Brief note

Novel class II- alpha *MHC* variability in a small peripheral Atlantic salmon population

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Running title: Novel *MHC* class II variability in salmon

Background: The highly polymorphic genes of the Major Histocompatibility Complex (*MHC*) are some of the most studied immune related genes in vertebrates [1]. *MHC* genes encode for proteins involved in the adaptive immune response by presenting pathogen-derived antigens to T-cells [2]. *MHC* diversity can be maintained by balancing selection driven by pathogens [3, 4] and/or by mate choice [5, 6]. Inbred populations tend to have a lower number of unique *MHC* genotypes and fewer alleles compared to their outbred counterparts [7, 8] but *MHC* diversity can be maintained even under inbreeding by the intensity of selection [9]. We analysed *MHC* class II alpha gene (*Sasa-DAA*) variation in a declining (Ne<200) peripheral Atlantic salmon (*Salmo salar*) population at the southern limit of the species' distribution (River Ason, Northern Spain). Peripheral populations are considered to be valuable for conservation to preserve the evolutionary potential of the species, as they can be genetically and morphologically different as a consequence of their isolation and (usually) smaller size than populations in the middle of the range [10, 11].

MHC sequencing: DNA was extracted from 47 scales taken from salmon caught by anglers during the period 1991-1993 using the Promega Wizard DNA extraction kit following the manufacturer's instructions. The primers (forward) 5'-GGGTTTCTTTTCTCAGTTCTGC-3' and (reverse) 5'-CTTCTCTCTCTTACCTATTTTCTCTTCTG-3' [12] were used to amplify a 244 base pair region including the exon 2 (α1 domain) of the class II *Sasa-DAA* locus. Reactions were performed as in [12], PCR products were purified using the Qiagen Qiaquick system and sequenced in a reaction including 3 µl of PCR template, 5 µl of Better Buffer™ (Microzone), 1 µl of BigDye (BigDye V3.1 Sequencing Kit™, ABI), 0.16 ρmol of primer and deionisied water to a total reaction volume of 15 µl. Sequences were aligned using Sequencher 3.1.1. and alleles were determined using the taxonomic based sequence analysis (TBSA)

methodology [13]. Only sequences found in at least 2 individuals and from independent PCRs were considered. Mega4 [14] was used to calculate the gamma distance from the amino acid sequences and to build a Neigbour-Joining phylogenetic tree with 10,000 bootstrap iterations. The tree was rooted using *Onchorynchus mykiss* class II α (*Onmy-DAA*) sequences (GenBank accession numbers: AJ251431–33).

MHC variation: Sequencing of 47 individuals revealed 23 individual amino acid sequences, 13 of which had been reported previously in Norwegian and Irish Atlantic salmon populations [12]. The ten new sequences have been deposited in Genbank under accession numbers FJ456877-FJ456885. There were a total of 26 polymorphic positions, 21 of which resulted in non-synonymous amino acid substitutions (Figure 1), all coincident with those reported in [12]. The phylogeny of amino acid sequences revealed three lineages with shallow branch lengths and moderate statistical support (Supplementary material, Figure 1S). Sequencing of class II alpha suggested that in the river Ason allelic diversity appears to be lower than values found previously found in Irish and Norwegian populations [12, 15]. Almost half of the *MHC*-related alleles found in Spain were unique and clustered mostly together, although with low branch support, suggesting an immunogenetic composition potentially different from that found in more northern populations.

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Table 1. Comparison of MHC class II alpha allele diversity among different Atlantic salmon populations.

Population	Origin	Sample size	MHC class II alpha alleles	Reference
Ireland	Wild	17	14	Consuegra *et al.* 2005[12]
Norway	Wild	20	14	Consuegra *et al.* 2005[12]
Spain	Wild	47	23	This work
Norway	Farmed	84	7	Stet *et al.* 2002[15]
Chile	Farmed	57	12	Gomez *et al.* 2011[16]

Figure 1. Alignment of class II *Sasa-DAA* α1 domain amino acid sequences. Those observed in this study ar

circles. A '.' indicates consensus; a '~' indicates a gap.

