Large scale gene duplication affected the European eel (*Anguilla anguilla*) after the 3R teleost duplication

Christoffer Rozenfeld[1*], Jose Blanca[2*], Victor Gallego[1], Víctor García-Carpintero[2], Juan Germán Herranz-Jusdado[1], Luz Pérez[1], Juan F. Asturiano[1▲], Joaquín Cañizares[2†], David S. Peñaranda[1†]

1 Grupo de Acuicultura y Biodiversidad. Instituto de Ciencia y Tecnología Animal. Universitat Politècnica de València. Camino de Vera s/n, 46022 Valencia, Spain
2 Instituto de Conservación y Mejora de la Agrodiversidad Valenciana, Universitat Politècnica de València, Camino de Vera 14, 46022, Valencia, Spain.

Authors marked with * or † contributed equally to this work, respectively.

Running title: Evidence of European eel large scale gene duplication

Keywords: European eel, PHYLDOG, 4dTv, whole genome duplication

▲ Corresponding author:

Dr. Juan F. Asturiano
Grupo de Acuicultura y Biodiversidad
Instituto de Ciencia y Tecnología Animal
Universitat Politècnica de València
Camino de Vera s/n 46022 Valencia (Spain)
E-mail: jfastu@dca.upv.es

Abstract

Genomic scale duplication of genes generates raw genetic material, which may facilitate new adaptations for the organism. Previous studies on eels have reported specific gene duplications, however a species-specific large-scale gene duplication has never before been proposed. In this study, we have assembled a *de novo* European eel transcriptome and the data show more than a thousand gene duplications that happened, according to a 4dTv analysis, after the teleost specific 3R whole genome duplication (WGD). The European eel has a complex and peculiar life cycle, which involves extensive migration, drastic habitat changes and metamorphoses, all of which could have been facilitated by the genes derived from this large-scale gene duplication.

Of the paralogs created, those with a lower genetic distance are mostly found in tandem repeats, indicating that they are young segmental duplications. The older eel paralogs showed a different pattern, with more extensive synteny suggesting that a Whole Genome Duplication (WGD) event may have happened in the eel lineage. Furthermore, an enrichment analysis of eel specific paralogs further revealed GO-terms typically enriched after a WGD. Thus, this study, to the best of our knowledge, is the first to present evidence indicating an Anguillidae family specific large-scale gene duplication, which may include a 4R WGD.

Introduction

Large-scale gene duplications can originate from one single event, like a whole genome duplication (WGD; Ohno, 1970) or from multiple smaller segmental duplication events (SDs; Gu et al. 2002). Any of these duplication events may contribute to species radiation, since both provide raw material for new genetic variation (Cañestro et al. 2013; Gu et al. 2002; Ohno 1970). It has been suggested that early in the vertebrate lineage two WGDs (1R and 2R) happened, resulting in species radiation and evolution of new traits (Cañestro et al. 2013; Dehal and Boore 2005; Gu et al. 2002; Ohno 1970). In teleosts, there is strong evidences to support an additional WGD, called the 3rd teleost specific WGD (3R), which occurred in the base of the teleost lineage, between 350 and 320 million years ago (MYA; Aparicio et al. 2002; Christoffels et al. 2004; Howe et al. 2013; Vandepoele et al. 2004; Jaillon et al. 2004; Kasahara et al. 2007; Meyer and Peer 2005; Schartl et al. 2013). Previous studies have proposed that this extra 3R WGD is one of the possible causes of the massive species radiation observed in teleosts (Hoegg et al. 2004; Santini et al. 2009). In addition to 3R, multiple genus or species specific WGDs have been documented in teleosts, e.g. in salmonids (order Salmoniformes; Allendorf and Thorgaard, 1984; Johnson et al. 1987), sturgeons (order Acipenseriform; Ludwig et al. 2001), common carp (*Cyprinus carpio*; Larhammar and Risinger, 1994), goldfish (*Carassius auratus*; Ohno, 1970), suckers (family Catastomidae; Uyeno and Smith, 1972), and loaches (*Botia macracantha* and *Botia modesta*; Ferris and Whitt 1977).

As mentioned previously, other mechanisms to WGDs can create large-scale gene duplications. Several species have shown a high occurrence of relatively recent segmental duplications (SD), often found in tandem, with segments spanning from a few hundred base pairs to several genes e.g. in yeast (Llorente et al. 2000), daphnia (Colbourne et al. 2011), humans (Bailey et al. 2002; Gu et al. 2002; Vallente Samonte and Eichler 2016) and teleosts (Blomme et al. 2006; David et al. 2003; Jaillon et al. 2004; Lu et al. 2012; Rondeau et al. 2014). It is quite common for one of

the copies of these SDs to get lost over time, possibly due to genetic drift or purifying selection. As a consequence, the genetic distance between two copies often tends to be quite small (Ohno, 1970). This process is known as the continuous mode hypothesis (Gu et al. 2002). In some cases however, these SDs have been conserved in high frequency at particular times, e.g. in yeast (Llorente et al. 2000), common carp (David et al. 2003) and humans (Asrar et al. 2013; Bailey et al. 2002; Gu et al. 2002; Hafeez et al. 2016). Some mechanisms, which could be facilitating this conservation include the processes of subfunctionalization, neofunctionalization or dosage selection (for review see Zhang, 2003). Furthermore, these processes have also been associated with the adaptation to new environments (Colbourne et al. 2011; Tautz and Domazet-lošo 2011).

The elopomorpha cohort, is one of the most basal teleost groups (Greenwood et al. 1966; Inoue et al. 2004). Elopomorphas are believed to originally be a marine species however, the 19 species of Anguillidae family, broke away from the ancestral trait and adapted a catadromous life style, migrating from their feeding grounds in freshwater rivers and lakes to their marine spawning grounds (Inoue et al. 2010; Munk et al. 2010; Schmidt 1923; Tsukamoto Katsumi, Nakai Izumi 1998). It is likely that species of the Anguillidae family originally performed relatively short reproductive migrations however, due to continental drift (Inoue et al. 2010; Tsukamoto et al. 2002) or changes in oceanic currents these migrations have since become vastly extensive (Jacobsen et al. 2014), with a total migrating distance of >6.000 km in the case of the European eel (Righton et al. 2016).

Several previous studies have revealed a high occurrence of duplicated genes in eels (Dufour et al. 2005; Henkel et al. 2012; Lafont et al. 2016; Maugars and Dufour 2015; Morini et al. 2015; Pasqualini et al. 2009; Pasquier et al. 2012; Rozenfeld et al. 2016; Morini et al. 2017). E.g. Lafont et al. (2016) found two paralog genes of *ift140*, *tleo2*, *nme4*, *xpo6*, and *unkl*, in the *gper* genomic regions of the eel. Only one copy of these genes has been observed in other teleosts. These results led Lafont et al. (2016) to hypothesize i) that the whole region containing *gper*

could have been duplicated in *Anguilla* eels, and maybe also in other teleosts, and ii) that the retention of duplicated genes may be higher in eels than in other teleosts.

For the present study, we assembled a *de novo* European eel transcriptome from Illumina RNA sequencing data. In order to study species-specific duplications and the timings of the events that created them, we ran phylogenetic reconstructions and calculated fourfold synonymous third-codon transversion (4dTv) distances. This analysis was performed on our transcriptome, and on multiple other fish transcriptomes and genomes. Our analysis revealed a high accumulation of duplicated genes in eel compared to other teleost species (which do not have a confirmed 4R duplication event in their linage). Many of these duplications are restricted to the eel lineage, and the 4dTv analyses suggested that these duplications happened much later than the 3R WGD shared by all teleosts. We will discuss in more depth if these eel specific duplications are the result of several SDs or one eel-specific 4R WGD. To our knowledge, this is the first published evidence of a large-scale lineage specific duplication in the elopomorpha cohort.

Results

Transcriptome assemblies and genomes

To assemble a *de novo* European eel transcriptome, we performed high quality RNA extractions from forebrain, pituitary, and testis samples, of one eel, following the protocol described by Peña-Llopis and Brugarolas (2013). The RNA was then quality tested on the Bio-Rad Bioanalyser, which yielded average RIN values of 8.90. From this RNA, in total 181 million Illumina reads, with a length of 101 bp, were produced. These reads were assembled by using the Trinity assembler after a digital normalization step that left 75 million representative reads. The transcriptomes of Northern pike (*Esox lucius*), elephantnose fish (*Gnathonemus petersii*) and silver arowana (*Osteoglossum bicirrhosum*) were also assembled by Trinity using Illumina reads from the Phylofish database (Pasquier et al. 2016). The resulting unigenes were clustered

by using a transitive clustering approach to create sets of very similar transcripts. The number of unigenes (henceforth referred to as transcripts) assembled ranged from 68489 to 78610 (table 1) and the number of transcript clusters from 49154 to 55667 (henceforth referred to as genes; table 2).

Table 1 Size and quality of included transcriptomes from: European eel (*Anguilla Anguilla*), Northern Pike (*Esox Lucius*), elephantnose fish (*Gnathonemus petersi*), and silver arowana (*Osteoglossum bicirrhosum*).

Species	N.º Reads	Q30	Transcripts
European eel	181322106	0.994	77247
Northern Pike	553710218	0.989	68489
Elephantnose fish	498451616	0.993	74642
Silver arowana	490649254	0.992	78610

Table 2 Quantities of included genes per included species: European eel (*Anguilla anguilla*), Zebrafish (*Danio rerio*), Northern pike (*Esox Lucius*), Elephantnose fish (*Gnathonemus petersi*), Spotted gar (*Lepisosteus oculatus*), Silver arowana (*Osteoglossum bicirrhosum*), Atlantic salmon (*Salmo salar*), Fugu (*Takifugu rubripes*), and Platyfish (*Xiphophorus maculatus*).

Species	Transcripts	Genes	Representative transcripts	Representative transcripts with predicted protein	Gene family transcripts	% of genes assigned to a gene family
European eel	77247	54879	54845	27696	25862	93.38
Zebrafish	58274	32189	32189	25790	22703	88.03
Northern pike	68489	49154	49154	23843	21696	90.99
Elephantnose fish	74642	50455	50455	24857	22036	88.65
Spotted gar	22483	18341	18341	18341	17872	97.44
Silver arowana	78610	55667	55667	24938	21604	86.63
Atlantic salmon	109584	55104	55104	48593	42625	87.72
Fugu	47841	18523	18523	18523	17698	95.55
Platyfish	20454	20379	20379	20379	19807	97.19

The genomes of zebrafish (*Danio rerio*), northern pike, spotted gar (*Lepisosteus oculatus*), fugu (*Takifugu rubripes*), and platyfish (*Xiphophorus maculatus*) were obtained from the ENSEMBL

database, the Atlantic salmon (*Salmo salar*) genome was downloaded from NCBI and the published eel genome was downloaded from the ZF-genomics web site (Henkel et al. 2012).

