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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, D₁ (former D_{1A}), D₅ (former
26 D_{1B}), D₆ (former D_{1C}) and D₇ (former D_{1E}), while the D2 family encloses five subtypes, D₂,
27 D₃, D₄, D₈ (former D_{2like} or D_{2l}) and D₉ (former D_{4-related sequence} or D_{4-rs}). 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 D₁, one D_{5a}, one
35 D_{6a} and one D_{6b} and to the D2 family: two spliced variants of D₂, one D₃, one D₈ and one D₉.
36 Unlike zebrafish, the subtypes D₄ and D₇ have not yet been isolated in pikeperch. As expected
37 D₁, D₃, D₈ and D₉ are mostly expressed in brain parts except for the cerebellum (D₁ and D₃).
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_{2long} (D_{2L}) and D_{2short} (D_{2S}) by an alternative splicing of the D₂ 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, D₁ (former D_{1A}), D₅ (former D_{1B}), D₆ (former
78 D_{1C}) and D₇ (former D_{1E}) while the D2 family would enclose five subtypes, D₂, D₃, D₄, D₈
79 (former D_{2I}) and D₉ (former D_{4rs}). In zebrafish, *Danio rerio*, these nine DA receptors subtypes
80 have been isolated (Yamamoto et al., 2015). In this species, except for D₇, D₈ (D_{2b} in
81 Boehmler et al., 2004), D₃ and D₉ (D_{4b} in Boehmler et al., 2007) encoded by a unique gene,
82 the D₁, D₅, D₆, D₂ and D₄ receptor subtypes are encoded by two paralogous genes giving two
83 distinct receptors per subtype, D_{1a} and D_{1b}, D_{5a} and D_{5b}, D_{6a} and D_{6b}, D_{2a} and D_{2b} (called D_{2a}
84 and D_{2c}, respectively in Boehmler et al., 2004), and D_{4a} and D_{4b} (called D_{4a} and D_{4c},
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 D₂ 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 D₂ 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 D₂ (D_{2a} and D_{2c}), D₃
106 and D₈ (D_{2b}) receptor subtypes may be involved in visual function. Also, they showed that
107 receptors D_{2a} and D₈ (or D_{2b}) 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 D₂ 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_{2A} and D_{2B}
115 in European eel and the D₂ 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_{2A}, but not D_{2B}, 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_{1,1} and D_{1,2} (D_{1A1} and D_{1A2}) 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, D₂ receptors are not detected in European eel
123 ovaries, liver, kidney, muscle and gills whereas in Nile tilapia, D₂ 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 2m³ 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 (D₁, D₅, D₆, D₇), and
230 75 osteichthyan receptors from the D2 family (D₂, D₃, D₄, D₈, D₉) 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_{1a}, D_{1b}, D₃, D₈ and D₉), 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 D₁ receptor (range between 72-73%). Relatively to teleost
308 species, D1_1 displayed about 80% of sequence identity with zebrafish and European eel D₁
309 receptors while it displayed the highest identity with the Nile tilapia D_{1a} receptor (93%).
310 Pikeperch D1_2 shared high percentage of sequence identity with zebrafish D_{1b} (84%), Nile
311 tilapia D_{1b} (93%) and European eel D_{1,1} (84%). D1_3 showed 66% of sequence identity with
312 the human D₅ receptor while D1_4 displayed 60% of sequence identity with the human D₁
313 receptor. Relatively to teleost receptors, D1_3 shared similar sequence identity with zebrafish
314 D_{1a} and D_{5a} (71-72%) and a high percentage of sequence identity with the Nile tilapia D_{5a}
315 (93%) and European eel D₅ (85%). D1_4 displayed the highest identity with the zebrafish and
316 Nile tilapia D_{6a} (66 and 79%, respectively) and European eel D₆ receptor (65%). Finally,
317 D1_5 sequence shared similar sequence identities with human D₁ and D₅ receptors (61-62%)
318 while it displayed the highest sequence identities with zebrafish D_{6a} and D_{6b} (76%), Nile
319 tilapia D_{6b} (82%) and European eel D₆ (76%) receptors. No pikeperch sequences identified in

