Supplementary Material and Methods

Database mining and sea bream tyrosinase (tyr) and dopachrome tautomerase (dct) sequence retrieval

Since members of the tyrosinase gene family are not described in gilthead sea bream, database mining and phylogenetic analysis were performed to identify them and confirm. The gilthead sea bream tyrosinase gene family members were identified using the zebrafish (Danio rerio) homologues (tyr: NM_131013; tyrp1: NM_001002749; dct: AF280090) and the basic local alignment search tool (BLAST) algorithm to search a sea bream transcriptome assembly prepared “in house” from multiple tissues (Louro et al. 2016). No orthologue of the teleost dct was retrieved. The sequence of zebrafish tyr family members were used to retrieve homologues from other teleosts with available sequenced genomes: two pufferfishes, Tetraodon nigroviridis and Takifugu rubripes; stickleback, Gasterosteus aculeatus; Nile tilapia, Oreochromis niloticus; medaka, Oryzias latipes; platyfish, Xiphophorus maculatusi; Atlantic cod, Gadus morhua; blind cavefish, Astyanax mexicanus and from the primitive freshwater ray-finned fish spotted gar (Lepisosteus oculatus), the lobe-finned fish coelacanth (Latimeria chalumnae) and the marine lamprey (Petromyzon marinus) genomes, all available from ENSEMBL (http://www.ensembl.org, accessed in September 2014). The genomes of the Atlantic salmon, (Salmon salar, http://salmondb.cmm.uchile.cl/), the cartilaginous elephant shark (Callorhinus milii, http://esharkgenome.imcb.a-star.edu.sg) and Japanese lamprey (Lethenteron japonicum, http://jlampreygenome.imcb.a-star.edu.sg) were also investigated.

The tetrapod genomes including two placental mammals human (Homo sapiens) and mouse (Mus musculus) and two non-placental mammals the marsupial gray short-tailed opossum (Monodelphis domestica) and the monotreme platypus (Ornithorhynchus anatinus); the bird chicken (Gallus gallus); two reptiles Anole lizard (Anolis carolinensis) and turtle (Pelodiscus sinensis) and the amphibian (Xenopus tropicalis) available from ENSEMBL (http://www.ensembl.org, accessed in September 2014) were also explored for tyrosinase gene
family members using a similar strategy to that used for the fish. Tyrosinase gene family members were also searched in early chordate genomes of the cephalochordate amphioxus (*Branchiostoma floridae*, [http://genome.jgi-psf.org/Brafl1/ Brafl1. home.html](http://genome.jgi-psf.org/Brafl1/ Brafl1. home.html)) and the tunicate Ciona (*Ciona intestinalis*, [http://www.ensembl.org/](http://www.ensembl.org/), accessed in September 2014).

**Phylogenetic analysis**

Phylogenetic analysis was performed using the deduced amino acid sequences of retrieved tyr family members. The metazoan sequences were aligned using CLUSTALW (1.83) (available from [http://www.genome.jp/tools/clustalw/](http://www.genome.jp/tools/clustalw/), Thompson *et al.* 1997) and the alignment was assessed using ProtTest (2.4) to select the best model for analysis of protein family evolution according to the Akaike Information Criterion (AIC) statistical model (Abascal *et al.* 2005). Phylogenetic trees were constructed using two methods, maximum likelihood (ML) and neighbor-joining (NJ) and the accuracy of the phylogenetic clades assessed using bootstrap analysis, 100 and 1000 for ML and NJ, respectively (Felsenstein 1985). ML analysis was constructed in the PhyML program (3.0) ([http://atgc.lirmm.fr/phyml/](http://atgc.lirmm.fr/phyml/)) using the JTT substitution model (Jones *et al.* 1992) including a fixed proportion of invariant sites (0.037), 4 gamma-distributed rate categories and gamma shape parameter (0.796). The NJ method (Saitou & Nei 1987) was implemented using the Mega 5.2 program ([http://tree.bio.ed.ac.uk/software/figtree/](http://tree.bio.ed.ac.uk/software/figtree/)) with the JTT substitution model and 4 gamma-distributed rate categories, and a fixed gamma parameter (0.796). ML trees were displayed and edited using FigTree software v.1.4.2 ([http://tree.bio.ed.ac.uk/software/figtree/](http://tree.bio.ed.ac.uk/software/figtree/)). Both methods generated trees with similar topologies and a hypothetical root was added to separate the Tyr and TYRP1/DCT branches.
Supplementary Figure Legends

Supplementary Figure 1: Different stage of development depending on the thermal regime. Photographed stages: lecitotrophic at 2 dph (presence of large yolk sac and oil globule, no exogenous feeding yet, no major differences among groups); notochord flexion at 19-24 dph (differences among groups); caudal fin formation at 34-39 dph; juvenile stage at 131 dph (all fish are fully scaled and pigmented). Temperature conditions experienced by each developmental stage up until metamorphosis are indicated at the top of each column. Fish from the final stage, the juvenile were all maintained from metamorphosis (Supplementary Table 1) until the experiment (9 months) at 22 ºC.

Supplementary Figure 2. Phylogeny of the gilthead sea bream Tyr members with the metazoan homologues. Tree was built using the ML method and reliability of internal branching was assessed with 100 bootstrap replicates. Only branch support values higher than 50% are represented. The 3 sea bream Tyr members identified clustered in different branches which confirmed their identity as tyra, tyrp1b and dct, respectively. Species names and accession numbers are available in Supplementary Table 3.
References