Genome and transcriptome quality assessment

In order to test the completeness of the transcriptomes and genomes we ran a BUSCO analysis in which we looked for a set of single-copy orthologues, typically found in fish genomes (Simão et al. 2015; Fig. 1). In general, genomes were more complete than transcriptomes according to the BUSCO assessment, and were thus preferred. However, in the cases of the pike and eel, the transcriptomes outperformed the genomes (Fig. 1), and these transcriptomes were therefore used for further analysis.

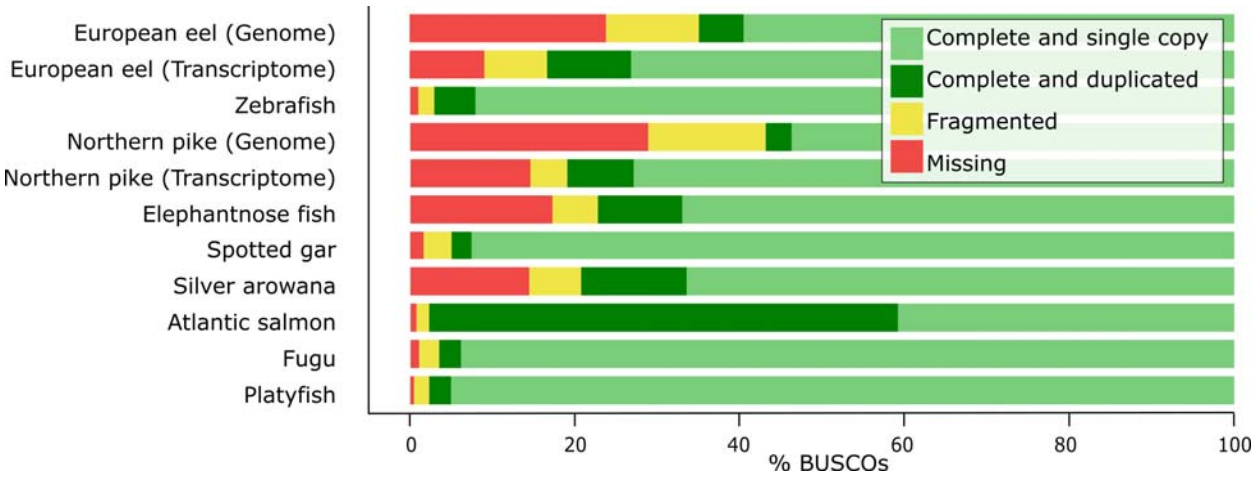

Figure 1
BUSCO (Benchmarking set of Universal Single-Copy Orthologues) result for every genome and transcriptome, one per row. The sequence of a BUSCO gene can be found complete or fragmented in each genome and it can be found once (single copy), more than once (duplicated) or not found (missing). Included genomes: European eel (*Anguilla anguilla*), zebrafish (*Danio rerio*), northern pike (*Esox lucius*), spotted gar (*Lepisosteus oculatus*), fugu (*Takifugu rubripes*), platyfish (*Xiphophorus maculatus*) and Atlantic salmon (*Salmo salar*). Included transcriptomes: European eel, northern pike, elephantnose fish (*Gnathonemus petersii*) and silver arowana (*Osteoglossum bicirrhosum*).

In order to further test the completeness of the eel and pike transcriptomes, we mapped the eel and pike RNA-seq reads to the transcriptome assembly using BWA-MEM (Li and Durbin 2010)

and to the genome using the software HISAT2 (Pertea et al. 2016). The percentages of reads that mapped concordantly against the genome and the transcriptome were 65.8 and 91.9% respectively for eel, and 44.6 and 85.8% for pike. Likewise, previous published eel RNA-sequencing experiments were also mapped to the eel genome and transcriptome. In this case, 52.2% (Coppe et al. 2010), 57.9% (Burgerhout et al. 2016), and 66.18% (Ager-Wick et al. 2013) reads mapped concordantly against the eel genome whereas 84.3% (Coppe et al. 2010), 69.5% (Burgerhout et al. 2016), and 87.32 % (Ager-Wick et al. 2013) mapped against the transcriptome.

Gene families

One representative transcript for each gene and species was selected; the longest one for genomes and the most expressed one for transcriptomes. The OrthoMCL web service (Li et al. 2003) assigned gene families to the genes. The percentage of genes assigned to a family ranged from 88.0% (zebrafish) to 97.4% (spotted gar; table 2). Overall, 17003 gene families were covered, from which 13823 protein and codon alignments were built. These families contained between 2 and 161 genes, with 9 genes per family being the mode (suppl. Fig. 1).

Phylogenetic reconstruction and duplication dating

PHYLDOG (Boussau et al. 2013) was run 10 independent times using 8,000 protein alignments chosen at random. Overall, PHYLDOG created trees for 10,352 gene families and, based on the tree topology, it labelled the branches in which gene duplication events had happened. All 10 runs produced a species tree that matched the species tree topology created by phylobayes (Lartillot et al. 2009) with a CAT-GTR model built with the concatenation of 100 protein alignments and a Neighbour-Joining tree built with a 4dTv distance matrix (Fig. 2).

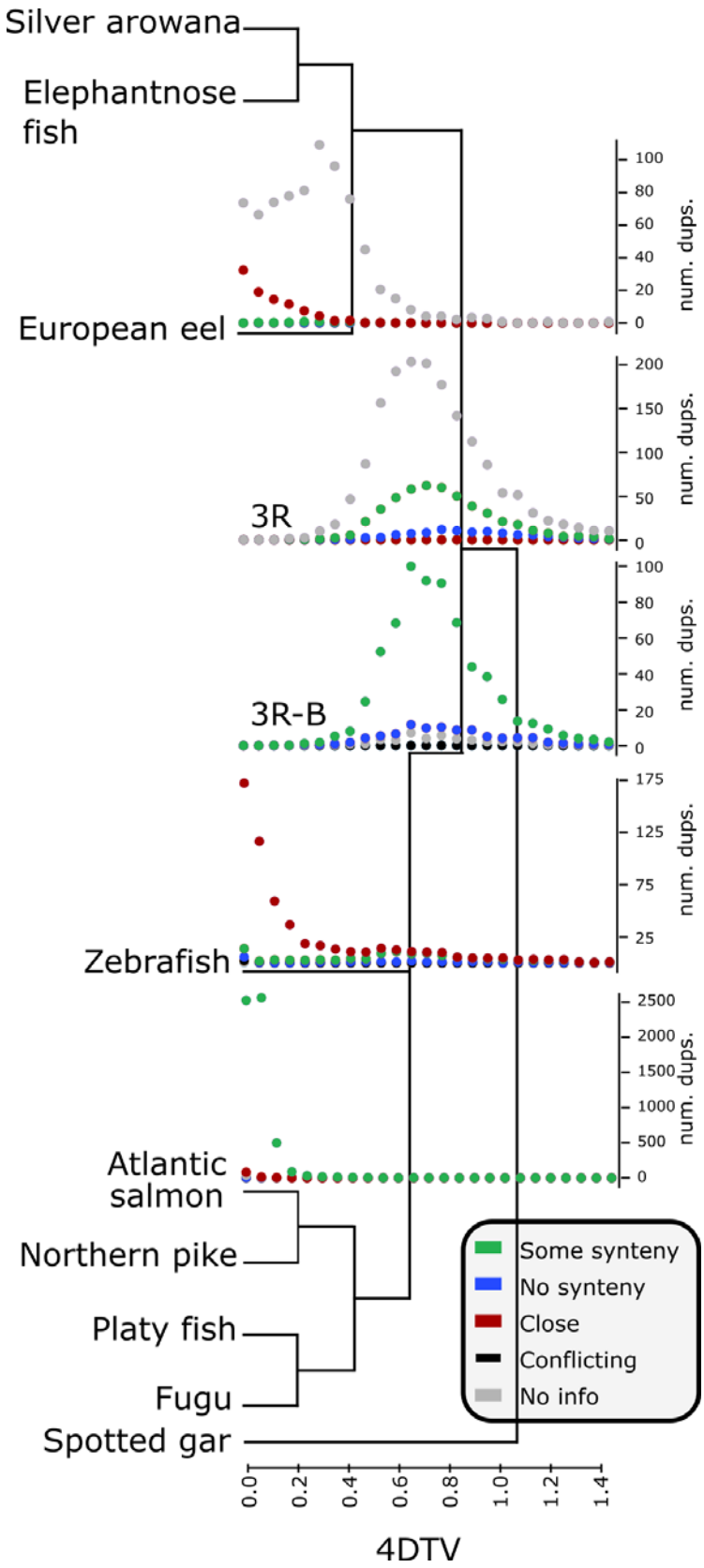

Figure 2
Species cladogram generated by PHYLDOG for the species included in this study: European eel (*Anguilla anguilla*), zebrafish (*Danio rerio*), northern pike (*Esox lucius*), spotted gar (*Lepisosteus oculatus*), fugu (*Takifugu rubripes*), platyfish (*Xiphophorus maculatus*), Atlantic salmon (*Salmo salar*), elephantnose fish (*Gnathonemus petersii*) and silver arowana (*Osteoglossum bicirrhosum*). PHYLDOG also determined the duplication events and for each of these events the 4dTv and the synteny type found around the gene was determined. Only the 4dTv distributions for the branches with most duplications are represented over the corresponding cladogram branch, for the distributions for all branches refer to supplementary figure 2. The synteny types are the following: close, the copies originated by the duplication are close in the genome; some synteny, some genes close to the one duplicated are also found to be duplicated close by; no synteny, there are no paralogs for others genes found close to the paralog copies created by the duplication; no information, the duplicated genes are located in small scaffolds with not enough genes close by; conflicting syntenies, different synteny classification found in the genomes of the different species affected by the duplication)

For each duplication found in each gene family tree, the 4dTv distance between the genes was calculated, and by grouping them according to the species tree branch in which it happened, the distribution of the 4dTvs for each lineage was built. Each 4dTv distribution was further divided according to the synteny type found in the region where the paralogs of each gene family were located (Fig. 2 and suppl. Fig. 2). The duplications were thus labeled according to the genomic region where the resulting paralogs were found. In some cases the paralog pairs were found close to each other (labelled as close), denoting a tandem SD, in other cases they were in syntenic regions where paralogs from other gene families were also located (labelled as “some synteny”), possibly denoting a WGD and, finally, in some other cases, there were not enough close genes (labelled as “no info”) in the genome assembly or conflicting evidence was found (labelled as “conflicting syntenies”).

