Aquaculture 455 (2016) 8–21

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

Characterization and refinement of growth related quantitative trait loci in European sea bass (*Dicentrarchus labrax*) using a comparative approach

Bruno Louro [a,b,*], Heiner Kuhl [c], Mbaye Tine [d,1], Dirk-Jan de Koning [b,2], Costas Batargias [e], Filip A.M. Volckaert [f], Richard Reinhardt [d], Adelino V.M. Canario [a], Deborah M. Power [a]

[a] Centre for Marine Sciences, CCMAR-CIMAR Laboratório Associado, Universidade do Algarve, Campus do Gambelas, 8005-139, Faro, Portugal
[b] The Roslin Institute and R(D)SVS, University of Edinburgh, The Roslin Building, Easter Bush, Midlothian, EH25 9RG, United Kingdom
[c] MPI Molecular Genetics, Ihnestrasse 63-73, D-14195 Berlin-Dahlem, Germany
[d] Max-Planck-Institut für Pflanzenzüchtungsforschung Carl-von-Linné-Weg 10, D-50829 Köln, Germany
[e] Laboratory of Applied Fish Genetics and Fish Breeding, Department of Aquaculture and Fisheries Management, Technological Educational Institute of Messolonghi, Nea Ktiria, 30200 Messolonghi, Greece
[f] Laboratory of Biodiversity and Evolutionary Genomics, University of Leuven, Ch. Deberiotstraat 32, B-3000 Leuven, Belgium

ARTICLE INFO

Article history:
Received 27 April 2015
Received in revised form 6 January 2016
Accepted 7 January 2016
Available online 11 January 2016

Keywords:
Comparative genomics
Convergent mapping
Fish
Growth QTL
Linkage map
Marker assisted selection

ABSTRACT

The identification of genetic markers for traits of interest for aquaculture, such as growth, is an important step for the establishment of breeding programmes. As more genomic information becomes available the possibility of applying comparative genomics to identify and refine quantitative trait locus (QTLs) and potentially identify candidate genes responsible for the QTL effect may accelerate genetic improvement in established and new aquaculture species. Here we report such an approach on growth related traits in the European sea bass (*Dicentrarchus labrax*), an important species for European aquaculture. A genetic map was generated with markers targeted to previously identified QTL for growth which reduced distance and improved resolution in these regions. A total of 36 significant QTLs were identified when morphometric traits were considered individually in maternal half sibs, paternal half sibs and sib-pair analysis. Twenty seven new markers targeted to the growth QTLs, obtained by comparative mapping, reduced the average distance between markers from 23.4, 9.1, and 5.8 cM in the previous map to 3.4, 2.2, and 5.2 cM, on linkage group (LG) LG4, LG6 and LG15 respectively. Lists of genes embedded in the QTL – 591 genes in LG4, 234 genes in LG6 and 450 genes in LG15 – were obtained from the European sea bass genome. Comparative mapping revealed conserved gene synteny across teleost fishes. Functional protein association network analysis with the gene products of the 3 linkage groups revealed a large global association network including 42 gene products. Strikingly the association network was populated with genes of known biological importance for growth and body weight in terrestrial farm animals, such as elements of the signaling pathways for Jak–STAT, MAPK, adipocytokine and insulin, growth hormone, IGFI and II. This study demonstrates the feasibility of a comparative genomics combined with functional gene annotation to refine the resolution of QTL and the establishment of hypothesis to accelerate discovery of putative responsible genes.

Statement of relevance: This study demonstrates the feasibility of a comparative genomics approach, combined with functional annotation to refine the resolution of QTL and establishment of hypothesis to accelerate discovery of candidate genes. As production of genomic data is becoming more accessible, the implementation of this strategy will rapidly and efficiently provide the tools required for genetic selection in new candidate aquaculture species.

1. Introduction

The impact of genomics on fish aquaculture remains limited probably as a consequence of the relatively recent adoption of intensive aquaculture, the diversity of species cultured and non-uniform nature of the industry (Power et al., 2011). Nonetheless, successful breeding programmes exist with the most advanced being for salmonids, such as Atlantic salmon (*Salmo salar*), and it has contributed to an increased

* Corresponding author at: Centre for Marine Sciences, CCMAR-CIMAR Laboratório Associado, Universidade do Algarve, Campus do Gambelas, 8005-139 Faro, Portugal.
E-mail address: blouro@ualg.pt (B. Louro).
[1] Current address: The Molecular Zoology Laboratory, University of Johannesburg, Kingsway Campus, Auckland Park 2006, South Africa.
[2] Current address: Swedish University of Agricultural Sciences, Department of Animal Breeding and Genetics, 750 07 Uppsala, Sweden.

http://dx.doi.org/10.1016/j.aquaculture.2016.01.004
0044-8486/

production of global significance, with large and sustained genetic gain in economically important traits, such as increased resistance to infectious pancreatic necrosis trough marker assisted selection (MAS) implementation (Fjalestad et al., 2003; Houston et al., 2008). Salmon breeding companies have now the resources to start implementing genomic selection (GS), a high density SNP chip (Houston et al., 2014) to initially genotype the reference population and confirm the expected potential of genetic gain shown in the simulation studies (Ødegård et al., 2014).

The European sea bass (*Dicentrarchus labrax* L.; Dicentrarchidae), heretoforth sea bass, is a gonochoristic marine teleost fish, distributed in temperate European coastal areas of the Atlantic Ocean and Mediterranean Sea. Its intensive exploitation as an aquaculture species is relatively recent and production is concentrated predominantly in the Mediterranean basin. Total production was 112,183 tons in 2009, with a market value of 500 million Euros (FAO, 2010). Current developments of selective breeding of European sea bass are being performed in several private companies and respective results are confidential. On the other hand, ongoing Aquaexcel2020 European project with results open to the scientific and producer communities, aim to implement and characterize new breeding lines for a stable improvement of production traits.

Several growth related QTLs were identified in two previous studies which analyzed sea bass body weight, morphometric and stress response traits (Chatziplis et al., 2007; Massault et al., 2010). Heritability values for morphometric growth related traits in sea bass are high (0.52–0.68) (Saillant et al., 2006; Volckaert et al., 2012), suggesting that there is considerable potential for increased growth rates if such traits can be selected during breeding. High heritability also suggests that genetic gain can be obtained by mass selection (Vandeputte et al., 2009), contrary to others traits of interest which require to sacrifice the fish for phenotyping, such as fillet quality or disease resistance. Future MAS or GS will aim at those more appropriate traits with lower heritability or requirement to sacrifice the fish, but should also have a multiple trait selection approach. The selection index should include those traits and growth related traits to ensure positive correlations of genetic gain, since growth is still the most desired trait in the producer point of view. In vertebrates the major regulatory factors of growth are conserved in the growth hormone/insulin-like growth factor-I (GH/IGF-I) axis. The importance of these factors in growth physiology is evident from observations of vertebrates carrying mutations or that are transgenic (Du et al., 1992; Hull and Harvey, 1999).

A recent approach in mammals to decipher QTL is to construct concordant QTL maps since orthologous genes are expected to have conserved function in biological and biochemical traits. Thus, those genes with a quantitative effect in one species may also be important in another species (Jiang et al., 2007; Kim et al., 2004; Rothschild et al., 2007). The construction of concordant maps for teleosts may prove to be a useful approach in aquaculture where many different species of fish are cultivated but relatively few have extensive molecular resources. However, such an approach is likely to be more challenging than in terrestrial animals because of their richer evolutionary diversity (Canario et al., 2008). Additional complexity in map construction may arise from the teleost specific whole genome duplication (Jaillon et al., 2004) and selective gene loss and divergence of duplicate gene function which presumably may modify selective pressures (Brunet et al., 2006), family specific partial chromosome duplications/rearrangements (Jaillon et al., 2004), and differing evolutionary pressures on the genome arising from adaptation to a specific ecological niche (Hoegg et al., 2004). Nonetheless there is evidence that the construction of concordant QTL maps and comparison of growth QTL genome regions at the taxonomic level of Family or Order in teleost fish may be feasible, as exemplified for the sea bass and gilthead sea bream (*Sparus auratus*) by Sarropoulou et al. (2008).

Several recent studies have used a comparative approach to better characterize identified QTL in aquaculture species. Similar to mammals they revealed the existence of concordant chromosomal regions. For example, co-segregating QTL for body growth and sex determination in the gilthead sea bream were established using synteny between the genome of sea bass and Asian sea bass (*Lates calcarifer*) (Loukovitis et al., 2011). A comparative approach between growth QTL identified in rainbow trout (*Oncorhynchus mykiss*), Arctic char (*Salvelinus alpinus*) and Atlantic salmon revealed several homologous linkage groups (Moghadam et al., 2007; Reid et al., 2005; Wringe et al., 2010). Moreover, the comparison of a genomic region from the spotted green pufferfish (*Tetraodon nigroviridis*) microsyntenic with a growth related QTL from turbot (*Scophthalmus maximus*) revealed a number of genes with ontology classification (GO) for growth regulation and cell proliferation (Sanchez-Molano et al., 2011).

In addition to the identified QTL, several genetic and genomic resources have also been developed in European sea bass. They include a medium dense linkage map with 190 microsatellites, 176 AFLP (amplified fragment length polymorphisms) and two single nucleotide polymorphisms (SNP) (Chistiakov et al., 2008) to which a further 35 SNP were added (Souche et al., in press), a 30,000 plus EST collection representing almost 18,000 unigenes (Louro et al., 2010), a comparative BAC (bacterial artificial chromosome) map (Kuhl et al., 2010), a radiation hybrid (RH) map (Guyon et al., 2010), and recently the fully assembled genome (Tine et al., 2014). The availability and status of current molecular tools and genomic data for European sea bass give an opportunity to apply genomics to assist selective breeding.

Taking into consideration the conservation of growth regulatory mechanisms between vertebrates and the synteny among related teleosts, here we take a comparative approach to narrow down the list of candidate genes located in growth QTL regions of the sea bass genome. Comparative genomics was used to link sea bass QTL and linkage map markers to the three-spined stickleback (*Gasterosteus aculeatus*) and green spotted puffer (*Tetraodon nigroviridis*) assembled genomes. To increase the resolution of the sea bass QTL map and identify putative markers targeted to QTL regions, candidate genome regions were functionally annotated. A complimentary approach was to conduct pathway analysis using genes from sea bass QTL regions to assess whether these regions were enriched with genes of functional relevance for growth. These approaches were taken to fulfill the two main objectives of this study: first to increase the resolution of previously identified sea bass growth related QTLs and second to obtain a short-list of candidate genes.

2. Materials and methods

2.1. Growth related genes bibliographic search

A bibliographic search was performed using the bibliosphere module of the Genomatix software suite (Genomatix Software GmbH, Munich, Germany). The human growth hormone (GH1) was used as keystone gene in the initial analysis of the bibliographic search of growth related genes. All co-citations were checked for functional relation in the hand-curated knowledgebase of Protein–Protein NetPro™ (http://www.interactions.net.in). Gene sequences were retrieved from the NCBI GenBank database, and those genes from species phylogenetically closer to sea bass were analyzed further.

