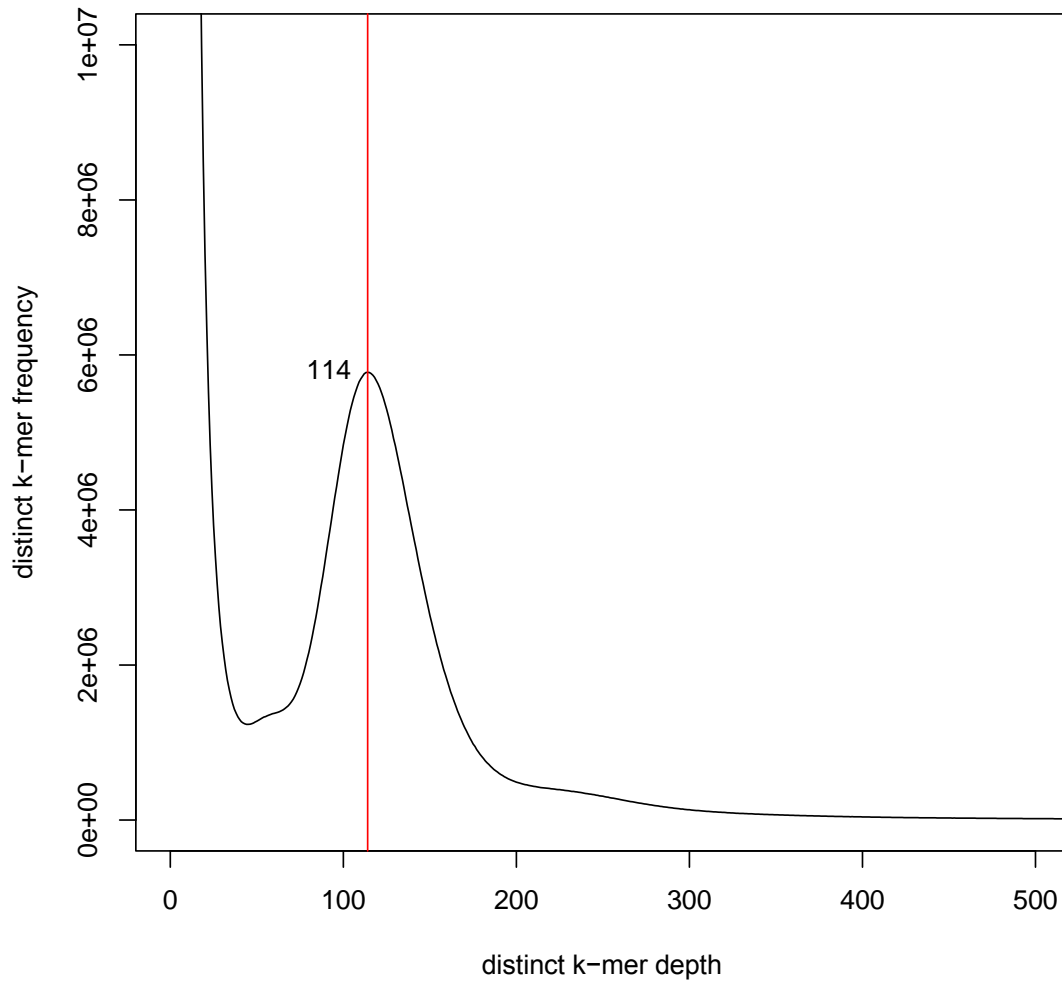


Supplementary Information

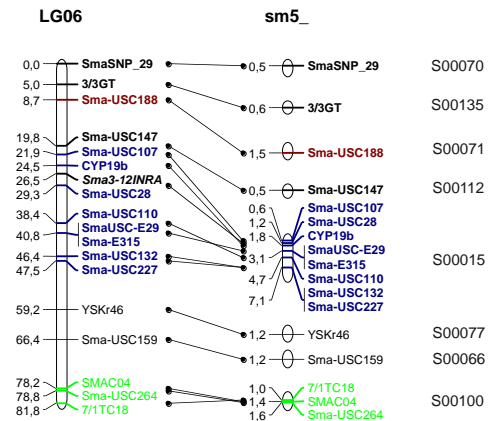
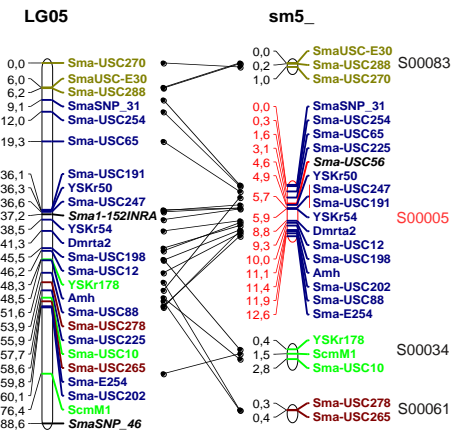
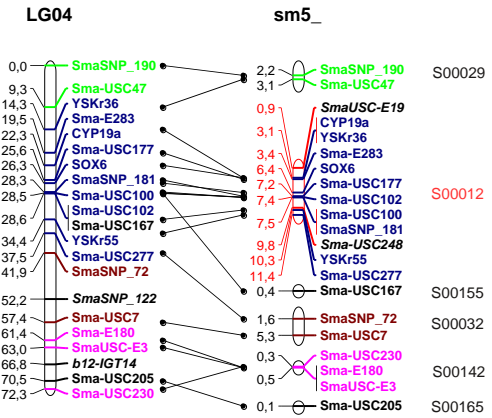
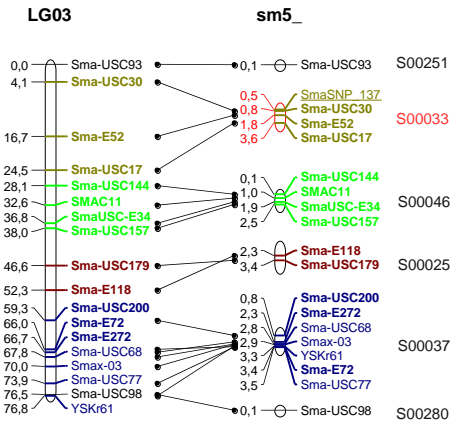
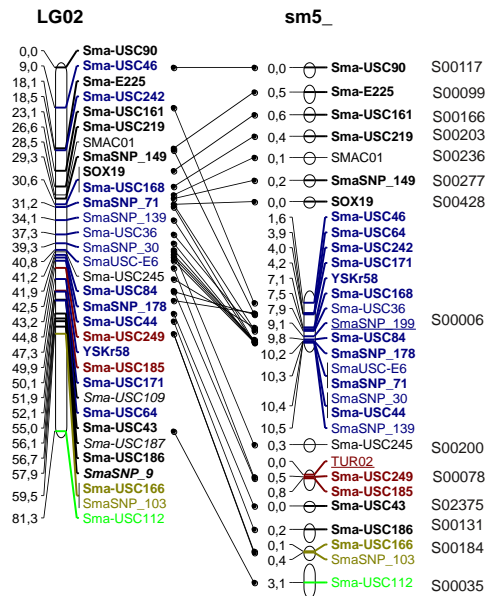
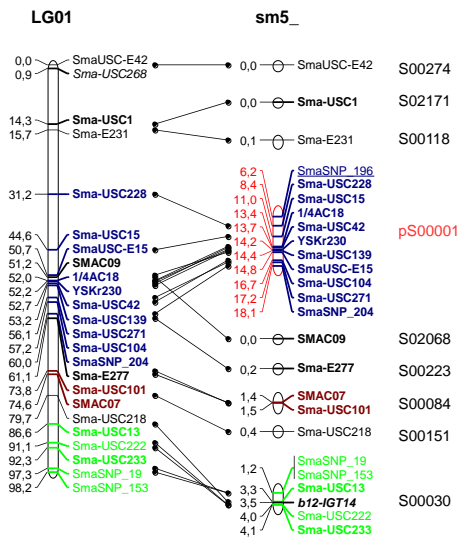
Whole genome sequencing of turbot (*Scophthalmus maximus*; Pleuronectiformes): a fish adapted to demersal life

