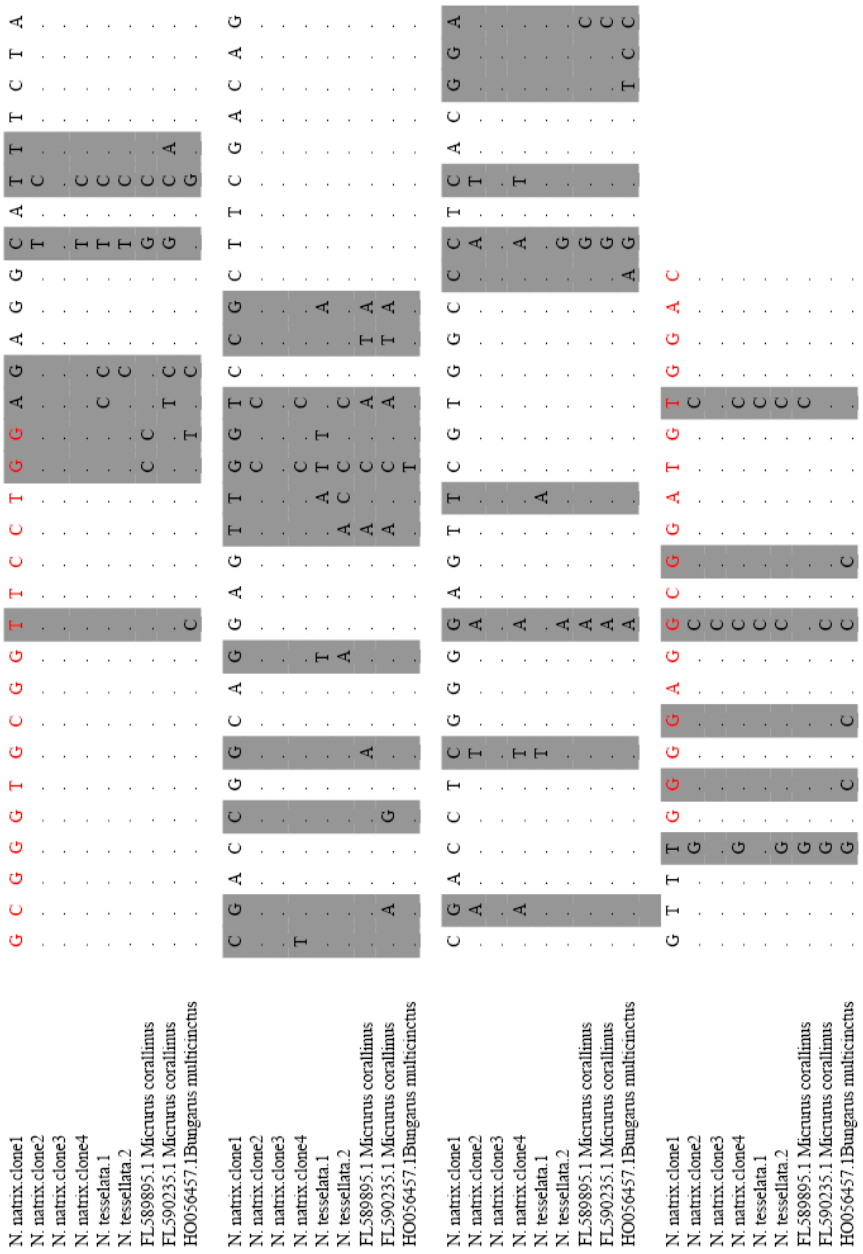

Figure 2: Alignment of the identified *Natrix tessellata* (N. tessellata 1 and N. tessellata 2) and *Natrix natrix* (N. natrix clone1-4) sequences with the homologue snake sequences from the NCBI (*Micrurus corallinus, Bungarus multicinctus*). Primer binding sites are marked with red while the variable nucleotide positions with grey.

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