2.2. Comparative genomic maps of QTL regions

All genetic marker sequences from the sea bass linkage map and candidate gene protein sequences identified previously were queried using, respectively, BLASTN and TBLASTN algorithms (Altschul et al., 1990), against the *G. aculeatus* and *T. nigroviridis* genome masked databases in Ensembl BLASTView (http://www.ensembl.org/Multi/blastview). The BLASTN query was performed with distant homology parameters (−E:10 –B:100 –filter:dust –RepeatMasker –W:9 –M:1 –N: 1 –Q:2 –R:1) for high sensitivity. The cut-off for positive hits was taken at *E*-value of $5e^{-3}$ for at least one hit per chromosome. TBLASTN

was used with near-exact matches parameters (−E:10^{-5} −B:100 –filter:seg –W:4 –hitdist:40 –matrix:BLOSUM80 –Q:3 –R:3 –T:16) for low sensitivity. The annotated genomic region in both *G. aculeatus* and *T. nigroviridis* genomes which contained orthologs of sea bass candidate genes and linkage markers was used as input data in the MAPCHART software (Voorrips, 2002) to design the "candidate genes comparative map."

2.3. Identification of QTL-related genomic and mRNA sequences

The sequences of linkage markers from the sea bass linkage groups (1, 4, 6 and 15) positioned within the sea bass growth related QTL were queried against the *G. aculeatus* genome using Ensembl BLASTView (www.ensembl.org). The BLASTN algorithm was used with distant homology parameters (−E:10 –B:100 –filter:dust –RepeatMasker –W:9 –M:1 –N:-1 –Q:2 –R:1) for high sensitivity and a cut-off *E*-value of $5e^{-3}$. The MartView tool from the BioMart system (www.biomart.org) was used to characterize the *G. aculeatus* genomic region orthologous to the sea bass QTL. This allowed the retrieval of the features as Ensembl gene ID plus the respective Gene Start (bp), Gene End (bp), and strand from the genomic region in question.

Genomic and mRNA databases were created with available sea bass genomic sequences (WGS and BAC ends) (Kuhl et al., 2010) and expressed sequence tags (ESTs) (Louro et al., 2010) using the formatDB executable within the NCBI blast-2.2.20 standalone pack (ftp://ftp.ncbi.nlm.nih.gov/blast/executables/release/2.2.20/). The sea bass genomic database was composed of 147,763 sequences and 157,675,311 letters. The *G. aculeatus* regions homologous to the flanking markers of sea bass QTL were queried against the sea bass genomic database using BLASTN (−e 10^{-5} −T F −v 10 −b 10) to identify the relevant genomic region in sea bass. All the identified genomic sequences were retrieved. If BAC ends from a unique sea bass BAC clone were both present in reverse orientation with a significant hit in the blast output, the genomic coordinates of the hits were annotated. The sequence of the *G. aculeatus* genomic region orthologous to the sea bass QTL was used to search BAC end sequences and identify BAC clones covering the QTL and to design a BAC tiling map of the genomic region.

The sea bass transcript database was formed of 17,716 sequences and 12,381,139 letters. The identified and retrieved *G. aculeatus* genes within the syntenic regions with the markers flanking the sea bass QTL were blasted against the sea bass transcript database using BLASTN (−e 10^{-5} −T F −v 10 −b 10). This BLASTN output of the available sea bass transcript sequences was used to identify genes in the target QTL. The sequences of all the identified transcripts were retrieved and sequence ID annotated in a table containing the corresponding genes identified in the orthologous region of the *G. aculeatus* genome.

2.4. Marker development and genotyping

All the identified genomic sequences were scanned for microsatellites using the FASTPCR software repeat search (default parameters) (Kalendar et al., 2009). Sequences containing microsatellites were BLAST queried against the whole *G. aculeatus* genome, in order to eliminate sequences with high similarity to multiple genomic regions. This approach ensured that duplicate genes present in multiple chromosomes were excluded from the analysis. The BLASTN algorithm was used to interrogate the target genomes (masked) database and distant homology parameters (−E:10 –B:100 –filter:dust –RepeatMasker –W:9 –M:1 –N:1 –Q:2 –R:1) were used to maximize sensitivity. The cut-off for a positive hit was taken at an *E*-value $5e^{-3}$. The sea bass genomic sequences that were restricted to sea bass QTL and contained microsatellites were selected for primer design. Criteria for selection included the presence of loci spread over as wide a range as possible of the target chromosome regions, potential for microsatellite development, potential for polymorphism, and primer design conditions.

PCR primers were designed to flank microsatellites of interest using PRIMER3 (http://frodo.wi.mit.edu/) (Rozen and Skaletsky, 2000). Compatibility among the primers designed was checked using the FastPCR "primers list test" option (all primers against all primers). The forward primer was fluorescently labeled for detection of PCR products, and the protocol was carried out with a Qiagen Multiplex PCR kit (QIAGEN) using the conditions recommended by the manufacturer. Thirty three primer pairs were designed from the 50 loci chosen within QTL for marker development. Six of the primer pairs developed for markers were rejected because either they produced a PCR product of a significantly different size from that predicted ($n = 2$), gave undefined alleles with stutter peaks ($n = 2$), failed to amplify a product ($n = 1$) or because the locus was homozygous ($n = 1$). Twenty-seven markers were selected for development of three PCR multiplex amplification reactions.

The genomic panel was genotyped for 27 microsatellites with the three developed PCR multiplexes, using an ABI3730xl DNA analyzer (Applied Biosystems) and an internal GeneScan 500-LIZ size standard (Applied Biosystems) to determine allele sizes. Genotyping output was obtained using the Genemapper v.4.0 software (Applied Biosystems). Markers sequence, allele variations and individual genotypes were submitted to the Probedb (http://www.ncbi.nlm.nih.gov/probe/) database with the same nomenclature used in this manuscript.

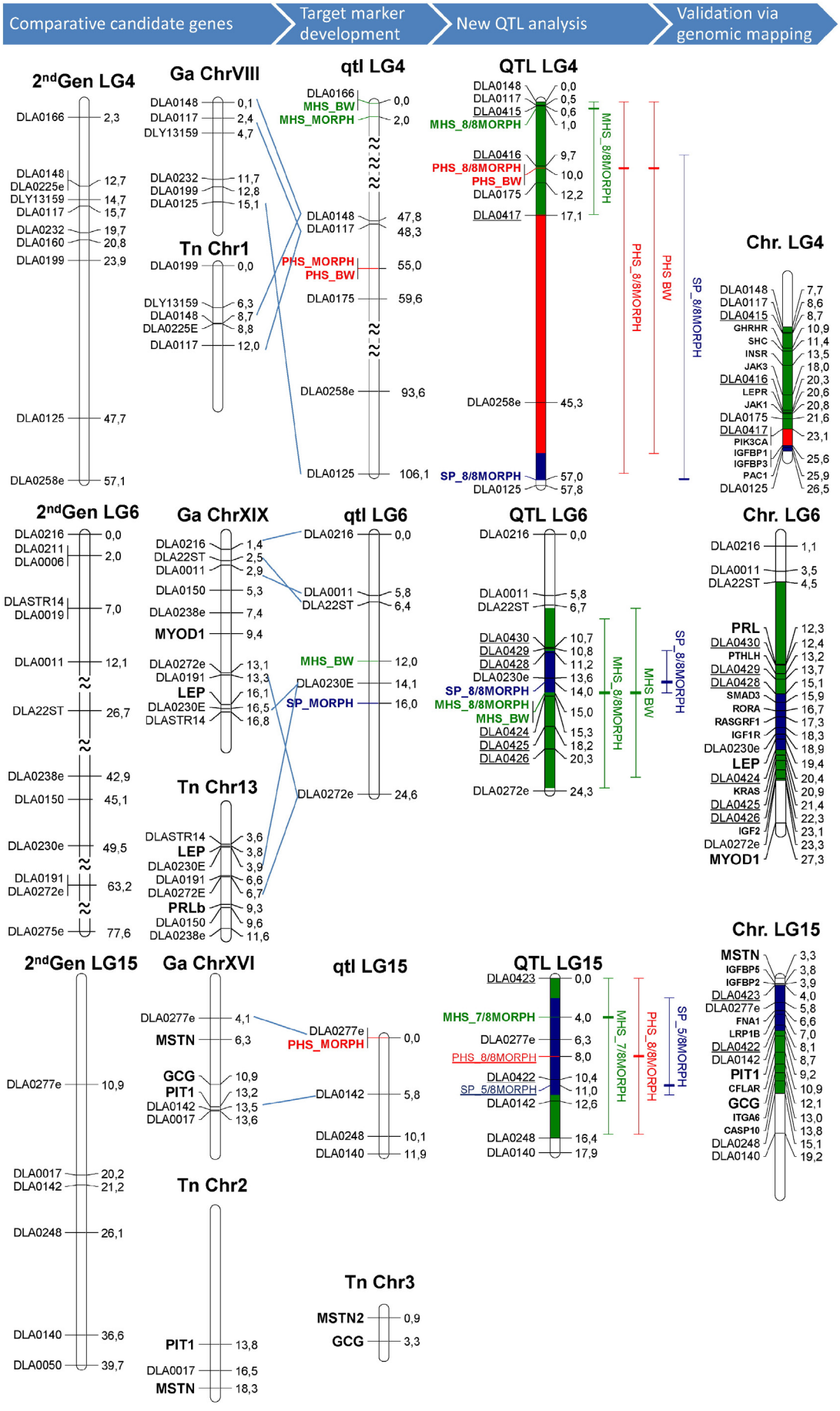
2.5. QTL mapping population

Massault et al. (2010) detected two significant QTL for body weight in linkage group (LG) 4 and LG6 and six significant QTL for morphometric traits in LG1B, LG4, LG6, LG7, LG15 and LG23. Chatziplis et al. (2007) using a different genomic panel described a growth QTL positioned in LG1. The sea bass genomic panel used in the present study for QTL targeted marker development and genotyping consisted of the same 5 full-sib families, comprised of 2 dams, 5 sires and their 8-month-old offspring ($n = 576$) of 40.6 ± 14.21 g body mass and 13.3 ± 1.61 cm standard length previously described by Massault et al. (2010). The population structure (Table 6) of the genomic panel was selected from a larger pool of families (56 parents and 1151 progeny), identified by parentage assignment (Volckaert et al., 2012) to optimize power of QTL detection (Massault et al., 2008). Individuals were phenotyped for 9 growth related traits: standard length (SL), head length (HL), body length (BL), pre-anal length (PRAL), abdominal length (AL), post-anal length (POAL), head depth (HD), body depth (BD) and body mass (BW) (see detailed description in Massault et al. (2010)).