320 this study were related to zebrafish and tilapia D_{5b} or to zebrafish D₇ 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 D₂ receptors (from 55% to
327 66%). Relatively to teleost species, D2_1 and D2_2 displayed the highest sequence identity
328 with teleost D₂ subtype, the Nile tilapia D₂ (95% and 89%, respectively), European eel D_{2A}
329 (78% and 73%, respectively), European eel D_{2B} (75% and 72%, respectively), zebrafish D_{2a}
330 (73% and 69%, respectively) and zebrafish D_{2b} receptors (73% and 71%, respectively). D2_3
331 shared 58% of sequence identity with human D₃ while he displayed the highest sequence
332 identities with zebrafish and Nile tilapia D₃ (75-90%). D2_4 shared highest sequence identity
333 with Nile tilapia D₈ (89%) and zebrafish D₈ (75%) receptors. Finally, D2_5 shared 68% of
334 sequence identity with human D₄ while he displayed the highest sequence identities with
335 zebrafish and Nile tilapia D₉ (71-76%) receptors, respectively.

336 Accordingly, we propose the following designation for those ten DA receptor sequences: D_{1a}
337 (D1_1), D_{1b} (D1_2), D_{5a} (D1_3), D_{6a} (D1_4), D_{6b} (D1_5), D₂ for two sequences sharing 100%
338 of identity (D2_1 and D2_2), D₃ (D2_3), D₈ (D2_4) and D₉ (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_{1a}, D_{1b} and both D₂ 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_{5a} and D_{6b} 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_{6b} 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_{6b}. 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 D₂ sequences, a gap in the protein sequence within the third intracellular loop (29
354 missing amino acids) was observed (Figure 2). The two D₂ sequences were renamed D_{2Long}
355 (D_{2L}) and D_{2Short} (D_{2S}), 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 D₆, D₁ and D₅
365 sequences in three well-supported clades, with bootstrap values of 91, 98 and 67%,
366 respectively. However, the few D₇ sequences retrieved from the available databases were not
367 encompassed in a single monophyletic clade. The D₁ 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 D₁ sequences were separated into two clades, named D_{1a} and
371 D_{1b}. Pikeperch D1_1 and D1_2 sequences branched individually within each one of these
372 clades, confirming their identification as D_{1a} and D_{1b}. D₅ sequences were separated into three
373 clades: a sarcopterygian clade, and two actinopterygian clades. The first actinopterygian clade
374 encompassed the teleost D_{5a} sequences with the spotted gar D₅ branching at its base, and the
375 second one encompassed the D_{5b} teleost sequences. The D1_3 pikeperch sequence branched
376 together with the D_{5a} teleost sequences, confirming its place in the nomenclature as D_{5a}.
377 Concerning D₆, the actinopterygian clade, with the spotted gar D₆ 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_{6a} and
380 D_{6b}, respectively.

381 Concerning the D2 family, phylogenetic analysis clustered all vertebrate sequences in two
382 strongly-supported clades, D₄/D₉ (100%) on one side and D₃/D₂/D₈ (91%) on the other side. A
383 clear demarcation was observed between D₄ (80%) and D₉ (89%) sequences within the D₄/D₉
384 clade. Within the D₃/D₂/D₈ clade, D₃ (90%), D₂ (78%) and D₈ (68%) sequences diverged into
385 three subclades. Into each one of the D₄, D₉, D₃, D₂ and D₈ subclades, a well-supported
386 actinopterygian branch was defined.

387 The spotted gar D₄ sequence branched at the base of the two teleost D₄ clades, D_{4a} and D_{4b},
388 while the spotted gar D₉ branched with a single teleost D₉ clade, including the pikeperch D2_5
389 sequence. The spotted gar D₃ sequence branched at the base of a single teleost D₃ clade,
390 including the D2_3 pikeperch sequence. Accordingly, these pikeperch sequences were
391 respectively named D₉ and D₃. Similarly, the spotted gar D₈ sequence branched together with
392 the coelacanth D₈ one, within a single teleost D₈ clade, including the D2_4 pikeperch
393 sequence, now called D₈. The spotted gar D₂ sequence branched at the base of two well-
394 supported clades, named D_{2a} (73%) and D_{2b} (60%). The pikeperch D2_L sequence branched

395 together with other teleost D_{2a} sequences. Thus, the two pikeperch D₂ 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 D₂ gene, hereby named D_{2a} (D_{2aL} and D_{2aS}). No pikeperch
398 sequences orthologous to vertebrate D₄ 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_{1a} and D_{1b}**

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_{1b} expression was also undetectable in liver and heart.