PHYLDOG assigned 4,308 duplications to the basal teleost branch, after the split of the spotted gar (Fig. 2 and Fig. 3), with a 4dTv mode of 0.8. Of the paralogs created by these duplications 63.1% were located in regions with some synteny, 2.4% were close to each other, and 32.3% had no synteny. These percentages are calculated without taking into account the duplications where no information regarding the physical location of the genes could be established. The following branch (directly following the split of the eel, arowana and elephantnose fish) was

assigned 1,525 duplications and showed very similar distributions with an overall 4dTv mode of 0.75. The eel specific branch was assigned 1460 duplications of which 16.5, 75.8, and 7.2% were labelled as some synteny, close and without synteny, respectively. Notably, most of the eel specific duplications lacked sufficient physical genomic location information. The duplications that generated close genes in tandem within the eel genome clearly showed a different distribution to the ones located in syntenic regions and the ones with no information. The tandem ones tended to be more recent according to their 4dTv (fig. 2) while the syntenic ones, and most of the duplications without sufficient genomic location information, showed a 4dTv mode of 0.4. In both the salmon and zebrafish specific branches most duplications seemed quite recent, according to their 4dTv values, with 8,712 and 1,452 branch specific duplications, respectively. In the case of the salmon, most duplications (80.6%) were characterized by paralogs located in syntenic regions whereas most zebrafish paralogues (54.6%) were close tandem SDs (Fig. 2 and Fig. 3).

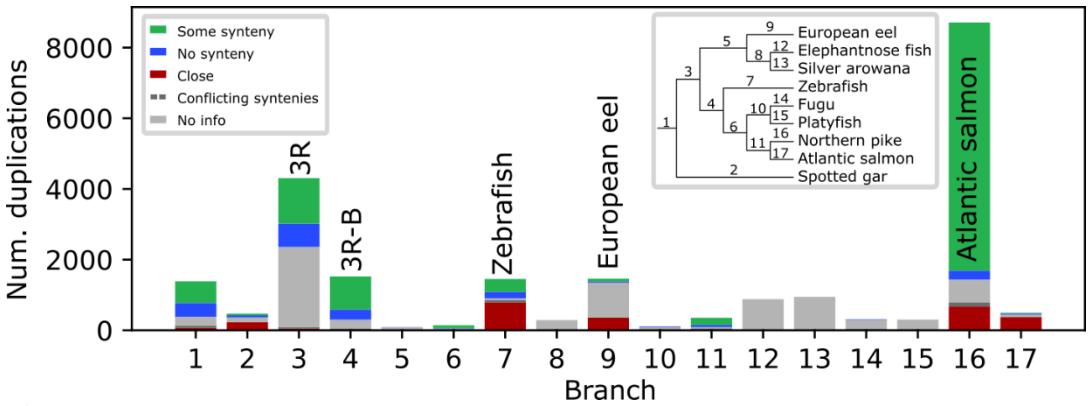

Figure 3
Barplot of the number of duplications PHYLDOG assigned to each branch of the species tree. Bars are numbered according to the cladogram in the upper righthand corner. 3R indicates the branch where the 3R teleost-specific whole genome duplication is hypothesized to have happened. 3R-B indicates the basal branch of the remaining teleosts after the split of the elopomorphas and osteoglossomorphas. Each bar is subdivided into the synteny types described in figure 2.

In order to investigate the timing of the main eel duplication event in greater depth, we compared the 4dTv distribution found for eel paralogs with the 4dTv distribution built for the eel orthologs with elephantnose fish, and arowana. The results showed a 4dTv maximum of 0.4 for the main eel paralog peak, and 0.5 for the peaks corresponding to the speciation event that separated elephantnose fish and arowana from eel (Fig. 4).

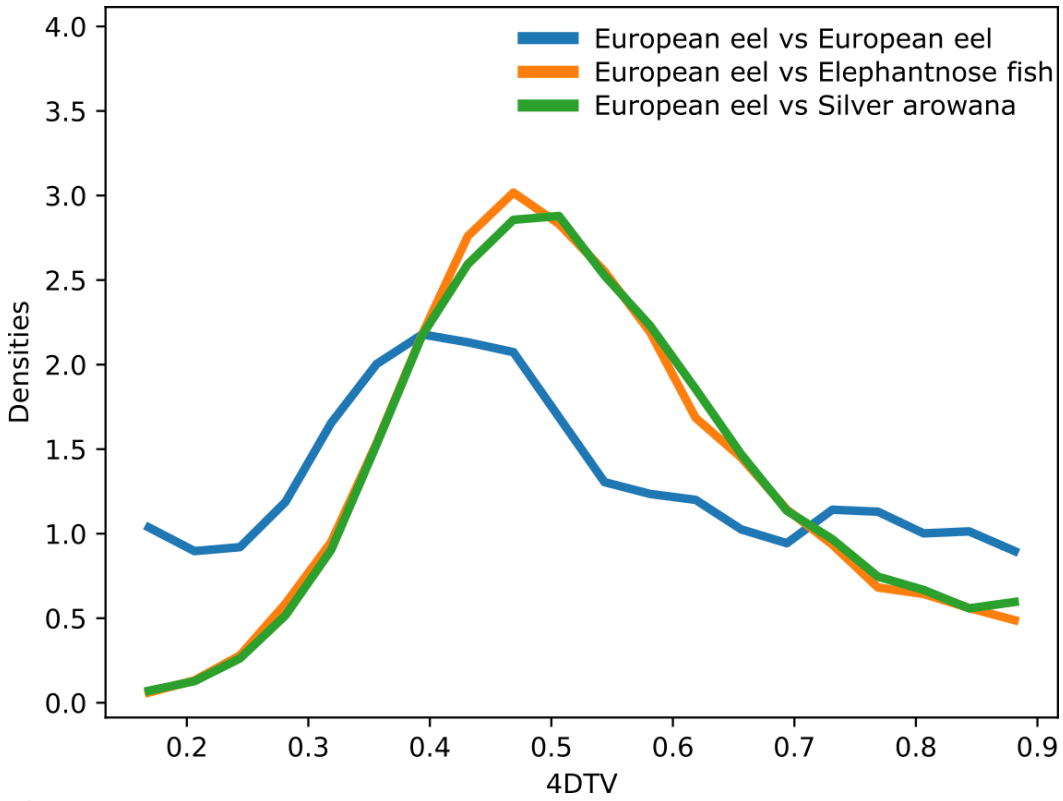

Figure 4
4dTv distribution of European eel (*Anguilla anguilla*) paralogs (blue), European eel and elephantnose fish (*Gnathonemus petersii*) orthologs (green), and European eel and silver arowana (*Osteoglossum bicirrhosum*) orthologs (orange). This analysis was carried out in the gene families without taking into account the PHYLDOG result.

Investigation of functional category enrichment

To investigate if some functional categories were overrepresented in the eel duplications, an enrichment test was carried out. GO terms were assigned to 9570 gene families by comparing them to the annotated EggNOG gene families (Huerta-Cepas et al. 2016). The GO annotation can be found in suppl. table 1. From these terms we performed an enrichment analysis with the

topGO R library (Alexa and Rahnenfuhrer 2016).

The resulting enriched GO-terms, are presented in the suppl. table 2. In most cases these terms are involved either in signal transduction (GTPase, MAPK, sphingosine-1-phosphate), morphological alterations (convergent extension involved in axis elongation and gastrulation, heart development, and pronephric glomerulus development, pigmentation), or forebrain development.

Additionally, KEGG terms were assigned to 16466 eel genes using BlastKOALA (Kanehisa et al. 2016) and the terms related to the genes involved in the eel duplication were mapped onto the KEGG pathways using the KEGG Mapper tool. A fisher test, corrected for multiple testing using False Discovery Rate, was used to look for enriched KEGG pathways in the eel branch (Suppl. Table 3). Most of the KEGG pathways found to be enriched were related to immune system, nervous system, oocyte, apoptosis, cell adhesion, amino acid metabolism, glycan biosynthesis, and signal transduction. Several key genes related to the immune system were also duplicated, including: Cytohesin-associated scaffolding protein (*casp*) c-jun N-terminal knase (*jnk*) and vav proto-oncogene (*vav*), involved in the lymphocyte differentiation and activation, the interleukine and T-cell receptors (*tcr*'s), major histone compatibility complex (*mch*) I, *mch* II, and several cytokines including *tgf*-beta (transforming growth factor beta) and B-cell activating factor (*baff*). There were also numerous duplications related to apoptosis, including activator protein 1 (*ap*-1), c-fos proto-oncogene (*c-fos*), *casp*, serine protease (*omi*), mitochondrial Rho GTPase (*miro*), Death executioner (*bcl*-2), Caspase Dredd, X-linked inhibitor of apoptosis protein (*xiap*), *casp8*, and Baculoviral inhibitor-of-apoptosis repeat (*bir*). In the nervous system tyrosine hydroxylase (*th*) and monoamine oxidase, as well as some receptors (*gaba-a*, *gaba-b*, *ampa*, *mglur*, *crfr1*, and *girk*) were duplicated, as well as several genes involved in synaptic exocytosis (*rab3a*, *munc-18*, syntaxin, *vgat*), and Golf and Transductin, which are involved in olfaction and phototransduction. In the oocyte more affected genes were found, including the progesterone receptor (*pgr*), early mitotic inhibitor 2 (*emi2*), aurora-B