2.6. Genetic map

The QTL population linkage map was generated using software CRIMAP 64-bits v. 2.503, a revised version of CRIMAP v. 2.4 (Green et al.,

Fig. 1. Sea bass LG4, LG6 and LG15 QTL comparative map workflow. The integration of all comparative maps starting with sea bass linkage map vs. *G. aculeatus* and *T. nigroviridis* genomes, vs. sea bass published QTL map, vs. present sea bass QTL map and ending in sea bass genome. The banner is the resumed objective across the comparative workflow. Sea bass 2nd generation linkage map groups are represented named "2nd Gen," *G. aculeatus* and *T. nigroviridis* chromosomes are named "Ga" and "Tn," respectively. Published and present sea bass QTL linkage groups are represented by "qtl" and "QTL" respectively, and sea bass chromosomes are named "Chr LG." All sea bass LG4, LG6 and LG15 linkage groups (cM) and *T. nigroviridis*, *G. aculeatus* and sea bass chromosomes (Mbp) are at a 1:1 scale, with the exception of bar breaks, which each represent a 10 cM distance. The three analytical methods are colour coded: red, green and blue are paternal (PHS), maternal (MHS) half-sib and sib-pair regression analysis, respectively. Confidence intervals are represented with also colour coded bars at right of the respective linkage group bar. The traits are MORPH for morphology and BW for body weight; full QTL nomenclature and significance levels are described in Table 2. All QTLs are 5% genome-wide, with exception of both underlined QTLs in LG15 that are 5% chromosome-wide. Sea bass orthologous markers to *G. aculeatus* and *T. nigroviridis* loci are linked with solid lines. For sake of clarity not all orthology was represented but all orthologous loci have the same nomenclature.


Comparative candidate genes
Target marker development
New QTL analysis
Validation via genomic mapping
2ndGen LG4
Ga ChrVIII
qtl LG4
QTL LG4
Chr. LG4
Tn Chr1
2ndGen LG6
Ga ChrXIX
qtl LG6
QTL LG6
Chr. LG6
Tn Chr13
2ndGen LG15
Ga ChrXVI
qtl LG15
QTL LG15
Chr. LG15
Tn Chr2
Tn Chr3

1990) modified by Jill Maddox and Ian Evans (Jill Maddox, University of Melbourne, personal communication). Retrieved data from ResSpecies (www.ResSpecies.org) (Law and Archibald, 2000) were checked for Mendelian errors prior to map construction. The data formatted in the ".gen" file consisted of 118 microsatellite marker genotypes of the 576 offspring from the five sires and two dams included in the pedigree. These 117 microsatellite marker genotypes consist of 27 new marker genotypes plus the previous 90 marker genotypes (87 markers in linkage) (Massault et al., 2010). The CRI-MAP options "two-points," "build," "flips" and "fixed" were used to obtain the sex specific and averaged linkage group maps with a LOD threshold of three. Figures of linkage groups were designed using software MapChart version 2.2 (Voorrips, 2002).

2.7. QTL analysis

Two methods were used to detect QTL, half-sib regression analysis (HSr) (Knott et al., 1996), and sib-pair regression analysis (SPr), both using the GridQTL web based interface (http://www.gridqtl.org.uk). Genotype, map and phenotype files were submitted to the half-sib and sib-pair portlets for the HSr and SPr analysis, respectively. One QTL and two QTL models per linkage group were tested at 1 cm step regressions. Significant QTLs were detected with standard *F*-test statistic as previously described (Knott et al., 1996), in which genome wide permutation tests with 10,000 iterations were made to define the significance threshold. Bootstrap with 10,000 resamplings was made to define a 95% confidence interval of the detected QTL. In the SPr analysis the residuals from the fixed effect analysis are used accordingly to Visscher and Hopper method (Visscher and Hopper, 2001).

2.8. QTL genes and annotation

The previous workflow carried out prior the availability of the sea bass genome assembly and annotation, through a comparative genomics approach for marker development, allowed the predictive characterization of the QTL regions using *G. aculeatus* (GAC) and *T. nigroviridis* (TNI) Ensembl proteins. QTL genomic characterization was confirmed using the annotated sea bass genome assembly (Tine et al., 2014). Previously published and newly developed microsatellite markers were queried via BLASTN against the recently sequenced sea bass genome in order to establish a physical map.

A tabulated list of genes within the QTL confidence intervals was retrieved (Additional file 5) based on the mapped microsatellite marker positions. The deduced gene products in the QTL confidence intervals were mapped to KEGG pathways via orthology assignment using KAAS (KEGG Automatic Annotation Server) (Moriya et al., 2007) (http://www.genome.jp/tools/kaas). The deduced protein sequences of genes in the QTL region were queried against a eukaryotic representative gene data set using the bi-directional best hit assignment method. BRITE hierarchy files were retrieved to visualize which sea bass genes are mapped to KEGG pathways using the KegHier software (http://www.genome.jp/download). Gene orthology (KEGG Orthology, KO) and KEGG pathway IDs were integrated with the tabulated list of genes.

The KOBAS v.2.0 (KEGG Orthology Based Annotation System) web server (Xie et al., 2011) (http://kobas.cbi.pku.edu.cn) was queried for annotation and identification of enriched pathways within the genomic regions of interest (QTL confidence intervals). KO terms of the annotated genes within the QTL were used to identify statistically enriched related pathways in the respective genome region, with default cut-offs and default hypergeometric statistical test and Benjamini–Hochberg FDR correction, using a whole genome set as background.

The STRING v. 9.0 protein interaction database (Szklarczyk et al., 2011) (http://string-db.org) was queried for gene network inferences within the genomic regions of interest (QTL confidence intervals). The interactions include direct (physical) and indirect (functional) associations derived from four sources: genomic context, high-throughput experiments, co-expression, and previous knowledge. The confidence of the interaction score was defined at maximum highest confidence interactions (>0.9) and the query was restricted to the population of genes in each of QTL confidence intervals.

To assess and validate the predictive power of the comparative approach interspecies synteny analysis was performed. Whole genome alignment files (.bed) of seven teleost reference genomes (*G. aculeatus*, *O. latipes*, *T. nigroviridis*, *T. rubripes*, *O. niloticus*, *G. morhua* and *D. rerio*) were retrieved from the sea bass genome browser (http://seabass.mpipz.de/). These whole genome alignments resulted from genomic pairwise alignments against sea bass LG4, LG6, and LG15 chromosomes and integration into blocks of shared collinearity. Circular genomic synteny representations were created using Circos (Krzywinski et al., 2009).

Table 1
Linkage groups (LG) of European sea bass used for QTL analysis. Number (#) of total and new markers per LG, and their average number of alleles (avg. # alleles); average information content for maternal (MHS avg. IC) and paternal (PHS avg. IC) half-sib method analysis; and average additive effect for sib-pair method analysis (SP avg. AE). Shaded in grey are the LG targeted for new markers.

LG	Length (cM)	# markers	New # markers	Avg # alleles	MHS avg IC	PHS avg IC	SP avg AE
LG1a	55	6	1	5.3	0.76	0.81	1.34
LG1b	11	6	1	5.2	0.95	0.97	1.82
LG2	40	9	2	4	0.83	0.87	1.43
LG3	14	4	–	3.5	0.81	0.83	1.20
LG4	58	8	3	4.1	0.84	0.81	1.38
LG5	46	6	–	4.2	0.91	0.88	1.58
LG6	24	11	6	4.7	0.87	0.92	1.60
LG7	34	4	–	6	0.84	0.89	1.52
LG8	43	7	–	4.3	0.88	0.85	1.50
LG9	–	1	–	–	–	–	–
LG10	36	4	–	4.3	0.80	0.83	1.40
LG11	5	2	–	3.5	0.67	0.86	1.36
LG12	14	4	–	5.5	0.90	0.92	1.68
LG13	15	3	–	5	0.88	0.92	1.65
LG14	60	6	–	3.8	0.77	0.79	1.23
LG15	18	6	2	3.5	0.93	0.90	1.65
LG17	17	4	–	4.8	0.92	0.90	1.67
LG18	–	1	–	–	–	–	–
LG19	46	4	1	4.3	0.73	0.85	1.22
LG20	47	5	2	3.8	0.70	0.55	0.89
LG23	9	2	–	4.5	0.92	0.88	1.56
LG24	6	2	–	7	0.97	0.93	1.79
LG25	45	10	9	3.9	0.60	0.66	0.90

3. Results

3.1. Genomic and genetic comparative maps

Sea bass 2nd generation linkage map vs. *G. aculeatus* and *T. nigroviridis* genomes comparative maps allowed the relative linkage of the candidate genes, previously identified by bibliographic search, with the sea bass genetic markers and respective linkage groups. Inversely the comparative mapping between the linkage map of the QTL population (Massault et al., 2010) allowed the relative linkage between the identified QTL loci and *G. aculeatus* and *T. nigroviridis* physical genomes to be made (Fig. 1). These comparative mappings were the scaffolds for new genetic marker development aimed to flank the previously identified QTLs in sea bass LG1, LG4, LG6 and LG15. Twenty-eight new microsatellites markers were designed based on these interspecies synteny blocks.

3.2. Genetic linkage map

A genetic map of sea bass representing 21 linkage groups (two or more markers in linkage) was made with 113 markers and incorporated the 27 new microsatellite markers developed in the current study (Table 1, Additional file 2). Only 1 of the 10 markers targeted a tentative growth QTL identified in LG1 (at present LG1a and LG1b) in a previous study with a different mapping population was linked (Chatziplis

Table 2
Linkage groups with significant QTL. Linkage groups with *F* value higher 5% than genome wide (GW) permutation threshold. Morphometric traits: SL, standard length; HL, head length; BL, body length; PRAL, pre-anal length; AL, abdominal length; POAL, post-anal length; HD, head depth; BD, body depth and trait body weight (BW). QTLs in bold are significant and QTL values showed are for the most significant trait effect which is underlined. QTLs shaded in grey correspond to significant QTL represented in Fig. 1. Method: maternal (MHS) and paternal (PHS) half sibs and sib pairs (SP). Thresholds: suggestive if above 5% chromosome wide (CW) permutations, significant if above 5% genome wide (GW) permutations thresholds.