411

412 **3.4.2. Gene expression of D₃, D₈ and D₉**

413 The three genes were mainly expressed in the brain region (Figures 5C, D and E). The D₃ was
414 mostly expressed in the telencephalon and rostral ORR, olfactory bulbs, hypothalamus/IL and
415 pituitary. The D₈ was predominantly expressed in the optic tectum, medulla/rostral spinal cord
416 and hypothalamus/IL while the D₉ was mostly expressed in olfactory bulbs, telencephalon and
417 rostral ORR, and optic tectum. In the ovaries, D₃ and D₈ gene expressions were above the
418 detection limit while the D₉ mRNA was undetectable. In the heart, D₃ had an intermediate
419 expression level. Finally, the gene expressions were above the detection limit in the liver for

420 D₃ and D₈, in the heart for D₈ and D₉, in the muscles for D₈, in the gills for D₃ 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 D₁ receptors and only 21-41% with vertebrate D₂
429 receptors. Besides the seven TMDs found in complete sequences and the DRY sequence
430 specific of GPCR, these receptors share common features with D₁ 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, D₁, D₅ and D₆ (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_{1a}, D_{1b}, D_{5a}, D_{6a} and D_{6b}.

444 These different D₁ receptor paralogs have already been identified in literature or have been
445 released in the relevant databases. A single D₁ 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 D₁ 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_{1A} paralogs, as most of the teleosts.

454 In the same way, two D₆ receptors were identified in pikeperch, as in other teleosts, such as
455 Atlantic herring, *Clupea harengus*, zebrafish, and Nile tilapia, while a single D₆ 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 D₆ 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 D₆ 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 D₅, a single receptor is present in sarcopterygians, while two D₅ 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 D₅ 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 D₅
471 branched at the base of the teleost D_{5a} clade, and in polytomy with the sarcopterygian and the
472 teleost D_{5b} (named D_{1X} in Hirano et al., 1998) clades. This result does not suggest that the two
473 teleost D₅ 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_{5b} 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 D₂, D₃, D₈, and D₉. 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 D₂, D₃, D₈, and D₉ during evolution in pikeperch.

491 All these D2 family receptors have been previously identified in vertebrates including several
492 teleost species. For instance, a D₈ 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 D₈ sequences clustered in a single clade distinct to the other vertebrate D₂. 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 D₈ sister clade, encompassing D₂ 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 D₂ 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 D₂ gene encodes for two identical receptors D_{2aL} and D_{2aS},
506 except for a 29 amino acid sequence missing in the third cytoplasmic loop of the D_{2aS}. 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_{2a} 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 D₂ 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_{2S} receptor could be presynaptically located while the D_{2L} 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 D₃, 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 D₃ paralogs may have been lost shortly
531 after the teleost emergence.

532 In the same way, a single copy of D₉ 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 D₉ 3R-paralogs during teleost radiation.

537 Concerning the D₄ 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 D₄ has
542 been evidenced in the pikeperch.

543 Few studies have investigated D₄ and D₉ subtypes in teleosts besides Boehmler et al. (2007),
544 which reported three distinct genes encoding those receptors, two genes encoding the D₄
545 subtype (D_{4a} and D_{4b} (or D_{4c})) and a single gene encoding the D₉ subtype. As suggested in
546 previous studies, the existence of D₄ and D₉ 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_{5b}, D_{2b}, both D₄ paralogs and D₇ in all
551 female pikeperch specimens analysed in the present study. First, some receptors like D_{2b} and
552 D₇ 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_{5b}, 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_{1a},
566 D_{1b}, D₃, D₈ and D₉.

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 D₁ DA receptors, D_{1a}, D_{1b}, D_{5a}, D_{6a} and D_{6b} and five D₂ DA receptors, D_{2L}
599 and D_{2S}, D₃, D₈ and D₉, sharing features specific to each DA receptor family, were identified
600 in pikeperch during the pre-ovulatory period. Presence of two receptor families (D₁ and D₂),
601 multiple receptor subtypes (D₁, D₅, D₆, D₂, D₃, D₈ and D₉), some teleost-specific paralogs for
602 some subtypes (D_{1a} and D_{1b}, D_{6a} and D_{6b}) and even spliced variants (D_{2aL} and D_{2aS})
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

