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1 **Title: First identification of dopamine receptors in pikeperch, *Sander lucioperca*, during**  
2 **the pre-ovulatory period.**

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22 **Abstract**

23 Dopamine (DA) is a ubiquitous neurotransmitter exerting a range of pleiotropic actions  
24 through two DA receptor families, the D1 and the D2. To date in vertebrates, a maximum of  
25 four receptor subtypes have been identified within the D1 family, D<sub>1</sub> (former D<sub>1A</sub>), D<sub>5</sub> (former  
26 D<sub>1B</sub>), D<sub>6</sub> (former D<sub>1C</sub>) and D<sub>7</sub> (former D<sub>1E</sub>), while the D2 family encloses five subtypes, D<sub>2</sub>,  
27 D<sub>3</sub>, D<sub>4</sub>, D<sub>8</sub> (former D<sub>2like</sub> or D<sub>2l</sub>) and D<sub>9</sub> (former D<sub>4-related sequence</sub> or D<sub>4-rs</sub>). In teleosts, no study  
28 has investigated in parallel all the DA receptors to identify and localize the whole receptor  
29 repertoire from both families. In pikeperch, *Sander lucioperca*, a species of interest for  
30 aquaculture development, the existence, number and location of the DA receptors are totally  
31 unknown. To address these questions, RNA-seq with *de novo* transcriptome reconstruction,  
32 functional annotation and phylogenetic analysis were performed to characterize the transcript  
33 repertoire of DA receptors in the brain of female pikeperch at the pre-ovulatory period. Ten  
34 different cDNA were identified and showed to belong to the D1 family: two D<sub>1</sub>, one D<sub>5a</sub>, one  
35 D<sub>6a</sub> and one D<sub>6b</sub> and to the D2 family: two spliced variants of D<sub>2</sub>, one D<sub>3</sub>, one D<sub>8</sub> and one D<sub>9</sub>.  
36 Unlike zebrafish, the subtypes D<sub>4</sub> and D<sub>7</sub> have not yet been isolated in pikeperch. As expected  
37 D<sub>1</sub>, D<sub>3</sub>, D<sub>8</sub> and D<sub>9</sub> are mostly expressed in brain parts except for the cerebellum (D<sub>1</sub> and D<sub>3</sub>).  
38 The inter-species differences in the number of DA receptors and the inter-organ differences in  
39 the gene expression of all receptors support the complexity of the dopaminergic actions in  
40 vertebrate.

41 **Keywords:** dopamine, RNA-sequencing, *de novo* transcriptome assembly, pikeperch

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## 47 **1. Introduction**

48 Dopamine (DA) is an ubiquitous neurotransmitter found in both central and peripheral  
49 nervous systems in many vertebrate species. In the central nervous system, it exerts a range of  
50 pleiotropic actions ranging from the control of locomotion, learning, emotion and sexual  
51 behaviour to the regulation of pituitary hormone release. This neuro-hormone also plays  
52 multiple roles in the peripheral tissues like regulation of the gastrointestinal motility,  
53 respiration, blood pressure and insulin secretion (Missale et al., 1998; Ben-Jonathan and  
54 Hnasko, 2001; Rubí and Maechler, 2010).

55 DA actions are mediated through seven transmembrane domain receptors (or G-protein  
56 coupled receptors GPCR) divided into two receptor families, namely D1 and D2 families.  
57 These receptor families are distinguished according to their intron-exon gene organization,  
58 their primary structure, their pharmacological properties and the stimulatory (D1 family) or  
59 inhibitory (D2 family) effects on adenylate cyclase throughout their signalling pathways.  
60 Notably, the D1 genes of the D1 family are intronless, their deduced protein structure exhibit  
61 a short third cytoplasmic loop and a long C-terminal tail while the genes of the D2 family  
62 contains introns, and the corresponding receptors a long third cytoplasmic loop and a short C-  
63 terminal tail (Missale et al., 1998). According to the authors and databases, different  
64 nomenclatures of genes and receptor subtypes exist, often leading to confusion when it comes  
65 to the designation of DA receptors in vertebrates (Yamamoto et al., 2015). Also, the set of  
66 genes and receptors existing into each family is dependent of vertebrate groups. During  
67 vertebrate evolution, many DA receptor genes and subtypes have been lost in mammals in  
68 comparison with non-mammalian species. Mammals kept only two subtypes in the D1 family  
69 ( $D_{1A}/D_1$  and  $D_{1B}/D_5$ ) and three subtypes in the D2 family ( $D_2$ ,  $D_3$  and  $D_4$ ) each encoded by  
70 one gene (Missale et al., 1998; Yamamoto et al., 2015). In addition, in this vertebrate  
71 subgroup, the presence of introns in the D2 family allowed the generation of two receptor

72 variants, D<sub>2long</sub> (D<sub>2L</sub>) and D<sub>2short</sub> (D<sub>2S</sub>) by an alternative splicing of the D<sub>2</sub> gene (Dal Toso et  
73 al., 1989). This phenomenon has already been described in an amphibian, the bullfrog, *Rana*  
74 *catesbeiana*, but has not yet been found in teleosts, except for the goldfish, *Carassius auratus*  
75 (Nakano et al., 2010; Popesku et al., 2011). To date, a maximum of nine DA receptor  
76 subtypes have been reported in the clade of Osteichthyes (Yamamoto et al., 2015). Four  
77 subtypes would be assigned to the D1 family, D<sub>1</sub> (former D<sub>1A</sub>), D<sub>5</sub> (former D<sub>1B</sub>), D<sub>6</sub> (former  
78 D<sub>1C</sub>) and D<sub>7</sub> (former D<sub>1E</sub>) while the D2 family would enclose five subtypes, D<sub>2</sub>, D<sub>3</sub>, D<sub>4</sub>, D<sub>8</sub>  
79 (former D<sub>2I</sub>) and D<sub>9</sub> (former D<sub>4rs</sub>). In zebrafish, *Danio rerio*, these nine DA receptors subtypes  
80 have been isolated (Yamamoto et al., 2015). In this species, except for D<sub>7</sub>, D<sub>8</sub> (D<sub>2b</sub> in  
81 Boehmler et al., 2004), D<sub>3</sub> and D<sub>9</sub> (D<sub>4b</sub> in Boehmler et al., 2007) encoded by a unique gene,  
82 the D<sub>1</sub>, D<sub>5</sub>, D<sub>6</sub>, D<sub>2</sub> and D<sub>4</sub> receptor subtypes are encoded by two paralogous genes giving two  
83 distinct receptors per subtype, D<sub>1a</sub> and D<sub>1b</sub>, D<sub>5a</sub> and D<sub>5b</sub>, D<sub>6a</sub> and D<sub>6b</sub>, D<sub>2a</sub> and D<sub>2b</sub> (called D<sub>2a</sub>  
84 and D<sub>2c</sub>, respectively in Boehmler et al., 2004), and D<sub>4a</sub> and D<sub>4b</sub> (called D<sub>4a</sub> and D<sub>4c</sub>,  
85 respectively in Boehmler et al., 2007; Yamamoto et al., 2015). As suggested by Yamamoto et  
86 al. (2015), these copies likely result from the 3R teleost-specific genome duplication.  
87 However, the duplicated copies resulting from 3R may have not been conserved in all teleost  
88 species. For example, in common carp, *Cyprinus carpio*, and in Nile tilapia, *Oreochromis*  
89 *niloticus*, only one copy of the D<sub>2</sub> receptor gene has been identified (Hirano et al., 1998;  
90 Levavi-Sivan et al., 2005) whereas in European eel, *Anguilla anguilla*, rainbow trout,  
91 *Oncorhynchus mykiss*, and goldfish, at least two D<sub>2</sub> receptor genes have been found (Vacher  
92 et al., 2003; Pasqualini et al., 2009; Popesku et al., 2011). In rainbow trout, these duplicated  
93 copies may result directly from the salmonid-specific genome duplication 4R (Dufour et al.,  
94 2010). As observed in vertebrate species, there is a high diversity and complexity in the  
95 transcript profile of DA receptors, which should be deciphered for each species separately.

96 This high complexity raises the question about the specificities of each receptor in their  
97 respective biological functions. The literature provides some indications that the DA receptors  
98 roles depend on the receptor subtype and its location. For instance, receptors of the D2 family  
99 seems to play an inhibitory or stimulatory role in mammal locomotion whereas receptors of  
100 the D1 family would have little or no effect (Jackson and Westlind-Danielsson, 1994; Missale  
101 et al., 1998). However, a synergistic effect between D2 and D1 receptor activation was  
102 suggested to increase forward locomotion (Missale et al., 1998). In pikeperch, *Sander*  
103 *luciperca*, *in vivo* experiments suggested that the D1 receptor family, but not D2 family, is  
104 involved in the regulation of sex-steroid production during the final oocyte meiotic maturation  
105 (Roche et al., 2018). In zebrafish, Boehmler et al. (2004) suggested that D<sub>2</sub> (D<sub>2a</sub> and D<sub>2c</sub>), D<sub>3</sub>  
106 and D<sub>8</sub> (D<sub>2b</sub>) receptor subtypes may be involved in visual function. Also, they showed that  
107 receptors D<sub>2a</sub> and D<sub>8</sub> (or D<sub>2b</sub>) are both expressed in the pineal gland but not at the same  
108 developmental stage, leading to the possibility of two different roles within this tissue  
109 (Boehmler et al., 2004). The study of DA receptor functions is often managed through the  
110 investigation of their central and peripheral distributions. However, unlike mammals, data  
111 regarding the location of DA receptors are very scarce in teleosts. Using *in situ* hybridization  
112 or q-PCR analyses, some studies showed that D<sub>2</sub> receptors are differentially expressed in the  
113 brain of species such as European eel, rainbow trout, Nile tilapia, and zebrafish (Vacher et al.,  
114 2003; Boehmler et al., 2004; Levavi-Sivan et al., 2005; Pasqualini et al., 2009). D<sub>2A</sub> and D<sub>2B</sub>  
115 in European eel and the D<sub>2</sub> receptors in rainbow trout display high expression levels in most  
116 of the brain areas such as the olfactory bulbs, the telencephalon, the optic tectum, and also in  
117 the pituitary (Vacher et al., 2003; Pasqualini et al., 2009). However, in European eel, only  
118 D<sub>2A</sub>, but not D<sub>2B</sub>, is expressed in the retina, the olfactory epithelium, the spinal cord and the  
119 adipose tissue (Pasqualini et al., 2009). Similarly, receptors from D1 family are differentially  
120 expressed according to the brain areas but only D<sub>1,1</sub> and D<sub>1,2</sub> (D<sub>1A1</sub> and D<sub>1A2</sub>) are expressed in

121 the pituitary (Kapsimali et al., 2000). Regarding the location in the peripheral tissues, there  
122 are also inter-species peculiarities. Indeed, D<sub>2</sub> receptors are not detected in European eel  
123 ovaries, liver, kidney, muscle and gills whereas in Nile tilapia, D<sub>2</sub> receptor gene expression  
124 was measured in these tissues, except for kidney and muscle (Levavi-Sivan et al., 2005;  
125 Pasqualini et al., 2009). More information about the presence and abundance level of all DA  
126 receptors would provide indications about their respective roles in teleost physiology. To date,  
127 experimental studies about DA receptors are very scarce, making genomic and protein  
128 databases the main informative sources. No investigation has aimed to identify and locate the  
129 full DA receptors repertoire for one given teleost species in a single study.

130 Pikeperch is a percid fish from the perciform order and a species of interest for aquaculture  
131 diversification (Kestemont et al., 2015). Contrary to the situation in cyprinids, salmonids, or  
132 anguillids, no data have been evidenced so far on the existence, number and location of DA  
133 receptors in percid fishes. Furthermore, few information exists on the expression profile of  
134 DA receptors during the final stages of reproduction, a period of huge behavioural and  
135 endocrine balance changes. To address these questions, we gathered the repertoire of DA  
136 receptors in the brain of maturing pikeperch using whole transcriptome reconstruction from  
137 RNA sequencing, analysed their cDNA, inferred protein sequences, compared them with  
138 other species and investigated a multi-tissue gene expression pattern of these receptors.

139

## 140 **2. Material & Methods**

### 141 **2.1. Animal and sampling procedures**

142 The experiment was performed according to the European and French legislation for fish  
143 welfare and approved by the institutional Ethics Committee (APAFIS3073-  
144 2015120813148770). Mature pikeperch females (n=9; 3-4 years old; 1.54 ± 0.07 kg) from  
145 Czech Republic (origin: production pond Bynovsky, Fishery Nove Hradky Ltd) were

146 transported to the Aquaculture Experimental Platform (AEP, registration number for animal  
147 experimentation C54-547-18) belonging to the URAFPA lab of the University of Lorraine  
148 (Nancy, France), acclimated into RAS systems containing 2m<sup>3</sup> tanks and killed by  
149 overexposure to anesthesia ethyl 3-aminobenzoate methanesulfonate (MS-222; 240 mg/L;  
150 Sigma-Aldrich, Lyon, France) and quickly sampled for different tissues. Brains from three  
151 females were collected and stored at -80°C for subsequent RNA sequencing. Six major brain  
152 structures were dissected out and used for the qPCR analysis (Figure 6): the 4 prominent lobes  
153 (telencephalon and the rostral Optic Recess Region (Tel/ORR), optic tectum (OT), cerebellum  
154 (Cb), hypothalamus/Inferior Lobe (Hyp/IL)), the olfactory bulbs (OB), the medulla/rostral  
155 spinal cord (Med/SC). The rest of the brain (including diencephalon, tegmentum, and the  
156 caudal spinal cord) was discarded. Pituitary, ovaries, heart, adipose tissue, muscle, gills,  
157 spleen and liver were also collected and also stored at -80°C for further DA receptor gene  
158 expression analysis.