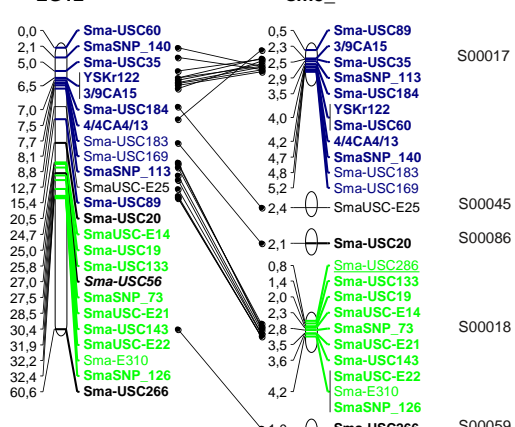
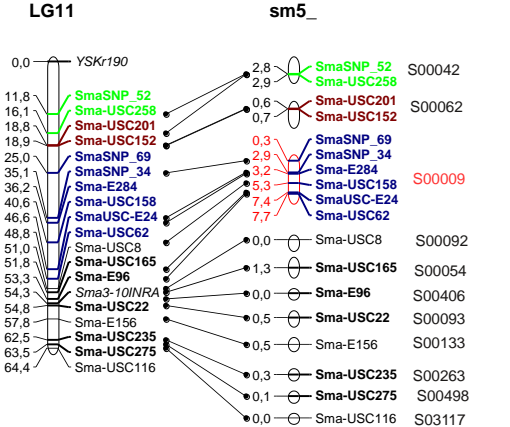
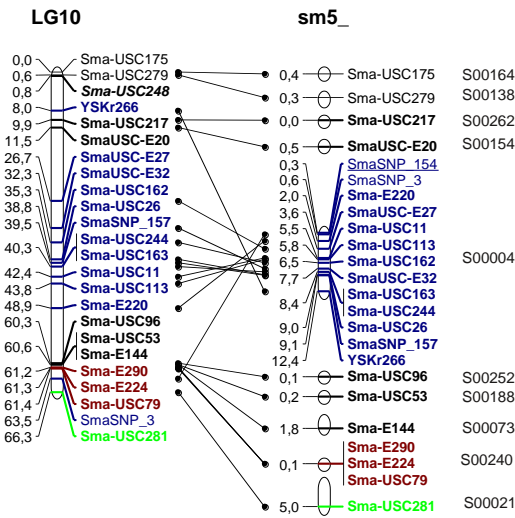
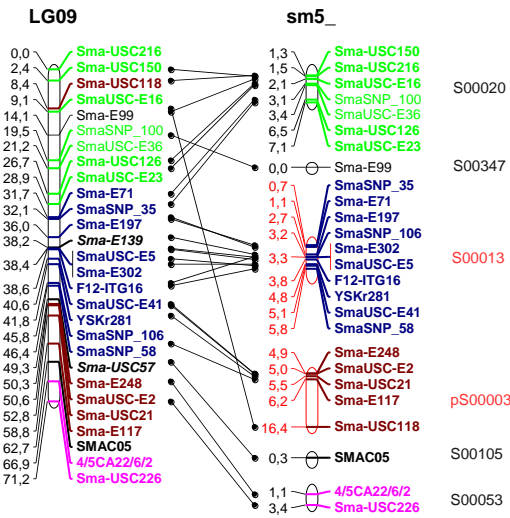
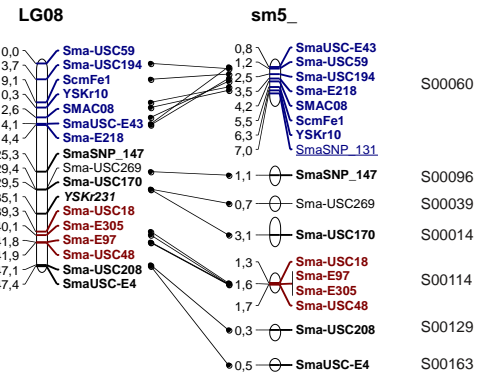
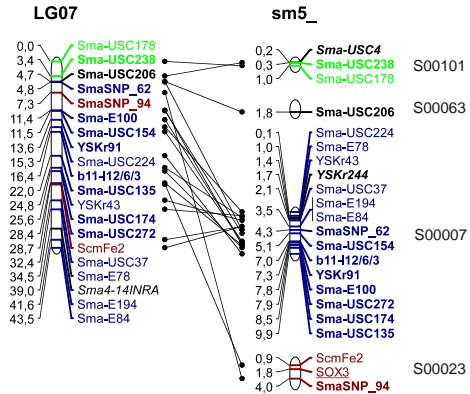
Antonio Figueras^{1,*}, Diego Robledo², André Corvelo^{3,4}, Miguel Hermida⁵, Patricia Pereiro¹, Juan A Rubiolo⁵, Jèssica Gómez-Garrido³, Laia Carreté³, Xabier Bello^{6,7}, Marta Gut³, Ivo Glynne Gut³, Marina Marcet-Houben³, Gabriel Forn-Cuní¹, Beatriz Galán⁸, José Luis García⁸, José Luis Abal-Fabeiro^{6,7}, Belen G Pardo⁵, Xoana Taboada², Carlos Fernández⁵, Anna Vlasova³, Antonio Hermoso-Pulido³, Roderic Guigó³, José Antonio Álvarez-Dios⁹, Antonio Gómez-Tato¹⁰, Ana Viñas², Xulio Maside^{6,7}, Toni Gabaldón^{3,11,12}, Beatriz Novoa¹, Carmen Bouza⁵, Tyler Alioto³, Paulino Martínez^{5,*}

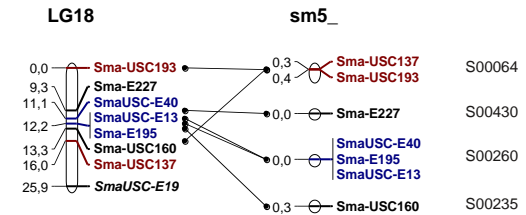
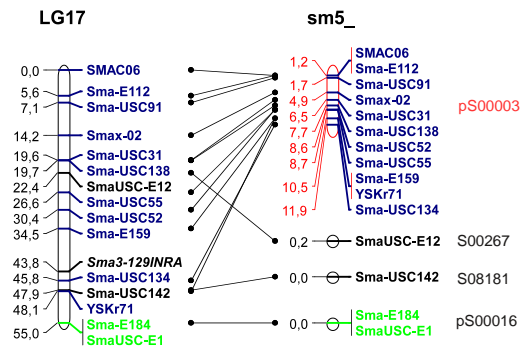
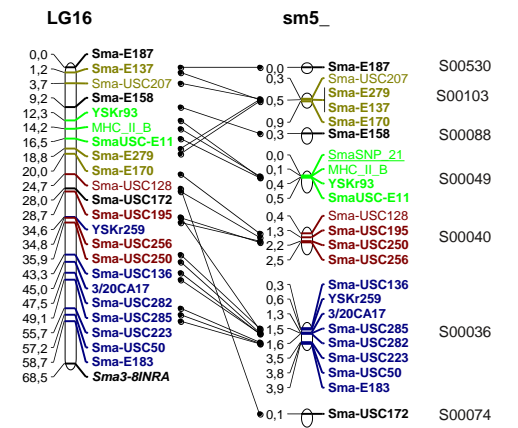
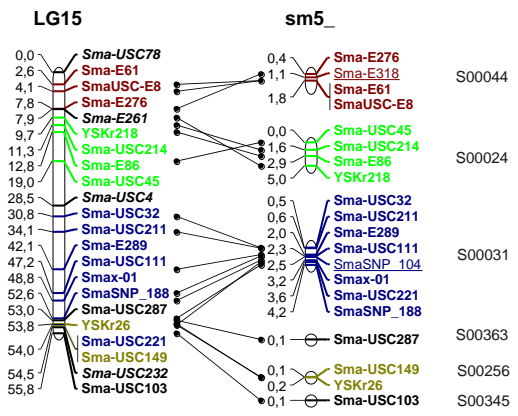
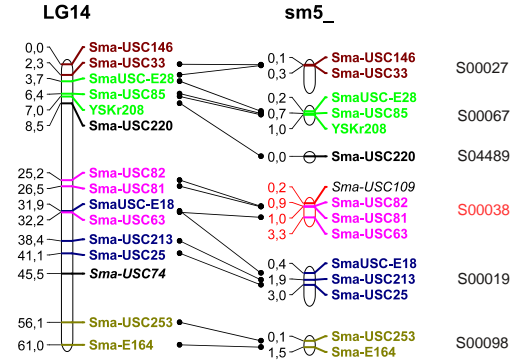
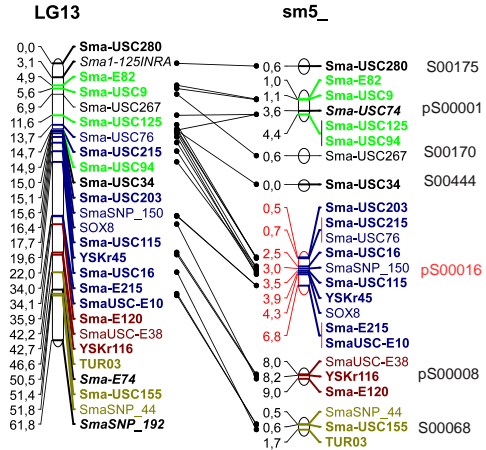
Supplementary Figures

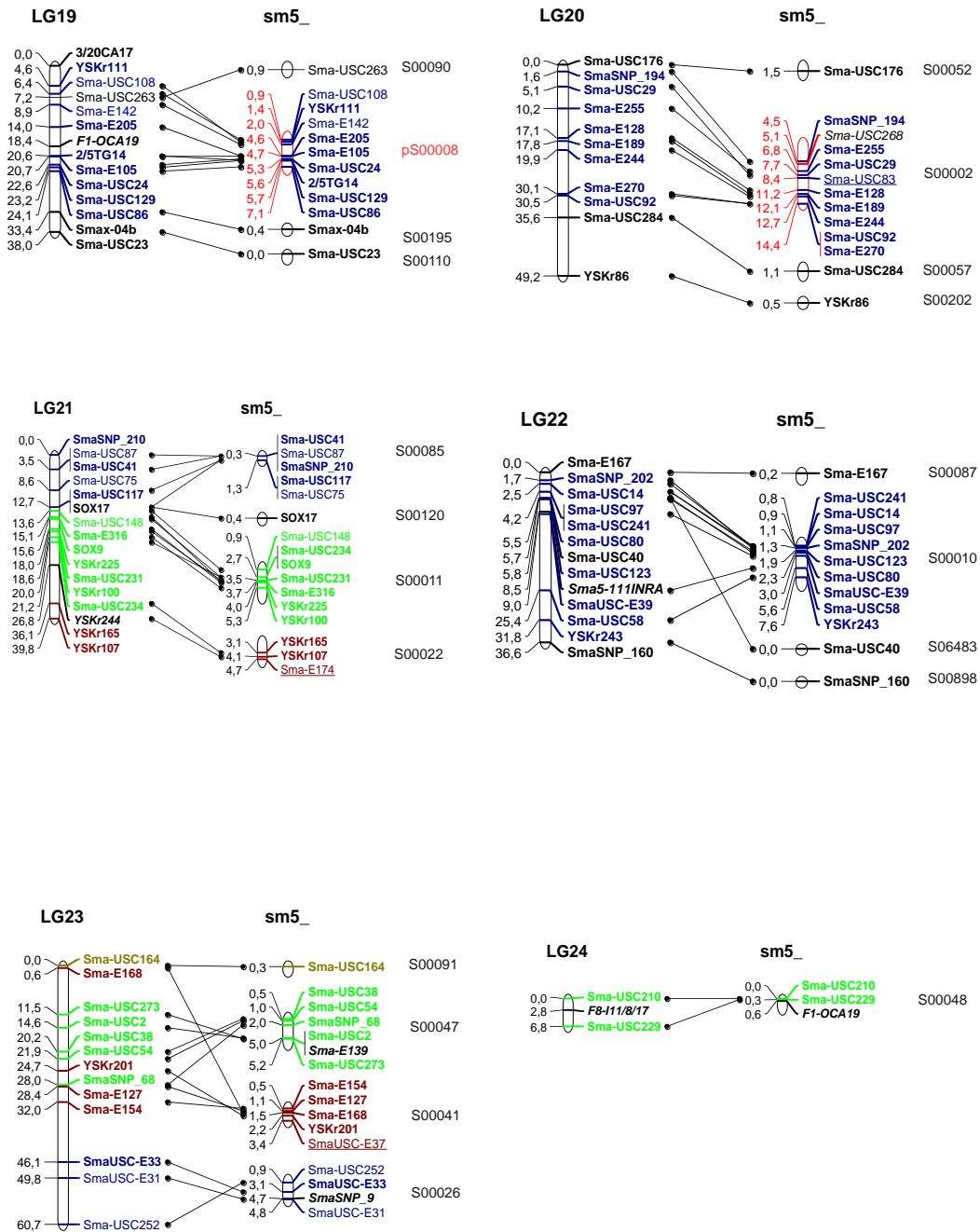


Supplementary Fig. S1. Estimation of turbot (*S. maximus*) genome size. Evaluation was performed through a kmer content analysis of the genome using the software Jellyfish v1.1.10¹. The number of distinct 17-mers are plotted as a function of k-mer multiplicity (depth).