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

Sequences (5' – 3')			
	Forward	Reverse	Size (bp)
D _{1a}	CTTCATCCTCAACTGCATGGT	CCTCCTGGATACAGTCTGTGG	204
D _{1b}	CCAGAGAGAGACTCGTCCAAA	TTGCAGAAAGCACCAAACGG	196
D ₃	TGGTGTGCTCCATCTCCAAC	GCCACAGGCAAACCTGACAAC	306
D ₈	TGCCATCTCCTGCCCTTTAC	CCATCGCCTCCTTTACCAGG	255
D ₉	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
H D₁	Id % (Sc)	72.75 (513)	72.15 (621)	57.30 (397)	59.53 (439)	61.94 (188)
	e-value	0.0	0.0	1.88e-140	1.28e-154	4.58e-62
H D₅	Id % (Sc)	67.45 (455)	56.76 (477)	66.13 (492)	51.84 (461)	61.24 (197)
	e-value	2.72e-162	1.54e-169	2.75e-177	1.79e-162	3.74e-65
ZF D_{1a}	Id % (Sc)	81.03 (570)	82.37 (575)	71.43 (382)	65.63 (435)	62.81 (189)
	e-value	0.0	0.0	1.14e-135	4.06e-154	2.95e-63
ZF D_{1b}	Id % (Sc)	79.95 (565)	84.13 (756)	59.07 (404)	62.97 (437)	66.46 (208)
	e-value	0.0	0.0	2.91e-143	1.88e-153	1.26e-69
ZF D_{5a}	Id % (Sc)	64.71 (272)	64.35 (286)	72.19 (421)	58.58 (290)	66.88 (199)
	e-value	2.40e-93	1.20e-97	1.80e-152	8.15e-99	3.73e-68
ZF D_{5b}	Id % (Sc)	61.45 (419)	62.08 (417)	59.13 (370)	57.65 (405)	59.24 (171)
	e-value	2.70e-149	1.75e-147	2.21e-130	7.51e-142	6.25e-56
ZF D_{6a}	Id % (Sc)	68.31 (476)	57.52 (495)	61.30 (420)	65.77 (597)	76.19 (224)
	e-value	4.59e-171	3.74e-177	2.24e-149	0.0	9.17e-76
ZF D_{6b}	Id % (Sc)	70.54 (475)	68.75 (480)	60.28 (409)	59.84 (533)	75.97 (228)
	e-value	4.77e-171	1.05e-171	2.08e-145	0.0	8.43e-78
ZF D₇	Id % (Sc)	62.82 (431)	55.71 (455)	56.43 (377)	59.84 (446)	62.18 (185)
	e-value	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

Pikeperch		D2_1	D2_2	D2_3	D2_4	D2_5
H D_{2L}	Id % (Sc)	66.10 (602)	63.23 (557)	53.76 (438)	56.47 (456)	38.48 (262)
	e-value	0.0	0.0	8.44e-155	2.94e-161	7.23e-87
H D_{2S}	Id % (Sc)	62.50 (559)	66.37 (571)	53.68 (440)	54.98 (436)	41.18 (274)
	e-value	0.0	0.0	4.47e-156	8.36e-154	1.13e-91
H D₃	Id % (Sc)	48.89 (388)	53.15 (403)	57.72 (449)	49.22 (368)	40.96 (276)
	e-value	1.32e-135	4.63e-142	9.23e-160	1.63e-127	1.47e-92
H D₄	Id % (Sc)	46.88 (152)	36.49 (228)	38.22 (232)	53.49 (150)	68.04 (228)
	e-value	2.33e-44	6.86e-73	2.22e-74	1.76e-43	2.33e-73
ZF D_{2a}	Id % (Sc)	73.35 (684)	69.44 (632)	51.45 (418)	54.96 (471)	33.63 (265)
	e-value	0.0	0.0	6.88e-147	1.92e-167	6.38e-88
ZF D_{2b}	Id % (Sc)	73.25 (677)	71.15 (617)	53.60 (438)	58.02 (500)	37.09 (276)
	e-value	0.0	0.0	6.56e-155	1.15e-178	4.04e-92
ZF D₃	Id % (Sc)	50.10 (447)	53.06 (456)	74.57 (666)	49.31 (386)	38.02 (270)
	e-value	3.87e-158	8.14e-162	0.0	5.62e-134	1.55e-89
ZF D_{4a}	Id % (Sc)	47.62 (155)	36.62 (228)	48.54 (139)	36.59 (222)	51.39 (343)
	e-value	6.50e-46	5.54e-74	2.30e-40	3.04e-71	2.33e-119
ZF D_{4b}	Id % (Sc)	35.28 (253)	37.53 (258)	36.96 (244)	35.85 (251)	54.10 (394)
	e-value	3.46e-83	2.12e-85	5.65e-80	1.90e-82	5.79e-139
ZF D₈	Id % (Sc)	59.23 (490)	58.47 (457)	51.64 (385)	74.61 (610)	41.06 (271)
	e-value	2.56e-175	7.06e-163	5.39e-134	0.0	1.71e-90
ZF D₉	Id % (Sc)	37.05 (249)	37.12 (253)	39.59 (262)	42.48 (274)	70.76 (535)
	e-value	3.94e-81	2.15e-83	1.35e-86	1.12e-90	0