159

## 160 **2.2. Evaluation of oocyte maturation stages**

161 Before tissue samplings, oocyte maturation stages were evaluated for each female according  
162 to the classification by Źarski et al. (2012). Briefly, oocytes were sampled using a catheter  
163 (CH06; 1.2 mm internal and 2 mm external diameter) and placed in Serra's solution  
164 (ethanol/formalin/glacial acetic acid, 6:3:1 v/v/v). After slowly mixing oocytes in Serra's  
165 solution and waiting (about 5 min) until the cytoplasm of the oocyte has become clarified, the  
166 oocyte maturation stage was evaluated under the binocular microscope, magnification  $\times 4$   
167 (Motic® SFC-11 Series, Motic Asia, Hong Kong, China). In pikeperch, the final stages of  
168 maturation were divided into seven morphological stages, from stage I to stage VII  
169 (ovulation; Źarski et al., 2012). This allowed the determination of the advancement of the

170 oocyte meiotic maturation at the sampling time. Females were evaluated between the stages II  
171 and V.

172

### 173 **2.3. RNA-seq & *de novo* transcriptome assembly**

#### 174 **2.3.1. RNA sequencing**

175 Total RNA from three brain samples was extracted using Isol-RNA reagent (VWR  
176 International SAS, Strasbourg, France) according to the manufacturer's protocol. The extracts  
177 were then sent to the McGill University and Genome Quebec Innovation Centre (Montreal,  
178 Québec, Canada) where RNA concentration was measured using a NanoDrop  
179 Spectrophotometer ND-1000 (NanoDrop Technologies, Inc.) and RNA integrity was assessed  
180 using a 2100 Bioanalyzer (Agilent Technologies). Libraries were generated from 250 ng of  
181 total RNA using the TruSeq stranded mRNA Sample Preparation Kit (Illumina), following the  
182 manufacturer's recommendations. Libraries were quantified using the Quant-iT™  
183 PicoGreen® dsDNA Assay Kit (Life Technologies) and the Kapa Illumina GA with Revised  
184 Primers-SYBR Fast Universal kit (Kapa Biosystems). Average fragment size was determined  
185 using a LabChip GX (PerkinElmer) instrument.

186 Whole transcriptome-Seq was then performed using a HiSeq 2500 instrument, generating  
187 125-nucleotide-long paired-end reads which were compiled in FASTQ files complying the  
188 format standard. Quality control of sequence reads was done using FastQC v0.11.5 software  
189 (<http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>). Illumina universal adapters  
190 were removed with cutadapt v1.11 (Martin, 2013) discarding trimmed reads shorter than 30  
191 nucleotides, with parameters “ -a AGATCGGAAGAGC -A AGATCGGAAGAGC -m 30 --  
192 no-indels -O 5 ”. Low quality reads were also filtered, discarding bases with a Phred quality  
193 score < 20.

194

### 195 **2.3.2. *De novo* transcriptome assembly**

196 The *de novo* transcriptome assembly was carried out using *Trinity* v2.2.0 (Grabherr et al.,  
197 2011; Haas et al., 2013) without a guiding genome, for stranded libraries (with option –  
198 ss\_lib\_type RF). Assemblies for each pikeperch sample were further evaluated (i) by  
199 assessing their read contents - mapping was achieved with *bowtie2* v2.2.8 (Langmead and  
200 Salzberg, 2012), (ii) by counting full-length transcripts by sequence alignment with high  
201 quality annotations for known proteins; *blastx* (Camacho et al., 2009) was used on the  
202 SwissProt database obtained from <http://www.uniprot.org>, and (iii) by describing them  
203 statistically with *Transrate* v1.0.1 (Smith-Unna et al., 2016). Transcripts were then quantified  
204 using *Kallisto* v0.43 (Bray et al., 2016). Identification of likely coding regions within each  
205 transcript was performed with *Transdecoder* v3.0.1 (<http://github.com/Transdecoder>), which  
206 makes use of *blastp* on SwissProt. Each putative peptide was aligned with *hmmer3.1* (Eddy,  
207 2011) on the PFAM database (Finn et al., 2016) to match with a protein domain. Signal  
208 peptide and transmembrane domain predictions were checked out with *signalP* v4.1. (Petersen  
209 et al., 2011) and TMHMM v2.0c (Krogh et al., 2001), respectively. *Rnammer-1.2* (Lagesen et  
210 al., 2007) was used to mark potential rRNA remaining transcripts. All the data produced was  
211 finally compiled and submitted to *Trinotate* v3.0.1 (<https://github.com/Trinotate>) to populate  
212 an SQLite database representing the functionally annotated transcriptome, along with blast  
213 homologies, as well as gene ontology (GO), orthology (eggNOG) and pathway (KEGG)  
214 informations (Ashburner et al., 2000; Huerta-Cepas et al., 2016; Kanehisa et al., 2017;  
215 The Gene Ontology Consortium, 2017). Annotations were further enriched with custom blasts  
216 on a subset of the trEMBL database (<http://www.uniprot.org>) containing all known and  
217 predicted DA receptor sequences.

218 These populated whole transcriptome databases (one for each sample) were extensively used  
219 as a dedicated tool for searching and identifying the DA receptors in pikeperch brain. Among

220 the three females, a total of 30 cDNA sequences were obtained. Whenever possible, each  
221 identified DA receptor was computationally confirmed using three ways: 1) multiple  
222 alignments with orthologous receptors of closely related species, 2) secondary structures  
223 alignments (RaptorX, Källberg et al., 2012) with well-characterized DA receptors of the same  
224 type were performed to validate and localize functional domains, and 3) the receptor should be  
225 found in at least two samples. The degree of identity (%), e-values and bit scores of DA  
226 receptor protein sequences between pikeperch and other species were given by *blastp*  
227 (Camacho et al., 2009).

#### 228 **2.4. Phylogenetic analysis**

229 Amino acid sequences of 74 osteichthyan receptors from the D1 family (D<sub>1</sub>, D<sub>5</sub>, D<sub>6</sub>, D<sub>7</sub>), and  
230 75 osteichthyan receptors from the D2 family (D<sub>2</sub>, D<sub>3</sub>, D<sub>4</sub>, D<sub>8</sub>, D<sub>9</sub>) were retrieved from NCBI  
231 (<https://www.ncbi.nlm.nih.gov>), Ensembl (<http://www.ensembl.org/index.html>) and Uniprot  
232 databases (Supplementary Table 3). These osteichthyan species included sarcopterygians  
233 (tetrapods and a basal sarcopterygian, the coelacanth, *Latimeria chalumnae*), and  
234 actinopterygians (teleosts and a non-teleost actinopterygian, the spotted gar, *Lepisosteus*  
235 *oculatus*). Human, *Homo sapiens*, adrenergic receptor sequences were used as outgroup.

236 Multiple sequence alignments of D1 and D2 protein families were created using Clustal  
237 Omega (Sievers et al., 2011) included in SeaView version 4.6.3 (Gouy et al., 2010) and  
238 manually adjusted. Calculation of the best amino acid substitution matrix was determined  
239 using the ProtTest software version 3.4.2 (Darriba et al., 2011). The JTT (Jones, Taylor and  
240 Thornton) protein substitution matrix was selected for both alignments. Phylogenetic analyses  
241 were performed with the resulting protein alignments using the Maximum Likelihood method  
242 with 1000 bootstrap replicates (RaxML software (Stamatakis, 2014), <http://www.phylo.org>).

243

#### 244 **2.5. Expression profile analysis of DA receptors**

245 After total RNA extraction using Isol-RNA reagent (VWR International SAS), a Dnase  
246 treatment was performed according to the manufacturer's procedure (Fisher Scientific,  
247 Illkirch, France). Reverse transcription (RT) was then achieved using 1 µg/µL of total RNA  
248 following a previously described procedure (Milla et al. 2010). To determine the multi-tissues  
249 mRNA profile of some DA representatives (D<sub>1a</sub>, D<sub>1b</sub>, D<sub>3</sub>, D<sub>8</sub> and D<sub>9</sub>), Real-time polymerase  
250 chain reaction (q-PCR) was carried out using a StepOne Plus system (Applied Biosystems,  
251 Fisher Scientific) as previously described (Milla et al. 2010). To validate the specificity of the  
252 amplification and the absence of amplified genomic DNA, different controls were performed:  
253 1) sample without Dnase treatment, 2) sample without reverse transcriptase and 3) negative  
254 controls with RNase-free water. Gene expression was normalized using the geometric mean  
255 of two housekeeping genes: Ribosomal Protein L8 (RPL8) and Adenosine Kinase like (AK),  
256 whose expressions were stable under these conditions (data not shown). Primers used for DA  
257 receptors and housekeeping genes were described in Table 1. To ensure that primers were  
258 specific of each DA receptor they were designed in unconservative regions such as intra and  
259 extracellular domains, specific to each receptor. All primer pairs were further checked for  
260 uniqueness and concordance within each transcriptome using *blastn* with 'word size 7' as  
261 parameter for small sequence matches.

262

## 263 **2.6. Statistical analysis**

264 Statistical analyses were performed using R version 3.4.0. For all dependent variables,  
265 homogeneity of variances was tested using Levene test (`leveneTest`, package 'car', Fox and  
266 Weisberg, 2011). For relative DA receptors gene expression, data were analysed by a linear  
267 mixed model (`lmer`, package 'lme4', Bates et al., 2015) with tissues as fixed effects, and  
268 either the fish (for repeated measures) and/or the maturation stage at the sampling time as  
269 random effects: `model=lmer(Y~tissues+(1|fish)+(1|maturation_stage)` with *Y*: dependent

270 variable. For model validation, residuals were tested for homogeneity and normality using  
271 residual vs fitted value and sample vs theoretical quantile (Q-Q) plots, respectively (plotresid,  
272 package ‘RVAideMemoire’, Hervé, 2016). If necessary, data were log-transformed or root-  
273 square-transformed. When the model was validated, an Anova table was performed to  
274 calculate F-tests (Anova, package ‘car’, Fox and Weisberg, 2011) followed by a Least-squares  
275 means (predicted marginal means) multiple comparison between tissues as *post-hoc* test  
276 (lsmeans, package ‘lsmeans’, Lenth, 2016). When data, even transformed, did not meet the  
277 assumptions for the linear mixed model, we used the Kruskal-Wallis test for non-parametric  
278 analysis with n variable (kruskal.test) followed by a pairwise comparison using Dunn test  
279 (posthoc.kruskal.dunn.test, package ‘PMCMR’, Pohlert, 2016) with Benjamini & Hochberg  
280 correction (BH; Benjamini and Hochberg, 1995). Data are expressed as mean ± SEM. The  
281 level of significance used in all tests was  $P < 0.05$ .

282

### 283 **3. RESULTS**

#### 284 **3.1. Evaluation of the transcriptome assembly**

285 After filtering and trimming low quality reads, sequencing generated an average of 79.9  
286 million read pairs per sample. This led to an average of 272,211 contigs per fish after the  
287 Trinity (Grabherr et al., 2011; Haas et al., 2013) assembly step. Re-mapping of the respective  
288 reads on each transcriptome reached 99.1% match and 97.4% complete, concordant pair re-  
289 alignment. N50 calculation, based on the longest transcript per “gene”, was evaluated at 1000,  
290 with an average of 659 nt length (median was at 375 nt). GC content was 44.67%. After  
291 estimating their relative abundance, transcripts were flagged for sufficient expression with a  
292 minimum of 20 reads per contig required. An average of 91,052 transcripts per sample passed  
293 this filter. For our purposes, all transcripts were retained anyway until the identification step.  
294 Putative proteomes were finally computed from each transcriptome with Transdecoder

295 (<http://github.com/Transdecoder>), which produced an average of 76,976 putative proteins per  
296 sample, 40,856 being complete (from the first methionine to the stop codon) and fully  
297 annotated. A comprehensive database compiling transcriptomic results was generated at this  
298 point (see Material and Methods) and was used throughout the rest of the study.

299

### 300 **3.2. Identification and sequence analyses of pikeperch DA receptors**

301 Database mining and pairwise comparisons led to the identification of ten distinct cDNAs  
302 corresponding to DA receptors. Their deduced amino acid sequences were aligned with  
303 human and some teleost and DA receptors from both D1 and D2 families.