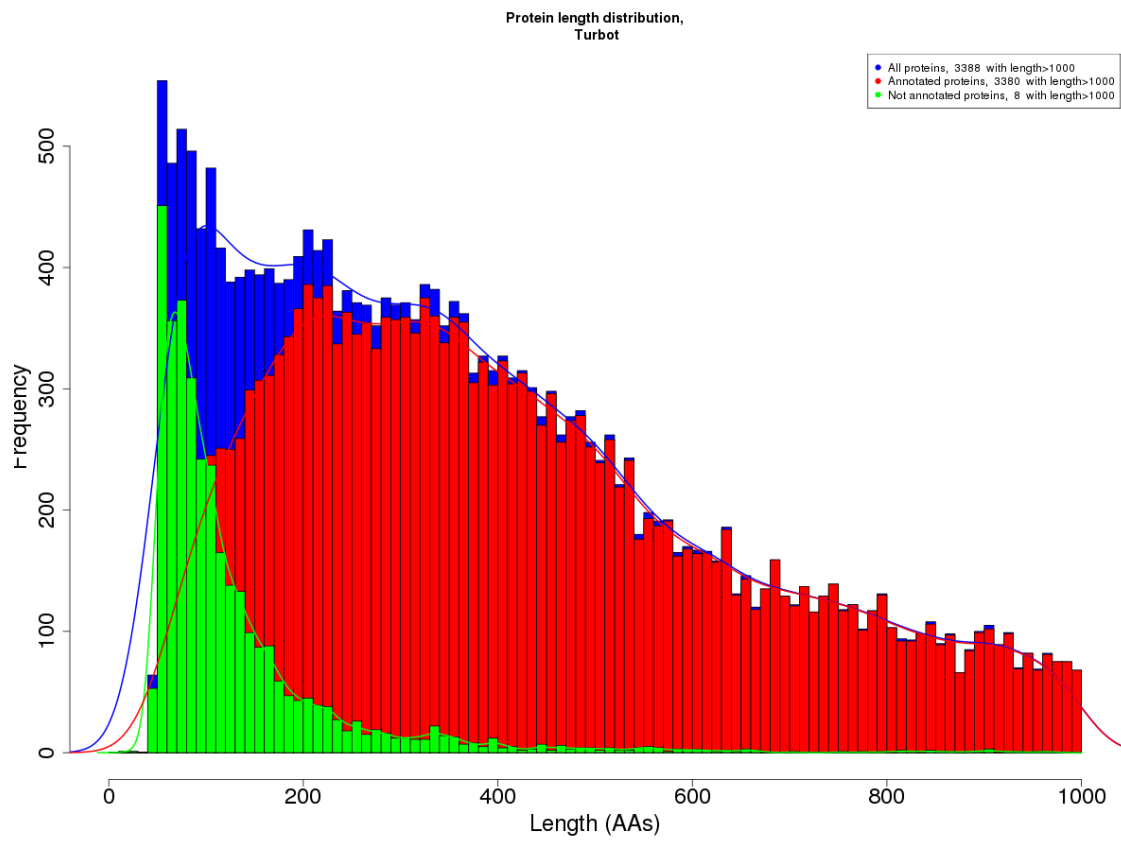




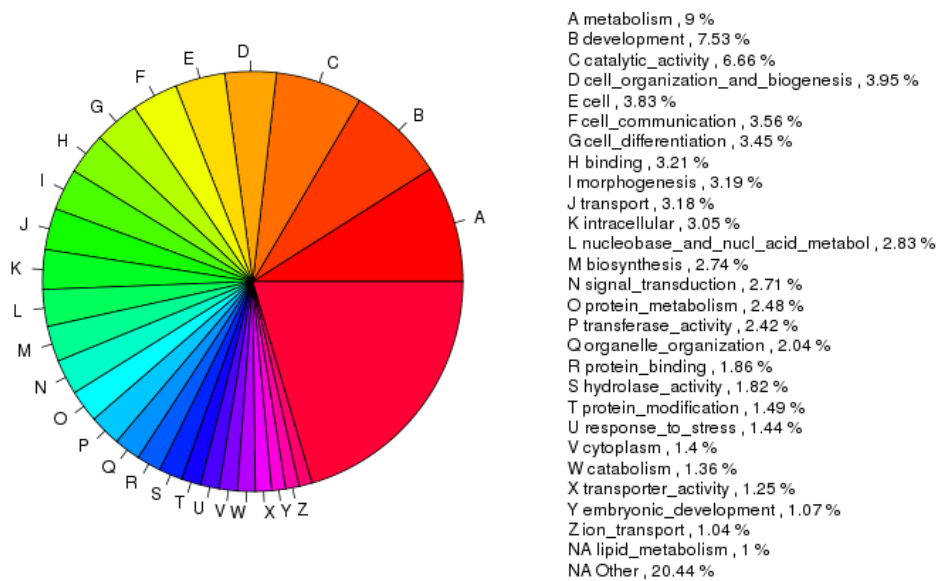


Supplementary Fig. S2. Anchoring of the turbot (*S. maximus*) genome assembly (right) on the turbot genetic map (left). The position of markers in the genetic map and in the scaffolds where their sequences matched is shown. Marker positions are represented in cM in the genetic map and in Mb in the genome. Scaffolds with two or more markers from the same LG are colored (colors are meaningful), whereas scaffolds with only one marker are in black. Framework markers are

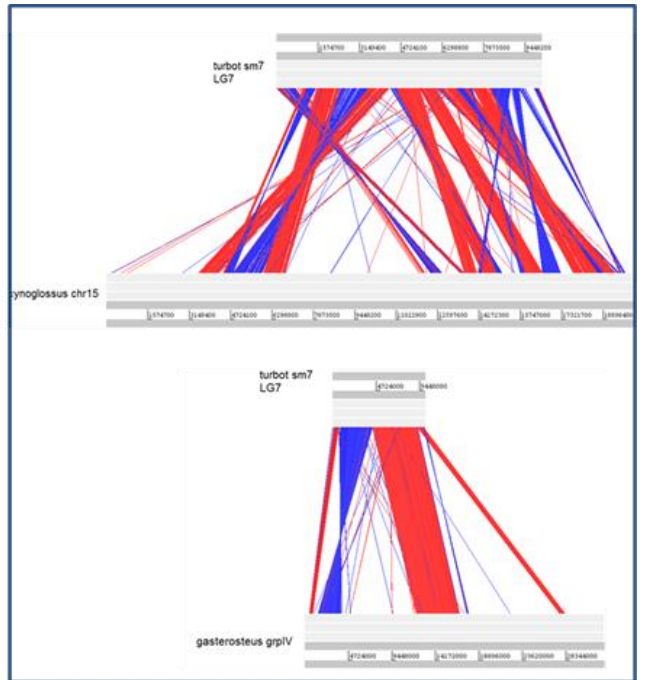
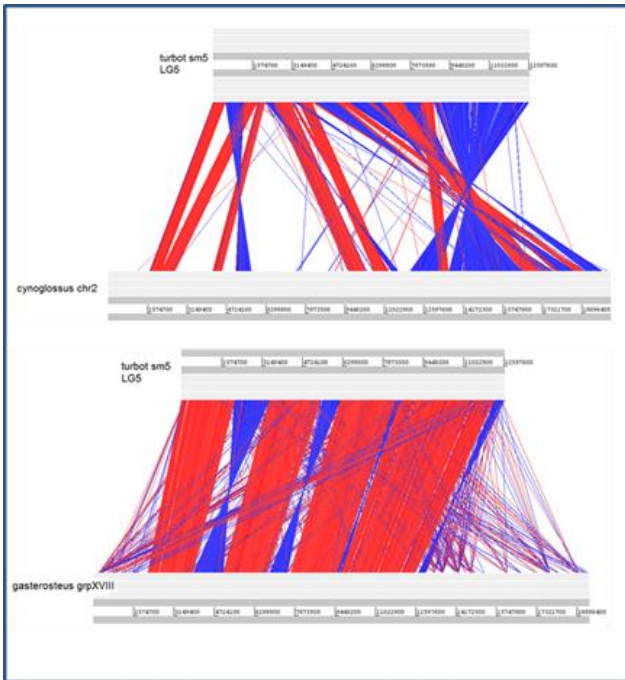
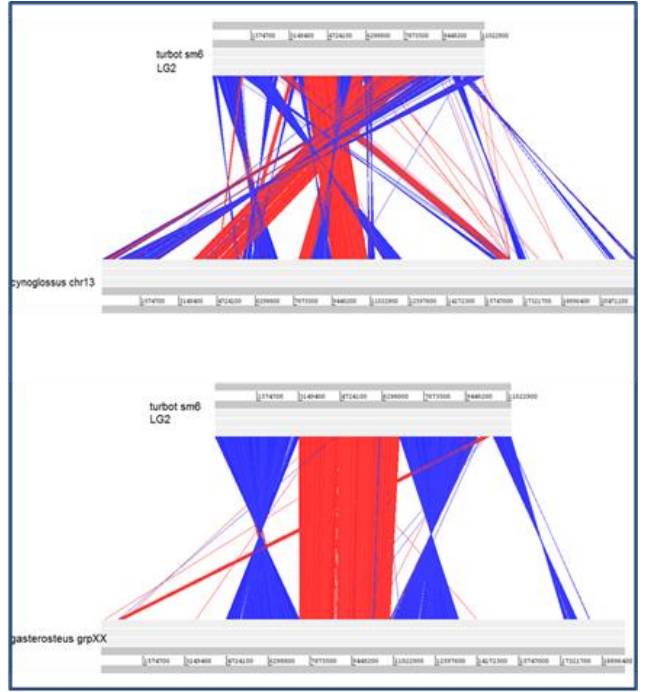
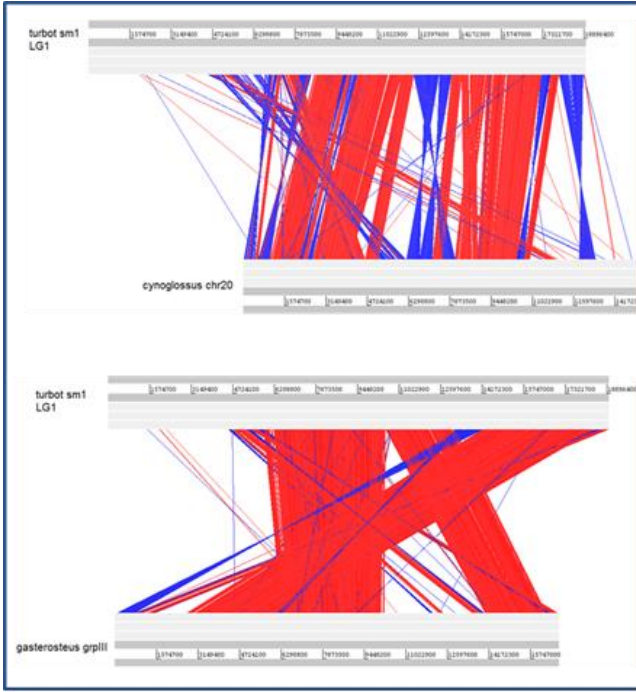
represented in bold type. Markers of the genetic map with no match in scaffolds, and *viceversa*, are in italic type. The marker closest to the centromere is underlined. The scaffolds with positions in red have been inverted only for representation purposes.