Traits	Method	Position (cM)	Linkage group	F	5% CW	5% GW	QTL effect (%)	ΔConf (cM)
BL	MHS	7	LG1b	3.62	**3.59**	6.99	3.86	0–11
SL, **HL**, PRAL, AL, HD, BD	PHS	9	LG1b	5.02	**2.44**	**4.24**	14.50	0–11
SL, **HL**, BL, PRAL, AL, HD, BD	SP	10	LG1b	21.5	**8.43**	**20.95**	–	0–11
BW	PHS	11	LG1b	3.52	**2.47**	4.60	8.82	0–11
BW	SP	11	LG1b	11.97	**8.63**	21.83	–	9–11
SL, BL, PRAL, **AL**, POAL, HD, BD	PHS	21	LG2	4.36	**2.74**	**4.31**	12.21	0–35
BW	PHS	33	LG2	3.84	**2.76**	4.60	9.88	9–39
SL, HL, PRAL, AL, POAL, HD, **BD**	MHS	1	LG4	7.15	**3.90**	**6.84**	8.96	0–17
SL, HL, **BL**, **PRAL**, AL, **POAL**, HD, **BD**	PHS	10	LG4	4.93	**2.93**	**4.30**	14.20	0–56
SL, **HL**, BL, PRAL, AL, POAL, HD, **BD**	SP	57	LG4	22.19	**8.38**	**20.42**	–	8–57
BW	MHS	1	LG4	4.53	**3.98**	7.13	4.95	0–46
BW	PHS	10	LG4	5.04	**2.90**	**4.60**	13.92	0–53
BW	SP	57	LG4	20.04	**9.02**	21.83	–	12–57
SL, **HL**, **BL**, **PRAL**, **AL**, **POAL**, HD, BD	MHS	15	LG6	20.4	**3.81**	**6.99**	27.00	8–24
SL, BL, AL, POAL	PHS	18	LG6	3.61	**2.58**	4.20	9.53	5–24
SL, HL, **BL**, **PRAL**, **AL**, **POAL**, **HD**, BD	SP	14	LG6	97.22	**5.42**	**20.03**	–	11–15
BW	MHS	15	LG6	9.76	**3.51**	**7.13**	12.07	7–23
BW	SP	14	LG6	7.48	**5.37**	20.42	–	11–24
SL, HL, **BL**, **PRAL**, **AL**, POAL, HD, BD	PHS	30	LG7	5.91	**2.65**	**4.31**	17.58	0–34
SL, HL, **BL**, PRAL, **AL**, POAL, HD, BD	SP	28	LG7	22.13	**5.16**	**21.40**	–	0–33
BW	MHS	9	LG7	4.46	**3.52**	7.13	4.86	0–34
BW	PHS	34	LG7	4.46	**2.63**	4.60	11.97	0–34
BW	SP	25	LG7	13.34	**5.17**	21.83	–	0–34
SL, **HL**, BL, **PRAL**, AL, **HD**, **BD**	MHS	4	LG15	7.87	**3.47**	**6.84**	9.98	0–17
SL, HL, BL, PRAL, AL, POAL, HD, BD	PHS	8	LG15	3.89	**2.63**	4.20	10.55	0–17
SL, BL, PRAL, AL, HD	SP	11	LG15	7.87	**5.13**	20.58	–	2–12
BW	MHS	3	LG15	4.06	**3.37**	7.13	4.06	0–16
BW	PHS	7	LG15	3.79	**2.71**	4.60	3.79	0–17
BW	SP	10	LG15	5.15	**4.71**	21.83	–	1–12
SL, HL, BL, PRAL, AL, POAL, HD, **BD**	MHS	46	LG20	7.39	**3.83**	**6.84**	9.30	0–46
SL, HL, PRAL, AL, HD, **BD**	SP	30	LG20	22.10	**6.10**	**20.42**	–	2–46
BW	MHS	46	LG20	4.92	**3.87**	7.13	5.49	0–46
BW	SP	37	LG20	10.78	**6.18**	21.83	–	3–46
SL, HL, **BL**, PRAL, **AL**, POAL, HD, BD	PHS	0	LG24	5.73	**2.50**	**4.30**	16.98	0–6
SL, HL, BL, PRAL, AL, POAL, HD, BD	SP	4	LG24	20.12	**4.38**	21.40	–	0–6
BW	PHS	0	LG24	3.69	**2.45**	4.60	9.40	0–6
BW	SP	4	LG24	17.66	**4.70**	21.83	–	5–6

et al., 2007) to LG1a. The remaining 17 new markers targeted the previously identified growth QTL on LG4 ($n = 8$), LG6 ($n = 7$) and LG15 ($n = 2$) (Massault et al., 2010) in the same QTL mapping population used in the present study (Additional file 2). In the targeted linkage groups, respectively, 3/8, 6/7 and 2/2 markers were in linkage in LG4, LG6 and LG15. The new markers which were not in linkage with the targeted LGs were dispersed among other LGs. The markers developed for LG4 which were not in linkage (5/8) were designed around a previously identified LG4 marker DLA0166 (Massault et al., 2010), which in the present study failed to reach the LOD 3 threshold.

The inclusion of further markers in the European sea bass linkage map modified the position of previously mapped markers. Genotype data of markers DLA0251e, DLA0402 retrieved from ResSpecies database, which were used in the construction of the first sea bass linkage map, were homozygous, and consequently were excluded from the present map. No linkage was found for the markers DLA0245 and DLA0037, previously placed in LG9 and LG18, respectively in a different mapping population (Chistiakov et al., 2005). LG16, LG21, and LG22 described in Chistiakov et al. (2005) are not represented because no markers from those linkage groups were used to genotype the sea bass QTL mapping population in the present study.

The genetic map covers 644.2 cM in total length. The addition of the new markers to the previous map generated from the same mapping population led to an increase in linkage groups from 20 to 21, but the total length remained similar changing from 639 to 644 cM. This resulted in a considerable decrease in the averaged distance between markers, changing from 7.7 to 5.7 cM. The average distance between markers in LG4, LG6 and LG15 was reduced from 21.2, 6.2, and 4.0 cM to 8.3, 2.4 and 3.6 cM, respectively. Furthermore, in the specific region targeted in LG4, LG6 and LG15, the average distance between markers decreased from 23.4, 9.1, and 5.8 cM to 3.4, 2.2, and 5.2 cM, respectively.

3.3. QTL analysis

Significant QTLs were detected for morphometric traits SL, HL, BL, PRAL, AL, POAL, HD, BD, and BW in both maternal and paternal half-sibs (HS) and sib-pairs (SP) regression analyses (Table 2; Fig. 1). Results values of analyses for all linkage groups are described in Additional file 3. The exception was trait HD in the paternal HS analysis which was only detected at a suggestive level (>5% chromosome-wide, CW). Significant QTLs (>5% genome-wide, GW) were detected for trait body weight (BW) in both the maternal (MHS) and paternal (PHS) half-sib analyses but was only at a suggestive level in the SP analysis (Table 2; Fig. 1). The average information content (IC) of the linkage analysis of the sea bass genome was 0.81 for the MHS and 0.82 for the PHS. The average IC of each linkage group for both MHS and PHS analysis is indicated in Table 1. None of the significant or suggestive QTL in the linkage map fitted a two QTL model in any of the regression analysis (*i.e.* MHS, PHS and SP).

Eight of the 21 linkage groups, LG1b, LG2, LG4, LG6, LG7, LG15, LG20, and LG24 contained one or more morphometric QTL that passed the significance threshold (>5% GW). In general if one morphometric QTL was detected at a significant level in an LG other morphometric QTLs in the same LG were also detected at a significant or a suggestive level. This could be considered as an indirect indication of high genetic correlations between morphometric traits, as already have been shown by others (Chatziplis et al., 2007; Volckaert et al., 2012) (Table 2). Likewise, in general, significant and suggestive QTL for BW were highly concordant with significant morphometric QTL. If all morphometric traits are

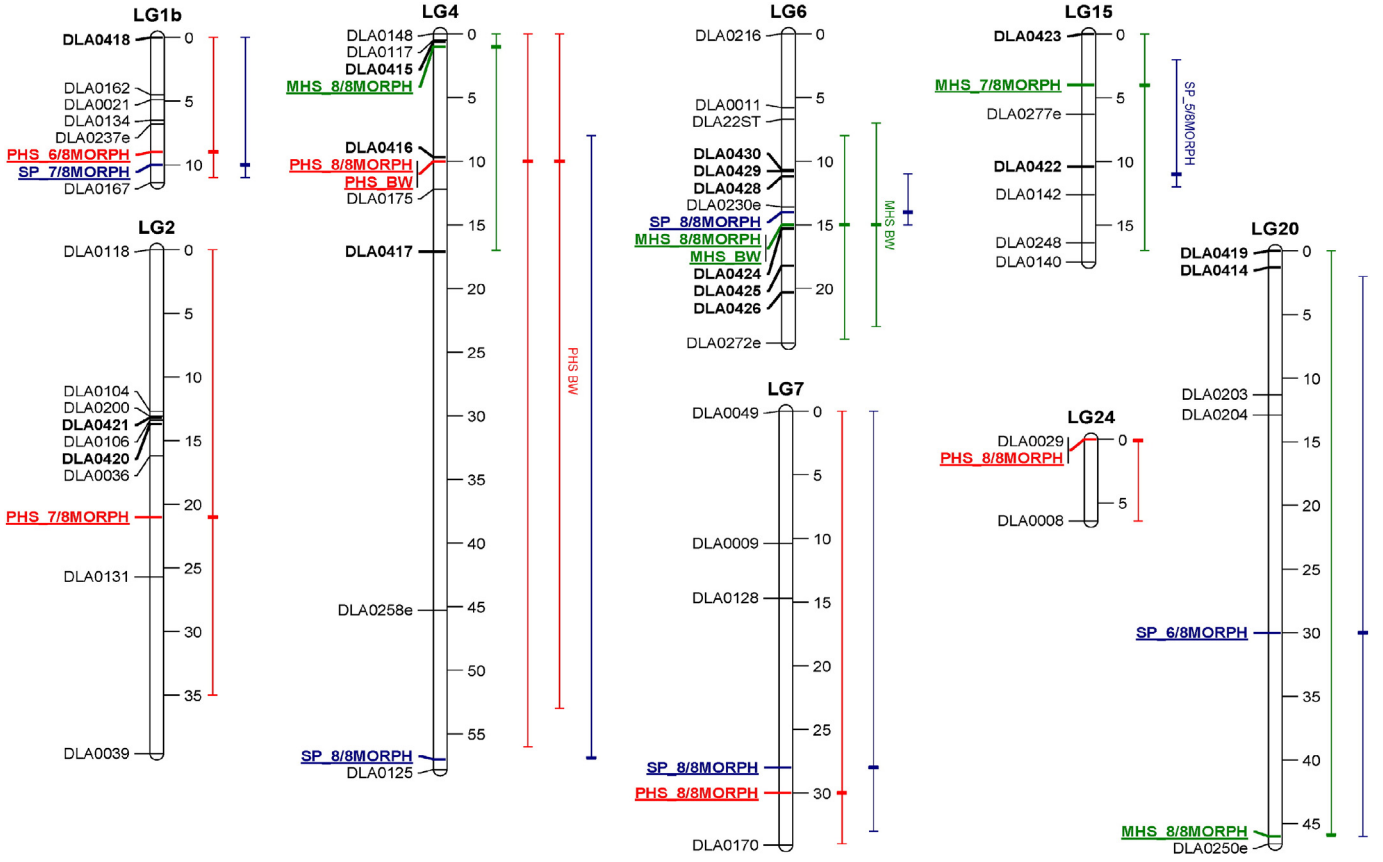


Fig. 2. Genetic linkage map of significant QTLs for BW and morphometric (MORPH) traits. All represented QTLs are at least 5% genome-wide. The three analytical methods are colour coded: red, green and blue are paternal (PHS), maternal (MHS) half-sib and sib-pair regression analysis, respectively. Confidence intervals are represented with also colour coded bars at right of the respective linkage group bar. The QTL designation describes method (MHS, or PHS, or SP), and number of detected traits up to eight (n/8). The full list of QTLs detected and respective details is in Table 2 and Additional file 3.

grouped as a single morphological trait (MORPH) following previous QTL nomenclature (Massault et al., 2010) assigning the MORPH QTL peak location to be the strongest of the overlapping morphometric QTL detected, then the results of MHS, PHS and SP analysis gave a total of 14 significant MORPH QTL in the 8 LGs. However, when morphometric QTL traits were considered individually (SL, HL, BL, PRAL, AL, POAL, HD and BD) in MHS, PHS and SP analysis then a total of 36 significant QTLs were identified (in bold in Table 2, trait column). The greatest MORPH QTL effect among the targeted LGs was for LG6 (27%), followed by LG4 (14%) and LG15 (10%). As previously reported by Massault et al. (2010) the present analysis of LG1a failed to identify a growth QTL and none of the markers developed were linked to this group. For this reason LG1a was excluded from further analysis.