304 Five DA receptors, namely D1\_1, D1\_2, D1\_3, D1\_4 and D1\_5 (Table 2 and Supplementary  
305 Table 1), belonged to the D1 family (only 21-41% of identity with human and teleost D2  
306 receptors family). Among these five receptors, D1\_1 and D1\_2 displayed the highest  
307 sequence identity with human D<sub>1</sub> receptor (range between 72-73%). Relatively to teleost  
308 species, D1\_1 displayed about 80% of sequence identity with zebrafish and European eel D<sub>1</sub>  
309 receptors while it displayed the highest identity with the Nile tilapia D<sub>1a</sub> receptor (93%).  
310 Pikeperch D1\_2 shared high percentage of sequence identity with zebrafish D<sub>1b</sub> (84%), Nile  
311 tilapia D<sub>1b</sub> (93%) and European eel D<sub>1,1</sub> (84%). D1\_3 showed 66% of sequence identity with  
312 the human D<sub>5</sub> receptor while D1\_4 displayed 60% of sequence identity with the human D<sub>1</sub>  
313 receptor. Relatively to teleost receptors, D1\_3 shared similar sequence identity with zebrafish  
314 D<sub>1a</sub> and D<sub>5a</sub> (71-72%) and a high percentage of sequence identity with the Nile tilapia D<sub>5a</sub>  
315 (93%) and European eel D<sub>5</sub> (85%). D1\_4 displayed the highest identity with the zebrafish and  
316 Nile tilapia D<sub>6a</sub> (66 and 79%, respectively) and European eel D<sub>6</sub> receptor (65%). Finally,  
317 D1\_5 sequence shared similar sequence identities with human D<sub>1</sub> and D<sub>5</sub> receptors (61-62%)  
318 while it displayed the highest sequence identities with zebrafish D<sub>6a</sub> and D<sub>6b</sub> (76%), Nile  
319 tilapia D<sub>6b</sub> (82%) and European eel D<sub>6</sub> (76%) receptors. No pikeperch sequences identified in

320 this study were related to zebrafish and tilapia D<sub>5b</sub> or to zebrafish D<sub>7</sub> receptors at these levels  
321 of identification.

322

323 The five remaining DA receptors, namely D2\_1, D2\_2, D2\_3, D2\_4 and D2\_5 (Table 3 and  
324 Supplementary Table 2), belonged to the D2 family (with only 29-46% of identity with  
325 human and other teleost D1 receptors family). Among these five receptors, D2\_1, D2\_2 and  
326 D2\_4 showed the highest degree of sequence identity with human D<sub>2</sub> receptors (from 55% to  
327 66%). Relatively to teleost species, D2\_1 and D2\_2 displayed the highest sequence identity  
328 with teleost D<sub>2</sub> subtype, the Nile tilapia D<sub>2</sub> (95% and 89%, respectively), European eel D<sub>2A</sub>  
329 (78% and 73%, respectively), European eel D<sub>2B</sub> (75% and 72%, respectively), zebrafish D<sub>2a</sub>  
330 (73% and 69%, respectively) and zebrafish D<sub>2b</sub> receptors (73% and 71%, respectively). D2\_3  
331 shared 58% of sequence identity with human D<sub>3</sub> while he displayed the highest sequence  
332 identities with zebrafish and Nile tilapia D<sub>3</sub> (75-90%). D2\_4 shared highest sequence identity  
333 with Nile tilapia D<sub>8</sub> (89%) and zebrafish D<sub>8</sub> (75%) receptors. Finally, D2\_5 shared 68% of  
334 sequence identity with human D<sub>4</sub> while he displayed the highest sequence identities with  
335 zebrafish and Nile tilapia D<sub>9</sub> (71-76%) receptors, respectively.

336 Accordingly, we propose the following designation for those ten DA receptor sequences: D<sub>1a</sub>  
337 (D1\_1), D<sub>1b</sub> (D1\_2), D<sub>5a</sub> (D1\_3), D<sub>6a</sub> (D1\_4), D<sub>6b</sub> (D1\_5), D<sub>2</sub> for two sequences sharing 100%  
338 of identity (D2\_1 and D2\_2), D<sub>3</sub> (D2\_3), D<sub>8</sub> (D2\_4) and D<sub>9</sub> (D2\_5).

339

340 Amino acid sequence alignments of the 10 putative pikeperch DA receptors with human and  
341 teleost DA receptors showed structure conservation. Two examples from D1 and D2 families:  
342 D<sub>1a</sub>, D<sub>1b</sub> and both D<sub>2</sub> are shown in Figures 1 and 2, respectively. The other sequence  
343 alignments are shown in Supplementary Figures 1, 2, 3, 4 and 5. All sequences are complete  
344 except for the predicted pikeperch D<sub>5a</sub> and D<sub>6b</sub> for which two and four of the seven putative

345 transmembrane domains (TMD) are missing, respectively. For both families, the TMD are  
346 highly conserved relatively to the TMD of other species. Also, the specific GPCR DRY motif  
347 is observed within all sequences, except for the predicted partial D<sub>6b</sub> polypeptide  
348 (Supplementary Material). Regarding the D1 family (Figure 1 and Supplementary Materials),  
349 we observed a short third cytoplasmic loop and a long and variable C-terminal tail enclosing a  
350 cysteine amino acid at its beginning, except for the partial D<sub>6b</sub>. In contrast, we noticed a long  
351 and variable third cytoplasmic loop and a short and relatively well-conserved cytoplasmic tail  
352 ending with a cysteine residue for all predicted receptors of the D2 family. Regarding one of  
353 both D<sub>2</sub> sequences, a gap in the protein sequence within the third intracellular loop (29  
354 missing amino acids) was observed (Figure 2). The two D<sub>2</sub> sequences were renamed D<sub>2Long</sub>  
355 (D<sub>2L</sub>) and D<sub>2Short</sub> (D<sub>2S</sub>), as in mammals. Highly conserved amino acid residues between  
356 pikeperch and other vertebrate species were also observed in TMD and extracellular and  
357 cytoplasmic loops. For instance, we observed one conserved aspartate in TMD II and III, one  
358 asparagine in TMD VII, two serines in TMD V and two cysteines in extracellular loops I and  
359 II (Figure 2).

360

### 361 **3.3. Phylogenetic analysis**

362 Phylogenetic analyses of actinopterygian and sarcopterygian DA receptors amino acid  
363 sequences were performed to confirm the identity of the predicted pikeperch sequences.

364 Regarding the D1 family (Figure 3), the phylogeny analysis clustered D<sub>6</sub>, D<sub>1</sub> and D<sub>5</sub>  
365 sequences in three well-supported clades, with bootstrap values of 91, 98 and 67%,  
366 respectively. However, the few D<sub>7</sub> sequences retrieved from the available databases were not  
367 encompassed in a single monophyletic clade. The D<sub>1</sub> clade was composed of two distinct  
368 subclades: a sarcopterygian and an actinopterygian, with the non-teleost actinopterygian  
369 spotted gar branching at the base of the actinopterygian clade, in accordance with its

370 phylogenetical position. Teleost D<sub>1</sub> sequences were separated into two clades, named D<sub>1a</sub> and  
371 D<sub>1b</sub>. Pikeperch D1\_1 and D1\_2 sequences branched individually within each one of these  
372 clades, confirming their identification as D<sub>1a</sub> and D<sub>1b</sub>. D<sub>5</sub> sequences were separated into three  
373 clades: a sarcopterygian clade, and two actinopterygian clades. The first actinopterygian clade  
374 encompassed the teleost D<sub>5a</sub> sequences with the spotted gar D<sub>5</sub> branching at its base, and the  
375 second one encompassed the D<sub>5b</sub> teleost sequences. The D1\_3 pikeperch sequence branched  
376 together with the D<sub>5a</sub> teleost sequences, confirming its place in the nomenclature as D<sub>5a</sub>.  
377 Concerning D<sub>6</sub>, the actinopterygian clade, with the spotted gar D<sub>6</sub> sequence branching at its  
378 base, encompassed two well-supported teleost clades, each one including a pikeperch  
379 sequence belonging to the D1 family. Pikeperch D1\_4 and D1\_5 were thus named D<sub>6a</sub> and  
380 D<sub>6b</sub>, respectively.

381 Concerning the D2 family, phylogenetic analysis clustered all vertebrate sequences in two  
382 strongly-supported clades, D<sub>4</sub>/D<sub>9</sub> (100%) on one side and D<sub>3</sub>/D<sub>2</sub>/D<sub>8</sub> (91%) on the other side. A  
383 clear demarcation was observed between D<sub>4</sub> (80%) and D<sub>9</sub> (89%) sequences within the D<sub>4</sub>/D<sub>9</sub>  
384 clade. Within the D<sub>3</sub>/D<sub>2</sub>/D<sub>8</sub> clade, D<sub>3</sub> (90%), D<sub>2</sub> (78%) and D<sub>8</sub> (68%) sequences diverged into  
385 three subclades. Into each one of the D<sub>4</sub>, D<sub>9</sub>, D<sub>3</sub>, D<sub>2</sub> and D<sub>8</sub> subclades, a well-supported  
386 actinopterygian branch was defined.

387 The spotted gar D<sub>4</sub> sequence branched at the base of the two teleost D<sub>4</sub> clades, D<sub>4a</sub> and D<sub>4b</sub>,  
388 while the spotted gar D<sub>9</sub> branched with a single teleost D<sub>9</sub> clade, including the pikeperch D2\_5  
389 sequence. The spotted gar D<sub>3</sub> sequence branched at the base of a single teleost D<sub>3</sub> clade,  
390 including the D2\_3 pikeperch sequence. Accordingly, these pikeperch sequences were  
391 respectively named D<sub>9</sub> and D<sub>3</sub>. Similarly, the spotted gar D<sub>8</sub> sequence branched together with  
392 the coelacanth D<sub>8</sub> one, within a single teleost D<sub>8</sub> clade, including the D2\_4 pikeperch  
393 sequence, now called D<sub>8</sub>. The spotted gar D<sub>2</sub> sequence branched at the base of two well-  
394 supported clades, named D<sub>2a</sub> (73%) and D<sub>2b</sub> (60%). The pikeperch D2<sub>L</sub> sequence branched

395 together with other teleost D<sub>2a</sub> sequences. Thus, the two pikeperch D<sub>2</sub> sequences (D2\_1 and  
396 D2\_2), differing only by a contiguous sequence of 29 amino acids, corresponded to the long  
397 and short isoforms of a single D<sub>2</sub> gene, hereby named D<sub>2a</sub> (D<sub>2aL</sub> and D<sub>2aS</sub>). No pikeperch  
398 sequences orthologous to vertebrate D<sub>4</sub> were evidenced in this study.

399

### 400 **3.4. Multi-tissue mRNA abundance of DA receptors from the D1 and D2 families in** 401 **pikeperch**

402 Over the ten sequences of DA receptors found by RNA-seq, the multi-tissue gene expression  
403 pattern focused on five receptor subtypes as we failed to design good primers for the five  
404 remaining ones.

#### 405 **3.4.1. Gene expression profile of D<sub>1a</sub> and D<sub>1b</sub>**

406 The same profile of expression was observed for both genes with a high expression in the  
407 brain (Figures 5A and B), predominantly in the olfactory bulbs, optic tectum, telencephalon  
408 and rostral ORR, medulla/rostral spinal cord and hypothalamus/IL (Figure 6). Both gene  
409 expressions were lower in the cerebellum and in the pituitary, and undetectable in muscle,  
410 adipose tissue, gills and spleen. D<sub>1b</sub> expression was also undetectable in liver and heart.

411

#### 412 **3.4.2. Gene expression of D<sub>3</sub>, D<sub>8</sub> and D<sub>9</sub>**

413 The three genes were mainly expressed in the brain region (Figures 5C, D and E). The D<sub>3</sub> was  
414 mostly expressed in the telencephalon and rostral ORR, olfactory bulbs, hypothalamus/IL and  
415 pituitary. The D<sub>8</sub> was predominantly expressed in the optic tectum, medulla/rostral spinal cord  
416 and hypothalamus/IL while the D<sub>9</sub> was mostly expressed in olfactory bulbs, telencephalon and  
417 rostral ORR, and optic tectum. In the ovaries, D<sub>3</sub> and D<sub>8</sub> gene expressions were above the  
418 detection limit while the D<sub>9</sub> mRNA was undetectable. In the heart, D<sub>3</sub> had an intermediate  
419 expression level. Finally, the gene expressions were above the detection limit in the liver for

420 D<sub>3</sub> and D<sub>8</sub>, in the heart for D<sub>8</sub> and D<sub>9</sub>, in the muscles for D<sub>8</sub>, in the gills for D<sub>3</sub> and  
421 undetectable in the remaining tissues.

422

#### 423 **4. Discussion**

424 In this study, we identified ten DA receptor mRNAs isolated from the brain of pikeperch at  
425 the pre-ovulatory period.

426 Five DA receptor sequences were attributed to the D1 family using amino acid sequence  
427 comparisons and phylogenetic analyses. These sequences share between 52% and 93% of  
428 sequence identity with other vertebrate D<sub>1</sub> receptors and only 21-41% with vertebrate D<sub>2</sub>  
429 receptors. Besides the seven TMDs found in complete sequences and the DRY sequence  
430 specific of GPCR, these receptors share common features with D<sub>1</sub> receptors characterized in  
431 other vertebrate species, including mammals and teleosts. Among these characteristics, we  
432 observed the short third intracellular cytoplasmic loop and a long unvariable C terminal  
433 cytoplasmic tail enclosing a conserved cysteine residue allowing to anchor the receptor to the  
434 membrane (Civelli et al., 1993; Missale et al., 1998; Ben-Jonathan and Hnasko, 2001). Some  
435 conserved amino acid residues allowing the receptor functionality and regulation were also  
436 observed. For instance, we found an aspartate in TMD 2 and 3 or two serines in TMD 5 that  
437 form the narrow pocket for the ligand binding, two cysteines in the first and second  
438 extracellular loop that allow the stabilization of the receptor conformation with a disulfide  
439 bond, or also some phosphorylation/glycosylation sites in the different cytoplasmic and  
440 extracellular loops (Civelli et al., 1993; Missale et al., 1998). Phylogenetic analysis assigned  
441 these five pikeperch sequences to specific DA receptor clades, D<sub>1</sub>, D<sub>5</sub> and D<sub>6</sub> (Cardinaud et  
442 al., 1997; Yamamoto et al., 2015). Thus, we assume that pikeperch possesses at least five D1  
443 family DA receptor genes that are orthologous to D<sub>1a</sub>, D<sub>1b</sub>, D<sub>5a</sub>, D<sub>6a</sub> and D<sub>6b</sub>.