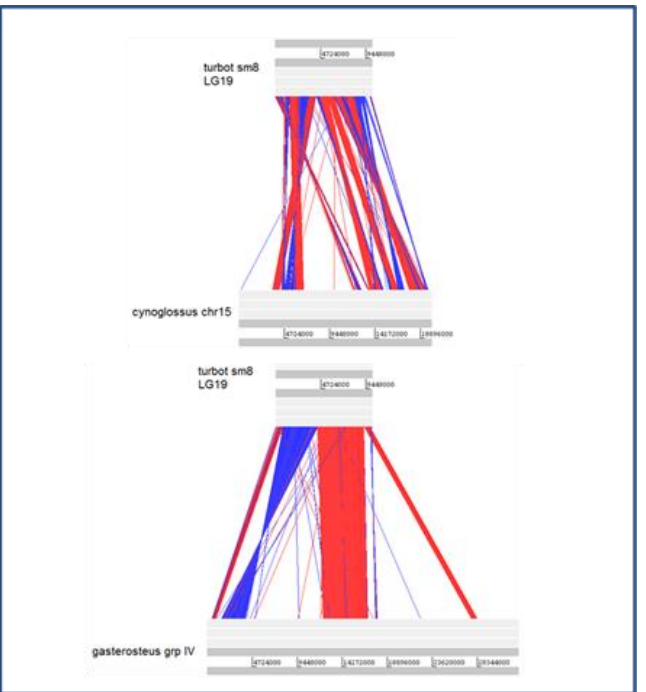
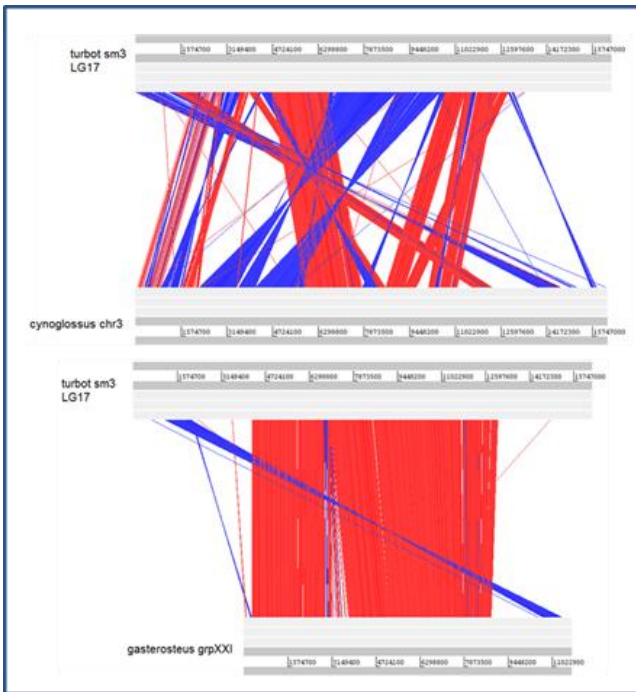
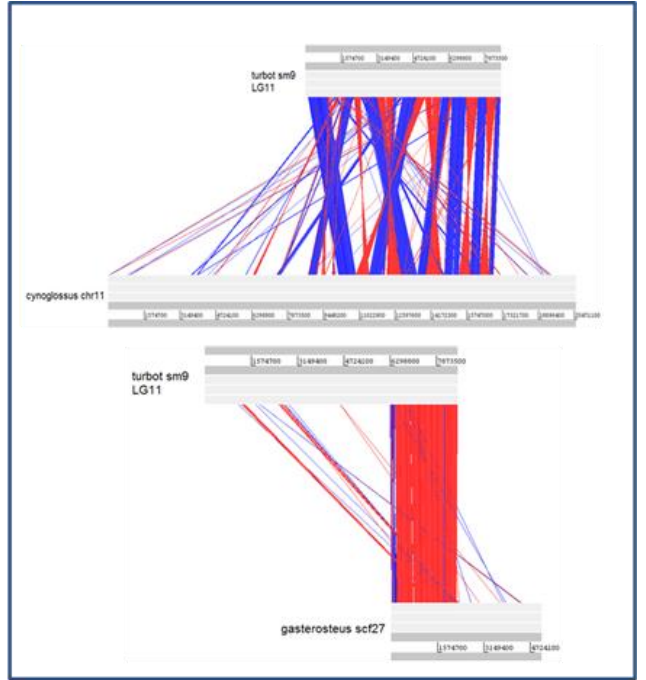
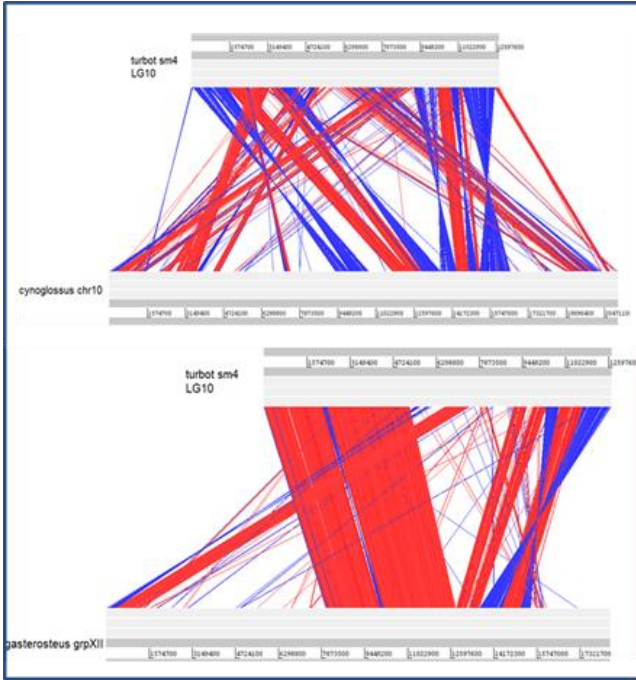


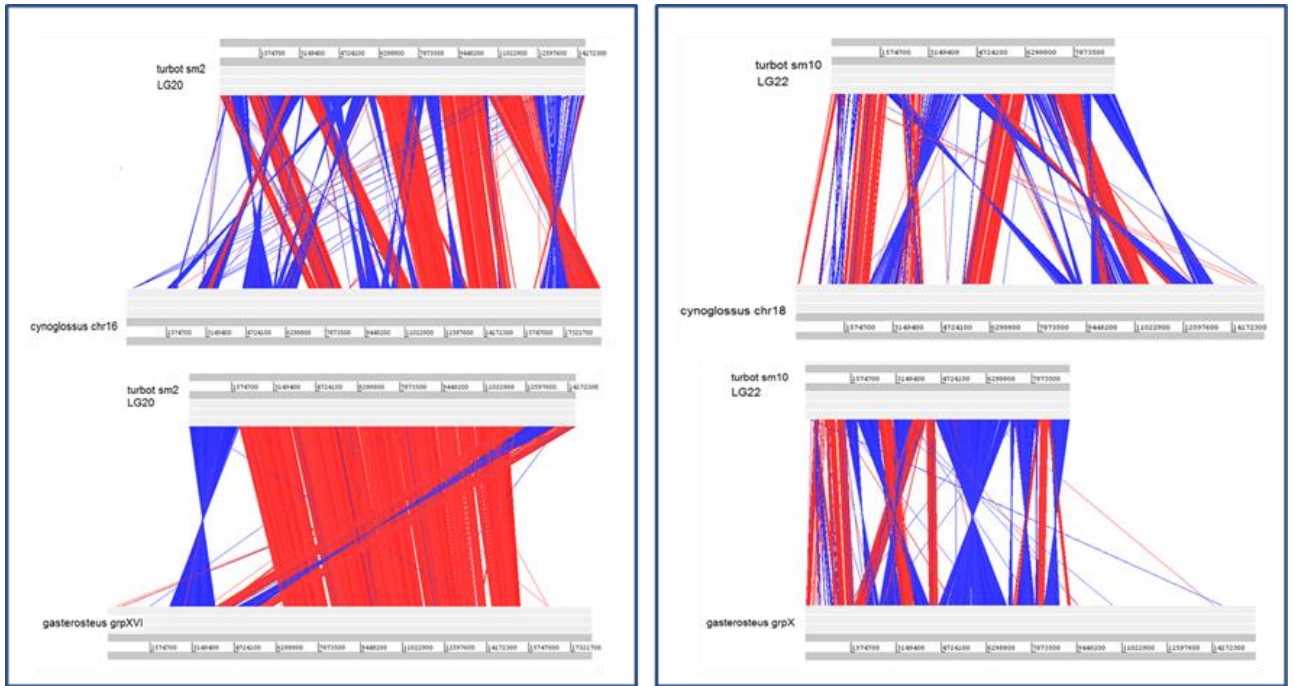
Supplementary Fig. S3. Size distribution and annotation of the turbot (*S. maximus*) transcriptome (protein coding genes). Blue: all mRNAs; red: annotated mRNAs; green: non-annotated mRNAs.