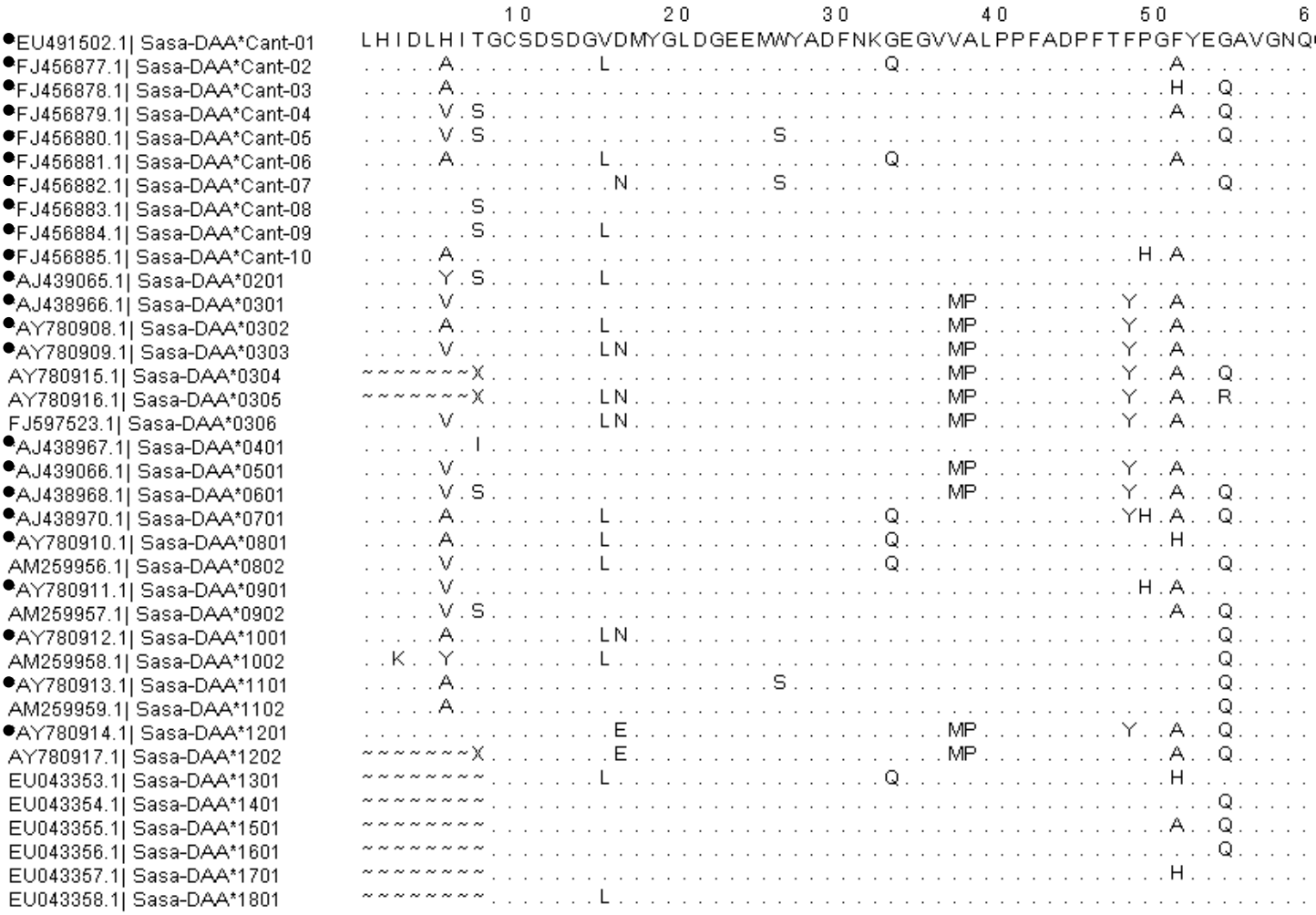

Figure S1. Neighbour-joining tree of known α1domain amino acid sequences of the MHC class II *Sasa-DAA* gene, including those from the River Asón Atlantic salmon (Sasa-DAA*Cant01-10). The analysis used 10,000 bootstrap iterations; bootstrap support is indicated at each node.