256 phosphatase (*glc7*), cullin F-box containing complex (*scf-skp*), insulin-like growth factor 1 (*igf1*),
257 and cytoplasmic polyadenylation element binding protein (*cpeb*).
258
259 **Discussion**
260 The observed data has shown, for the first time to our knowledge, that in the eel lineage more
261 than a thousand gene families have genes that are the result of a large-scale gene duplication
262 that happened after the teleost specific 3R WGD. Only Atlantic salmon, due to its salmonid-
263 specific 4R WGD, showed a higher amount of conserved duplicates, whereas the number of
264 duplicated gene families found in eel and zebrafish were similar. Furthermore, the paralog 4dTv
265 distribution shows a unique pattern in the case of the eel branch when compared to the other
266 teleosts.
267 The duplications assigned by the phylogenetic analysis to the eel specific branch, after the split
268 of arowana and elephantnose fish, showed a distribution of 4dTv distances younger than those
269 corresponding to the 3R duplication and older than the salmonid duplications. This result was
270 replicated in an independent analysis, not based on phylogenetic tree topologies. In this case
271 the distributions of the 4dTv distances (Fig. 4) were calculated between the eel genes found in
272 the gene families (eel paralogs) and between the eel sequences and the arowana and
273 elephantnose fish sequences (orthologs). Both the phylogenetic topologies and the 4dTv
274 distances corroborate the hypothesis that the main eel duplication happened after the teleost
275 specific 3R duplication event (320-350 MYA; Vandepoele et al. 2004 and Christoffels et al.
276 2004), and after the arowana and elephantnose fish split, but before the salmonid duplication
277 (80-100 MYA; Macqueen et al. 2014). Given the 4dTv distribution found, we could assume that
278 this duplication event is shared by all members of the Anguilla genus, as they are estimated to
279 first appear 20-50 MYA (Minegishi et al. 2005). To be more precise about the timing of the main
280 eel duplication event it would be advisable to study other elopomorpha transcriptomes or
281 genomes to analyze if they share the same duplication.

Not all the PHYLDOG duplication assignments to the basal branches of the species tree are as trustworthy as the lineage specific ones. According to PHYLDOG the duplications usually associated with the 3R WGD would be split into two events, one would correspond to the 3R branch and the other with the one that gave rise to zebrafish, salmon and fugu (indicated as 3R-B; Fig. 2). If PHYLDOG is right and there were really two genome duplications the 4dTv distributions of the events should be different. These distributions are quite similar, although not completely identical. Nevertheless, duplications this old are almost completely saturated in terms of 4dTv, decreasing the accuracy of the measurement. Alternatively, the PHYLDOG split of the 3R duplication could be explained as an artifact. Only 3 species make up the daughter clade of the 3R branch at one side of the split and some gene families will only include representatives from the species of the other daughter clade. This could make the phylogenetic based assignment of the gene duplication to a particular branch more error prone. Thus, some gene family duplications created by 3R might end up wrongly assigned by PHYLDOG to 3R-B. This might have happened even with some zebrafish lineage specific duplications. The 4dTv distribution for the zebrafish branch shows a bump that overlaps with the 3R duplication. That might be due to a real old duplication in the zebrafish lineage, but it is also possible that it is a PHYLDOG artifact. The zebrafish genome is among the most complete genomes, with the most well-supported protein coding gene annotation, used in this study. Therefore, it might be the case that some duplicated genes could be found only in this species making the PHYLDOG assignment more difficult.

In previous studies, data consistent with an eel specific duplication event has been reported, although it has never been interpreted in this way. Recently, the transcriptome of several teleosts was sequenced (Pasquier et al. 2016), and the European eel was the species with the highest number of contigs, expect for species with a documented 4R WGD in their lineage. In the additional data included by Inoue et al. (2015), in their analysis of the gene loss process that followed the teleost 3R WGD, both the eel and zebrafish are the species with the highest

percentage of duplications (36.6 and 31.9%, respectively). Thus, these two studies are in concordance with our analysis.

Previously, several lineage specific gene duplications have been found and studied in eel, in studies focusing on particular genes. For instance, Morini et al. (2015) found that the leptin receptors were duplicated in the eel. However, their phylogeny did not include other basal teleosts and was compatible with both the hypothesis that those genes were duplicated in an eel specific duplication event or in the teleost 3R WGD. Similarly, Lafont et al. (2016) found several species-specific duplicated genes in the eel genome, but they attributed it to less extensive loss of genes in the eel lineage after the 3R duplication. Furthermore, several other analyses of single genes have reached the same conclusion (Dufour et al. 2005; Maugars and Dufour 2015; Pasqualini et al. 2009; Pasquier et al. 2012; Morini et al. 2017; Henkel et al. 2012). In general, these studies were based on tree topologies that did not include other basal teleost species and often did not report the genetic distances.

The eel duplication was also not detected in the analysis of the published eel genome (Henkel et al. 2012). This genome was quite fragmented and, according to our BUSCO analysis, it was more incomplete than our transcriptome, thus including less duplications (Fig. 1). Moreover, no global 4dTv or Ks distribution was calculated, but a remarkably high number of Hox genes (73) were found, thus these results are also compatible with our current analysis.

In the proposed species phylogeny (Fig. 2) the eel is located in a basal position as found in other previous phylogenies. The location of the osteoglossomorphes order, represented by the elephantnose fish and arowana is, however, in disagreement with previous phylogenies based on elopomorpha mitochondrial genomes (Inoue et al. 2003) and on the nuclear arowana genome (Austin et al. 2015). In these phylogenies arowana is the basal node to the teleosts, whereas it is grouped with the eel in ours. In the transcriptome based phylogeny proposed by the PhyloFish project (Pasquier et al. 2016) the topology is reversed and the Anguilliformes appear as the basal teleosts whereas the Osteoglossiformes appear to have split more recently.

The branch that separated eel and arowana, in the arowana genome paper, was one of the shortest and had a lower posterior probability than the others; this could be the reason why these different phylogenies disagree. This disparity in the results is unlikely to be due to the phylogenetic methods used. In this study we have used the neighbor joining, maximum likelihood and bayesian approaches, and all of them agree that arowana and eel form two sister clades. The difference might be due to the extra species that we are including, the elephantnose fish. To be more confident of the topology of these events in the base of the teleosts, it would be advisable to include other basal species.

Two different mechanisms can create genomic scale duplications: WGDs or many small-scale SDs, a process referred to as the continuous mode hypothesis (Gu et al. 2002). The latter process has been observed in many species, including yeast (Llorente et al. 2000), fruit flies (Zhou et al. 2008), water fleas (Colbourne et al. 2011), humans (Bailey et al. 2002; Gu et al. 2002; Vallente Samonte and Eichler 2016), several plant species (Cui et al. 2006) and teleosts (Blomme et al. 2006; David et al. 2003; Jaillon et al. 2004; Lu et al. 2012; Rondeau et al. 2014). Usually, most of these SDs are lost soon after they are generated. Therefore, in the genome, many young paralog pairs, and few old, can be found. In a 4dTv distribution this pattern would be detected as a peak of 4dTv values with a mode close to 0. Moreover, SDs are usually the result of tandem duplications, therefore very similar paralogs located in tandem are likely to be the result of this process. Patterns compatible with this scenario were seen in several species in our analysis, including: zebrafish, elephantnose fish, arowana, pike, fugu, spotted gar, salmon and eel (suppl. Fig. 2). The high amount of SDs that we detected in zebrafish has been previously documented (Blomme et al. 2006; Lu et al. 2012). In some other species it has been shown that these SDs could be retained at specific points in time, possibly during specific evolutionary events e.g. in yeast (Llorente et al. 2000), common carp (David et al. 2003) and humans (Bailey et al. 2002; Hughes et al. 2001). These events have been linked to the

359 adaptation of a species to a new environment (Chain et al. 2014; Colbourne et al. 2011; Tautz
360 and Domazet-lošo 2011).
361 In eel, the 4dTv distribution pattern found is quite distinct as it reflects two modes; one of
362 younger and one of older duplications. Of these duplications, the older ones are clearly older
363 than those found, for example, in zebrafish or salmon. Furthermore, the genomic surroundings
364 of the paralogs of the younger duplications are quite different from those of the older
365 duplications. The younger duplications, which have a lower 4dTv, tend to be located close
366 together in the genome and are likely to have been generated recently by tandem SDs, whereas
367 the older duplications are not usually found in tandem. A WGD should have left behind blocks of
368 syntenic regions similar to those detected in our analysis for the 3R teleost and the salmon
369 WGD. In the case of the eel, an increase in these syntenic blocks was also detected in the older
370 duplications. However most genomic regions are very fragmented in the genome assembly and
371 thus, we lack physical genomic location information for many genes. However, from the
372 evidence available we can hypothesize that most of the older duplications are likely to be the
373 result of a WGD which occurred in the eel lineage, and that an analysis with the latest eel
374 genome assembly published (Jansen et al. 2017), but not available without restrictions, would
375 detect more syntenic regions. In other words, the numerous duplications found in eel are likely
376 to have been generated by a WGD followed by many SDs, a pattern which has also been
377 observed in primates (Gu et al. 2002), and common carp (David et al. 2003).
378 In this study, several gene function analyses were carried out to study overrepresented
379 functions among the eel specific duplications. These overrepresentations could be linked to
380 several adaptations that have taken place throughout eel evolution, e.g. the inclusion of a
381 leptocephali larvae stage to their life history (Inoue et al. 2004), the adaptation to a catadromous
382 lifecycle (Inoue et al. 2010), and the adaptation to withhold maturation until after the extensive
383 reproductive migration (Righton et al. 2016; van Ginneken et al. 2005). Other mechanisms
384 which perhaps influence the conservation of paralogs are: dosage selection (Glasauer and