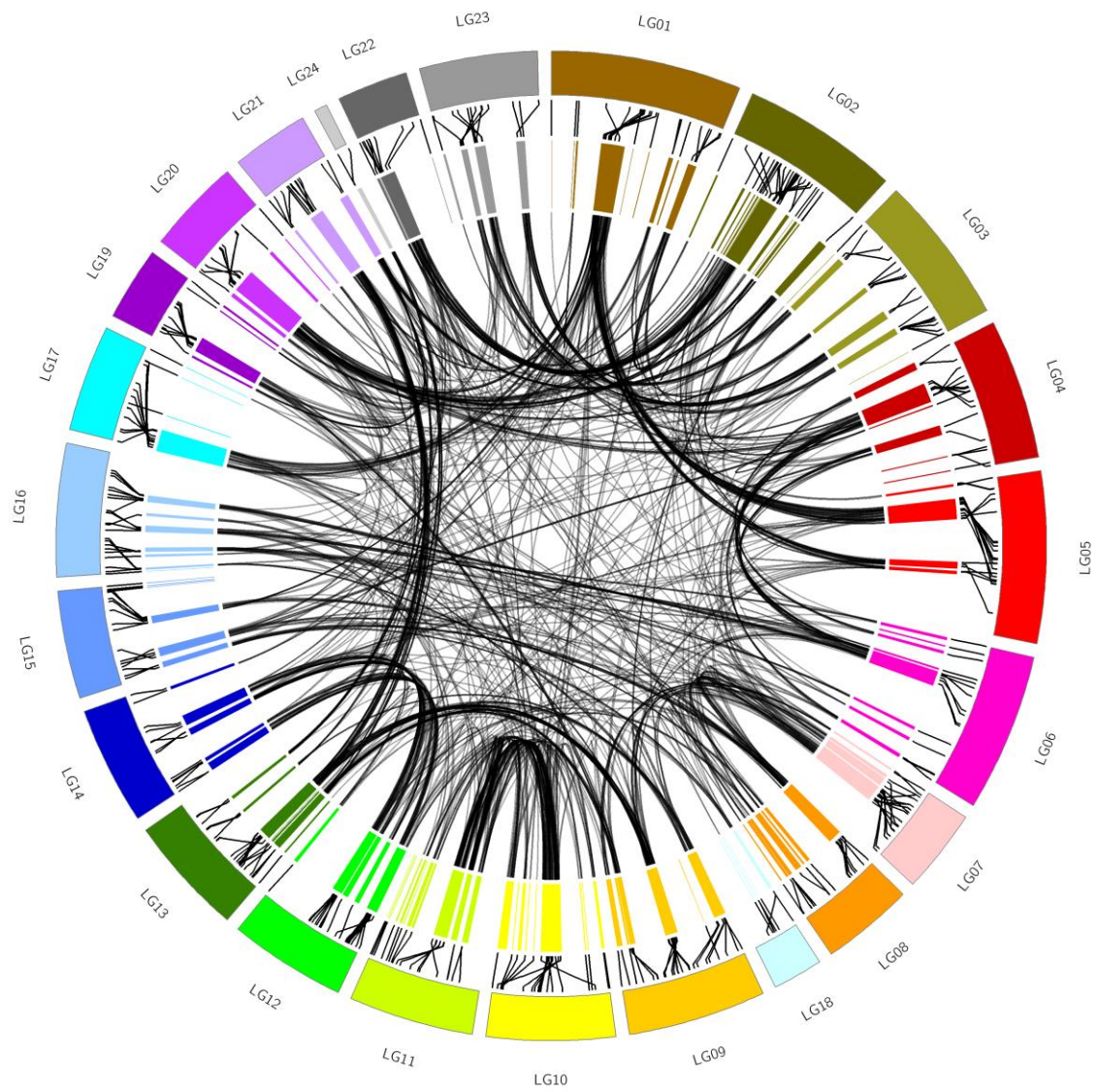
Supplementary Fig. S4. Distribution of gene ontology (GO) functional terms of the turbot (*S. maximus*) proteome.







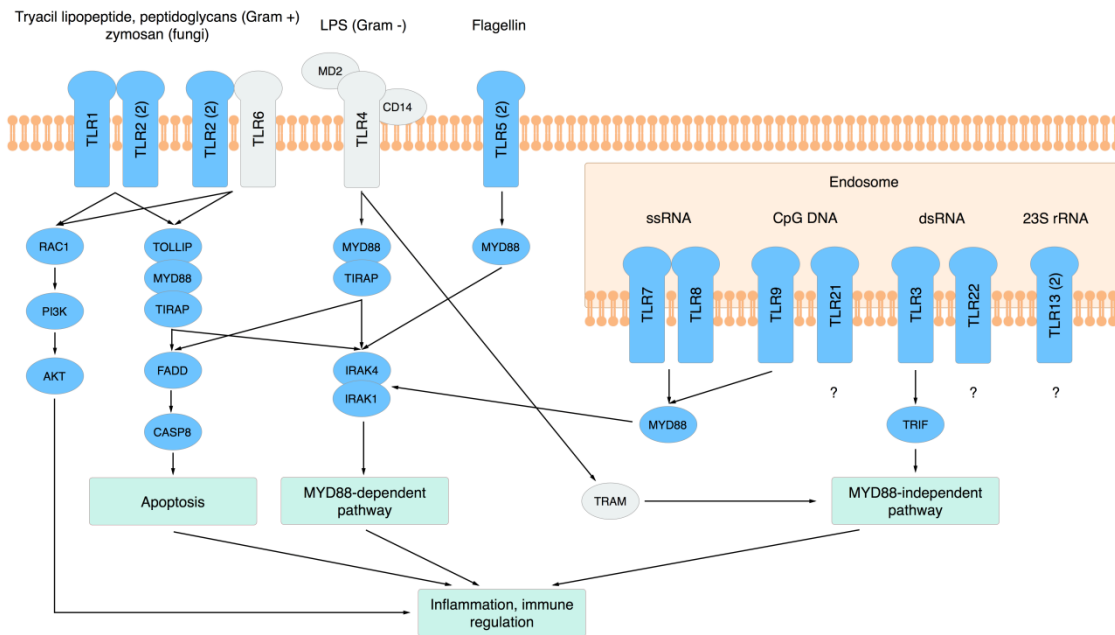
Supplementary Fig. S5. Microreorganizations in the turbot (*S. maximus*) genome when compared with the genomes of the two closest fish species, stickleback (*G. aculeatus*) and tongue sole (*C. semilaevis*). The sequences of the 10 biggest scaffolds from turbot genome were compared (E-100) against the genomes of the closest species. Positions within scaffolds or chromosomes are indicated in bp.



Supplementary Fig. S6. Circle diagram showing the syntenic pattern within the turbot (*S. maximus*) genome from paralogous relationships.

| Turbot LG | LG01 | LG02 | LG03 | LG04 | LG05 | LG06 | LG07 | LG08+18 | LG09 | LG10 | LG11 | LG12 | LG13 | LG14 | LG15 | LG16 | LG17 | LG19 | LG20 | LG21+24 | LG22 | LG23 | |
|-----------|------|------|------|------|------|------|------|---------|------|------|------|------|------|------|------|------|------|------|------|---------|------|------|--|
| LG01 | | | | | | | | | | | | | | | | | | | | | | | |
| LG02 | 12 | | | | | | | | | | | | | | | | | | | | | | |
| LG03 | 9 | 1 | | | | | | | | | | | | | | | | | | | | | |
| LG04 | 3 | 6 | | | | | | | | | | | | | | | | | | | | | |
| LG05 | 73 | 8 | 2 | 3 | | | | | | | | | | | | | | | | | | | |
| LG06 | 1 | 3 | | 48 | | | | | | | | | | | | | | | | | | | |
| LG07 | 1 | 2 | 3 | 4 | 1 | | | | | | | | | | | | | | | | | | |
| LG08+18 | 1 | 1 | | 3 | 3 | 5 | 25 | | | | | | | | | | | | | | | | |
| LG09 | 1 | 6 | 1 | | 4 | 3 | 22 | 1 | | | | | | | | | | | | | | | |
| LG10 | 8 | 1 | | 1 | 1 | 2 | | | 1 | | | | | | | | | | | | | | |
| LG11 | 2 | 1 | | | | 2 | 1 | 1 | | 93 | | | | | | | | | | | | | |
| LG12 | 3 | 1 | 3 | 3 | 3 | 1 | 2 | 9 | 3 | | | | | | | | | | | | | | |
| LG13 | 2 | | 1 | | | | | | 78 | | 1 | 3 | | | | | | | | | | | |
| LG14 | 5 | 1 | | 2 | 7 | 1 | 1 | 1 | 2 | 1 | | 73 | | | | | | | | | | | |
| LG15 | 12 | | 3 | 6 | 1 | 2 | 3 | 24 | | | | 1 | | 1 | | | | | | | | | |
| LG16 | 2 | 1 | | 1 | | 35 | | 3 | 10 | 4 | 8 | | 1 | | 1 | | | | | | | | |
| LG17 | 21 | | 2 | 1 | | | 1 | 6 | 2 | 2 | | | 1 | | | 1 | | | | | | | |
| LG19 | 1 | 2 | 3 | | | 1 | | 6 | 11 | | 1 | 2 | | 1 | | 1 | | | | | | | |
| LG20 | 12 | 38 | 7 | 12 | | | 1 | 1 | 2 | 3 | 2 | | 1 | | 2 | 1 | 3 | 3 | | | | | |
| LG21+24 | 7 | 19 | 3 | | | | 2 | 5 | 1 | 2 | | | 60 | | 1 | 1 | | 15 | 1 | | | | |
| LG22 | | 47 | | 2 | 1 | 1 | | 2 | | 3 | | | 2 | 6 | | | | 1 | | | | | |
| LG23 | 1 | 5 | 52 | 8 | | 2 | | 2 | | 3 | | | 16 | 3 | | | 1 | 5 | 1 | | | 4 | |

Supplementary Fig. S7. Oxford plot showing paralogous relationships in the turbot (*S. maximus*) genome. In yellow the highest figures for each linkage group (LG).