In LG6, all 8 morphometric traits considered had a QTL peak between 10 and 15 cM. The most significant MORPH QTL in LG6 with MHS analysis had a QTL effect explaining up to 27% of the phenotypic variation (BL trait, $F = 20.4$ with 5% GW threshold of 6.99) with a peak located at 15 cM and confidence interval spanning from 8 to 24 cM. SP analysis also gave a significant MORPH QTL in LG6 (trait POAL, $F = 97.22$ with 5% GW threshold of 20.03) with a peak located at 14 cM and confidence interval spanning from 11 to 15 cM. No significant MORPH QTL was detected by PHS analysis; however, a suggestive MORPH QTL (SL, BL, AL,

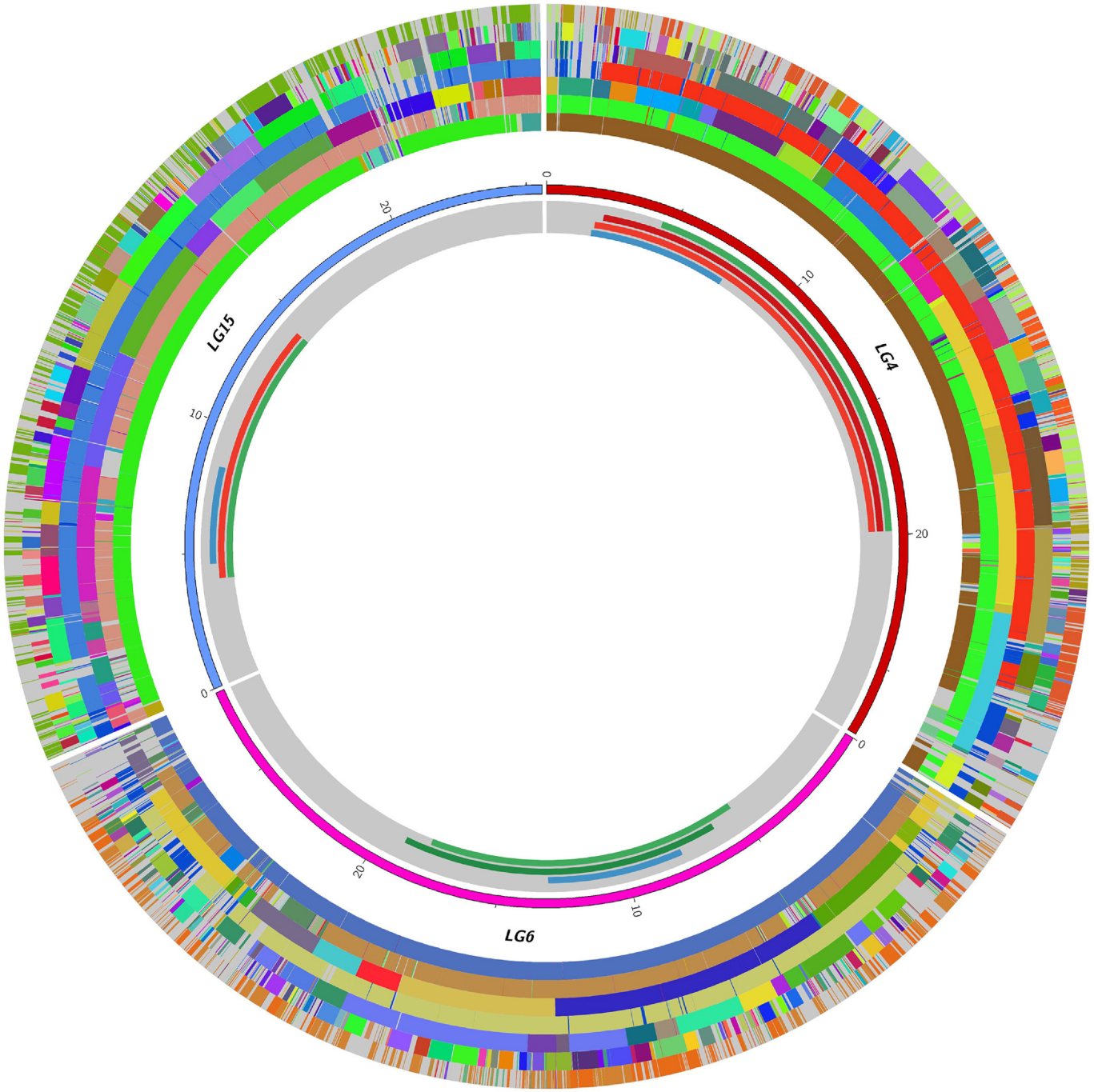


Fig. 3. Synteny of sea bass chromosomes LG4, LG6, and LG15 with teleost genomes. Exterior tiles represent collinear blocks of the overall degree of synteny between the sea bass (*Dicentrarchus labrax*) LG4, LG6, LG15 chromosomes and seven other publicly available teleost genomes. From the inner to the outer layer: *G. aculeatus*, *O. latipes*, *T. nigroviridis*, *D. rerio*, *O. niloticus*, *T. rubripes* and *G. morhua*. The respective colour code is species and chromosomal specific. The central ring represents the sea bass chromosomes scaled in Mbp. The interior tiles represent the genomic span corresponding to the confidence interval of the identified QTL for BW and morphometric traits. Light and dark green are maternal half-sib MORPH and BW QTL, respectively; light and dark red are paternal half-sib MORPH and BW QTL, respectively; and blue is sib-pair MORPH QTL.

Table 3
Top 5 enriched KEGG orthology (KO) pathways in QTL confidence intervals in LG4, LG6, and LG15 of European sea bass. Sample number, number of input genes mapped/number of total input genes; background number, number of total pathway genes/number of total genes. Corrected *P*-value after false discovery rate (FDR) test.

Linkage group	KEGG reference pathway (KO) Term	KO ID	Sample number	Background number	Corrected *P*-value
LG4	Amino sugar and nucleotide sugar metabolism	ko00520	6/179	48/6148	0.25
	VEGF signaling pathway	ko04370	7/179	73/6148	0.25
	Cholinergic synapse	ko04725	9/179	112/6148	0.25
	Non-small cell lung cancer	ko05223	5/179	54/6148	0.34
	Fructose and mannose metabolism	ko00051	4/179	36/6148	0.34
LG6	Axon guidance	ko04360	12/177	127/6148	0.04
	PPAR signaling pathway	ko03320	7/177	69/6148	0.23
	Cholinergic synapse	ko04725	9/177	112/6148	0.23
	Primary bile acid biosynthesis	ko00120	3/177	16/6148	0.23
	Focal adhesion	ko04510	12/177	199/6148	0.23
LG15	Protein digestion and absorption	ko04974	7/165	79/6148	0.15
	Galactose metabolism	ko00052	4/165	27/6148	0.15
	Mucin type O-glycan biosynthesis	ko00512	4/165	30/6148	0.15
	Hedgehog signaling pathway	ko04340	5/165	56/6148	0.25
	ECM-receptor interaction	ko04512	6/165	85/6148	0.36

and POAL) was identified between 14 and 23 cM and had a confidence intervals of 5–24 cM. Additional suggestive QTLs were found by MHS analysis (HD, and BD, 1%CW, Table 2) and SP analysis (HL, and BD, 1%CW, Table 2).

In linkage group LG4, significant MORPH QTLs were detected with all three regression analysis (MHS, PHS and SP). The peak of MHS QTL was at the beginning of the linkage group (1 cM), PHS QTL at 10 cM and SP QTL at the end of the LG (57 cM). Six MHS morphometric QTLs (SL,

Table 4
European sea bass genes identified within QTL confidence intervals. Gene names and KO reference identified as members of the adipocytokine, MAPK, Jak–STAT, and insulin signaling KEGG orthology reference pathways in linkage groups LG4, LG6 and LG15.

Gene name	KO ID	Sea bass gene	LG	Adipocytokine	Jak–STAT	Insulin	MAPK
NR2B3	K08526	DLA_IV_004730	LG4	x			
		DLA_VI_001610	LG6				
CPT1	K08765	DLA_VI_001620	LG6	x			
		DLA_VI_002600	LG6				
NR1C1	K07294	DLA_VI_006190	LG6	x			
JAK2	K04447	DLA_IV_002540	LG4	x	x		
LEPR	K05062	DLA_IV_001550	LG4	x	x		
JAK1	K11217	DLA_IV_001520	LG4		x		
TPOR	K05082	DLA_IV_001330	LG4		x		
IL12RB2	K05064	DLA_IV_006120	LG4		x		
		DLA_IV_006130	LG4				
PIAS	K04706	DLA_IV_005420	LG4		x		
		DLA_VI_004200	LG6				
TPO	K00431	DLA_VI_004520	LG6		x		
PIK3R	K02649	DLA_IV_002230	LG4		x	x	
PIK3C	K00922	DLA_IV_000980	LG4		x	x	
SHC	K06279	DLA_IV_004570	LG4			x	
INSR	K04527	DLA_IV_003780	LG4			x	
PRKAR	K04739	DLA_VI_005350	LG6			x	
PDE3	K13296	DLA_VI_005300	LG6			x	
PPP1R3	K07189	DLA_VI_006300	LG6			x	
IRS	K07187	DLA_XV_004720	LG15			x	
GSK3B	K03083	DLA_XV_004610	LG15			x	
MKNK	K04372	DLA_IV_001840	LG4			x	x
		DLA_IV_004870	LG4				
PKA	K04345	DLA_IV_003220	LG4			x	x
MAP2K2	K04369	DLA_IV_005430	LG4			x	x
KRAS	K07827	DLA_VI_002050	LG6			x	x
MAP2K1	K04368	DLA_VI_004100	LG6			x	x
HRAS	K02833	DLA_VI_001720	LG6			x	x
JUND	K04449	DLA_IV_002180	LG4				x
FGF	K04358	DLA_IV_005620	LG4				x
CACNA1E	K04852	DLA_IV_004010	LG4				x
PLA2G	K01047	DLA_IV_001700	LG4				x
DUSP	K04459	DLA_IV_000480	LG4				x
		DLA_VI_001510	LG6				
MAPK8IP2	K04435	DLA_VI_002650	LG6				x
RASGRF1	K04349	DLA_VI_003460	LG6				x
CACNA2D1	K04858	DLA_VI_002850	LG6				x
PTP	K04458	DLA_VI_001520	LG6				x
FLNA	K04437	DLA_VI_005400	LG6				x
MAP3K13	K04422	DLA_XV_002600	LG15				x
MRAS	K07831	DLA_XV_001360	LG15				x
ZAK	K04424	DLA_XV_003630	LG15				x
ATF2	K04450	DLA_XV_003750	LG15				x

HL, PRAL, AL, POAL, and HD) were detected with only one at a significant level (trait BD, $F = 7.15$ with 5% GW threshold of 6.84), which explained up to 9% of the phenotypic variance (Table 2). In PHS analysis, 8 morphometric QTLs were detected, four of which were significant (SL, BL, PRAL, POAL, and BD, Table 2). The highest QTL, trait BL ($F = 4.93$ with 5% GW threshold of 4.30) explained up to 14% of the phenotypic variance. In the SP analysis, 8 morphometric QTLs were detected, two (HL, and BD) at a significant level (Table 2). Confidence interval for the MSH MORPH QTL spanned from 0 to 17 cM and for PHS and SP analysis covered most of LG4.