444 These different D<sub>1</sub> receptor paralogs have already been identified in literature or have been  
445 released in the relevant databases. A single D<sub>1</sub> receptor was identified in sarcopterygian  
446 species, such as mammals, birds, and amphibians, as well as in the spotted gar, a non-teleost  
447 actinopterygian. Two D<sub>1</sub> paralogs were found in several teleost species, including European  
448 eel, common carp, zebrafish, and Nile tilapia (Sugamori et al., 1994; Demchyshyn et al.,  
449 1995; Macrae and Brenner, 1995; Cardinaud et al., 1997; Hirano et al., 1998; Missale et al.,  
450 1998; Yamamoto et al., 2015; NCBI, Ensembl and Uniprot databases), probably as a  
451 consequence of the teleost-specific genome duplication (3R). In some species such as in  
452 Atlantic herring (Figure 3), one paralog is likely to be secondarily lost. But the pikeperch  
453 would have conserved these two D<sub>1A</sub> paralogs, as most of the teleosts.

454 In the same way, two D<sub>6</sub> receptors were identified in pikeperch, as in other teleosts, such as  
455 Atlantic herring, *Clupea harengus*, zebrafish, and Nile tilapia, while a single D<sub>6</sub> is present in  
456 sarcopterygians and in non-teleost actinopterygian (Yamamoto et al., 2015). In European eel,  
457 Japanese medaka, *Oryzias latipes*, Japanese pufferfish (fugu), *Takifugu rubripes*, and three-  
458 spined stickleback, *Gasterosteus aculeatus*, only one D<sub>6</sub> paralog was identified (Sugamori et  
459 al., 1994; Macrae and Brenner, 1995; Lamers et al., 1996; Cardinaud et al., 1997; Hirano et  
460 al., 1998). These results suggest that the two D<sub>6</sub> identified in the pikeperch are issued from  
461 3R, and that one copy may have been lost in some species throughout teleost radiation.

462 Concerning D<sub>5</sub>, a single receptor is present in sarcopterygians, while two D<sub>5</sub> receptors were  
463 evidenced in some teleosts species, such as Japanese medaka, three-spined stickleback, Nile  
464 tilapia, fugu and Atlantic herring (Sugamori et al., 1994; Demchyshyn et al., 1995; Cardinaud  
465 et al., 1997; Hirano et al., 1998; Missale et al., 1998; Yamamoto et al., 2015 NCBI, Ensembl  
466 and Uniprot databases). In contrast, only one D<sub>5</sub> receptor, orthologous to the pikeperch D1\_3,  
467 was evidenced in European eel, Asian bonytongue, *Scleropages formosus*, zebrafish and  
468 European seabass, *Dicentrarchus labrax* (Sugamori et al., 1994; Demchyshyn et al., 1995;

469 Cardinaud et al., 1997; Hirano et al., 1998; Missale et al., 1998; Yamamoto et al., 2015 NCBI,  
470 Ensembl and Uniprot databases). From the phylogenetic analysis, the single spotted gar D<sub>5</sub>  
471 branched at the base of the teleost D<sub>5a</sub> clade, and in polytomy with the sarcopterygian and the  
472 teleost D<sub>5b</sub> (named D<sub>1X</sub> in Hirano et al., 1998) clades. This result does not suggest that the two  
473 teleost D<sub>5</sub> paralogs result from 3R. As this study was based on brain transcriptome analysis,  
474 and with the absence of a pikeperch genome in the available databases, we cannot ascertain  
475 the absence of a D<sub>5b</sub> paralog in this species.

476

477 Five receptor sequences, belonging to the D2 receptor family, have herein been identified in  
478 pikeperch. These sequences share from 33% to 95% identity with other receptors of D2  
479 family in vertebrates and only 29-46% with receptors of D1 family. The molecular structure is  
480 also consistent with the one described in other vertebrate species: the seven TMDs, the long  
481 variable third cytoplasmic loop and a short and conserved C terminal tail ending with a  
482 conserved cysteine residue. Similarly to the receptors of D1 family, some conserved amino  
483 acid residues were also observed in pikeperch sequences of the D2 family. For instance, the  
484 primary structure possesses the aspartate in TMD 2 and 3, the two serines in TMD 5, the two  
485 cysteines in the first and second extracellular loops or the phosphorylation/glycosylation sites  
486 in the different loops (Civelli et al., 1993; Missale et al., 1998; Levavi-Sivan et al., 2005).  
487 Phylogenetic analysis revealed that the pikeperch receptors of the family D2 are orthologous  
488 to D<sub>2</sub>, D<sub>3</sub>, D<sub>8</sub>, and D<sub>9</sub>. From our study based on brain transcriptome analysis, we could not  
489 evidence in the pikeperch the presence of 3R-paralogs belonging to the D2 receptor family,  
490 suggesting the loss of a 3R-duplicated D<sub>2</sub>, D<sub>3</sub>, D<sub>8</sub>, and D<sub>9</sub> during evolution in pikeperch.

491 All these D2 family receptors have been previously identified in vertebrates including several  
492 teleost species. For instance, a D<sub>8</sub> sequence was cloned in zebrafish (Boehmler et al., 2004),  
493 and identified *in silico* in other actinopterygians, such as spotted gar, Nile tilapia, Japanese

494 medaka and a sarcopterygian, the coelacanth (Yamamoto et al., 2015; NCBI, Ensembl and  
495 Uniprot databases). Phylogenetic analysis clearly revealed that the actinopterygian and  
496 sarcopterygian D<sub>8</sub> sequences clustered in a single clade distinct to the other vertebrate D<sub>2</sub>. Our  
497 results are consistent with Boehmler et al. (2004) and Yamamoto et al. (2015), suggesting that  
498 this receptor could be the product of an early gene duplication during vertebrate evolution,  
499 prior to divergence of the Osteichthyes group.

500 In the D<sub>8</sub> sister clade, encompassing D<sub>2</sub> sequences, two paralogs have been evidenced in  
501 European eel, Asian bonytongue, Atlantic herring and zebrafish, likely resulting from 3R  
502 (Vacher et al., 2003; Boehmler et al., 2004; Pasqualini et al., 2009; Popesku et al., 2011;  
503 NCBI database). In contrast, only one D<sub>2</sub> receptor gene could be identified in pikeperch, as in  
504 other teleost, such as Japanese medaka, tilapia and fugu, suggesting the loss of the duplicated  
505 paralog in these species. This D<sub>2</sub> gene encodes for two identical receptors D<sub>2aL</sub> and D<sub>2aS</sub>,  
506 except for a 29 amino acid sequence missing in the third cytoplasmic loop of the D<sub>2aS</sub>. This  
507 has already been reported in some mammals including human and rat (Dal Toso et al., 1989;  
508 Monsma et al., 1989; Missale et al., 1998) but also in bullfrog (Nakano et al., 2010). Taking  
509 advantage of the phylogenetic results, regarding the mammalian model and the full identity  
510 between both sequences, we suggest an alternative splicing of the pre-messenger of the  
511 unique D<sub>2a</sub> gene in pikeperch. This phenomenon has been described before in goldfish  
512 (Popesku et al., 2011), but to date this has never been confirmed in another teleost.  
513 Nevertheless, no alternative splicing has been highlighted in a wide range of vertebrate  
514 species including xenopus (Martens et al., 1993), fugu (Macrae and Brenner, 1995), common  
515 carp (Hirano et al., 1998), rainbow trout (Vacher et al., 2003), zebrafish (Boehmler et al.,  
516 2004), Nile tilapia (Levavi-Sivan et al., 2005) and in European eel (Pasqualini et al., 2009).  
517 Taking into account the involvement of the third cytoplasmic loop in the coupling to the  
518 protein G, it has been proposed that this splicing could be involved in the functional diversity

519 of the D<sub>2</sub> receptors (Missale et al., 1998; Ben-Jonathan and Hnasko, 2001; Callier et al.,  
520 2003). Indeed, Senogles et al., (2004) suggested that the splice variants could be coupled to a  
521 different Gi protein. Other authors showed that, besides their distinct expression in the brain,  
522 the D<sub>2S</sub> receptor could be presynaptically located while the D<sub>2L</sub> may be found in the post-  
523 synaptic region in mammals (Khan et al., 1998). Conversely, Tress et al. (2017) suggested  
524 that most annotated splice variants could lead to unfunctional proteins at a cellular level and a  
525 debate remains open about the physiological roles of these variants.

526

527 Relatively to D<sub>3</sub>, a single copy has been evidenced in some mammals, sauropsids and some  
528 teleosts such as fugu, zebrafish, Japanese medaka, European seabass, and Nile tilapia (Macrae  
529 and Brenner, 1995; Missale et al., 1998; Boehmler et al., 2004; Yamamoto et al., 2015; NCBI  
530 database). This suggests that one of the 3R duplicated D<sub>3</sub> paralogs may have been lost shortly  
531 after the teleost emergence.

532 In the same way, a single copy of D<sub>9</sub> was cloned in zebrafish (Boehmler et al., 2007) and was  
533 identified in databases for some other teleost species such as Nile tilapia, Japanese medaka, in  
534 the non-teleost actinopterygian spotted gar and the sarcopterygian coelacanth (Yamamoto et  
535 al., 2015; NCBI, Ensembl and Uniprot databases). This again suggests an early loss of one of  
536 the D<sub>9</sub> 3R-paralogs during teleost radiation.

537 Concerning the D<sub>4</sub> subtype, 3R duplicated paralogs are present in some teleosts, such as  
538 zebrafish, Atlantic herring, Asian bonytongue and European eel, while a single copy has been  
539 evidenced in Nile tilapia, platyfish, Japanese medaka, and fugu (Boehmler et al., 2007; NCBI,  
540 Ensembl and Uniprot databases). This suggests that different loss events may have occurred  
541 during teleost radiation. In the present transcriptome, no DA receptor orthologous to D<sub>4</sub> has  
542 been evidenced in the pikeperch.

543 Few studies have investigated D<sub>4</sub> and D<sub>9</sub> subtypes in teleosts besides Boehmler et al. (2007),  
544 which reported three distinct genes encoding those receptors, two genes encoding the D<sub>4</sub>  
545 subtype (D<sub>4a</sub> and D<sub>4b</sub> (or D<sub>4c</sub>)) and a single gene encoding the D<sub>9</sub> subtype. As suggested in  
546 previous studies, the existence of D<sub>4</sub> and D<sub>9</sub> subtypes would result from a duplication event  
547 that occurred before the split of sarcopterygians and actinopterygians (Boehmler et  
548 al., 2007; Yamamoto et al., 2015).

549

550 Several hypotheses might explain the absence of D<sub>5b</sub>, D<sub>2b</sub>, both D<sub>4</sub> paralogs and D<sub>7</sub> in all  
551 female pikeperch specimens analysed in the present study. First, some receptors like D<sub>2b</sub> and  
552 D<sub>7</sub> may have been lost during vertebrate evolution in most of the teleost lineages (Dufour et  
553 al., 2010; Yamamoto et al., 2015). Other paralogs, like D<sub>5b</sub>, may have been lost only in some  
554 perciform species, including pikeperch. Second, we could also speculate that the genes  
555 encoding some of these receptors in fact correspond to unfunctional pseudogenes (Prince and  
556 Pickett, 2002) whose transcripts remain undetectable in the brain. To address these questions,  
557 an exhaustive genome sequencing in pikeperch would be useful. Third, the present study  
558 analysed the transcripts during the prespawning period, a highly specific physiological  
559 situation. This event of final oocyte maturation is synchronous with huge alterations of the  
560 brain and gonad transcriptomes. We may preclude low and barely detectable gene expression  
561 of these receptors before ovulation in pikeperch (Aegerter et al., 2004). Fourth, we cannot rule  
562 out the fact that the lack of identification may stem from methodological limitations (e.g.  
563 assembly, detection thresholds, incomplete references/databases).

564 All DA receptors do not have an undetectable gene expression in pikeperch. Indeed, all the  
565 multi-tissues gene expression patterns revealed a specific and measurable expression for D<sub>1a</sub>,  
566 D<sub>1b</sub>, D<sub>3</sub>, D<sub>8</sub> and D<sub>9</sub>.

567 Despite the rough examination, the gene expression study showed relatively high expression  
568 of DA receptors in brain regions compared to peripheral organs. This would be in accordance  
569 with the importance of DA in neurophysiological functions (Missale et al., 1998; Dufour et  
570 al., 2010). The brain profile also showed a part-specific gene expression of these receptors.  
571 For instance, the two  $D_{1A}$  are ubiquitously expressed in the brain except for the cerebellum  
572 where the expression is slightly lower. These low levels of  $D_{1A}$  mRNA in the cerebellum have  
573 previously been reported in mammals and European eel (Mansour et al., 1991; Mengod et al.,  
574 1992; Laurier et al., 1994; Cardinaud et al., 1997; Kapsimali et al., 2000). On the contrary,  
575 some receptor mRNA are present in the brain parts related to sensory perception (e.g.  
576 olfactory bulb) or reproductive function (e.g. optic recess region). *In situ* hybridization would  
577 be useful to locate the DA receptor genes more precisely. For example, if a DA receptor gene  
578 is colabelled with GnRH transcripts, it indicates a possibility that DA directly interacts with  
579 GnRH neurons.