385 Neuhauss 2014) and segregation avoidance (Hahn 2009). It has been suggested that these
386 mechanisms conserve duplicated genes related to specific biological processes, such as
387 development, signaling, ion transport, metabolism and neuronal function after WGDS (Berthelot
388 et al. 2014; Blomme et al. 2006; Brunet et al. 2006; Kassahn et al. 2009).
389 Specifically, 54 GO-terms were found to be enriched among the eel specific duplications (suppl.
390 table 2). Interestingly, several of the GO-terms found to be enriched among the eel specific
391 duplications form part of some of the aforementioned processes, including; development, ion
392 transport, signaling, neuronal function, and metabolism. The high number of enriched GO-
393 terms, which are part of processes that are often conserved after WGDs, suggests that the
394 duplication event here described is a WGD. It is likely that they have been conserved due to the
395 mechanisms regulating gene conservation after WGD rather than due to specific necessities of
396 the *Anguilla* species. Other GO terms that were found to be duplicated in eel are not usually
397 found in other WGDs, for example “pigmentation”. As the eel has incorporated several
398 pigmentation alterations into its lifecycle, it is likely that the genes associated with this GO-term
399 are conserved due to new functions acquired in the *Anguilla* species. Most of the pigmentation
400 changes undergone by the eel are linked to the transition between the marine and freshwater
401 environment, therefore the duplication of these genes might have generated the necessary raw
402 genetic material for adaptation to the catadromous lifecycle.
403 Furthermore, 54 KEGG pathways were also found to be enriched among the eel specific
404 duplications (suppl. table 3). As in the case of the GO-terms, several of these pathways are
405 involved in signaling, metabolism and neuronal function. Additionally, olfactory transduction and
406 several pathways involved in immune response e.g. Tuberculosis, Th1 and Th2 cell
407 differentiation, Bacterial invasion of epithelial cells, Th17 cell differentiation, and others were
408 found to be enriched. Lu et al. (2012) also found that immune response pathways and olfactory
409 receptor activity were enriched among the recent segmental duplications found in zebrafish.
410 Several studies have also found immune response genes to be enriched among other recent

411 SDs (Conrad and Antonarakis 2007; Kasahara et al. 2007; She et al. 2008; Stein et al. 2007;
412 Wang et al. 2012). Thus this enrichment in immune response genes could be linked to eel SDs.
413 However, in these studies, the recent duplications were found to be mostly in the components
414 interacting with pathogens, possibly to contribute to the response against different pathogens,
415 as opposed to the components downstream of the receptors, which make up most of the
416 enriched pathways found in our study. Also, among the eel specific duplications were the
417 progestin receptors which have recently been characterized in eel (Morini et al. 2017).
418 Progestins are known as maturation-inducing steroids promoting sperm maturation and
419 spermiation (for review see Scott et al. 2010), and the two paralogs do show differential
420 expression during maturation (Morini et al. 2017).
421 The most significantly enriched pathway found in the eel duplications is the dopaminergic
422 synapse pathway. Dopamine (DA) is an essential neurotransmitter in vertebrates, with several
423 functions (Davila et al. 2003; Hsia et al. 1999). DA has been proven to be important in teleosts,
424 where DA has been found to have an inhibitory role on the gonadotropic activity of the pituitaries
425 (Dufour et al. 2005; Peter et al. 1986). In the case of the eel in particular, DA inhibition
426 completely arrests puberty before their oceanic migration (Vidal et al. 2004), indicating that DA
427 has a much stronger inhibitor effect in eel compared to most other teleosts (Dufour 1988; Vidal
428 et al. 2004). This suggests that the duplicated genes involved in the dopaminergic synapse
429 pathway may have been conserved during the adaptation to block maturation until after the
430 extensive reproductive migration. Among the duplicated genes assigned to the dopaminergic
431 synapse pathway, we found tyrosine hydroxylase (TH). TH is the rate limiting enzyme of the DA
432 biosynthesis (Nagatsu et al. 1964), and is therefore often used as an indicator of DA tone in eel
433 (Davila et al. 2003; Weltzien et al. 2015). As genes are rarely conserved without a specific
434 function or necessity, the presence of two TH genes in the eel encourages suspicion of potential
435 differential expression or function between the two, which may prove important for the regulation
436 of the DA induced inhibition of puberty observed in pre-migration eels.

437 In conclusion, the data presented strongly suggest that a vast amount of genes have been
438 duplicated specifically in the eel lineage. Furthermore, the synteny, 4dTv, and enrichment
439 analyses suggest that these genes derive both from a WGD as well as continuously created
440 SDs, and that they are related to the eel specific physiology. To our knowledge this is thus the
441 first evidence published suggesting a possible eel lineage specific 4R WGD.
442
443 **Materials and methods**
444 *Fish husbandry*
445 Ten immature farm eel males (mean body weight 96.7±3.6 g±SEM) supplied by Valenciana de
446 Acuicultura S.A. (Puzol, Valencia, Spain) were transported to the Aquaculture Laboratory at the
447 Universitat Politècnica de València, Spain. The fish were kept in a 200-L tank, equipped with
448 individual recirculation systems, a temperature control system (with heaters and, coolers), and
449 aeration. The fish were gradually acclimatized to sea water (final salinity 37 ± 0.3‰), over the
450 course of two weeks. The temperature, oxygen level and pH of rearing were 20 ºC, 7-8 mg/L
451 and ~ 8.2, respectively. The tank was covered to maintain, as much as possible, a constant
452 dark photoperiod and the fish were starved throughout the holding period. After acclimation, the
453 fish were sacrificed in order to collect samples of forebrain, pituitary, and testis tissues.
454
455 *Human and Animal Rights*
456 This study was carried out in strict accordance with the recommendations given in the Guide for
457 the Care and Use of Laboratory Animals of the Spanish Royal Decree 53/2013 regarding the
458 protection of animals used for scientific purposes (BOE 2013), and in accordance with the
459 European Union regulations concerning the protection of experimental animals (Dir
460 86/609/EEC). The protocol was approved by the Experimental Animal Ethics Committee from
461 the Universitat Politècnica de València (UPV) and final permission was given by the local

462 government (Generalitat Valenciana, Permit Number: 2014/VSC/PEA/00147). The fish were
463 sacrificed using anesthesia and all efforts were made to minimize suffering.
464
465 *RNA extraction and sequencing*
466 High quality RNA was extracted from forebrain, pituitary, and testis samples following the
467 protocol developed by Peña-Llopis and Brugarolas (2013). Quantity and quality were tested on
468 a Bio-Rad Bioanalyser (Bio-Rad Laboratories, Hercules, CA, USA), selecting the samples with
469 RIN values and amounts higher than >8 >3 µg of total RNA, respectively. Total RNA samples
470 were shipped to the company Macrogen Korea (Seoul, South Korea). Then, a mRNA
471 purification was carried out using Sera-mag Magnetic Oligo (dT) Beads, followed by buffer
472 fragmentation. Reverse transcription was followed by PCR amplification to prepare the samples
473 for sequencing. The strand information was kept in an Illumina Hiseq-2000 sequencer (Illumina,
474 San Diego, USA). Resulting raw sequences are available at the NCBI Sequence Read Archive
475 (SRA) as stated in the section titled “Data accessibility”.
476
477 *Transcriptome assemblies and genomes*
478 The software FastQC (Andrews 2010) was used to assess the quality of the raw reads
479 generated by Macrogen. Thereafter, trimmomatic (Bolger et al. 2014) was used to trim the
480 reads, eliminating known adaptor sequences, and low quality regions. Finally, trimmed reads
481 shorter than 50 bp were filtered out. Eel reads were digitally normalized before assembly by
482 khmer software (Crusoe et al. 2015) using a k-mer length of 25 and a coverage of 100. Further,
483 The RNA-Seq raw reads for pike, arowana and elephantnose fish were downloaded from the
484 PhyloFish project (Pasquier et al. 2016). All transcriptomes were then assembled using Trinity
485 software (Haas et al. 2013), with the read orientation and sense (in the eel case) into account.
486 The transcripts assembled were filtered according to their complexity (with a DUST score
487 threshold of 7 and a DUST window of 64), length (with a minimum length of 500 bp), and level

of expression (with a TPM threshold of 1). After assembly, the CDSs and proteins were annotated using the Trinotate functional annotation pipeline (Haas et al. 2013).

Transcripts that share k-mers are clustered by Trinity, however, these transcripts might correspond to different transcript forms of the same gene or to closely related genes from a gene family. We split these transcripts into genes by running a transitive clustering based on a blast search. In this clustering transcripts which shared at least 100 bp with a minimum identity of 97% were considered to be isoforms of the same gene. Thus, some Trinity clusters were split into several genes. For each gene, the most expressed transcript, according to Salmon (Patro et al. 2017), was chosen as its representative.

The available eel genome was downloaded from the ZF-Genomics web site (Henkel et al. 2012). The salmon genome assembled by the International Cooperation to Sequence the Atlantic Salmon Genome was downloaded from NCBI (Lien et al. 2016). The genomes of zebrafish (Howe et al. 2013), fugu (Kai et al. 2011), spotted gar (Braasch et al. 2016), and platyfish (Schartl et al. 2013) were downloaded from ENSEMBL (release 87). The pike genome (Rondeau et al. 2014) was downloaded from the Northern Pike Genome web site (Genbank accession GCA_000721915.1). For each gene in the genomes, the longest transcript was chosen as the representative.

Genome and transcriptome quality assessment

In order to check the quality of the transcriptomes and genomes we looked for the BUSCO conserved gene set in them (Simão et al. 2015). BUSCOs are conserved proteins, and are expected to be found in any complete genome or transcriptome. Therefore, the number of present, missing, or fragmented BUSCOs can be used as a quality control of a genome or transcriptome assembly. For this assessment the Actinopterygii (*odb9*) gene set, which consists of 4584 single-copy genes that are present in at least 90% of Actinopterygii species was used.

As an additional comparison between the transcriptome and genomes of pike and eel, the RNA-

514 seq reads were mapped both to the genome and transcriptome assemblies using the softwares
515 HISAT2 (Pertea et al. 2016) and BWA-MEM (Li and Durbin 2010), respectively.
516
517 *Gene families*
518 Genes were clustered into gene families by the OrthoMCL web service (Li et al. 2003). For each
519 gene family a multiple protein alignment was built. To avoid transcriptome assembly artifacts
520 proteins longer than 1,500 amino acids, transcripts with a DUST score higher than 7 or
521 sequences with more than 40% of gaps in the alignment were filtered out. The software Clustal
522 Omega (Sievers et al. 2011) carried out the protein multiple alignment and trimAl (Capella-
523 Gutiérrez et al. 2009) removed the regions with too many gaps or those difficult to align. The
524 protein alignment was used as a template to build the codon alignment by aligning the transcript
525 sequences against the corresponding protein using the protein2dna exonerate algorithm (Slater
526 and Birney 2005).
527
528 *Phylogenetic reconstruction and duplication dating*
529 The resulting protein alignments were used by PHYLDOG (Boussau et al. 2012) software to
530 generate a species tree as well as a family tree corresponding to each alignment. Due to the
531 high memory requirements of PHYLDOG not all gene families could be run in the same analysis
532 so 10 analyses were carried out, choosing 8000 protein alignments at random for each. Once all
533 runs were finished, we checked that the species tree topology of all the 10 species trees,
534 matched exactly. . PHYLDOG uses a maximum likelihood approach to simultaneously co-
535 estimate the species and gene family trees from all individual alignments. From the topology of
536 the gene family trees, it is capable of inferring when the duplications in each family happened.
537 Alternatively, the species phylogeny was also reconstructed using a bayesian approach by
538 using PhyloBayes MPI version 1.7 (Lartillot et al. 2009). From the gene families that had one
539 gene for each species, 100 were chosen at random to create a concatenated alignment of

43566 aminoacids. The model used was CAT-GTR and three independent MCMC chains were run for 39872, 56328, and 39285 iterations.