D_{2S} = D_{2Short}; D_{2L} = D_{2Long}; 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

Pikeperch		D1_1	D1_2	D1_3	D1_4	D1_5
Tilapia D_{1a}	Id % (Sc)	92.74 (674)	79.30 (562)	66.91 (368)	62.19 (434)	61.05 (203)
	e-value	0.0	0.0	5.42e-130	1.46e-153	1.50e-68
Tilapia D_{1b}	Id % (Sc)	79.36 (583)	92.66 (883)	57.14 (405)	62.40 (463)	64.29 (206)
	e-value	0.0	0.0	2.01e-143	1.53e-163	1.60e-68
Tilapia D_{5a}	Id % (Sc)	67.89 (468)	59.02 (500)	93.47 (685)	55.39 (478)	61.21 (194)
	e-value	7.05e-168	6.48e-179	0.0	1.63e-169	2.48e-64
Tilapia D_{5b}	Id % (Sc)	60.21 (437)	61.11 (443)	62.14 (368)	58.07 (426)	54.75 (174)
	e-value	1.94e-155	1.22e-156	6.11e-129	2.81e-149	1.82e-56
Tilapia D_{6a}	Id % (Sc)	63.01 (461)	55.82 (481)	56.17 (395)	78.91 (758)	61.70 (207)
	e-value	1.21e-164	4.80e-171	8.39e-139	0.0	1.08e-68
Tilapia D_{6b}	Id % (Sc)	68.27 (468)	66.30 (481)	58.97 (391)	59.25 (531)	82.47 (254)
	e-value	5.17e-168	1.27e-171	5.26e-138	0.0	1.02e-87
Eel D_{1,1}	Id % (Sc)	80.00 (565)	83.62 (735)	62.60 (427)	58.50 (452)	66.06 (207)
	e-value	0.0	0.0	2.95e-152	1.08e-159	2.56e-69
Eel D_{1,2}	Id % (Sc)	80.05 (598)	80.13 (731)	59.33 (408)	62.40 (467)	63.47 (201)
	e-value	0.0	0.0	7.38e-145	2.28e-165	5.22e-67
Eel D₅	Id % (Sc)	68.73 (464)	59.91 (496)	85.27 (620)	55.18 (478)	63.19 (197)
	e-value	2.03e-166	1.94e-177	0.0	2.17e-169	2.02e-65
Eel D₆	Id % (Sc)	70.21 (484)	69.60 (492)	63.23 (429)	65.37 (594)	75.80 (246)
	e-value	3.19e-174	4.87e-176	7.73e-153	0.0	2.22e-84
Id	=	Identity;	Sc	=	bit	Score