580 Pituitary showed intermediate and low transcript abundance for the two  $D_{1A}$ , and an increased  
581 expression level for the D2 family receptors. Using specific DA receptor antagonists, it was  
582 demonstrated that the D2 family receptors, rather than the D1 ones, might be involved in  
583 gonadotropin secretion (Dufour et al., 2010; Fontaine et al., 2015). Nevertheless, we recently  
584 showed that *in vivo* treatment with an antagonist of the family D1 increased the T and E2  
585 plasmatic levels, while no effect was observed with an antagonist of the family D2 (Roche et  
586 al., 2018). We thus imagine that this divergence between both DA receptor families  
587 localization in the pituitary is not linked to the activation of the gonadotropic axis in  
588 pikeperch at this pre-ovulatory period. In the gonad, the gene expression is either just above  
589 the quantification limit ( $D_{1a}$  and  $D_9$ ) or simply low ( $D_{1b}$ ,  $D_3$ ,  $D_8$ ). Given this level of detection,  
590 we cannot rule out any direct effect of DA through its receptors on ovarian mechanisms  
591 (Isobe, 1994; Venegas-Meneses et al., 2015).

592 Finally, depending on the receptor type, the expression level is also just detectable in heart,  
593 liver or gills, which might be put in relation with involvements of DA in osmoregulatory  
594 mechanisms, heart beats and energetic metabolism in teleost fish (Missale et al., 1998; Rubí  
595 and Maechler, 2010). In the spleen, muscle and adipose tissue, no expression was observable,  
596 which allows us to conclude on the non-involvement of these receptors in these tissues.

597

598 In summary, five D<sub>1</sub> DA receptors, D<sub>1a</sub>, D<sub>1b</sub>, D<sub>5a</sub>, D<sub>6a</sub> and D<sub>6b</sub> and five D<sub>2</sub> DA receptors, D<sub>2L</sub>  
599 and D<sub>2S</sub>, D<sub>3</sub>, D<sub>8</sub> and D<sub>9</sub>, sharing features specific to each DA receptor family, were identified  
600 in pikeperch during the pre-ovulatory period. Presence of two receptor families (D<sub>1</sub> and D<sub>2</sub>),  
601 multiple receptor subtypes (D<sub>1</sub>, D<sub>5</sub>, D<sub>6</sub>, D<sub>2</sub>, D<sub>3</sub>, D<sub>8</sub> and D<sub>9</sub>), some teleost-specific paralogs for  
602 some subtypes (D<sub>1a</sub> and D<sub>1b</sub>, D<sub>6a</sub> and D<sub>6b</sub>) and even spliced variants (D<sub>2aL</sub> and D<sub>2aS</sub>)  
603 highlight the complexity of DA receptors in teleost fish. These receptors are differentially  
604 expressed in different brain areas and in the pituitary, but also in some peripheral tissues  
605 (gonads, heart, liver, gills) indicating roles of DA through one of its receptors in different  
606 physiological functions.

607

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614

## 615 **Bibliography**

616 Aegerter, S., Jalabert, B., Bobe, J., 2004. Messenger RNA stockpile of cyclin B, insulin-like

617 growth factor I, insulin-like growth factor II, insulin-like growth factor receptor Ib, and  
618 p53 in the rainbow trout oocyte in relation with developmental competence. *Mol.*  
619 *Reprod. Dev.* 67, 127–135.

620 Ashburner, M., Ball, C.A., Blake, J.A., Botstein, D., Butler, H., Cherry, J.M., Davis, A.P.,  
621 Dolinski, K., Dwight, S.S., Eppig, J.T., Harris, M.A., Hill, D.P., Issel-Tarver, L.,  
622 Kasarskis, A., Lewis, S., Matese, J.C., Richardson, J.E., Ringwald, M., Rubin, G.M.,  
623 Sherlock, G., 2000. Gene Ontology: tool for the unification of biology. *Nat. Genet.* 25,  
624 25–29.

625 Bates, D., Maechler Martin, Bolker, B., Walker, S., 2015. Fitting Linear Mixed-Effects  
626 Models using lme4. *J. Stat. Softw.* 67, 1–48.

627 Ben-Jonathan, N., Hnasko, R., 2001. Dopamine as a prolactin (PRL) inhibitor. *Endocr. Rev.*  
628 22, 724–763.

629 Benjamini, Y., Hochberg, Y., 1995. Controlling the false discovery rate: a practical and  
630 powerful approach to multiple testing. *J. R. Stat. Soc. Ser. B.*

631 Boehmler, W., Obrecht-Pflumio, S., Canfield, V., Thisse, C., Thisse, B., Levenson, R., 2004.  
632 Evolution and expression of D2 and D3 dopamine receptor genes in zebrafish. *Dev. Dyn.*  
633 230, 481–493.

634 Boehmler, W., Carr, T., Thisse, C., Thisse, B., Canfield, V.A., Levenson, R., 2007. D4  
635 dopamine receptor genes of zebrafish and effects of the antipsychotic clozapine on larval  
636 swimming behaviour. *Genes, Brain Behav.* 6, 155–166.

637 Bray, N.L., Pimentel, H., Melsted, P., Pachter, L., 2016. Near-optimal probabilistic RNA-seq  
638 quantification. *Nat. Biotechnol.* 34, 525–527.

639 Callier, S., Snapyan, M., Crom, S., Prou, D., Vincent, J.-D., Vernier, P., 2003. Evolution and  
640 cell biology of dopamine receptors in vertebrates. *Biol. Cell* 95, 489–502.

641 Camacho, C., Coulouris, G., Avagyan, V., Ma, N., Papadopoulos, J., Bealer, K., Madden,

642 T.L., 2009. BLAST+: architecture and applications. *BMC Bioinformatics* 10, 421.

643 Cardinaud, B., Sugamori, K.S., Coudouel, S., Vincent, J.-D., Niznik, H.B., Vernier, P., 1997.

644 Early emergence of three dopamine D1 receptor subtypes in vertebrates. *J. Biol. Chem.*

645 272, 2778–2787.

646 Civelli, O., Bunzow, J.R., Grandy, D.K., 1993. Molecular diversity of the dopamine receptors.

647 *Annu. Rev. Pharmacol. Toxicol.* 33, 281–307.

648 Dal Toso, R., Sommer, B., Ewert, M., Herb, A., Pritchett, D.B., Bach, A., Shivers, B.D.,

649 Seeburg, P.H., 1989. The dopamine D2 receptor: two molecular forms generated by

650 alternative splicing. *EMBO J.* 8, 4025–4034.

651 Darriba, D., Taboada, G.L., Doallo, R., Posada, D., 2011. ProtTest 3: fast selection of best-fit

652 models of protein evolution. *Bioinforma. Appl. NOTE* 27, 1164–1165.

653 Demchyshyn, L.L., Sugamori, K.S., Lee, F.J., Hamadanizadeh, S.A., Niznik, H.B., 1995. The

654 dopamine D1D receptor. Cloning and characterization of three pharmacologically

655 distinct D1-like receptors from *Gallus domesticus*. *J. Biol. Chem.* 270, 4005–12.

656 Dufour, S., Sebert, M.E., Weltzien, F.A., Rousseau, K., Pasqualini, C., 2010. Neuroendocrine

657 control by dopamine of teleost reproduction. *J. Fish Biol.* 76, 129–160.

658 Eddy, S.R., 2011. Accelerated Profile HMM Searches. *PLoS Comput. Biol.* 7, e1002195.

659 Finn, R.D., Coghill, P., Eberhardt, R.Y., Eddy, S.R., Mistry, J., Mitchell, A.L., Potter, S.C.,

660 Punta, M., Qureshi, M., Sangrador-Vegas, A., Salazar, G.A., Tate, J., Bateman, A., 2016.

661 The Pfam protein families database: towards a more sustainable future. *Nucleic Acids*

662 *Res.* 44, D279–D285.

663 Fontaine, R., Affaticati, P., Bureau, C., Colin, I., Demarque, M., Dufour, S., Vernier, P.,

664 Yamamoto, K., Pasqualini, C., 2015. The dopaminergic neurons controlling anterior

665 pituitary functions: anatomy and ontogenesis in zebrafish. *Endocrinology* 156, 2934–

666 2948.

667 Fox, J., Weisberg, S., 2011. An R companion to applied Regression, Second. ed. SAGE  
668 Publications, Inc.

669 Frail, D.E., Manelli, A.M., Witte, D.G., Lin, C.W., Steffey, M.E., Mackenzie, R.G., 1993.  
670 Cloning and characterization of a truncated dopamine D1 receptor from goldfish retina:  
671 stimulation of cyclic AMP production and calcium mobilization. Mol. Pharmacol. 44.

672 Gouy, M., Guindon, S., Gascuel, O., 2010. SeaView Version 4: A Multiplatform Graphical  
673 User Interface for Sequence Alignment and Phylogenetic Tree Building. Mol. Biol. Evol  
674 27, 221–224.

675 Grabherr, M.G., Haas, B.J., Yassour, M., Levin, J.Z., Thompson, D.A., Amit, I., Adiconis, X.,  
676 Fan, L., Raychowdhury, R., Zeng, Q., Chen, Z., Mauceli, E., Hacohen, N., Gnirke, A.,  
677 Rhind, N., di Palma, F., Birren, B.W., Nusbaum, C., Lindblad-Toh, K., Friedman, N.,  
678 Regev, A., 2011. Full-length transcriptome assembly from RNA-Seq data without a  
679 reference genome. Nature biotechnology 29, 644-652.

680 Haas, B.J., Papanicolaou, A., Yassour, M., Grabherr, M., Blood, P.D., Bowden, J., Couger,  
681 M.B., Eccles, D., Li, B., Lieber, M., MacManes, M.D., Ott, M., Orvis, J., Pochet, N.,  
682 Strozzi, F., Weeks, N., Westerman, R., William, T., Dewey, C.N., Henschel, R., LeDuc,  
683 R.D., Friedman, N., Regev, A., 2013. *De novo* transcript sequence reconstruction from  
684 RNA-seq using the Trinity platform for reference generation and analysis. Nat. Protoc. 8,  
685 1494–1512.

686 Hervé, M., 2016. RVAideMemoire: diverse basic statistical and graphical functions. R  
687 package version 0.9-61.

688 Hirano, J., Archer, S.N., Djamgoz, M.B., 1998. Dopamine receptor subtypes expressed in  
689 vertebrate (carp and eel) retinae: cloning, sequencing and comparison of five D1-like and  
690 three D2-like receptors. Receptors Channels 5, 387–404.

691 Huerta-Cepas, J., Szklarczyk, D., Forslund, K., Cook, H., Heller, D., Walter, M.C., Rattei, T.,

692 Mende, D.R., Sunagawa, S., Kuhn, M., Jensen, L.J., von Mering, C., Bork, P., 2016.  
693 eggNOG 4.5: a hierarchical orthology framework with improved functional annotations  
694 for eukaryotic, prokaryotic and viral sequences. *Nucleic Acids Res.* 44, D286–D293.

695 Isobe, S., 1994. The role of the dopaminergic system in the rat ovary. *Folia Endocrinol. Jpn.*  
696 70, 457–464.

697 Jackson, D.M., Westlind-Danielsson, A., 1994. Dopamine receptors: molecular biology,  
698 biochemistry and behavioural aspects. *Pharmacol. Ther* 64, 291–369.

699 Källberg, M., Wang, H., Wang, S., Peng, J., Wang, Z., Lu, H., Xu, J., 2012. Template-based  
700 protein structure modeling using the RaptorX web server. *Nat. Protoc.* 7, 1511–1522.

701 Kanehisa, M., Furumichi, M., Tanabe, M., Sato, Y., Morishima, K., 2017. KEGG: new  
702 perspectives on genomes, pathways, diseases and drugs. *Nucleic Acids Res.* 45, D353–  
703 D361.

704 Kapsimali, M., Vidal, B., Gonzalez, A., Dufour, S., Vernier, P., 2000. Distribution of the  
705 mRNA encoding the four dopamine D1 receptor subtypes in the brain of the European  
706 eel (*Anguilla anguilla*): comparative approach to the function of D1 receptors in  
707 vertebrates. *J. Comp. Neurol.* 419, 320–343.

708 Kestemont, P., Dabrowski, K., Summerfelt, R.C., 2015. Biology and culture of percid fishes:  
709 Principles and practices, *Biology and Culture of Percid Fishes: Principles and Practices.*  
710 Springer.

711 Khan, Z.U., Mrzljak, L., Gutierrez, A., de la Calle, A., Goldman-Rakic, P.S., 1998.  
712 Prominence of the dopamine D2 short isoform in dopaminergic pathways. *Proc. Natl.*  
713 *Acad. Sci. U. S. A.* 95, 7731–7736.

714 Krogh, A., Larsson, B., von Heijne, G., Sonnhammer, E.L., 2001. Predicting transmembrane  
715 protein topology with a hidden markov model: application to complete genomes. *J. Mol.*  
716 *Biol.* 305, 567–580.