Finally, a neighbor joining tree based on the fourfold synonymous third-codon transversion distances (4dTv) was also calculated (Tang et al. 2008). Between any pair of sequences the number of transversions found in the third base of the codon was divided by the number of four-fold degenerated codons. A correction to the 4dTv was applied: ln(1 - 2 * distance) / -2. For each pair of species a 4dTv distance was calculated. 4dTvs were calculated between the sequences of those species found in each gene family codon alignment. The distribution of those 4dTvs was fitted with a log normal mixture model using the scikit-learn Gaussian Mixture class. The number of components required was one for all the species pairs, except for those where a bimodal distribution was found due to a recent speciation event. The distance between any two species was the mode of the fitted model. The neighbor joining tree was built using the BioPython Tree Construction class. This process is implemented in the fit_fdtv_distributions module found in the Python scripts (suppl. Material 1).

The 4dTv was calculated for each duplication tagged by PHYLDOG within any gene family. A duplication event defines a subtree in the gene family tree, and this subtree defines two child branches, so the 4dTv calculated for that event was the mean of the 4dTv between all combinations of sequences found between those branches. These calculations are implemented by the functions calculate_4dTv and calculate_mean_fdtv_for_tree found in the Python scripts (suppl. Material 1).

Synteny

Furthermore, the kind of event that created each duplication was characterized by analyzing the conserved synteny between the paralogs created by that duplication within a particular genome. A duplication may derive from a SD that could have occurred in tandem or not, or from a WGD, among others. Tandem SDs would create paralogs found close to each other in the genome,

whereas the paralogs created by a WGD would be far away, but surrounded by similar genes in each of the duplicated regions. We also have to consider that several phylogenetically close species can be affected by the same older duplication event. Therefore, these traces of the duplication event could be found in the genomes of those different species and, if no other genomic rearrangement happened since, these traces should match each other and convey the same information. With this in mind, we categorized duplications as one of 4 classes: i) the paralog genes that were found close to each other in the genome, within a 50 gene distance were labelled as close, ii) the paralogs which were found in syntenic regions where 2 or more paralogous from other gene families were located within a 50 genes distance, not necessarily in the same colineal order, were labelled as “some synteny”, iii) the cases in which fewer than 2 gene families could be identified within a 50 gene distance from both of the paralogous genes were labelled as “no info”, and iv) the cases in which conflicting evidence was found in the genomes of the different species affected by the duplication were labelled as “conflicting syntenies”. This labelling of the duplications was carried out by the Python function determine_if_pair_is_close_or_synthenic and the Python class GenomeLocator, found in the scripts (suppl. material 1). The location of each gene in a genome was obtained by performing a BLAST search with its representative transcript against the genome.

Investigation of functional category enrichment

The EggNOG database has GO annotations for each of its gene families (Huerta-Cepas et al. 2016). To match our gene families with those from the EggNOG database, the protein sequence with least gaps for each of our families was selected and a HMMER search (Finn et al. 2011) was carried out against the EggNOG position weight matrices with an e-value threshold of 0.0001. The GO annotation of the best EggNOG hit in this search was transferred to our family. The enrichment analysis was carried out using the fisher statistic and the weight algorithm of the topGO library (Alexa and Rahnenfuhrer 2016) from the bioconductor project. The R script

592 go_enrichment_analysis found in the scripts (suppl. Material 1) implements this analysis. Eel
593 transcripts were annotated using the BlastKOALA KEGG service (Kanehisa et al. 2016) and a
594 fisher exact test was carried out, using the scipy implementation, to look for overrepresented
595 KEGG pathways in the eel duplications.
596
597 **Disclosure declaration**
598 The authors declare no potential conflicts of interest with respect to the authorship, research,
599 and/or publication of this article.
600
601 **Data accessibility**
602 The raw RNA-sequencing reads from brain, pituitary, and testis samples from European eel
603 (*Anguilla anguilla*) have been deposited at GenBank (http://www.ncbi.nlm. nih.gov/genbank)
604 under accession no. XX. Deposition and acquiring of accession number was not finished at the
605 time of first submission but will be settled within a few days.
606
607 **Acknowledgements**
608 This study received funding from the project REPRO-TEMP (AGL2013-41646-R) funded by the
609 Spanish Ministry of Economy and Competitiveness, and from the European Union's Horizon
610 2020 research and innovation program under the Marie Skłodowska-Curie grant agreement No
611 642893 (IMPRESS). V. Gallego has a postdoc grant from the UPV (PAID-10-16).
612
613 **References**
614 Ager-Wick E, Dirks RP, Burgerhout E, Nourizadeh-Lillabadi R, de Wijze DL, Spaink HP, van den
615 Thillart GEEJM, Tsukamoto K, Dufour S, Weltzien FA, et al. 2013. The pituitary gland of
616 the European eel reveals massive expression of genes involved in the melanocortin

617 system. *PLoS One* **8**: 1–12.

618 Alexa A, Rahnenfuhrer J. 2016. topGO: Enrichment analysis for gene ontology. R package
619 version 2.29.0.

620 Allendorf F, Thorgaard G. 1984. Tetraploidy and the evolution of salmonid fishes. In
621 *Evolutionary Genetics of Fishes*, pp. 1–53.

622 Andrews S. 2010. FastQC: A quality control tool for high throughput sequence data.
623 http://www.bioinformatics.babraham.ac.uk/ projects.

624 Aparicio S, Chapman J, Stupka E, Putnam N, Chia J, Dehal P, Christoffels A, Rash S, Hoon S,
625 Smit A, et al. 2002. Whole-Genome shotgun assembly and analysis of the genome of fugu
626 rubripes. *Science* **297**: 1301–1310.

627 Asrar Z, Haq F, Abbasi AA. 2013. Molecular Phylogenetics and Evolution Fourfold paralogy
628 regions on human HOX-bearing chromosomes : Role of ancient segmental duplications in
629 the evolution of vertebrate genome. *Mol Phylogenet Evol* **66**: 737–747.

630 Austin CM, Tan MH, Croft LJ, Hammer MP, Gan HM. 2015. Whole genome sequencing of the
631 asian arowana of ray-finned fishes. *Genome Biol Evol* **7**: 2885–2895.

632 Bailey JA, Gu Z, Clark RA, Reinert K, Samonte R V, Schwartz S, Adams MD, Myers EW, Li PW,
633 Eichler EE. 2002. Recent segmental duplications in the human genome. *Science* **297**:
634 1003–1007.

635 Berthelot C, Brunet F, Chalopin D, Juanchich A, Bernard M, Noël B, Bento P, Da Silva C,
636 Labadie K, Alberti A, et al. 2014. The rainbow trout genome provides novel insights into
637 evolution after whole-genome duplication in vertebrates. *Nat Commun* **5**: 3657.

638 Blomme T, Vandepoele K, De Bodt S, Simillion C, Maere S, Van de Peer Y. 2006. The gain and
639 loss of genes during 600 million years of vertebrate evolution. *Genome Biol* **7**: 1–12.

640 Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: A flexible trimmer for Illumina sequence
641 data. *Bioinformatics* **30**: 2114–2120.

642 Boussau B, Szöll GJ, Duret L, Gouy M, Tannier E, Daubin V, Lyon U De, Lyon U. 2012.

643 Genome-scale coestimation of species and gene trees. *Life Sci* **23**: 323–330.

644 Brunet FG, Crollius HR, Paris M, Aury JM, Gibert P, Jaillon O, Laudet V, Robinson-Rechavi M.
645 2006. Gene loss and evolutionary rates following whole-genome duplication in teleost
646 fishes. *Mol Biol Evol* **23**: 1808–1816.

647 Braasch I, Gehrke AR, Smith JJ, Kawasaki K, Manousaki T, Pasquier J, Amores A, Desvignes
648 T, Batzel P, Catchen J, et al. 2016. Corrigendum: The spotted gar genome illuminates
649 vertebrate evolution and facilitates human-teleost comparisons. *Nat Genet* **48**: 700–700.

650 Burgerhout E, Minegishi Y, Brittijn SA, de Wijze DL, Henkel C V., Jansen HJ, Spaink HP, Dirks
651 RP, van den Thillart GEEJM. 2016. Changes in ovarian gene expression profiles and
652 plasma hormone levels in maturing European eel (Anguilla anguilla); Biomarkers for
653 broodstock selection. *Gen Comp Endocrinol* **225**: 185–196.

654 Cañestro C, Albalat R, Irimia M, Garcia-Fernàndez J. 2013. Impact of gene gains, losses and
655 duplication modes on the origin and diversification of vertebrates. *Semin Cell Dev Biol* **24**:
656 83–94.

657 Capella-gutiérrez S, Silla-martínez JM, Gabaldón T. 2009. trimAl: a tool for automated
658 alignment trimming in large-scale phylogenetic analyses. *Bioinformatics* **25**: 1972–1973.

659 Chain FJJ, Feulner PGD, Panchal M, Eizaguirre C, Samonte IE, Kalbe M, Lenz TL, Stoll M,
660 Bornberg-Bauer E, Milinski M, et al. 2014. Extensive copy-number variation of young
661 genes across stickleback populations. *PLoS Genet* **10**: e1004830.

662 Christoffels A, Koh EGL, Chia JM, Brenner S, Aparicio S, Venkatesh B. 2004. Fugu genome
663 analysis provides evidence for a whole-genome duplication early during the evolution of
664 ray-finned fishes. *Mol Biol Evol* **21**: 1146–1151.

665 Colbourne JK, Pfrender ME, Gilbert D, Thomas WK, Tucker A, Oakley TH, Tokishita S, Aerts A,
666 Arnold GJ, Basu MK, et al. 2011. The ecoresponsive genome of daphnia pulex. *Science*
667 **331**: 555–562.