Supplementary Fig. S8. Toll-like receptor pathways inferred from genome information of turbot (*S. maximus*). Although most of the mammalian TLRs were detected in the turbot genome, three of them (*tlr4*, *tlr6* and *tlr10*) are apparently missing. In agreement with published teleost genomes to date (with the exception of Cyprinidae), *tlr4* seems not to exist in teleost. The genes encoding its accessory molecules *cd14* and *md2*, constituting the lipopolysaccharide receptor complex *tlr4/cd14/md2*, are also missing. These observations suggest an alternative LPS-recognition pathway in fish.

Supplementary Tables

Supplementary Table S1. List of species used in the phylome reconstruction. TaxId: taxon identification code.

| TaxID | Species name | Source of protein coding sequences |
|--------------|-------------------------------|---|
| 7868 | <i>Callorhinchus milii</i> | Ensembl_75 |
| 7897 | <i>Latimeria chalumnae</i> | Ensembl_69 |
| 7918 | <i>Lepisosteus oculatus</i> | Ensembl_75 |
| 7955 | <i>Danio rerio</i> | Quest for Orthologs 2012_05 |
| 7994 | <i>Astyanax mexicanus</i> | Ensembl_75 |
| 8022 | <i>Oncorhynchus mykiss</i> | Genoscope |
| 8049 | <i>Gadus morhua</i> | Ensembl_75 |
| 8083 | <i>Xiphophorus maculatus</i> | Ensembl_75 |
| 8090 | <i>Oryzias latipes</i> | Ensembl_75 |
| 8128 | <i>Oreochromis niloticus</i> | Ensembl_75 |
| 8364 | <i>Xenopus tropicalis</i> | Quest for Orthologs 2012_05 |
| 10090 | <i>Mus musculus</i> | Quest for Orthologs 2012_05 |
| 28377 | <i>Anolis carolinensis</i> | Ensembl_69 |
| 31033 | <i>Takifugu rubripes</i> | Ensembl_75 |
| 52904 | <i>Scophthalmus maximus</i> | Sequencing project |
| 69293 | <i>Gasterosteus aculeatus</i> | Ensembl_75 |
| 99883 | <i>Tetraodon nigroviridis</i> | Ensembl_75 |
| 244447 | <i>Cynoglossus semilaevis</i> | NCBI |

Supplementary Table S2. Characteristics of the libraries constructed for assembling the turbot (*S. maximus*) genome.

| Type | Insert size | Read length | Number of clones | Number of reads | Number of bases | Sequencing depth |
|-------|-------------|-------------|------------------|---------------------|-----------------|------------------|
| PE | 200 bp | 150 | | 142x10 ⁶ | 42.5 Gb | 60x |
| PE | 500 bp | 150 | | 142*10 ⁶ | 42.5 Gb | 60x |
| MP | 3.0 kb | 100 | | 145*10 ⁶ | 29.0 Gb | 41x |
| MP | 5.0 kb | 100 | | 164*10 ⁶ | 33.0 Gb | 47x |
| FE | 40 kb | 100 | 140,000 | 60*10 ⁶ | 12.0 Gb | 17x |
| Total | | | | 653*10 ⁶ | 159 Gb | 219x |

Supplementary Table S3. Summarized (a) and detailed (b) description of TE-derived sequence and other simple repeats in the turbot (*S. maximus*) genome. N.A.- Not available. Superfamilies contributing < 1kb of genomic sequence were not included.

a

| Class | Order | Superfamily | % genome |
|------------------|----------|----------------|----------|
| RTs | | | 2.29 |
| | DIRS | | 0.05 |
| | LINE | | 1.21 |
| | | L2 | 0.58 |
| | | L1 | 0.20 |
| | | RTE | 0.17 |
| | LTR | | 0.62 |
| | | Gypsy | 0.34 |
| | | ERV | 0.21 |
| | PLE | | 0.03 |
| | SINE | | 0.37 |
| DNA | | | 2.56 |
| | Helitron | Helitron | 0.10 |
| | TIR | | 2.46 |
| | | hAT | 0.47 |
| | | Tc1-Mariner | 0.36 |
| | | MITEs | 1.33 |
| Unclassified | | | 0.15 |
| Total TE-derived | | | 5.0 |
| Othermotifs | | | |
| | | SmallRNAs | 0.03 |
| | | Satellites | 0.13 |
| | | Simple repeats | 3.01 |
| | | Lowcomplexity | 0.35 |

b

| Class | Order | Clade/Superfamily | No. fragments | Total length (kbp) | Genome fraction (%) |
|--------------|--------------|--------------------------|----------------------|---------------------------|----------------------------|
| RTs | | | 76621 | 12136 | 2.29 |
| | DIRS | | 2231 | 274 | 0.05 |
| | | Ngaro | 1344 | 154 | 0.03 |
| | | DIRS | 887 | 120 | 0.02 |
| | LINE | | 28674 | 6436 | 1.21 |
| | | L2 | 14588 | 3055 | 0.58 |
| | | L1 | 5290 | 1051 | 0.20 |
| | | RTE | 3801 | 908 | 0.17 |
| | LTR | | 28206 | 3291 | 0.62 |
| | | Gypsy | 9027 | 1781 | 0.34 |
| | | ERV1 | 8540 | 690 | 0.13 |
| | | ERVK | 6018 | 389 | 0.07 |
| | | Pao | 1283 | 256 | 0.05 |
| | | Copia | 96 | 20 | 0.00 |
| | | ERVL | 332 | 19 | 0.00 |
| | | ERV4 | 200 | 12 | 0.00 |
| | | Ginger | 32 | 5 | 0.00 |
| | | ERVL-MaLR | 32 | 2 | 0.00 |
| | PLE | Penelope | 1613 | 183 | 0.03 |
| | SINE | | 15897 | 1952 | 0.01 |
| | | tRNA-V | 6438 | 1175 | 0.22 |
| | | MIR | 3990 | 458 | 0.09 |
| | | tRNA-Core | 3384 | 441 | 0.08 |
| | | Mermaid | 642 | 55 | 0.01 |
| | | L2 | 875 | 50 | 0.01 |
| | | tRNA | 606 | 45 | 0.01 |
| | | tRNA-Core-L2 | 606 | 45 | 0.01 |
| | | tRNA-V-CR1 | 373 | 37 | 0.01 |
| | | 5S-Deu-L2 | 358 | 23 | 0.00 |
| | | 5S-Sauria-RTE | 135 | 15 | 0.00 |
| | | tRNA-V-Core-L2 | 122 | 13 | 0.00 |
| | | tRNA-L2 | 148 | 8 | 0.00 |
| | | tRNA-C | 126 | 7 | 0.00 |
| | | B4 | 63 | 4 | 0.00 |
| | | ID | 43 | 2 | 0.00 |
| | | tRNA-Deu-L2 | 35 | 2 | 0.00 |
| | | 7SL | 7 | 1 | 0.00 |
| | | tRNA-RTE | 22 | 1 | 0.00 |
| DNA | | | N.A. | 13557 | 2.56 |

| | | | | |
|---------------------------|----------------|--------|-------|------|
| Helitron | Helitron | 5573 | 513 | 0.10 |
| TIR | | 60743 | 13041 | 2.12 |
| | hAT | 31015 | 2473 | 0.47 |
| | Tc1-Mariner | 10861 | 1931 | 0.36 |
| | EnSpm | 7360 | 605 | 0.11 |
| | Maverick | 4183 | 371 | 0.07 |
| | PIF-Harbinger | 1875 | 208 | 0.04 |
| | Kolobok-T2 | 2213 | 156 | 0.03 |
| | Dada | 2160 | 156 | 0.03 |
| | PiggyBac | 825 | 68 | 0.01 |
| | Academ | 113 | 17 | 0.00 |
| | Harbinger | 80 | 8 | 0.00 |
| | PIF-ISL2EU | 23 | 2 | 0.00 |
| | MITEs | N.A. | 7043 | 1.33 |
| | Unclassified | 9102 | 793 | 0.15 |
| Total interspersedrepeats | | N.A. | 26486 | 5.00 |
| Other motifs | SmallRNAs | 2065 | 156 | 0.03 |
| | Satellites | 5432 | 664 | 0.13 |
| | Simple repeats | 358944 | 15954 | 3.01 |
| | Lowcomplexity | 33492 | 1860 | 0.35 |

Supplementary Table S7. Functional annotation sources for the turbot (*S. maximus*) protein-coding gene annotation.

| InterPro member database | Number of proteins |
|---------------------------------|---------------------------|
| PANTHER | 22,132 |
| Pfam | 21,247 |
| SUPERFAMILY | 17,477 |
| Gene3D | 16,659 |
| ProSiteProfiles | 12,010 |
| SMART | 11,279 |
| ProSitePatterns | 7,260 |
| PRINTS | 6,466 |
| Coils | 5,892 |
| TIGRFAM | 1,570 |
| PIRSF | 1,527 |
| Hamap | 472 |

Supplementary Methods

Genome Sequencing

Genomic DNA (2 µg) was sheared on a Covaris™ E220 and size selected on 2% agarose gel to obtain two insert sizes of 480-770 bp and 220-430 bp. The size selected DNA was end-repaired, adenylated, and ligated to Illumina specific indexed paired-end adaptors. Each library was run multiplexed on the GAIIx platform in 2x151 bp read length runs according to standard Illumina operation procedures. Primary data analysis was carried out with the standard Illumina pipeline. A total of 284 million paired end reads (> 85 Gb of raw sequence or 120x coverage) were produced.