Two significant BW QTLs were found in LG4 and LG6 explaining approximately 14% and 12% of phenotypic variance in the PHS and MHS analysis, respectively. Moreover, the BW QTL peak (10 cM) in LG4 coincided with the MORPH QTL peak. The peak of the BW QTL in LG6 (15 cM) is in exactly the same position as the MHS and SP MORPH QTL. In general suggestive BW QTLs in LG4 (MHS and SP) and LG6 (SP) were highly correlated with significant MORPH QTL.

In LG15 significant MORPH QTLs were only detected with MHS regression analysis (Table 2). Four morphometric MHS QTL (HL, PRAL, HD and BD) were significant with the strongest trait, BD ($F = 7.87$ with 5% GW threshold of 6.84), explaining up to 10% of the phenotypic variance. MORPH QTLs were detected at a suggestive level with PHS and SP analysis (Table 2). Suggestive BW QTLs detected with MHS, PHS and SP analysis were closely linked with the respective MORPH QTL.

MORPH QTLs were also detected in LG1b, LG2, LG7, LG20 and LG24 but were not targeted for marker development (Table 2). In brief, LG1b PHS and SP analysis detected a MORPH QTL for the trait HL which explained up to 15% of the phenotypic variance in the PHS analysis. In LG2, a single significant QTL was detected by PHS analysis for trait AL, with an effect explaining up to 12% of the phenotypic variance. In LG7 both PHS and SP analysis gave significant MORPH QTL (SL, BL, AL) at 30 cM and 28 cM, respectively, which explained up to 18% of the phenotypic variance in the PHS analysis. In LG20, trait BD gave a significant QTL in MHS and SP analysis, which explained 9% of the phenotypic variance in MHS analysis. In LG24, significant MORPH QTLs were only detected with PHS analysis.

Suggestive MORPH and BW QTLs (Additional file 4) were detected in other linkage groups (LG3, LG5, LG8, LG9, LG11, LG12, LG13, LG14, LG17, and LG19), and less overlap was found between QTL in those linkage groups.

3.4. QTL gene annotation and pathway analysis

Microsatellite markers in targeted linkage groups (LG4, LG6 and LG15) were physically mapped in the sea bass genomic assembly (Fig. 1). The genomic regions containing microsatellites corresponding to growth QTL confidence intervals in LG4, LG6 and LG15, were extracted from the annotated sea bass genome assembly. A list of genes within the genomic regions of interest (QTL confidence intervals) was retrieved and corresponded to 518 (MHS MORPH), 594 (PHS MORPH and BW) and 149 (SP MORPH) genes in LG4, 528 (MHS MORPH and BW) and 233 (SP MORPH) genes in LG6 and 347 (MHS MORPH), and 110 (SP MORPH) genes in LG15 (Fig. 3, Additional file 5). No significantly enriched KEGG ortholog (KO) reference pathways were identified in LG4 or LG15. One significantly enriched pathway was identified in LG6, which had a *P*-value of 0.04 and corresponded to "the axon guidance (ko04360) pathway" (Table 3). Of the 177 genes with KO annotation in LG6, 12 were present in the 127 genes comprising the axon guidance (ko04360) pathway (Table 3). Adipocytokine, MAPK, Jak–STAT, and insulin KO reference signaling pathways, although not statistically significantly enriched for the traits in question, were the three most relevant for the trait KO pathways which appeared in the top pathways with their elements mapped with the genes present within the QTL confidence intervals (Table 4).

Using the maximum confidence interaction score stringent identification of interacting gene products from LG4, LG6 and LG15 were identified by STRING analysis (Table 5). The most significant association networks constructed included: 13 genes for LG4, 25 genes for LG6 and 15 genes for LG15 (confidence view, Fig. 3a). A large global association network including 42 gene products arises when the gene products of the 3 linkage groups are analyzed together (Fig. 4b, Table 5). Strikingly the three association networks arising for LG4, LG6 and LG15 are populated with genes of biological importance for growth and body weight. For example, elements of the signaling pathways for Jak–STAT, insulin, MAPK and adipocytokine are previously identified in the KO pathways analysis (Table 4). In addition, endocrine factors such as insulin, growth hormone, IGFI and II are also present in the association networks. The largest association network with the greatest number of gene products interlinked is obtained with the genes mapped in the LG4 confidence interval. In LG6 two main association networks were evident that merge

Table 5
List of genes of European sea bass identified by STRING. List of genes from in the QTL confidence intervals from linkage groups LG4, LG6 and LG15, identified by STRING confidence view network displayed in Fig. 2.

Linkage group	Gene	Protein
LG4	JAK1	Janus kinase 1
	PLCG1	Phospholipase C, gamma 1
	SHC2	SHC (Src homology 2 domain) transforming protein 2
	INSR	Insulin receptor
	EPHB1	EPH receptor B1
	PIK3R2	Phosphoinositide-3-kinase, regulatory subunit 2 (β)
	LEPR	Leptin receptor
	IGFBP1	Insulin-like growth factor binding protein 1
	MPL	Myeloproliferative leukemia virus oncogene
	JAK3	Janus kinase 3
	IRF4	Interferon regulatory factor 4
	ZAP70	Zeta-chain (TCR) associated protein kinase 70 kDa
	PIK3CA	Phosphoinositide-3-kinase, catalytic, alpha polypeptide
LG6	KCNQ1	Potassium voltage-gated channel, KQT-like, member 1
	BHLHE41	Basic helix–loop-helix family, member e41
	MCHR1	Melanin-concentrating hormone receptor 1
	KRAS	v-Ki-ras2 Kirsten rat sarcoma viral oncogene homologue
	RORA	RAR-related orphan receptor A
	PPARA	Peroxisome proliferator-activated receptor alpha
	CPT1A	Carnitine palmitoyltransferase 1 A (liver)
	ARNTL2	Aryl hydrocarbon receptor nuclear translocator-like 2
	IGF1R	Insulin-like growth factor 1 receptor
	MAP2K1	Mitogen-activated protein kinase kinase 1
	HRAS	v-Ha-ras Harvey rat sarcoma viral oncogene homologue
	CPT1B	Carnitine palmitoyltransferase 1B (muscle)
	LEP	Leptin
	MORF4	Mortality factor 4
	SMAD3	SMAD family member 3
	NR2C1	Nuclear receptor subfamily 2, group C, member 1
	PNPLA2	Patatin-like phospholipase domain containing 2
	IGF2	Insulin-like growth factor 2 (somatomedin A)
	GNAI1	G protein, alpha inhibiting activity polypeptide 1
	RASSF7	Ras association (RalGDS/AF-6) domain family member 7
	SIN3A	SIN3 homologue A, transcription regulator (yeast)
	MRPL23	Mitochondrial ribosomal protein L23
	PTHLH	Parathyroid hormone-like hormone
	RASGRF1	Ras protein-specific guanine nucleotide-releasing factor 1
	CDKN1C	Cyclin-dependent kinase inhibitor 1C (p57, Kip2)
LG15	COL4A1	Collagen, type IV, alpha 1
	IGFBP2	Insulin-like growth factor binding protein 2, 36 kDa
	IGFBP5	Insulin-like growth factor binding protein 5
	GSK3B	Glycogen synthase kinase 3 β
	ARHGEF7	rho guanine nucleotide exchange factor (GEF) 7
	CFLAR	CASP8 and FADD-like apoptosis regulator
	FN1	Fibronectin 1
	COL4A2	Collagen, type IV, alpha 2
	EDNRB	Endothelin receptor type B
	ITGA6	Integrin, alpha 6
	CASP10	Caspase 10, apoptosis-related cysteine peptidase
	IRS2	Insulin receptor substrate 2
	APP	Amyloid β (A4) precursor protein
	CASP8	Caspase 8, apoptosis-related cysteine peptidase
	LRP1B	Low density lipoprotein-related protein 1B
	MSTN	Myostatin

via SMAD family member 3 (SMAD3) when the confidence threshold is lowered slightly (high confidence, >0.7). In LG15 a single large and highly significant association network is identified with myostatin and insulin growth factors binding proteins (IGFBP). Merging of the gene lists from the 3 LGs and using the highest confidence score for network analysis generates a single network with very strong associations (Fig. 4b). The genes represented are good candidate genes for the morphometric growth-related QTL.

The synteny analysis results are concordant with the previous comparative mapping results that allowed the development of QTL flanking markers, candidate genes mapping and predictive QTL characterization. In the overall synteny analysis shows that toward the tipping of the chromosomes the rate of synteny tends to diminish and blocks of synteny are smaller and with more mixed origin from different chromosomes (Fig. 3). This might be the reason why most new markers aimed for the tip of LG1a group ended up all linkage in LG25 (Additional file 2), as well as markers aimed to be in close linkage with marker DLA0166 also located at the tip of LG4.

4. Discussion

4.1. Delimiting the QTL

We have narrowed down the confidence interval of the QTL regions for growth in sea bass through a comparative approach. To that purpose we developed markers to increase the resolution within genomic regions flanking the QTL peaks in the sea bass genetic map. This was done by linking the genomic data and genetic information of sea bass with an assembled and annotated genome of a closely related model species. Although it did not lead to the identification of a specific growth related candidate gene it was possible, by taking advantage of demonstrated synteny in advanced teleosts (Sarropoulou et al., 2007; Shimizu and Purugganan, 2005), to extract a list of candidate genes. Their functional analysis revealed to be consistent with growth related traits.

Identification of the causal gene for any trait is clearly a limiting step; even in the pig, in which over 1675 candidate QTL regions have been identified over the past 15 years, only in a few cases has the causal mutation been identified (Rothschild et al., 2007). Nevertheless, in the present study, the confidence interval of the growth QTL regions on sea bass on LG4, LG6 and LG15 was significantly reduced in comparison with previous studies (Chatziplis et al., 2007; Massault et al., 2010). Subsequent access to the sea bass draft genome confirmed the validity of the comparative mapping approach using genetic markers flanking the QTL and revealed that LG4, LG6 and LG15 contain approximately 871, 915 and 774 genes, respectively. A similar approach in rainbow trout using comparative genomic analysis of linkage groups containing growth QTL identified syntenic blocks in the zebrafish (*Danio rerio*), medaka (*Oryzias latipes*) and stickleback genomes with 61%, 56.5% and 53.6% gene homologies, respectively. This is high considering their evolutionary distance and differing ecological niche (Wringe et al., 2010). A higher gene homology exists in our study, however, between sea bass and medaka and/or stickleback with large conserved blocks of synteny in the QTL interval regions (Additional file 6).