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

Pikeperch		D2_1	D2_2	D2_3	D2_4	D2_5
Tilapia D₂	Id % (Sc)	94.88 (918)	89.13 (844)	52.16 (429)	57.02 (496)	35.98 (271)
	e-value	0.0	0.0	5.51e-151	7.78e-177	7.16e-90
TilapiaD₃	Id % (Sc)	51.16 (435)	52.69 (441)	89.50 (832)	51.13 (388)	37.34 (275)
	e-value	2.78e-153	4.53e-156	0.0	1.42 e-134	8.97e-92
TilapiaD_{4a}	Id % (Sc)	34.73 (238)	37.62 (259)	43.72 (173)	36.13 (256)	53.39 (407)
	e-value	4.31e-77	1.39e-85	1.09e-52	5.51e-84	8.35e-144
Tilapia D_{4b}	Id % (Sc)	33.04 (223)	34.66 (225)	36.22 (231)	45.55 (164)	51.68 (363)
	e-value	1.01e-71	9.68e-73	4.72e-75	3.74e-49	7.81e-127
Tilapia D₈	Id % (Sc)	57.27 (489)	57.64 (460)	49.77 (387)	88.77 (842)	41.76 (283)
	e-value	3.30e-174	4.22e-163	4.96e-134	0.0	1.76e-94
Tilapia D₉	Id % (Sc)	36.74 (250)	38.12 (253)	39.17 (255)	41.95 (263)	75.71 (599)
	e-value	9.26e-82	4.82 e-83	8.90e-84	1.52e-86	0.0
Eel D_{2A}	Id % (Sc)	77.83 (729)	73.13 (666)	50.52 (427)	58.96 (497)	37.01 (273)
	e-value	0.0	0.0	2.45e-150	1.88 e-177	4.40e-91
Eel D_{2B}	Id % (Sc)	75.48 (686)	71.73 (635)	51.50 (441)	59.04 (492)	36.77 (268)
	e-value	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 sequences¹ 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 _{1a}	XP_018611872.1	D _{2a}	XP_018598250.1
		D _{1b}	XP_018607352.1	D _{2b}	XP_018610682.1
		D _{5a}	XP_018590910.1		
		D _{6a}	KPP63084.1		
		D _{6b}	KPP64030.1		
Atlantic herring	<i>Clupea harengus</i>	D _{1b}	XP_012693211.1	D _{2b}	XP_012677409.1
		D _{5a}	XP_012696109.1	D ₃	XP_012671515.1
		D _{5b}	XP_012686723.1	D ₉	XP_012687376.1
		D _{6a}	XP_012685303.1		
		D _{6b}	XP_012672788.1		
Cattle	<i>Bos taurus</i>	D ₁	Q95136	D ₂	P20288
		D ₅	G3X8D2	D ₃	NP_001179824.1
				D ₄	XP_024843396.1
Chicken	<i>Gallus gallus</i>	D ₁	B8YLW8	D ₂	A9YZQ5
		D ₅	XP_015141299.1	D ₃	C5HV40
		D ₇	XP_004947622.1	D ₄	B6UVA0
Clown fish (Sebae anemonefish)	<i>Amphiprion sebae</i>			D _{2a}	A0A0C5LBQ6
Coelacanth	<i>Latimeria chalumnae</i>	D ₁	XP_005992583.1	D ₂	H3BGA1
		D ₅	H2ZWY6	D ₈	XP_006010333.1
		D ₆	H3BEK6	D ₃	XP_014350627.1
		D ₇	XP_005995307.1	D ₄	H3B3R1

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

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

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

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

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

Figure captions

Figure 1: Alignment of the two deduced pikeperch D₁ 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*) D₁, P21728; Zebrafish (*Danio rerio*) D_{1a}, E7F359; Zebrafish D_{1b}, B6E506; Nile tilapia (*Oreochromis niloticus*) D_{1a}, XP_013127197; Nile tilapia D_{1b}, I3KZ49; European eel (*Anguilla Anguilla*) D_{1,1}, Q98841; European eel D_{1,2}, Q98842.

Figure 2: Alignment of the two deduced pikeperch D₂ 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_{2S} sequences. Sequences were extracted from Ensembl and UniProt with following sequence IDs: Human (*Homo sapiens*) D_{2L}, P14416-1; Human D_{2S}, P14416-2; Zebrafish (*Danio rerio*) D_{2a}, Q8AWE0; Zebrafish D_{2b}, Q7T1A1; Nile tilapia (*Oreochromis niloticus*) D₂, Q5Y5R4; European eel (*Anguilla Anguilla*) D_{2A}, A1XYV7; European eel D_{2B}, A1XYV8

Figure 3: Consensus phylogenetic tree of vertebrate D₁ 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_{1a}, (B) D_{1b}, (C) D₃, (D) D₈ and (E) D₉ 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 D₅ 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*) D₅, P21918; Zebrafish (*Danio rerio*) D_{5b}, XP_005159907.1; Nile tilapia (*Oreochromis niloticus*) D_{5a}, I3KYB9; Nile tilapia D_{5b}, XP_013131143; European eel (*Anguilla anguilla*) D₅, Q98843.

Supplementary Figure 2: Alignment of the deduced pikeperch D₆ 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_{6a}, XP_005158584.1; Zebrafish D_{6b}, F1QPK9; Nile tilapia D_{6a}, XP_005473811; Nile tilapia D_{6b}, XP_003449479; European eel D₆, Q98844.

Supplementary Figure 3: Alignment of the deduced pikeperch D₃ 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*) D₃, P35462; Zebrafish D₃, Q8AWE1; Nile tilapia D₃, I3K990.

Supplementary Figure 4: Alignment of the deduced pikeperch D₈ 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 D₈, Q7T1A2; Nile tilapia D₈, XP_005455936.1.

Supplementary Figure 5: Alignment of the deduced pikeperch D₉ 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 D₉, Q5DJ15; Nile tilapia D₉, I3JL32.