Two mate pair (MP) libraries, with 3 and 5 kb fragment sizes, were constructed according to a modified Illumina protocol incorporating a biotinylated 454 linker at the junction. The resulting libraries were run on the HiSeq2000 platform in 2x101 bp read length runs as above. In total, 145 million PE reads (29 Gb, 41x GeC) and 164 million PE reads (33 Gb, 47x GeC) of raw sequence were produced, respectively. Post-processing of sequence reads involved trimming of the linker sequence. Only pairs for which at least one mate was trimmed (i.e. contained the linker and was thus a true MP and not PE contamination) were kept for scaffolding.

A fosmid library of 140,000 clones was constructed (CIB-CSIC) in the pNGS vector (Lucigen Corp.). The DNA was processed for end-sequencing (4-cutter digest, intramolecular ligation, PCR amplification of truncated insert including standard Illumina adaptors) according to the Lucigen protocol and the resulting library was run on the HiSeq2000 in PE mode, 2*101+7 bp, in one sequencing lane following Illumina instructions for the custom recipe with 4 initial dark cycles in order to overcome possible sequencing errors due to the presence of leftover of the restriction site situated after the Illumina sequencing primer position. Primary data analysis was carried out with the standard Illumina pipeline. A total of 60 million paired end reads (>12 Gb raw data) were produced.

To estimate the genome size we performed an analysis of the kmer content of the genome. Using the software Jellyfish v1.1.10¹, 17mers were extracted from the WGS PE reads and unique kmers were counted and plotted according to kmer depth (multiplicity).

Genome Assembly

Paired end reads were first filtered for contaminating sequences (phiX, *Escherichia coli* and other vector sequences) using GEM² with -m 0.02 (2% mismatches). Then, reads were assembled into unitigs using ABySS v1.3.5³ with parameters: -s 300 -n 8 -k 96 -q 15. The unitigs were removed of contaminating sequences again (using BLAST+⁴ and custom scripts), the ends trimmed by 50 bp and then subjected to a misassembly detection routine that detects potential misassemblies by inconsistency with the 200 bp and 500 bp PE reads. Inconsistent segments were removed from the contigs, leaving only consistent contigs, which were then scaffolded with SSPACE⁵ as follows. The 200 and 500 bp fragment size PE libraries were trimmed to 75 bp and mapped with GEM with parameters: -m 0.04 --unique-mapping. The resulting mappings were converted to tab format files

for input to SSPACE, which was run with the parameters: -x 0 -z 0 -k 5 -a 0.7 -n 15 -T 1 -p 1. The library insert sizes provided as parameters to SSPACE were 215 and 480 +/- 33%. The scaffolded assembly was gap-filled using GapFiller⁶ with the parameters: -m 30 -o 2 -r 0.6 -n 10 -d 100 -t 15 -g 0 -T 8 -i 5. The assembly was then scaffolded with the two mate pair libraries using ABySS v1.3.4 with parameters: -n 5 -s 200 -N 10 -S 200-2000 -k 96 -l 36 -q 10. Again the assembly was gap-filled as above but with the addition of the two mate pair libraries 3 and 5 kb +/- 33% in RF orientation. Scaffolds were then broken at gaps greater than 6 kb in length (resulting from scaffolding with ABySS) and then the fosmid end library was used to do a final scaffolding using SSPACE with the same settings as before.

Comparative mapping

Genomic sequences containing the microsatellite/SNP loci used for linkage mapping⁷ were searched against the turbot genome using the BLAST algorithm and the best hit with E-value <1e-20 was retained. This approach enabled us to anchor a set of scaffolds covering a large fraction of the genome to the turbot map. This correspondence was used as a reference to refine the relationship between physical and genetic map in turbot when a scaffold (in particular the larger ones) matched to more than one LG. The relationship between genetic and physic maps was drawn with MAPCHART 2.2⁸.

Then, to identify syntenic patterns with closely related species within Percomorpha, the sequences of orthologous genes in these anchored scaffolds were compared by NCBI-BLAST with updated versions of model fish genomes downloaded from ftp://ftp.ensembl.org: *T. nigroviridis* v.8.61, *O. latipes* v.1.61 and *C. semilaevis*. BLAST⁹ searching was performed by using an E-value threshold <1e-05^{10,11}. Orthologous relationships among species and paralagous relationships within the turbot genome were represented with circle diagrams using CIRCOS¹².

Unanchored scaffolds of length > 150 kb covering altogether more than 96% of the turbot genome were predictively assigned to turbot LGs by doing a sequence search (BLAST) of the > 10,000 orthologous genes identified against *C. semilaevis*, *T. nigroviridis* and *O. latipes* proteomes and integrating these results with the already established collinearity between turbot and fish genomes. Additionally, we performed a sequence search using BLAST of the nucleotide sequence of the same turbot scaffolds against *G. aculeatus* genome and retained only single 1:1 matches for the same analysis. In this case, we used DNA sequence due to the poorer annotation of the stickleback genome. Those scaffolds matching in the same homologous position with at least three reference species were predictively assigned to turbot LGs.

Repetitive elements

Low complexity sequences and short repetitive motifs were analyzed using DUST v.1¹³ and TRF v.4.07b¹⁴. RepeatMasker v.4.0.5¹⁵ was used to screen for interspersed repeats, using the RepBase database (v.20.40 Apr 2015; available from the Genetic Information Research Institute)¹⁶. The program was run using the WU-BLAST¹⁷ engine with default settings and a custom library including

repetitive sequences from vertebrate species only. The genome sequence was further analyzed with the *blastn* and *tblastn* programs in WU-BLAST. The whole RepBase database was used as a query with *blastn* and all hits with higher than 70% nucleotide sequence homology with any query sequence was masked. In a second run, *tblastn* was used to search the amino acid sequences of all ORFs defined in RepBase elements against the masked genome. The combined output of these two programs was processed to extract the nucleotide sequences of the putatively full-length insertions (>80% length of the closest canonical query sequence), as described elsewhere¹⁸: i) the maximum number of unique hits per element were identified; ii) hits of the same element in a single contig were joined to build the longest chain, using the Chao and Miller algorithm¹⁹; iii) all chains were sorted by contig, direction, insertion point and score, and pooled in a single file. All chains embedded in other elements and the region of the chain with lower score where two chains overlapped were removed, thus leaving only the best unique hit per subject point; and iv) TE matches with > 70% of similarity over 100 bp were filtered and retained. The MITE-Hunter program²⁰ was used to identify putative miniature inverted repeat elements (MITEs). TE-derived sequences were named according to the RepBase element sequence and grouped following a standard TE classification proposed by Wicker *et al.*²¹. All sequences were edited with Bioedit v7.0.4.1²² and aligned with MUSCLE²³, using -600 as gap penalty. The average number of substitutions per site between sequences was estimated using the Tamura-Nei model, assuming a gamma distribution of the rate of variation among sites (shape parameter = 1), with the aid of MEGA v6.06²⁴.

Protein-coding gene annotation

Transcript and protein alignment

Transcripts for assembly with PASA²⁵ were obtained as follows: first, reads from two 454 Roche rRNA-seq studies^{26,27} were aligned to the final *S. maximus* assembly, “sm5”, with GEM². Transcript models were subsequently generated using the standard Cufflinks²⁸ pipeline and then added to the PASA database. In addition, 15,559 turbot ESTs^{27,29}, 404 CDS (July 24, 2013) and 53,749 mRNAs (Jan 29, 2014) present in NCBI were also added to PASA using GMAP³⁰ as the alignment engine. All of the above transcript alignments, in total 1,947,260, were then assembled by PASA, resulting in 105,044 PASA assembled transcripts.

We aligned Percomorpha proteins present in Uniprot to the turbot genome with SPALN³¹, resulting in 1,857,695 CDS alignments.

Ab initio gene predictions

Ab initio gene predictions were performed on the sm5 assembly, which was masked for repeats by RepeatMasker¹⁵ using the custom repeat library that we constructed. Low complexity repeats were left unmasked for this purpose.

GeneID³² *ab initio* gene predictions were obtained by running GeneID v1.4 with the parameter file specific for the *Tetraodon* genus. *S. maximus* protein-coding gene annotations were also obtained using the gene prediction tool Augustus³³ v2.5.5. For this purpose we used the program's pre-existing *Homo sapiens* parameter file. GeneMark-ES³⁴ v2.3e gene predictions were obtained using its self-training mode.

Hence, GeneID, Augustus and GeneMark were subsequently used to predict genes on the repeat-masked sm5 assembly of the turbot genome made up of 16,463 scaffolds. The number of predicted gene models ranged from 28,826 (Augustus) to 187,934 (GeneMark), while GeneID predicted 39,351 genes.

Generation of consensus gene models

Evidence Modeler (EVM r2012-06-25)³⁵ was used to obtain consensus coding sequence (CDS) models using three main sources of evidence: aligned transcripts, aligned proteins, and gene predictions. EVM was run with six different sets of weights and the resulting consensus models with the best specificity and sensitivity as determined by intersection (BEDTools³⁶ intersect) with the transcript mappings were chosen for the final annotation.

The EVM models were cleaned of transposon sequence by using BLAST⁹ to search the gene models produced by EVM against the RepeatMasker database of proteins encoded by transposable elements (TEs). All of the gene models that had a full-length hit against a repeat were discarded from the annotation. Those that had only a partial match to a TE were kept but modified to remove the sequence corresponding to the transposable element.

The consensus CDS models were then updated with UTRs and alternative exons through two rounds of PASA's annotation updates. A final round of quality control was performed, fixing reading frames and intron phases, and then the resulting transcripts were clustered into genes using shared splice sites or significant sequence overlap as criteria for designation as the same gene. Systematic identifiers with the prefix "SMAX5B" were assigned to the genes, transcripts and protein products derived from them.

Support by source of evidence at the gene and exon level was determined *a posteriori* using BEDTools³⁶ intersect and multiinter programs.