The largest effect BW and MORPH QTL co-localized in locus LG6 in sea bass which has a homologues in rainbow trout RT-27q (Drew et al., 2007; O'Malley et al., 2003; Wringe et al., 2010) and Artic charr AC-4 (Kuttner et al., 2011) linkage groups with detected BW and K (condition factor) QTL. This genomic region was shown both in the Wringe et al. (2010) study and our own to be homologue with stickleback chromosome group XIX and medaka chromosome 13. The results support the strategy of using comparative genomics to accelerate the

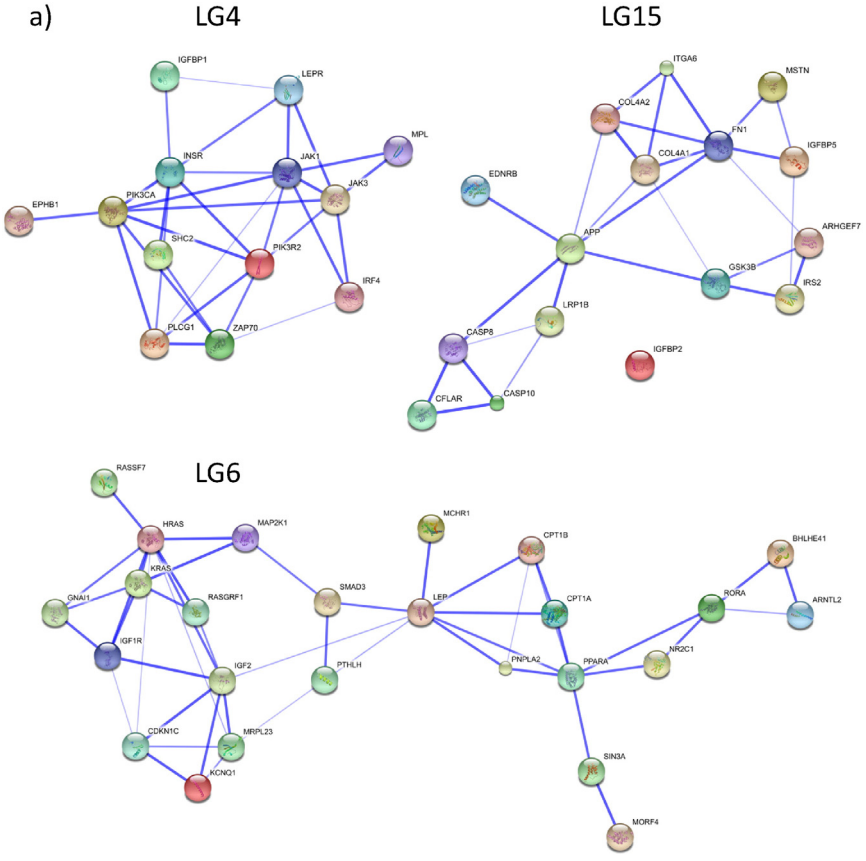


Fig. 4. String confidence view network display. A) The gene networks identified for the growth QTL in LG4, LG6, and LG15 were constructed separately and the largest networks are shown. B) The gene networks for the growth QTL in LG4, LG6, and LG15 in A) are integrated. Genes with stronger associations (edges) are represented by thicker lines.

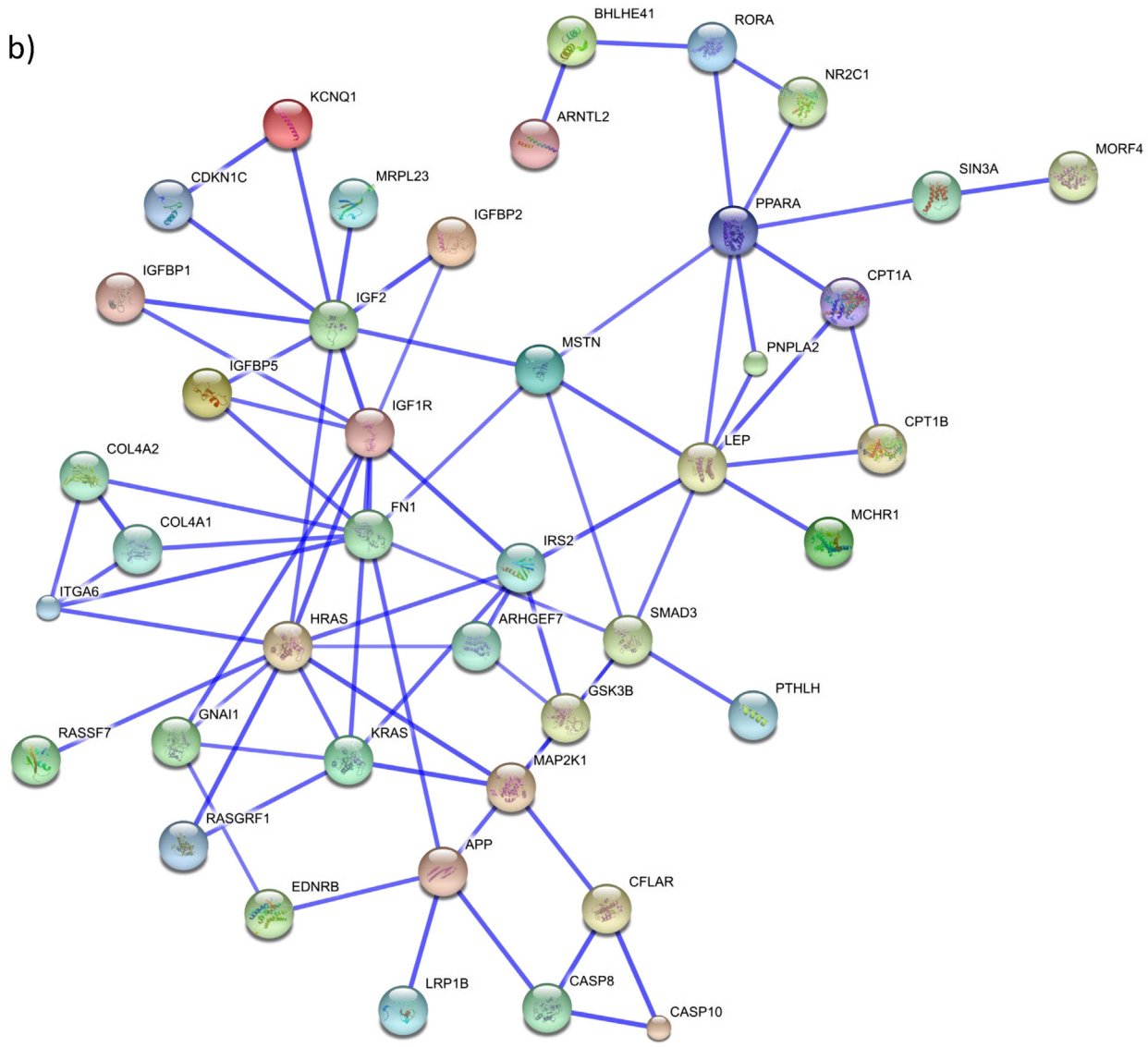


Fig. 4 (*continued*).

identification of genomic regions with higher probability of having trait effect in particular in species which have undergone little selection.

4.2. Search for candidate genes

Minor divergence exists in the morphometric traits linked with the growth QTL between the three LGs in sea bass. For example LG15 lacked the trait BW while LG4 and LG6 BW had a significant association but the latter LG differed in relation to body length and body depth. Overall LG6 had the greatest QTL effect of 26%. In the search for possible candidate genes the enrichment of QTL regions with growth associated genes was assessed by KEGG and revealed pathway enrichment for sugar, amino acid and nucleotide metabolism in LG4, PPAR signaling and focal adhesion in LG6, and protein digestion, hedgehog signaling and ECM receptor interaction in LG15, although not significantly enriched. Gene by gene analysis of the enriched pathway did not reveal any outstanding candidates with a potential quantitative effect on growth.

An alternative approach to identify candidate growth genes is to select genes reported in the literature to be causal or strong candidate genes for growth QTL in other vertebrates. Taking such an approach, LG4 was found to contain the leptin receptor which has previously been associated with backfat thickness in pigs (*Sus scrofa*) (Mackowski et al., 2005; Munoz et al., 2009), and insulin-like growth factor binding protein-1 and -3, polymorphisms of which in chicken are associated with body weight at 10 weeks (Ou et al., 2009).

The sea bass LG6 has a high quantitative effect on growth and contains several genes, such as insulin-like growth factor 2 (IGF2), cathepsin D and leptin, with a proven causal effect on growth in other vertebrates. Of particular interest is IGF2 which in pigs is linked to a significant increase in muscle mass (30%) when a G > A substitution occurs in intron 3 (Van Laere et al., 2003). Cursory analysis of LG15 immediately yielded an apparently strong candidate gene for growth, growth differentiation factor 8 (GDF 8) also known as myostatin. It is a negative

Table 6
QTL population structure of European sea bass. WTL population structure represents five full-sib families, one dam mated with two sires and the other dam mated with three different sires, resulting in two dam half sib and five sire half-sib families (Volckaert et al., 2012).

Number of offspring	Sire1	Sire2	Sire3	Sire4	Sire5
Dam1	98	93			
Dam2			92	143	142
Dam half sib 1	191				
Dam half sib 2			377		

regulator of muscle growth mutations which are responsible for double muscling in cattle breeds (Kambadur et al., 1997; McPherron and Lee, 1997; Wiener et al., 2002) and it has a causal effect on carcass yield in cattle (Sellick et al., 2007) and sheep (Hadjipavlou et al., 2008; Walling et al., 2004). Myostatin is relatively well conserved from mammals to fish (Pie and Alvares, 2006) but the effect of knockdown or knock-out in teleosts is variable. In medaka only expression of a dominant negative forms causes double muscling (Sawatari et al., 2010), but in zebrafish injection of antisense RNA-expressing vector to establish a myostatin gene knockdown is enough to cause double muscling phenotype (Lee et al., 2009). The variable effect of myostatin knockouts on teleost fish may be related to the presence of duplicate and even quadruplicate genes (2 in most osteichthyans and 4 in salmonids; Rodgers and Garikipati, 2008) as a consequence of teleost specific whole genome duplications (Jaillon et al., 2004).

Identification of causal genes for a given trait remains a key challenge in QTL analysis and is costly and laborious. Selection of candidate genes through knowledge about their biological function gleaned through bibliographic searches is one way of establishing gene lists but this approach is limited by the relatively small number of genes identified and validated, even in terrestrial farm animals. The development of concordant QTL maps offers an alternative approach but their validity and cross species applicability remains to be established. Yet another approach applicable to organisms with a sequenced genome, from which it is possible to draw up gene lists for a given QTL region, is to apply in silico methods such as STRING analysis to identify protein–protein interactions. Such an approach permits genetic data to be integrated with the wealth of functional information in public domain databases on human and model species. In fact in the present study STRING queries yielded a list of highly interlinked candidate genes for growth related traits which will be exploited in future studies. Genes identified included growth hormone (Besson et al., 2005; McCormack et al., 2009), insulin (Edghill et al., 2008), and leptin (Halaas et al., 1995; Mammes et al., 2000) which encode key hormones modulating growth, development, and energy metabolism. They have known allelic variations linked to growth and body weight-related traits variations and related diseases in mammals. Other genes in theses pathways, upstream or downstream to these key hormones, have known allelic variation linked to growth and body weight related traits such as IGF2 (Van Laere et al., 2003), and mutations with major QTL effects on muscle growth in pig and sheep (Clop et al., 2006). Overall the results point to a rapid means of identifying candidates for further investigation.