717 Lagesen, K., Hallin, P., Rødland, E.A., Stærfeldt, H.-H., Rognes, T., Ussery, D.W., 2007.  
718 RNAmmr: consistent and rapid annotation of ribosomal RNA genes. *Nucleic Acids*  
719 *Res.* 35, 3100–3108.

720 Lamers, A.E., Groneveld, D., De, D.P. V, Felix, K., Geeraedts, C.G., Leunissen B', J.A.M.,  
721 Flik, G., Sjoerd, \, Wendelaar Bonga, E., Martens, G.J.M., 1996. Cloning and sequence  
722 analysis of a hypothalamic cDNA encoding a D1C dopamine receptor in tilapia.  
723 *Biochim. cl Biophys. Acta* 1308, 17–22.

724 Langmead, B., Salzberg, S.L., 2012. Fast gapped-read alignment with Bowtie 2. *Nat. Methods*  
725 9, 357–359.

726 Laurier, L.G., O'dowd, B.F., George, S.R., 1994. Heterogeneous tissue-specific transcription  
727 of dopamine receptor subtype messenger RNA in rat brain. *Mol. Brain Res.* 25, 344–351.

728 Lenth, R., 2016. Least-Squares Means: The R package lsmeans. *J. Stat. Softw.* 69, 1–33.

729 Levavi-Sivan, B., Aizen, J., Avitan, A., 2005. Cloning, characterization and expression of the  
730 D2 dopamine receptor from the tilapia pituitary. *Mol. Cell. Endocrinol.* 236, 17–30.

731 Macrae, A.D., Brenner, S., 1995. Analysis of the dopamine receptor family in the compact  
732 genome of the puffer fish *Fugu rubripes*. *Genomics* 25, 436–446.

733 Mansour, A., Meador-Woodruff J.H., Zhou, Q.-Y., Civelli, O., Akil, H., Watson, S.J., 1991.  
734 A comparison of D1 receptor binding and mRNA in rat brain using receptor  
735 autoradiography and in situ hybridization techniques. *Neuroscience* 45, 359–371.

736 Martens, G.J.M., Groenen, P.M.A., Van Riel, M.C.H.M., Martens, G., 1993. Expression of  
737 the *Xenopus* D2, dopamine receptor. Tissue-specific regulation and two transcriptionally  
738 active genes but no evidence for alternative splicing. *Eur. J. Biochem* 213, 1349–1354.

739 Martin, M., 2013. Cutadapt removes adapter sequences from high-throughput sequencing  
740 reads. *EMBnet.journal* 17, 10–12.

741 Mengod, G., Villaro, M.T., Landwehrmeyer, G.B., Martinez-Mir, M.I., Niznik, H.B.,

742 Sunahara, R.K., Seeman, P., O'dowd, B.F., Probst, A., Palacios, J.M., Research, P.,  
743 1992. Visualization of dopamine D1, D2 and D3 receptor mRNA's in human and rat  
744 brain. *Neurochem. Int* 20, 33–43.

745 Milla, S., Mathieu, C., Wang, N., Lambert, S., Nadzialek, S., Massart, S., Henrotte, E.,  
746 Douxfils, J., Mélard, C., Mandiki, S.N.M., Kestemont, P., 2010. Spleen immune status is  
747 affected after acute handling stress but not regulated by cortisol in Eurasian perch, *Perca*  
748 *fluviatilis*. *Fish Shellfish Immunol.* 28, 931–941.

749 Missale, C., Nash, S.R., Robinson, S.W., Jaber, M., Caron, M.G., 1998. Dopamine receptors:  
750 from structure to function. *Physiol. Rev.* 78, 189–225.

751 Monsma, F.J., McVittie, L.D., Gerfen, C.R., Mahan, L.C., Sibley, D.R., 1989. Multiple D2  
752 dopamine receptors produced by alternative RNA splicing. *Nature* 342, 926–929.

753 Nakano, M., Hasunuma, I., Okada, R., Yamamoto, K., Kikuyama, S., Machida, T.,  
754 Kobayashi, T., 2010. Molecular cloning of bullfrog D2 dopamine receptor cDNA: tissue  
755 distribution of three isoforms of D2 dopamine receptor mRNA. *Gen. Comp. Endocrinol.*  
756 168, 143–148.

757 Pasqualini, C., Weltzien, F.-A., Vidal, B., Baloché, S., Rouget, C., Gilles, N., Servent, D.,  
758 Vernier, P., Dufour, S., 2009. Two distinct dopamine D2 receptor genes in the European  
759 eel: molecular characterization, tissue-specific transcription, and regulation by sex  
760 steroids. *Endocrinology* 150, 1377–1392.

761 Petersen, T.N., Brunak, S., von Heijne, G., Nielsen, H., 2011. SignalP 4.0: discriminating  
762 signal peptides from transmembrane regions. *Nat. Methods* 8, 785–786.

763 Pohlert, T., 2016. The Pairwise Multiple Comparisons of Mean Ranks package (PMCMR), R  
764 Package.

765 Popesku, J.T., Navarro-Martín, L., Trudeau, V.L., 2011. Evidence for alternative splicing of a  
766 dopamine D2 receptor in a teleost. *Physiol. Biochem. Zool.* 84, 135–46.

767 Prince, V.E., Pickett, F.B., 2002. Splitting pairs: the diverging fates of duplicated genes. Nat.  
768 Rev. 3, 827–837.

769 Roche, J., Źarski, D., Khendek, A., Ben Ammar, I., Broquard, C., Depp, A., Ledoré, Y.,  
770 Policar, T., Fontaine, P., Milla, S., 2018. D1, but not D2, dopamine receptor regulates  
771 steroid levels during the final stages of pikeperch gametogenesis. Animal 1–11.

772 Rubí, B., Maechler, P., 2010. Minireview: New roles for peripheral dopamine on metabolic  
773 control and tumor growth: let's seek the balance. Endocrinology 151, 5570–5581.

774 Senogles, S.E., Heimert, T.L., Odife, E.R., Quasney, M.W., 2004. A region of the third  
775 intracellular loop of the short form of the D2 dopamine receptor dictates Gi coupling  
776 specificity. J. Biol. Chem. 279, 1601–6.

777 Sievers, F., Wilm, A., Dineen, D., Gibson, T.J., Karplus, K., Li, W., Lopez, R., McWilliam,  
778 H., Remmert, M., Söding, J., Thompson, J.D., Higgins, D.G., 2011. Fast, scalable  
779 generation of high-quality protein multiple sequence alignments using Clustal Omega.  
780 Mol. Syst. Biol. 7, 539.

781 Smith-Unna, R., Boursnell, C., Patro, R., Hibberd, J.M., Kelly, S., 2016. TransRate:  
782 reference-free quality assessment of *de novo* transcriptome assemblies. Genome Res. 26,  
783 1134–44.

784 Stamatakis, A., 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of  
785 large phylogenies. Bioinforma. Appl. 30, 1312–1313.

786 Sugamori, K.S., Demchyshyn, L.L., Chung, M., Niznik, H.B., 1994. D1A, D1B, and D1C  
787 dopamine receptors from *Xenopus laevis*. Proc. Natl. Acad. Sci. USA 91, 10536–10540.

788 The Gene Ontology Consortium, 2017. Expansion of the Gene Ontology knowledgebase and  
789 resources. Nucleic Acids Res. 45, D331–D338.

790 Tress, M.L., Abascal, F., Valencia, A., 2017. Alternative splicing may not be the key to  
791 proteome complexity. Trends Biochem. Sci. 42, 98–110.

792 Vacher, C., Pellegrini, E., Anglade, I., Ferrière, F., Saligaut, C., Kah, O., 2003. Distribution of  
793 dopamine D 2 receptor mRNAs in the brain and the pituitary of female rainbow trout: an  
794 *in situ* hybridization study. *J. Comp. Neurol.* 458, 32–45.

795 Venegas-Meneses, B., Padilla, J.F., Juarez, C.E., Moran, J.L., Moran, C., Rosas-Murrieta,  
796 N.H., Handal, A., Dominguez, R., 2015. Effects of ovarian dopaminergic receptors on  
797 ovulation. *Endocrine* 50, 783–796.

798 Yamamoto, K., Fontaine, R., Pasqualini, C., Vernier, P., 2015. Classification of dopamine  
799 receptor genes in vertebrates: nine subtypes in Osteichthyes. *Brain Behav Evol* 86, 164–  
800 175.

801 Źarski, D., Kucharczyk, D., Targonska, K., Palińska, K., Kupren, K., Fontaine, P., Kestemont,  
802 P., 2012. A new classification of pre-ovulatory oocyte maturation stages in pikeperch,  
803 *Sander lucioperca* (L.), and its application during artificial reproduction. *Aquac. Res.* 43,  
804 713–721.

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806 **Table 1: Primers used for real-time PCR**

<b>Sequences (5' – 3')</b>			
	<b>Forward</b>	<b>Reverse</b>	<b>Size (bp)</b>
D <sub>1a</sub>	CTTCATCCTCAACTGCATGGT	CCTCCTGGATACAGTCTGTGG	204
D <sub>1b</sub>	CCAGAGAGAGACTCGTCCAAA	TTGCAGAAAGCACCAAACGG	196
D <sub>3</sub>	TGGTGTGCTCCATCTCCAAC	GCCACAGGCAAACCTGACAAC	306
D <sub>8</sub>	TGCCATCTCCTGCCCTTTAC	CCATCGCCTCCTTTACCAGG	255
D <sub>9</sub>	CGTCGTAGGGGTTTTTCTAGC	GCAGAGCAGCTTGTGAAAGAC	199
RPL8	GTTATCGCCTCTGCCAC	ACCGAAGGGATGCTCAAC	163
AK	CTTCCTGACCGTCTCTTTGG	CCTTAGTCTCGAAGTCTTGC	209

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**Table 2:** Pairwise comparisons within the D1 receptor family between pikeperch, zebrafish (ZF) and human (H) receptor protein sequences

Pikeperch		D1_1	D1_2	D1_3	D1_4	D1_5
<b>H D<sub>1</sub></b>	<b>Id % (Sc)</b>	72.75 (513)	72.15 (621)	57.30 (397)	59.53 (439)	61.94 (188)
	<b>e-value</b>	0.0	0.0	1.88e-140	1.28e-154	4.58e-62
<b>H D<sub>5</sub></b>	<b>Id % (Sc)</b>	67.45 (455)	56.76 (477)	66.13 (492)	51.84 (461)	61.24 (197)
	<b>e-value</b>	2.72e-162	1.54e-169	2.75e-177	1.79e-162	3.74e-65
<b>ZF D<sub>1a</sub></b>	<b>Id % (Sc)</b>	81.03 (570)	82.37 (575)	71.43 (382)	65.63 (435)	62.81 (189)
	<b>e-value</b>	0.0	0.0	1.14e-135	4.06e-154	2.95e-63
<b>ZF D<sub>1b</sub></b>	<b>Id % (Sc)</b>	79.95 (565)	84.13 (756)	59.07 (404)	62.97 (437)	66.46 (208)
	<b>e-value</b>	0.0	0.0	2.91e-143	1.88e-153	1.26e-69
<b>ZF D<sub>5a</sub></b>	<b>Id % (Sc)</b>	64.71 (272)	64.35 (286)	72.19 (421)	58.58 (290)	66.88 (199)
	<b>e-value</b>	2.40e-93	1.20e-97	1.80e-152	8.15e-99	3.73e-68
<b>ZF D<sub>5b</sub></b>	<b>Id % (Sc)</b>	61.45 (419)	62.08 (417)	59.13 (370)	57.65 (405)	59.24 (171)
	<b>e-value</b>	2.70e-149	1.75e-147	2.21e-130	7.51e-142	6.25e-56
<b>ZF D<sub>6a</sub></b>	<b>Id % (Sc)</b>	68.31 (476)	57.52 (495)	61.30 (420)	65.77 (597)	76.19 (224)
	<b>e-value</b>	4.59e-171	3.74e-177	2.24e-149	0.0	9.17e-76
<b>ZF D<sub>6b</sub></b>	<b>Id % (Sc)</b>	70.54 (475)	68.75 (480)	60.28 (409)	59.84 (533)	75.97 (228)
	<b>e-value</b>	4.77e-171	1.05e-171	2.08e-145	0.0	8.43e-78
<b>ZF D<sub>7</sub></b>	<b>Id % (Sc)</b>	62.82 (431)	55.71 (455)	56.43 (377)	59.84 (446)	62.18 (185)
	<b>e-value</b>	2.50e-153	1.22e-161	1.49e-132	4.07e-157	8.21e-61

Id = Identity; Sc = bit Score

**Table 3:** Pairwise comparisons within the D2 receptor family between pikeperch, zebrafish (ZF) and human (H) protein sequences