Proteome functional annotation

We used InterPro³⁷, KEGG³⁸ and Blast2GO³⁹ databases for functional annotation. InterProScan v.5⁴⁰ was used to scan through all available InterPro databases, including the most important ones - PANTHER⁴¹, Pfam⁴², TIGRFAM⁴³, HAMAP⁴⁴ and SUPERFAMILY⁴⁵. BLAST search against NCBI non-redundant (NR) collection of protein sequences (release 2014-02) was used as input to the local Blast2GO software p2gpipe version 2.5.0. KEGG orthology (KO) groups were assigned by KEGG

Automatic Annotation Server (KAAS)⁴⁶ using bi-directional best hit (BBH) method against a representative gene set from 28 different species, including *D. rerio*. KO identifiers were then used to retrieve using the KEGG REST-based API service the KEGG relevant functional annotation, such as metabolic pathways and external database references. Distribution of GO terms grouped by the different functional categories was done with CateGORizer⁴⁷ by using GOSlim without top-level categories

Phylogenomics

Phylome reconstruction

Turbot phylome was reconstructed using the phylomeDB pipeline⁴⁸. For each turbot gene, a search was performed against a database containing the proteomes of the 17 selected species (Table MS5). We used an e-value threshold $<1e-05$ and a continuous overlap of 50% over the query sequence for the detection of homologues, and limited the number of hits to the closest 150 homologues per gene. Multiple sequence alignments of homologous sequences were built using three different aligners, which were used in forward and reverse orientations (MUSCLE²³, MAFFT⁴⁹ and KALIGN⁵⁰). The resulting six alignments were combined using M-COFFEE⁵¹, and then, trimAl v1.4⁵² was used to trim the alignment (consistency cut-off of 0.16667 and -gt >0.1). Subsequently, trees were constructed using PhyML v3⁵³ using the best fitting model, four rate categories with rates and fraction of invariant sites estimated from the data. Branch support was analyzed using an aLRT (approximate likelihood ratio test) non-parametric test based on a chi-square distribution.

Prediction of orthology and paralogy relationships

Paralogy and orthology predictions were analyzed based on phylogenetic evidence from the turbot phylome. ETE⁵⁴ was used to infer gene duplication and speciation events with a species overlap approach (species overlap score of 0). Orthologous genes are those who the last common ancestor is represented by a speciation event, and paralogous genes are those that diverge from duplication events⁵⁵. All trees, alignments, and information about orthology and paralogy relationships are available in phylomeDB⁵⁶ with the PhylomeID code 18.

Gene duplications

The turbot phylome was analyzed to detect genes that had undergone duplications in lineages leading to this species using a previously-described algorithm of duplication detection and dating⁵⁷. Gene enrichment was analyzed using FatiGO⁵⁸ by comparing annotations of the proteins involved in a duplication at a given age against all the others encoded in the genome.

Species tree reconstruction

A total of 389 genes with one-to-one orthologues in each studied species were selected and their trimmed alignments concatenated. We then used RAxML v7.2.6, model Protgamma⁵⁹ to derive the species tree. Bootstrap supports were calculated by creating 100 alignments using Phylip's

SeqBoot⁶⁰. Finally, we reconstructed a super-tree from all single gene trees in the turbot phylome using a gene tree parsimony strategy as implemented in duptree⁶¹.

Adaptation to benthic life

Functional enrichment analysis of paralogous genes were carried out using the KOBAS web server (<http://kobas.cbi.pku.edu.cn/help.do>). For phylogenetic analysis of selected genes, protein sequences from sequenced fishes and those corresponding to *C. semilaevis* were downloaded from ENSEMBL and the NCBI databases respectively. ClustalW⁶² using Gonnet's protein weight matrix was used to produce alignments which were then visually inspected and filtered using Guidance2⁶³. Alignments were trimmed using trimAL⁵² to remove poorly aligned regions, and phylogenetic reconstruction was performed by the neighbor joining method using the JTT aminoacid substitution model in MEGA6²⁴. Statistical support of the trees was obtained using 1,000 bootstrap replicates. The ratio Ka/Ks was calculated following the Nei-Gojobori model⁶⁴. All ambiguous positions were removed for each sequence pair. Evolutionary analyses were conducted in MEGA6²⁴.

Genetic architecture of growth, resistance to diseases and sex determination

Mining analysis of the turbot genome was performed to characterize previously detected QTL regions and to identify candidate genes influencing biological pathways related to sex, growth and disease resistance traits. A set of selected QTL markers related to these three features were located in the turbot genome using BLAST⁹. Candidate genes for growth-, sex- and disease resistance-related traits in fish and vertebrates were selected based on previous reports and our own data and then mapped to the turbot genome taking QTL markers as a reference (Supplementary Tables 12 and 13). For this, we used the relationship between physical and genetic maps established in our work (Supplementary Table 3; Supplementary Fig. 2). Gene lists were extracted from conservative 2-Mb windows surrounding each selected QTL for all traits (closest associated marker position \pm 1 Mb), assuming an average genomic relationship of 0.5 Mb/cM¹¹ (Supplementary Table 14). To identify suggestive candidate genes and pathways within the extracted gene lists, we performed Gene ontology (GO)⁶⁵ enrichment analysis using BLAST2GO⁶⁶ and KEGG³⁸ pathway enrichment using KOBAS⁶⁷, against the turbot proteome (22,751 genes) as background (Supplementary Table 15). Enrichment probability values were adjusted for multiple testing (False Discovery Rate (FDR)-corrected P-values < 0.05). All analyses were focused on major QTL, either associated with sex determination, growth or disease resistance, but also on overlapping QTL for different traits⁶⁸ (Supplementary Table 16). A large set of QTL markers related to sex determination-SD (4), growth traits (12 for body weight-BW, 9 for length-L and 6 for Fulton's factor-FK), and disease resistance (7 for *Aeromonas salmonicida*-AS, 19 for *Philasterides dicentrarchi*-PD and 16 for Viral Hemorrhagic Septicaemia Virus-VHSV) were physically mapped to scaffolds of the turbot genome and mined. Selected candidate genes identified across QTL regions and traits were represented into the turbot genetic map⁷ using Mapchart 2.2⁸.

Supplementary Results Genetic architecture of sex determination, growth and resistance to diseases

The highest concentration of candidate genes related to sex determination was detected on LG5 (12 genes), LG6 (5), LG8+18 (7) and LG21+24 (9). Additionally, 12 genes were found on LG1, not previously related to SD. The mining strategy around SD-QTL revealed 23 additional genes involved in sex differentiation: *lhx9*, *bcar3* and *dmrt2b* on LG5; *cyp19a1* and *cyp11a* on LG6; *ar*, *lhx1* and *foxo1* at LG8; and *ryr2a*, *sox17*, *sox8a*, *sox9a* and *rara* on LG21+24 (Supplementary Table S15A).

Genetic factors (Supplementary Table S15B) and functional enrichment related to regulation of muscle development and growth (Supplementary Table S16B) were found at specific QTL. Different pathways related to L (mucin O-glycan biosynthesis) and BW (arachidonic acid metabolism and taste transduction) were identified at different QTL supporting distinct genetic mechanisms underlying growth traits. The most significant pathway involved in muscle differentiation and lipid metabolism was extracellular matrix communication (ECM)-receptor interaction (*gh1*, *lamb1*, *itga11*), associated to a L-QTL (LG6). ECM has been associated with growth in fish and other vertebrates^{69,70}. Pathway enrichment for taste transduction and arachidonic acid metabolism was detected within a BW-QTL (LG11), which includes candidate genes (*tas1r3* and *gpx1*) associated with lipid metabolism and growth effects of aquaculture diets⁷¹. Non-homologous end-joining and mucin type O-glycan biosynthesis pathways found in L-QTL (LG17 and LG20, respectively), also pinpoint candidate genes (*fen1*, *galnt3*, *galnt5*, *galnt13*) previously associated to differential growth in vertebrates^{72,73}.

Relevant immune genes were identified within VHS-, AS-, and PD-QTL (Supplementary Table S15C). Virus defense and clearance related genes were detected within VHS-QTL: i) genes implicated in T-cell proliferation, differentiation, maturation or activation on several LGs (*nlrc3*, *malt1*, *vav*, *nfkbid*, *irf4*); ii) genes involved in the blood coagulation cascade (*thpo*, *lrp8*, *f3* at LG1; *clec3b*, *thbs1*, *plgrkt*, *ptgds*, *plek* on LG2; *pip5k1c*, *bsg* at LG5; *vwf*, *cd9*, *calu*, *slc7a5*, *ranbp10* at LG6; and *plscr1*, *serpinb6* on LG17) likely related to the important hemorrhagic activity of this virus; and iii) genes related to iron homeostasis and scavenging⁷⁴, like some transferrin related genes (*tf*, *tfrc*; LG1) and hepcidin (*hamp*; LG2). In fact, hepcidin was previously associated with resistance to VHSV in turbot families⁷⁵. Typical antibacterial and bacterial recognition genes were found around AS-QTL: peptidoglycan recognition protein 1 (*pglyrp1*), g-type lysozyme (*lyg1*) and macrophage mannose receptor 1-like (*mrc1*) (tightly linked on LG9). Finally, important immune genes were also detected at PD-QTL: interleukin-17 (*il17f*; LG16) and its receptor (*il17re*; LG23); galectin-8 (*lgals8*; LG3), also reported as candidate for ISAV-resistance in *Salmo salar*⁷⁶; perforin-1 precursor (*prf1*; LG9), related to a broad antimicrobial spectrum⁷⁷; and two toll-like receptors (TLR) (*tlr2* and *tlr3*; LG9). The activity of TLRs in response to parasitic infections has been widely documented⁷⁸.

Finally, we detected genes and functions underlying genomic regions associated to different traits in turbot (Supplementary Table S17; Fig. 5). Among them, we identified in an overlapping BW- and VHSV-QTL region (LG1) genes associated with growth like *myod1*, a gene which plays a central role in the development of the skeletal muscle in fish and vertebrates⁷⁹ and recently associated with meat quality in rainbow trout^{80,81}, and genes involved in the TNF signaling pathway (*tab2*) which are associated with immune response in fish⁸². Also, the BW- and BL-QTL on LG5 include a sex-associated marker (ScmM1)⁸³ linked to *tgfbr3* and *sox14*, genes related to reproduction and cell proliferation in fish⁸⁴. The relationship between sex and growth in fish has been widely documented and it is of special interest in turbot considering its sexual dimorphism in growth⁸⁵.

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