668 Conrad B, Antonarakis SE. 2007. Gene Duplication: A drive for phenotypic diversity and cause

669 of human disease. *annurev.genom* **8**: 17–35.

670 Coppe A, Pujolar JM, Maes GE, Larsen PF, Hansen MM, Bernatchez L, Zane L, Bortoluzzi S.
671 2010. Sequencing, de novo annotation and analysis of the first Anguilla anguilla
672 transcriptome: EeelBase opens new perspectives for the study of the critically endangered
673 european eel. *BMC Genomics* **11**: 635.

674 Crusoe MR, Alameldin HF, Awad S, Boucher E, Caldwell A, Cartwright R, Charbonneau A,
675 Constantinides B, Edvenson G, Fay S, et al. 2015. The khmer software package: enabling
676 efficient nucleotide sequence analysis. *F1000Research* **4**: 900.

677 Cui L, Wall PK, Leebens-mack JH, Lindsay BG, Soltis DE, Doyle JJ, Soltis PS, Carlson JE,
678 Arumuganathan K, Barakat A, et al. 2006. Widespread genome duplications throughout the
679 history of flowering plants. *Genome Res* **814**: 738–749.

680 David L, Blum S, Feldman MW, Lavi U, Hillel J. 2003. Recent duplication of the common carp
681 (Cyprinus carpio L.) Genome as revealed by analyses of microsatellite loci. *Mol Biol Evol*
682 **20**: 1425–1434.

683 Davila NG, Blakemore LJ, Trombley PQ, Nestor G, Blakemore LJ, Trombley PQ. 2003.
684 Dopamine modulates synaptic transmission between rat olfactory bulb neurons in culture. *j*
685 *Neurophysiol* **90**: 395–404.

686 Dehal P, Boore JL. 2005. Two rounds of whole genome duplication in the ancestral vertebrate.
687 *PLoS Biol* **3**: 1700–1708.

688 Dufour S. 1988. Stimulation of gonadotropin release and of ovarian development, by the
689 administration of a gonadoliberin agonist and of dopamine antagonists, in female silver eel
690 pretreated with estradiol. *Gen Comp Endocrinol* **70**: 20–30.

691 Dufour S, Weltzien F, Sebert M-E, Le Belle N, Vidal B, Vernier P, Pasqualini C. 2005.
692 Dopaminergic inhibition of reproduction in teleost fishes: ecophysiological and evolutionary
693 implications. *Ann N Y Acad Sci* **1040**: 9–21.

694 Ferris SD, Whitt GS. 1977. Duplicate gene expression in diploid and tetraploid loaches

(Cypriniformes, Cobitidae). *Biochem Genet* **15**: 1097–1112.

Finn RD, Clements J, Eddy SR. 2011. HMMER web server: Interactive sequence similarity searching. *Nucleic Acids Res* **39**: 29–37.

Glasauer SMK, Neuhauss SCF. 2014. Whole-genome duplication in teleost fishes and its evolutionary consequences. *Mol Genet Genomics* **289**: 1045–1060.

Greenwood PH, Rosen DE, Weitsman SH MG. 1966. *Phyletic studies of teleostean fishes, with a provisional classification of living forms*. bull am Mus nat.

Gu X, Wang Y, Gu J. 2002. Age distribution of human gene families shows significant roles of both large- and small-scale duplications in vertebrate evolution. *Nat Genet* **31**: 205–209.

Hafeez M, Shabbir M, Altaf F, Abbasi AA. 2016. Phylogenomic analysis reveals ancient segmental duplications in the human genome. *Mol Phylogenet Evol* **94**: 95–100.

Hahn MW. 2009. Distinguishing among evolutionary models for the maintenance of gene duplicates. *J Hered* **100**: 605–617.

Henkel C V., Burgerhout E, de Wijze DL, Dirks RP, Minegishi Y, Jansen HJ, Spaink HP, Dufour S, Weltzien FA, Tsukamoto K, et al. 2012. Primitive duplicate hox clusters in the european eel's genome. *PLoS One* **7**.

Hoegg S, Brinkmann H, Taylor JS, Meyer A. 2004. Phylogenetic timing of the fish-specific genome duplication correlates with the diversification of teleost fish. *J Mol Evol* **59**: 190–203.

Howe K, Clark MD, Torroja CF, Torrance J, Berthelot C, Muffato M, Collins JEJE, Humphray S, McLaren K, Matthews L, et al. 2013. The zebrafish reference genome sequence and its relationship to the human genome. *Nature* **496**: 498–503.

Hsia AY, Vincent J, Lledo P, National C, Recherche D, Fessard IA. 1999. Dopamine depresses synaptic inputs into the olfactory bulb. *J Physiol* **82**: 1082–1085.

Huerta-cepas J, Szklarczyk D, Forslund K, Cook H, Heller D, Walter MC, Rattei T, Mende DR, Sunagawa S, Kuhn M, et al. 2016. eggNOG 4.5: a hierarchical orthology framework with

721 improved functional annotations for eukaryotic, prokaryotic and viral sequences. *Nucleic*
722 *Acids Res* **44**: 286–293.
723 Hughes AL, Silva J, Friedman R. 2001. Ancient genome duplications did not structure the
724 human hox -bearing chromosomes. *Genome Res* **11**: 771–780.
725 Haas BJ, Papanicolaou A, Yassour M, Grabherr M, Blood PD, Bowden J, Couger MB, Eccles D,
726 Li B, Lieber M, et al. 2013. De novo transcript sequence reconstruction from RNA-seq
727 using the Trinity platform for reference generation and analysis. *Nat Protoc* **8**: 1494–1512.
728 Inoue JG, Miya M, Miller MJ, Sado T, Hanel R, Hatooka K, Aoyama J, Minegishi Y, Nishida M,
729 Tsukamoto K. 2010. Deep-ocean origin of the freshwater eels. *Biol Lett* **6**: 363–366.
730 Inoue JG, Miya M, Tsukamoto K, Nishida M. 2003. Basal actinopterygian relationships: A
731 mitogenomic perspective on the phylogeny of the “ancient fish.” *Mol Phylogenet Evol* **26**:
732 110–120.
733 Inoue JG, Miya M, Tsukamoto K, Nishida M. 2004. Mitogenomic evidence for the monophyly of
734 elopomorph fishes (Teleostei) and the evolutionary origin of the leptocephalus larva. *Mol*
735 *Phylogenet Evol* **32**: 274–286.
736 Jacobsen MW, Pujolar JM, Gilbert MTP, Moreno-Mayar J V, Bernatchez L, Als TD, Lobon-
737 Cervia J, Hansen MM. 2014. Speciation and demographic history of Atlantic eels (Anguilla
738 anguilla and A. rostrata) revealed by mitogenome sequencing. *Heredity (Edinb)* **113**: 1–11.
739 Jaillon O, Aury J, Brunet F, Petit J-L, Stange-Thomann N, Mauceli E, Bouneau L, Fischer C,
740 Ozouf-costaz C, Bernot A, et al. 2004. Genome duplication in the teleost fish Tetraodon
741 nigroviridis reveals the early vertebrate proto-karyotype. *Nature* **431**: 946–957.
742 Jansen HJ, Liem M, Jong-raadsen SA, Dufour S, Swinkels W, Koelewijn A, Palstra AP, Pelster
743 B, Herman P, Thillart GE Van Den, et al. 2017. Rapid de novo assembly of the European
744 eel genome from nanopore sequencing reads. *Sci Rep* **7**: 7213.
745 Johnson KR, Wright JE, May B. 1987. Linkage relationships reflecting ancestral tetraploidy in
746 salmonid fish. *Genetics* **116**: 579–591.

747 Kai W, Kikuchi K, Tohari S, Chew AK, Tay A, Fujiwara A, Hosoya S, Suetake H, Naruse K,
748 Brenner S, et al. 2011. Integration of the genetic map and genome assembly of evolution in
749 teleosts and mammals. *Genome Biol Evol* **3**: 424–442.
750 Kanehisa M, Sato Y, Morishima K. 2016. BlastKOALA and GhostKOALA: KEGG tools for
751 functional characterization of genome and metagenome sequences. *J Mol Biol* **428**: 726–
752 731.
753 Kasahara M, Naruse K, Sasaki S, Nakatani Y, Qu W, Ahsan B, Yamada T, Nagayasu Y, Doi K,
754 Kasai Y, et al. 2007. The medaka draft genome and insights into vertebrate genome
755 evolution. *Nature* **447**: 714–719.
756 Kassahn KS, Dang VT, Wilkins SJ, Kassahn KS, Dang VT, Wilkins SJ, Perkins AC, Ragan MA.
757 2009. Evolution of gene function and regulatory control after whole-genome duplication :
758 Comparative analyses in vertebrates. *Genome Res* **19**: 1404–1418.
759 Lafont AG, Rousseau K, Tomkiewicz J, Dufour S. 2016. Three nuclear and two membrane
760 estrogen receptors in basal teleosts, Anguilla sp.: Identification, evolutionary history and
761 differential expression regulation. *Gen Comp Endocrinol* **235**: 177–191.
762 Larhammar D, Risinger C. 1994. Molecular genetic aspects of tetraploidy in the common carp
763 Cyprinus carpio. *Mol Phylogenet Evol* **3**: 59–68.
764 Lartillot N, Lepage T, Blanquart S. 2009. PhyloBayes 3: A Bayesian software package for
765 phylogenetic reconstruction and molecular dating. *Bioinformatics* **25**: 2286–2288.
766 Li H, Durbin R. 2010. Fast and accurate long-read alignment with Burrows-Wheeler transform.
767 *Bioinformatics* **26**: 589–595.
768 Li L, Stoeckert CJJ, Roos DS. 2003. OrthoMCL: identification of ortholog groups for eukaryotic
769 genomes. *Genome Res* **13**: 2178–2189.
770 Lien S, Koop BF, Sandve SR, Miller JR, Matthew P, Leong JS, Minkley DR, Zimin A, Grammes
771 F, Grove H, et al. 2016. The Atlantic salmon genome provides insights into rediploidization.
772 *Nature* **533**: 200–205.