<b>Pikeperch</b>		<b>D2_1</b>	<b>D2_2</b>	<b>D2_3</b>	<b>D2_4</b>	<b>D2_5</b>
<b>H D<sub>2L</sub></b>	<b>Id % (Sc)</b>	66.10 (602)	63.23 (557)	53.76 (438)	56.47 (456)	38.48 (262)
	<b>e-value</b>	0.0	0.0	8.44e-155	2.94e-161	7.23e-87
<b>H D<sub>2S</sub></b>	<b>Id % (Sc)</b>	62.50 (559)	66.37 (571)	53.68 (440)	54.98 (436)	41.18 (274)
	<b>e-value</b>	0.0	0.0	4.47e-156	8.36e-154	1.13e-91
<b>H D<sub>3</sub></b>	<b>Id % (Sc)</b>	48.89 (388)	53.15 (403)	57.72 (449)	49.22 (368)	40.96 (276)
	<b>e-value</b>	1.32e-135	4.63e-142	9.23e-160	1.63e-127	1.47e-92
<b>H D<sub>4</sub></b>	<b>Id % (Sc)</b>	46.88 (152)	36.49 (228)	38.22 (232)	53.49 (150)	68.04 (228)
	<b>e-value</b>	2.33e-44	6.86e-73	2.22e-74	1.76e-43	2.33e-73
<b>ZF D<sub>2a</sub></b>	<b>Id % (Sc)</b>	73.35 (684)	69.44 (632)	51.45 (418)	54.96 (471)	33.63 (265)
	<b>e-value</b>	0.0	0.0	6.88e-147	1.92e-167	6.38e-88
<b>ZF D<sub>2b</sub></b>	<b>Id % (Sc)</b>	73.25 (677)	71.15 (617)	53.60 (438)	58.02 (500)	37.09 (276)
	<b>e-value</b>	0.0	0.0	6.56e-155	1.15e-178	4.04e-92
<b>ZF D<sub>3</sub></b>	<b>Id % (Sc)</b>	50.10 (447)	53.06 (456)	74.57 (666)	49.31 (386)	38.02 (270)
	<b>e-value</b>	3.87e-158	8.14e-162	0.0	5.62e-134	1.55e-89
<b>ZF D<sub>4a</sub></b>	<b>Id % (Sc)</b>	47.62 (155)	36.62 (228)	48.54 (139)	36.59 (222)	51.39 (343)
	<b>e-value</b>	6.50e-46	5.54e-74	2.30e-40	3.04e-71	2.33e-119
<b>ZF D<sub>4b</sub></b>	<b>Id % (Sc)</b>	35.28 (253)	37.53 (258)	36.96 (244)	35.85 (251)	54.10 (394)
	<b>e-value</b>	3.46e-83	2.12e-85	5.65e-80	1.90e-82	5.79e-139
<b>ZF D<sub>8</sub></b>	<b>Id % (Sc)</b>	59.23 (490)	58.47 (457)	51.64 (385)	74.61 (610)	41.06 (271)
	<b>e-value</b>	2.56e-175	7.06e-163	5.39e-134	0.0	1.71e-90
<b>ZF D<sub>9</sub></b>	<b>Id % (Sc)</b>	37.05 (249)	37.12 (253)	39.59 (262)	42.48 (274)	70.76 (535)
	<b>e-value</b>	3.94e-81	2.15e-83	1.35e-86	1.12e-90	0

D<sub>2S</sub> = D<sub>2Short</sub>; D<sub>2L</sub> = D<sub>2Long</sub>; Id = Identity; Sc = bit Score

**Supplementary Table 1:** Pairwise comparisons within the D1 receptor family between pikeperch, Nile tilapia and European eel receptor protein sequences

<b>Pikeperch</b>		<b>D1_1</b>	<b>D1_2</b>	<b>D1_3</b>	<b>D1_4</b>	<b>D1_5</b>
<b>Tilapia D<sub>1a</sub></b>	<b>Id % (Sc)</b>	92.74 (674)	79.30 (562)	66.91 (368)	62.19 (434)	61.05 (203)
	<b>e-value</b>	0.0	0.0	5.42e-130	1.46e-153	1.50e-68
<b>Tilapia D<sub>1b</sub></b>	<b>Id % (Sc)</b>	79.36 (583)	92.66 (883)	57.14 (405)	62.40 (463)	64.29 (206)
	<b>e-value</b>	0.0	0.0	2.01e-143	1.53e-163	1.60e-68
<b>Tilapia D<sub>5a</sub></b>	<b>Id % (Sc)</b>	67.89 (468)	59.02 (500)	93.47 (685)	55.39 (478)	61.21 (194)
	<b>e-value</b>	7.05e-168	6.48e-179	0.0	1.63e-169	2.48e-64
<b>Tilapia D<sub>5b</sub></b>	<b>Id % (Sc)</b>	60.21 (437)	61.11 (443)	62.14 (368)	58.07 (426)	54.75 (174)
	<b>e-value</b>	1.94e-155	1.22e-156	6.11e-129	2.81e-149	1.82e-56
<b>Tilapia D<sub>6a</sub></b>	<b>Id % (Sc)</b>	63.01 (461)	55.82 (481)	56.17 (395)	78.91 (758)	61.70 (207)
	<b>e-value</b>	1.21e-164	4.80e-171	8.39e-139	0.0	1.08e-68
<b>Tilapia D<sub>6b</sub></b>	<b>Id % (Sc)</b>	68.27 (468)	66.30 (481)	58.97 (391)	59.25 (531)	82.47 (254)
	<b>e-value</b>	5.17e-168	1.27e-171	5.26e-138	0.0	1.02e-87
<b>Eel D<sub>1,1</sub></b>	<b>Id % (Sc)</b>	80.00 (565)	83.62 (735)	62.60 (427)	58.50 (452)	66.06 (207)
	<b>e-value</b>	0.0	0.0	2.95e-152	1.08e-159	2.56e-69
<b>Eel D<sub>1,2</sub></b>	<b>Id % (Sc)</b>	80.05 (598)	80.13 (731)	59.33 (408)	62.40 (467)	63.47 (201)
	<b>e-value</b>	0.0	0.0	7.38e-145	2.28e-165	5.22e-67
<b>Eel D<sub>5</sub></b>	<b>Id % (Sc)</b>	68.73 (464)	59.91 (496)	85.27 (620)	55.18 (478)	63.19 (197)
	<b>e-value</b>	2.03e-166	1.94e-177	0.0	2.17e-169	2.02e-65
<b>Eel D<sub>6</sub></b>	<b>Id % (Sc)</b>	70.21 (484)	69.60 (492)	63.23 (429)	65.37 (594)	75.80 (246)
	<b>e-value</b>	3.19e-174	4.87e-176	7.73e-153	0.0	2.22e-84
<b>Id</b>	<b>=</b>	<b>Identity;</b>	<b>Sc</b>	<b>=</b>	<b>bit</b>	<b>Score</b>

**Supplementary Table 2:** Pairwise comparisons within the D2 receptor family between pikeperch, Nile tilapia and European eel receptor protein sequences

<b>Pikeperch</b>		<b>D2_1</b>	<b>D2_2</b>	<b>D2_3</b>	<b>D2_4</b>	<b>D2_5</b>
<b>Tilapia D<sub>2</sub></b>	<b>Id % (Sc)</b>	94.88 (918)	89.13 (844)	52.16 (429)	57.02 (496)	35.98 (271)
	<b>e-value</b>	0.0	0.0	5.51e-151	7.78e-177	7.16e-90
<b>TilapiaD<sub>3</sub></b>	<b>Id % (Sc)</b>	51.16 (435)	52.69 (441)	89.50 (832)	51.13 (388)	37.34 (275)
	<b>e-value</b>	2.78e-153	4.53e-156	0.0	1.42 e-134	8.97e-92
<b>TilapiaD<sub>4a</sub></b>	<b>Id % (Sc)</b>	34.73 (238)	37.62 (259)	43.72 (173)	36.13 (256)	53.39 (407)
	<b>e-value</b>	4.31e-77	1.39e-85	1.09e-52	5.51e-84	8.35e-144
<b>Tilapia D<sub>4b</sub></b>	<b>Id % (Sc)</b>	33.04 (223)	34.66 (225)	36.22 (231)	45.55 (164)	51.68 (363)
	<b>e-value</b>	1.01e-71	9.68e-73	4.72e-75	3.74e-49	7.81e-127
<b>Tilapia D<sub>8</sub></b>	<b>Id % (Sc)</b>	57.27 (489)	57.64 (460)	49.77 (387)	88.77 (842)	41.76 (283)
	<b>e-value</b>	3.30e-174	4.22e-163	4.96e-134	0.0	1.76e-94
<b>Tilapia D<sub>9</sub></b>	<b>Id % (Sc)</b>	36.74 (250)	38.12 (253)	39.17 (255)	41.95 (263)	75.71 (599)
	<b>e-value</b>	9.26e-82	4.82 e-83	8.90e-84	1.52e-86	0.0
<b>Eel D<sub>2A</sub></b>	<b>Id % (Sc)</b>	77.83 (729)	73.13 (666)	50.52 (427)	58.96 (497)	37.01 (273)
	<b>e-value</b>	0.0	0.0	2.45e-150	1.88 e-177	4.40e-91
<b>Eel D<sub>2B</sub></b>	<b>Id % (Sc)</b>	75.48 (686)	71.73 (635)	51.50 (441)	59.04 (492)	36.77 (268)
	<b>e-value</b>	0.0	0.0	3.74e-156	1.63e-175	9.44e-89

Id = Identity; Sc = bit Score

**Supplementary Table 3:** ID correspondence and nomenclature for DA receptor sequences<sup>1</sup> used in the phylogenetic analysis and the manuscript

Vertebrate species	Latin names	D1 family		D2 family	
		Receptor names	Protein ID	Receptor names	Protein ID
Asian bonytongue (Arowana)	<i>Scleropages formosus</i>	D <sub>1a</sub>	XP_018611872.1	D <sub>2a</sub>	XP_018598250.1
		D <sub>1b</sub>	XP_018607352.1	D <sub>2b</sub>	XP_018610682.1
		D <sub>5a</sub>	XP_018590910.1		
		D <sub>6a</sub>	KPP63084.1		
		D <sub>6b</sub>	KPP64030.1		
Atlantic herring	<i>Clupea harengus</i>	D <sub>1b</sub>	XP_012693211.1	D <sub>2b</sub>	XP_012677409.1
		D <sub>5a</sub>	XP_012696109.1	D <sub>3</sub>	XP_012671515.1
		D <sub>5b</sub>	XP_012686723.1	D <sub>9</sub>	XP_012687376.1
		D <sub>6a</sub>	XP_012685303.1		
		D <sub>6b</sub>	XP_012672788.1		
Cattle	<i>Bos taurus</i>	D <sub>1</sub>	Q95136	D <sub>2</sub>	P20288
		D <sub>5</sub>	G3X8D2	D <sub>3</sub>	NP_001179824.1
				D <sub>4</sub>	XP_024843396.1
Chicken	<i>Gallus gallus</i>	D <sub>1</sub>	B8YLW8	D <sub>2</sub>	A9YZQ5
		D <sub>5</sub>	XP_015141299.1	D <sub>3</sub>	C5HV40
		D <sub>7</sub>	XP_004947622.1	D <sub>4</sub>	B6UVA0
Clown fish (Sebae anemonefish)	<i>Amphiprion sebae</i>			D <sub>2a</sub>	A0A0C5LBQ6
Coelacanth	<i>Latimeria chalumnae</i>	D <sub>1</sub>	XP_005992583.1	D <sub>2</sub>	H3BGA1
		D <sub>5</sub>	H2ZWY6	D <sub>8</sub>	XP_006010333.1
		D <sub>6</sub>	H3BEK6	D <sub>3</sub>	XP_014350627.1
		D <sub>7</sub>	XP_005995307.1	D <sub>4</sub>	H3B3R1

				D <sub>9</sub>	H3A1X7
European eel	<i>Anguilla</i>	D <sub>1,1</sub>	Q98841	D <sub>2a</sub> (D <sub>2A</sub> )	A1XYV7
	<i>anguilla</i>	D <sub>1,2</sub>	Q98842	D <sub>2b</sub> (D <sub>2B</sub> )	A1XYV8
		D <sub>5a</sub> (D <sub>1Ba</sub> )	Q98843		
		D <sub>6b</sub> (D <sub>6</sub> )	Q98844		
European seabass	<i>Dicentrarchus</i>			D <sub>3</sub>	E6ZIT8
	<i>labrax</i>				
Flathead grey mullet	<i>Mugil cephalus</i>			D <sub>2a</sub>	Q5Y5R5
Fugu (Japanese pufferfish)	<i>Takifugu</i>	D <sub>1a</sub>	XP_003970582.1	D <sub>2a</sub>	P53453
	<i>rubripes</i>	D <sub>1b</sub> (D <sub>1.4</sub> )	P53452	D <sub>3</sub>	XP_003967971.1
		D <sub>5a</sub>	XP_003973600.1	D <sub>4a</sub>	XP_003967634.1
		D <sub>5b</sub>	XP_011610919.1	D <sub>4b</sub>	XP_003969784.1
		D <sub>6b</sub> (DL)	P53454		
Golden-line (Golden-line barbel)	<i>Sinocyclocheilus</i>	D <sub>7</sub>	XP_016140732.1		
	<i>grahami</i>				
Goldfish	<i>Carassius</i>	D <sub>7</sub>	XP_026112506.1	D <sub>2a1</sub>	XP_026138158.1
	<i>auratus</i>			D <sub>2a2</sub>	XP_026082110.1
				D <sub>2b</sub>	XP_026109573.1
				D <sub>3_1</sub>	XP_026057526.1
				D <sub>3_2</sub>	XP_026093276.1
				D <sub>4a1</sub>	XP_026057689.1
				D <sub>4a2</sub>	XP_026111096.1
				D <sub>4b</sub>	XP_026122146.1
				D <sub>9,1</sub>	XP_026088236.1
				D <sub>9,2</sub>	XP_026066271.1
Human	<i>Homo sapiens</i>	D <sub>1</sub>	P21728	D <sub>2</sub>	P14416
		D <sub>5</sub>	P21918	D <sub>3</sub>	P35462

				D4	P21917
	<i>OUTGROUPS</i>	ADRA1A	NP_000671.2		
		ADRA2B	NP_000673.2		
Lizard	<i>Anolis</i>	D <sub>1</sub>	XP_008102998.1	D <sub>2</sub>	H9GEE4
	<i>carolinensis</i>	D <sub>5</sub>	H9GGW7	D <sub>3</sub>	G1KJF9
		D <sub>7</sub>	XP_003228657.1		
Medaka (Japanese medaka)	<i>Oryzias latipes</i>	D <sub>1a</sub>	XP_011478663.1	D <sub>2a</sub>	XP_004075461.1
		D <sub>1b</sub>	XP_004076181.1	D <sub>8</sub>	NP_001292346.1
		D <sub>5a</sub>	XP_004068909.1	D <sub>3</sub>	XP_004081111.1
		D <sub>5b</sub>	XP_011473350.1	D <sub>4a</sub>	XP_023811246.1
		D <sub>6a</sub>	XP_020555836.1	D <sub>4b</sub>	XP_023807515.1
				D <sub>9</sub>	XP_004085072.1
Mozambique tilapia	<i>Oreochromis</i>	D <sub>6b</sub>	P47800		
	<i>mossambicus</i>				
Nile tilapia	<i>Oreochromis</i>	D <sub>1a</sub>	XP_013127197	D <sub>2a</sub>	Q5Y5R4
	<i>niloticus</i>	D <sub>1b</sub>	I3KZ49	D <sub>8</sub>	XP_005455936.1
		D <sub>5a</sub>	I3KYB9	D <sub>3</sub>	I3K990
		D <sub>5b</sub>	XP_013131143.1	D <sub>4a</sub>	I3J7W8
		D <sub>6a</sub>	XP_005473811.1	D <sub>4b</sub>	I3KEQ9
		D <sub>6b</sub>	XP_003449479.1	D <sub>9</sub>	I3JL32
	<i>Paramormyrops kingsleyae</i>			D <sub>2b</sub>	XP_023672044.1
Platyfish	<i>Xiphophorus</i>			D <sub>2a</sub>	M4A3B9
	<i>maculatus</i>			D <sub>3</sub>	M4A6N4
				D <sub>4b</sub>	M4AVC2
				D <sub>8</sub>	M3ZW15
				D <sub>9</sub>	M4AF30

Rainbow trout	<i>Oncorhynchus</i>			D <sub>2a1</sub>	Q90WQ7
	<i>mykiss</i>			D <sub>2a2</sub>	Q90WQ6
Rat	<i>Rattus</i>	D <sub>1</sub>	P18901	D <sub>2</sub>	P61169
	<i>norvegicus</i>	D <sub>5</sub>	P25115	D <sub>3</sub>	P19020
				D <sub>4</sub>	P30729
Spotted gar	<i>Lepisosteus</i>	D <sub>1</sub>	W5MY24	D <sub>2</sub>	XP_006642348.1
	<i>oculatus</i>	D <sub>5</sub>	W5NNC6	D <sub>8</sub>	XP_015224221.1
		D <sub>6</sub>	XP_006630355.1	D <sub>3</sub>	W5M5T7
		D <sub>7</sub>	XP_006625630.1	D <sub>4</sub>	XP_006642527.1
				D <sub>9</sub>	XP_006633286.1
Stickleback (Three-spined stickleback)	<i>Gasterosteus</i>	D <sub>1a</sub>	G3Q397		
	<i>aculeatus</i>	D <sub>1b</sub>	G3QBX5		
		D <sub>5a</sub>	G3P395		
		D <sub>5b</sub>	G3PUT1		
		D <sub>6a</sub>	G3PAM4		
Tetraodon (Spotted green pufferfish)	<i>Tetraodon</i>			D <sub>2a</sub>	H3CQR4
	<i>nigroviridis</i>			D <sub>3</sub>	H3D5B2
Turkey	<i>Meleagris</i>			D <sub>2</sub>	O73810
	<i>gallopavo</i>			D <sub>3</sub>	G1NND1
Turtle	<i>Chelonia mydas</i>	D <sub>7</sub>	XP_007068400.1		
Zebrafish	<i>Danio rerio</i>	D <sub>1a</sub>	E7F359	D <sub>2a</sub>	Q8AWE0
		D <sub>1b</sub>	B6E506	D <sub>2b</sub> (D <sub>2c</sub> )	Q7T1A1
		D <sub>5b</sub>	XP_005159907.1	D <sub>3</sub>	Q8AWE1
		D <sub>6a</sub>	XP_005158584.1	D <sub>4a</sub>	NP_001012634.2
		D <sub>6b</sub>	F1QPK9	D <sub>4b</sub> (D <sub>4c</sub> )	Q5DJ14
		D <sub>7</sub>	A3KPR9	D <sub>8</sub>	Q7T1A2
		D <sub>9</sub> (D <sub>4b</sub> )	Q5DJ15		

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Xenopus	<i>Xenopus laevis</i>	D <sub>1</sub>	P42289	D <sub>2</sub>	P24628
		D <sub>5</sub>	P42290	D <sub>4</sub>	XP_002937535.2
		D <sub>6</sub>	P42291		

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\*correspond to incomplete sequences not included in the phylogenetic analyses.

## Figure captions

**Figure 1: Alignment of the two deduced pikeperch D<sub>1</sub> receptors with those of other vertebrates.** The dark amino acids shared residues across all compared sequences. TMDs (Transmembrane Domains) are indicated with a horizontal line and numbered. Cytoplasmic loops are numbered. The DRY sequence and the conserved cysteine residue in the C-terminal tail are boxed. Sequences were extracted from NCBI, Ensembl and UniProt with following sequence IDs: Human (*Homo sapiens*) D<sub>1</sub>, P21728; Zebrafish (*Danio rerio*) D<sub>1a</sub>, E7F359; Zebrafish D<sub>1b</sub>, B6E506; Nile tilapia (*Oreochromis niloticus*) D<sub>1a</sub>, XP\_013127197; Nile tilapia D<sub>1b</sub>, I3KZ49; European eel (*Anguilla Anguilla*) D<sub>1,1</sub>, Q98841; European eel D<sub>1,2</sub>, Q98842.

**Figure 2: Alignment of the two deduced pikeperch D<sub>2</sub> receptors with those of other vertebrates.** The dark amino acids shared residues across all compared sequences. TMDs (Transmembrane Domains) are indicated with a horizontal line and numbered. Cytoplasmic loops are numbered. The DRY sequence and the conserved cysteine residue in the C-terminal tail are boxed. \* indicates the 29 missing amino acids in the pikeperch D<sub>2S</sub> sequences. Sequences were extracted from Ensembl and UniProt with following sequence IDs: Human (*Homo sapiens*) D<sub>2L</sub>, P14416-1; Human D<sub>2S</sub>, P14416-2; Zebrafish (*Danio rerio*) D<sub>2a</sub>, Q8AWE0; Zebrafish D<sub>2b</sub>, Q7T1A1; Nile tilapia (*Oreochromis niloticus*) D<sub>2</sub>, Q5Y5R4; European eel (*Anguilla Anguilla*) D<sub>2A</sub>, A1XYV7; European eel D<sub>2B</sub>, A1XYV8

**Figure 3: Consensus phylogenetic tree of vertebrate D<sub>1</sub> receptor family.** This phylogenetic tree was constructed using the Maximum Likelihood method using 1000 bootstrap replicates. The number above the branch represents the bootstrap value (%). Only values and branching above 50% are shown. The consensus tree was rooted on the human,

*Homo sapiens*, adrenergic receptors. Red arrows indicate pikeperch sequences. Sequences used for this analyse are in Supplementary Table 3.

**Figure 4: Consensus phylogenetic tree of vertebrate D2 receptor family.** This phylogenetic tree was constructed using the Maximum Likelihood method using 1000 bootstrap replicates. The number above the branch represents the bootstrap value (%). Only values and branching above 50% are shown. The consensus tree was rooted on the human, *Homo sapiens*, adrenergic receptors. Red arrows indicate pikeperch sequences. Sequences used for this analyse are in Supplementary Table 3

**Figure 5: Relative gene expression of (A) D<sub>1a</sub>, (B) D<sub>1b</sub>, (C) D<sub>3</sub>, (D) D<sub>8</sub> and (E) D<sub>9</sub> in different pikeperch tissues.** Data are normalized on the geometric mean of housekeeping genes AK and RPL8. OB, olfactory bulbs; Tel/ORR, Telencephalon and the rostral Optic Recess Region (ORR); OT, Optic Tectum; Cb, Cerebellum; Hyp/IL, Hypothalamus/Inferior Lobe; Med/SC, Medulla/Spinal Cord; Pit, Pituitary; Gon, Gonads; L, Liver; H, Heart; Mu, Muscle; AT, Adipose Tissue; G, Gills; Spl, Spleen. Values are means  $\pm$  SEM. Different lowercase letters indicate significant differences between tissues.

**Figure 6: Dissection of the pikeperch brain showing the 6 parts where the gene expression pattern of the DA receptors was measured.** OB, olfactory bulb; Tel/ORR, Telencephalon and the rostral Optic Recess Region (ORR); OT, Optic Tectum; Cb, Cerebellum; Hyp/IL, Hypothalamus/Inferior Lobe; Med/SC, Medulla/Spinal Cord. The rostral part of the brain is on the bottom of the figure. The left picture shows the dorsal view, while the right picture shows the ventral view of the brain.

**Supplementary Figure 1: Alignment of the deduced pikeperch D<sub>5</sub> receptors with those of other vertebrates.** The dark amino acids shared residues across all compared sequences. TMDs (Transmembrane Domains) are indicated with a horizontal line and numbered. Cytoplasmic loops are numbered. The DRY sequence and the conserved cysteine residue in the C-terminal tail are boxed. Sequences were extracted from NCBI, Ensembl and UniProt with following sequence IDs: Human (*Homo sapiens*) D<sub>5</sub>, P21918; Zebrafish (*Danio rerio*) D<sub>5b</sub>, XP\_005159907.1; Nile tilapia (*Oreochromis niloticus*) D<sub>5a</sub>, I3KYB9; Nile tilapia D<sub>5b</sub>, XP\_013131143; European eel (*Anguilla anguilla*) D<sub>5</sub>, Q98843.

**Supplementary Figure 2: Alignment of the deduced pikeperch D<sub>6</sub> receptors with those of zebrafish, *Danio rerio*, Nile tilapia, *Oreochromis niloticus*, and European eel, *Anguilla anguilla*.** The dark amino acids shared residues across all compared sequences. TMDs (Transmembrane Domains) are indicated with a horizontal line and numbered. Cytoplasmic loops are numbered. The DRY sequence and the conserved cysteine residue in the C-terminal tail are boxed. Sequences were extracted from NCBI, Ensembl and UniProt with following sequence IDs: Zebrafish D<sub>6a</sub>, XP\_005158584.1; Zebrafish D<sub>6b</sub>, F1QPK9; Nile tilapia D<sub>6a</sub>, XP\_005473811; Nile tilapia D<sub>6b</sub>, XP\_003449479; European eel D<sub>6</sub>, Q98844.

**Supplementary Figure 3: Alignment of the deduced pikeperch D<sub>3</sub> receptor with those of other vertebrates.** The dark amino acids shared residues across all compared sequences. TMDs (Transmembrane Domains) are indicated with a horizontal line and numbered. Cytoplasmic loops are numbered. The DRY sequence and the conserved cysteine residue in the C-terminal tail are boxed. Sequences were extracted from NCBI, Ensembl and UniProt

with following sequence IDs: Human (*Homo sapiens*) D<sub>3</sub>, P35462; Zebrafish D<sub>3</sub>, Q8AWE1; Nile tilapia D<sub>3</sub>, I3K990.

**Supplementary Figure 4: Alignment of the deduced pikeperch D<sub>8</sub> receptor with those of zebrafish, *Danio rerio*, and Nile tilapia, *Oreochromis niloticus*.** The dark amino acids shared residues across all compared sequences. TMDs (Transmembrane Domains) are indicated with a horizontal line and numbered. Cytoplasmic loops are numbered. The DRY sequence and the conserved cysteine residue in the C-terminal tail are boxed. Sequences were extracted from NCBI, Ensembl and UniProt with following sequence IDs: Zebrafish D<sub>8</sub>, Q7T1A2; Nile tilapia D<sub>8</sub>, XP\_005455936.1.

**Supplementary Figure 5: Alignment of the deduced pikeperch D<sub>9</sub> receptor with those of zebrafish, *Danio rerio*, and Nile tilapia, *Oreochromis niloticus*.** The dark amino acids shared residues across all compared sequences. TMDs (Transmembrane Domains) are indicated with a horizontal line and numbered. Cytoplasmic loops are numbered. The DRY sequence and the conserved cysteine residue in the C-terminal tail are boxed. Sequences were extracted from NCBI, Ensembl and UniProt with following sequence IDs: Zebrafish D<sub>9</sub>, Q5DJ15; Nile tilapia D<sub>9</sub>, I3JL32.