773 Llorente B, Malpertuy A, Neuv¡église C, De Montigny J, Aigle M, Artiguenave F, Blandin G,
774 Bolotin-Fukuhara M, Bon E, Brottier P, et al. 2000. Genomic exploration of the
775 hemiascomycetousyYeasts: 18. comparative analysis of chromosome maps and synteny
776 with Saccharomyces cerevisiae. *FEBS Lett* **487**: 101–112.
777 Lu J, Peatman E, Tang H, Lewis J, Liu Z. 2012. Profiling of gene duplication patterns of
778 sequenced teleost genomes: evidence for rapid lineage-specific genome expansion
779 mediated by recent tandem duplications. *BMC Genomics* **13**: 246.
780 Ludwig A, Belfiore NM, Pitra C, Svirsky V, Jenneckens I. 2001. Genome duplication events and
781 functional reduction of ploidy levels in sturgeon (Acipenser, Huso and Scaphirhynchus).
782 *Genetics* **158**: 1203–1215.
783 Macqueen DJ, Johnston IA, Macqueen DJ. 2014. A well-constrained estimate for the timing of
784 the salmonid whole genome duplication reveals major decoupling from species
785 diversification. *Proc R Soc B* **281**: 20132881.
786 Maugars G, Dufour S. 2015. Demonstration of the Coexistence of Duplicated LH Receptors in
787 Teleosts, and Their Origin in Ancestral Actinopterygians. *PLoS One* **10**: e0135184.
788 Meyer A, Peer Y Van De. 2005. From 2R to 3R: evidence for a fish-specific genome duplication
789 (FSGD). *BioEssays* **27**: 937–945.
790 Minegishi Y, Aoyama J, Inoue JG, Miya M. 2005. Molecular phylogeny and evolution of the
791 freshwater eels genus Anguilla based on the whole mitochondrial genome sequences. *Mol*
792 *Phylogenet Evol* **34**: 134–146.
793 Morini M, Pasquier J, Dirks R, Van Den Thillart G, Tomkiewicz J, Rousseau K, Dufour S, Lafont
794 AG. 2015. Duplicated leptin receptors in two species of eel bring new insights into the
795 evolution of the leptin system in vertebrates. *PLoS One* **10**: 1–31.
796 Morini M, Peñaranda DS, Vílchez MC, Nourizadeh-Lillabadi R, Lafont AG, Dufour S, Asturiano
797 JF, Weltzien FA, Pérez L. 2017. Nuclear and membrane progestin receptors in the
798 European eel: Characterization and expression in vivo through spermatogenesis. *Comp*

Biochem Physiol -Part A Mol Integr Physiol **207**: 79–92.

Munk P, Hansen MM, Maes GE, Nielsen TG, Castonguay M, Riemann L, Sparholt H, Als TD, Aarestrup K, Andersen NG, et al. 2010. Oceanic fronts in the Sargasso Sea control the early life and drift of Atlantic eels. *Proc Biol Sci* **277**: 3593–3599.

Nagatsu T, Levitt M, Udenfriemd S. 1964. Tyrosine Hydroxylase: The initial step in norepinephrine biosynthesis. *J Biol Chem* **239**: 2910–2917.

Ohno S. 1970. *Evolution by Gene Duplication*. Springer-Verlag, New york.

Pasqualini C, Weltzien FA, Vidal B, Baloche S, Rouget C, Gilles N, Servent D, Vernier P, Dufour S. 2009. Two distinct dopamine D2 receptor genes in the european eel: Molecular characterization, tissue-specific transcription, and regulation by sex steroids. *Endocrinology* **150**: 1377–1392.

Pasquier J, Cabau C, Nguyen T, Jouanno E, Severac D, Braasch I, Journot L, Pontarotti P, Klopp C, Postlethwait JH, et al. 2016. Gene evolution and gene expression after whole genome duplication in fish: the PhyloFish database. *BMC Genomics* **17**: 368.

Pasquier J, Lafont AG, Jeng SR, Morini M, Dirks R, van den Thillart G, Tomkiewicz J, Tostivint H, Chang CF, Rousseau K, et al. 2012. Multiple kisspeptin receptors in early osteichthyans provide new insights into the evolution of this receptor family. *PLoS One* **7**: e48931.

Patro R, Duggal G, Love MI, Irizarry RA, Kingsford C. 2017. Salmon provides accurate, fast, and bias-aware transcript expression estimates using dual-phase inference. *bioRxiv* **14**: 21592.

Peña-Llopis S, Brugarolas J. 2013. Simultaneous isolation of high-quality DNA, RNA, miRNA and proteins from tissues for genomic applications. *Nat Protoc* **8**: 2240–55.

Pertea M, Kim D, Pertea GM, Leek JT, Salzberg SL. 2016. Transcript-level expression analysis of RNA-seq experiments with HISAT, StringTie and Ballgown. *Nat Protoc* **11**: 1650–1667.

Peter RE, Chang JP, Nahorniak RJ, Sokolowska OM, Shih SH, Billard R. 1986. Interactoin of catecholamin and GnRH in regulation of gonadotropin secretion in teleost fish. *Recent*

Prog Horm Res **42**: 513–548.

Righton D, Westerberg H, Feunteun E, Okland F, Gargan P, Amilhat E, Metcalfe J, Lobon-Cervia J, Sjo berg N, Simon J, et al. 2016. Empirical observations of the spawning migration of European eels: The long and dangerous road to the Sargasso Sea. *Sci Adv* **2**: e1501694.

Rondeau EB, Minkley DR, Leong JS, Messmer AM, Jantzen JR, Von Schalburg KR, Lemon C, Bird NH, Koop BF. 2014. The genome and linkage map of the northern pike (Esox lucius): Conserved synteny revealed between the salmonid sister group and the neoteleostei. *PLoS One* **9**: e102089.

Rozenfeld C, Butts IAE, Tomkiewicz J, Zambonino-Infante J-L, Mazurais D. 2016. Abundance of specific mRNA transcripts impacts hatching success in European eel, Anguilla anguilla L. *Comp Biochem Physiol A Mol Integr Physiol* **191**: 59–65.

Santini F, Harmon LJ, Carnevale G, Alfaro ME. 2009. Did genome duplication drive the origin of teleosts? A comparative study of diversification in ray-finned fishes. *BMC Evol Biol* **9**: 194.

Schartl M, Walter RB, Shen Y, Garcia T, Catchen J, Amores A, Braasch I, Chalopin D, Volff J-N, Lesch K-P, et al. 2013. The genome of the platyfish, Xiphophorus maculatus, provides insights into evolutionary adaptation and several complex traits. *Nat Genet* **45**: 567–572.

Schmidt J. 1923. The breeding places of the eel. *Philos Trans R Soc B Biol Sci* **211**: 179–208.

Scott a P, Sumpter JP, Stacey N. 2010. The role of the maturation-inducing steroid, 17,20beta-dihydroxypregn-4-en-3-one, in male fishes: a review. *J Fish Biol* **76**: 183–224.

She X, Cheng Z, Zöllner S, Church DM, Eichler EE. 2008. Mouse segmental duplication and copy number variation. *Nat Genet* **40**: 909–14.

Sievers F, Wilm A, Dineen D, Gibson TJ, Karplus K, Li W, Lopez R, McWilliam H, Remmert M, Söding J, et al. 2011. Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. *Mol Syst Biol* **7**: 539.

Simão FA, Waterhouse RM, Ioannidis P, Kriventseva E V., Zdobnov EM. 2015. BUSCO:

Assessing genome assembly and annotation completeness with single-copy orthologs. *Bioinformatics* **31**: 3210–3212.

Slater GSC, Birney E. 2005. Automated generation of heuristics for biological sequence comparison. *BMC Bioinformatics* **6**: 31.

Stein C, Caccamo M, Laird G, Leptin M. 2007. Conservation and divergence of gene families encoding components of innate immune response systems in zebrafish. *Genome Biol* **8**: R251.

Tang H, Wang X, Bowers JE, Ming R, Alam M, Paterson AH. 2008. Unraveling ancient hexaploidy through multiply-aligned angiosperm gene maps. *Genome Res* **18**: 1944–1954.

Tautz D, Domazet-lošo T. 2011. The evolutionary origin of orphan genes. *Nat Publ Gr* **12**: 692–702.

Tsukamoto K, Aoyama J, Miller MJ. 2002. Migration, speciation, and the evolution of diadromy in anguillid eels. *Can J Fish Aquat Sci* **59**: 1989–1998.

Tsukamoto Katsumi, Nakai Izumi TW-. V. 1998. Do all freshwater eels migrate? *Nature* **396**: 635–636.

Uyeno T, Smith GR. 1972. Tetraploid origin of the karyotype of catostomid fishes. *Science* **175**: 644–646.

Vallente Samonte R, Eichler EE. 2016. Segmental duplications and the evolution of the primate genome. *Nat Rev Genet* **3**: 65–72.

van Ginneken V, Antonissen E, Müller UK, Booms R, Eding E, Verreth J, van den Thillart G. 2005. Eel migration to the Sargasso: remarkably high swimming efficiency and low energy costs. *J Exp Biol* **208**: 1329–35.

Vandepoele K, De Vos W, Taylor JS, Meyer A, Van de Peer Y. 2004. Major events in the genome evolution of vertebrates: paranome age and size differ considerably between ray-finned fishes and land vertebrates. *Proc Natl Acad Sci U S A* **101**: 1638–1643.

Vidal B, Pasqualini C, Le Belle N, Holland MCH, Sbaihi M, Vernier P, Zohar Y, Dufour S. 2004.

Dopamine inhibits luteinizing hormone synthesis and release in the juvenile European eel: a neuroendocrine lock for the onset of puberty. *Biol Reprod* **71**: 1491–500.

Wang J, Li J, Zhang X, Sun X. 2012. Transcriptome analysis reveals the time of the fourth round of genome duplication in common carp (Cyprinus carpio). *BMC Genet* **13**: 96.

Weltzien F, Pasqualini C, Se M, Vidal B, Belle N Le, Kah O, Vernier P, Dufour S. 2015. Androgen-dependent stimulation of brain dopaminergic systems in the female European eel (Anguilla anguilla). *Recent Prog Horm Res* **146**: 2964–2973.

Zhang J. 2003. Evolution by gene duplication: An update. *Trends Ecol Evol* **18**: 292–298.

Zhou Q, Zhang G, Zhang Y, Xu S, Zhao R, Zhan Z, Li X, Ding Y, Yang S, Wang W. 2008. On the origin of new genes in Drosophila. *Genome Res* **18**: 1446–1455.