5. Conclusions

A comparative genomics approach, combined with functional gene annotation, provided an identification of candidate genes responsible for growth related QTL in sea bass. This study demonstrates the feasibility of such an approach to refine the resolution of QTL and the establishment of hypothesis to accelerate discovery of putative responsible genes. As production of genomic data is becoming more accessible, the implementation of this strategy will rapidly and efficiently provide the tools required for genetic selection in new candidate aquaculture species.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.aquaculture.2016.01.004.

Acknowledgements

This study has been funded by the European Commission (project AQUAFIRST contract number FP6-STREP-2004-513692) and the Portuguese National Science Foundation (Pluriannual funds). D.J.K. acknowledges financial support from the BBSRC. B.L. acknowledges fellowships from FCT (SFRH/BD/29171/2006, SFRH/BPD/89889/2012) and SABRETRAIN Marie Curie EST, and mobility and resource exchange grants from the Aquagenome project (FP6 contract number 44481).

References

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J., 1990. Basic local alignment search tool. J. Mol. Biol. 215, 403–410.
Besson, A., Salemi, S., Deladoey, J., Vuissoz, J.M., Eble, A., Bidlingmaier, M., Burgi, S., Honegger, U., Fluck, C., Mullis, P.E., 2005. Short stature caused by a biologically inactive mutant growth hormone (GH-C53S). J. Clin. Endocrinol. Metab. 90, 2493–2499.
Brunet, F.G., Roest Crollius, H., Paris, M., Aury, J.M., Gibert, P., Jaillon, O., Laudet, V., Robinson-Rechavi, M., 2006. Gene loss and evolutionary rates following whole-genome duplication in teleost fishes. Mol. Biol. Evol. 23, 1808–1816.
Canario, A.V.M., Bargelloni, L., Volckaert, F., Houston, R.D., Massault, C., Guiguen, Y., 2008. Genomics toolbox for farmed fish. Rev. Fish. Sci. 16, 3–15.
Chatziplis, D., Batargias, C., Tsigenopoulos, C.S., Magoulas, A., Kollias, S., Kotoulas, G., Volckaert, F.A.M., Haley, C.S., 2007. Mapping quantitative trait loci in European sea bass (*Dicentrarchus labrax*): the BASSMAP pilot study. Aquaculture 272, S172–S182.
Chistiakov, D., Tsigenopoulos, C., Lagnel, J., Guo, Y., Hellemans, B., Haley, C., Volckaert, F., Kotoulas, G., 2008. A combined AFLP and microsatellite linkage map and pilot comparative genomic analysis of European sea bass *Dicentrarchus labrax* L. Anim. Genet. 39, 623–634.
Chistiakov, D.A., Hellemans, B., Haley, C.S., Law, A.S., Tsigenopoulos, C.S., Kotoulas, G., Bertotto, D., Libertini, A., Volckaert, F.A., 2005. A microsatellite linkage map of the European sea bass *Dicentrarchus labrax* L. Genetics 170, 1821–1826.
Clop, A., Marcq, F., Takeda, H., Pirottin, D., Tordoir, X., Bibe, B., Bouix, J., Caiment, F., Elsen, J.M., Eychenne, F., Larzul, C., Laville, E., Meish, F., Milenkovic, D., Tobin, J., Charlier, C., Georges, M., 2006. A mutation creating a potential illegitimate microRNA target site in the myostatin gene affects muscularity in sheep. Nat. Genet. 38, 813–818.
Drew, R.E., Schwabl, H., Wheeler, P.A., Thorgaard, G.H., 2007. Detection of QTL influencing cortisol levels in rainbow trout (*Oncorhynchus mykiss*). Aquaculture 272, S183–S194.
Du, S.J., Gong, Z., Fletcher, G.L., Shears, M.A., King, M.J., Idler, D.R., Hew, C.L., 1992. Growth enhancement in transgenic Atlantic salmon by the use of an "all fish" chimeric growth hormone gene construct. Nat. Biotechnol. 10, 176–181.
Edghill, E.L., Flanagan, S.E., Patch, A.M., Boustred, C., Parrish, A., Shields, B., Shepherd, M.H., Hussain, K., Kapoor, R.R., Malecki, M., MacDonald, M.J., Stoy, J., Steiner, D.F., Philipson, L.H., Bell, G.I., Neonatal Diabetes International Collaborative, G., Hattersley, A.T., Ellard, S., 2008. Insulin mutation screening in 1,044 patients with diabetes: mutations in the INS gene are a common cause of neonatal diabetes but a rare cause of diabetes diagnosed in childhood or adulthood. Diabetes 57, 1034–1042.
FAO, 2010. The State of the World Fisheries and Aquaculture. Food and Agriculture Organization of the United Nations, Rome.
Fjalestad, K.T., Moen, T., Gomez-Raya, L., 2003. Prospects for genetic technology in salmon breeding programmes. Aquac. Res. 34, 397–406.
Green, P., Falls, K., Crooks, S., 1990. Documentation for CRI-MAP, version 2.4. Washington University, St. Louis, Missouri USA.
Guyon, R., Senger, F., Rakotomanga, M., Sadequi, N., Volckaert, F.A., Hitte, C., Galibert, F., 2010. A radiation hybrid map of the European sea bass (*Dicentrarchus labrax*) based on 1581 markers: synteny analysis with model fish genomes. Genomics 96, 228–238.
Hadjipavlou, G., Matika, O., Clop, A., Bishop, S.C., 2008. Two single nucleotide polymorphisms in the myostatin (GDF8) gene have significant association with muscle depth of commercial Charollais sheep. Anim. Genet. 39, 346–353.
Halaas, J.L., Gajiwala, K.S., Maffei, M., Cohen, S.L., Chait, B.T., Rabinowitz, D., Lallone, R.L., Burley, S.K., Friedman, J.M., 1995. Weight-reducing effects of the plasma protein encoded by the obese gene. Science 269, 543–546.
Hoegg, S., Brinkmann, H., Taylor, J.S., Meyer, A., 2004. Phylogenetic timing of the fish-specific genome duplication correlates with the diversification of teleost fish. J. Mol. Evol. 59, 190–203.
Houston, R., Taggart, J., Cezard, T., Bekaert, M., Lowe, N., Downing, A., Talbot, R., Bishop, S., Archibald, A., Bron, J., Penman, D., Davassi, A., Brew, F., Tinch, A., Gharbi, K., Hamilton, A., 2014. Development and validation of a high density SNP genotyping array for Atlantic salmon (*Salmo salar*). BMC Genomics 15, 90.
Houston, R.D., Haley, C.S., Hamilton, A., Guy, D.R., Tinch, A.E., Taggart, J.B., McAndrew, B.J., Bishop, S.C., 2008. Major quantitative trait loci affect resistance to infectious pancreatic necrosis in Atlantic salmon (*Salmo salar*). Genetics 178, 1109–1115.
Hull, K.L., Harvey, S., 1999. Growth hormone resistance: clinical states and animal models. J. Endocrinol. 163, 165–172.
Jaillon, O., Aury, J.M., Brunet, F., Petit, J.L., Stange-Thomann, N., Mauceli, E., Bouneau, L., Fischer, C., Ozouf-Costaz, C., Bernot, A., Nicaud, S., Jaffe, D., Fisher, S., Lutfalla, G., Dossat, C., Segurens, B., Dasilva, C., Salanoubat, M., Levy, M., Boudet, N., Castellano, S., Anthouard, V., Jubin, C., Castelli, V., Katinka, M., Vacherie, B., Biemont, C., Skalli, Z., Cattolico, L., Poulain, J., De Berardinis, V., Cruaud, C., Duprat, S., Brottier, P., Coutanceau, J.P., Gouzy, J., Parra, G., Lardier, G., Chapple, C., McKernan, K.J., McEwan, P., Bosak, S., Kellis, M., Volff, J.N., Guigo, R., Zody, M.C., Mesirov, J., Lindblad-Toh, K., Birren, B., Nusbaum, C., Kahn, D., Robinson-Rechavi, M., Laudet, V., Schachter, V., Quetier, F., Saurin, W., Scarpelli, C., Wincker, P., Lander, E.S., Weissenbach, J., Roest Crollius, H., 2004. Genome duplication in the teleost fish *Tetraodon nigroviridis* reveals the early vertebrate proto-karyotype. Nature 431, 946–957.
Jiang, Z., Pappu, S.S., Rothschild, M.F., 2007. Hitting the jackpot twice: identifying and patenting gene tests related to muscle lipid accumulation for meat quality in animals and type 2 diabetes/obesity in humans. Recent patents on DNA & gene sequences. 1, 100–111.
Kalendar, R., Lee, D., Schulman, A., 2009. FastPCR software for PCR primer and probe design and repeat search. Genes Genomes Genomics. 3, 1–14.
Kambadur, R., Sharma, M., Smith, T.P., Bass, J.J., 1997. Mutations in myostatin (GDF8) in double-muscled Belgian Blue and Piedmontese cattle. Genome Res. 7, 910–916.
Kim, K.S., Thomsen, H., Bastiaansen, J., Nguyen, N.T., Dekkers, J.C., Plastow, G.S., Rothschild, M.F., 2004. Investigation of obesity candidate genes on porcine fat deposition quantitative trait loci regions. Obes. Res. 12, 1981–1994.

Knott, S.A., Elsen, J.M., Haley, C.S., 1996. Methods for multiple-marker mapping of quantitative trait loci in half-sib populations. Theor. Appl. Genet. 93, 71–80.
Krzywinski, M., Schein, J., Birol, İ., Connors, J., Gascoyne, R., Horsman, D., Jones, S.J., Marra, M.A., 2009. Circos: an information aesthetic for comparative genomics. Genome Res. 19, 1639–1645.
Kuhl, H., Beck, A., Wozniak, G., Canario, A., Volckaert, F., Reinhardt, R., 2010. The European sea bass *Dicentrarchus labrax* genome puzzle: comparative BAC-mapping and low coverage shotgun sequencing. BMC Genomics 11, 68.
Kuttner, E., Moghadam, H.K., Skulason, S., Danzmann, R.G., Ferguson, M.M., 2011. Genetic architecture of body weight, condition factor and age of sexual maturation in Icelandic Arctic charr (*Salvelinus alpinus*). Mol. Gen. Genet. MGG 286, 67–79.
Law, A.S., Archibald, A.L., 2000. Farm animal genome databases. Brief. Bioinform. 1, 151–160.
Lee, C.Y., Hu, S.Y., Gong, H.Y., Chen, M.H., Lu, J.K., Wu, J.L., 2009. Suppression of myostatin with vector-based RNA interference causes a double-muscle effect in transgenic zebrafish. Biochem. Biophys. Res. Commun. 387, 766–771.
Loukovitis, D., Sarropoulou, E., Tsigenopoulos, C.S., Batargias, C., Magoulas, A., Apostolidis, A.P., Chatziplis, D., Kotoulas, G., 2011. *Quantitative trait loci* involved in sex determination and body growth in the gilthead sea bream (*Sparus aurata* L.) through targeted genome scan. PloS One 6, e16599.
Louro, B., Passos, A.L., Souche, E.L., Tsigenopoulos, C., Beck, A., Lagnel, J., Bonhomme, F., Cancela, L., Cerda, J., Clark, M.S., Lubzens, E., Magoulas, A., Planas, J.V., Volckaert, F.A., Reinhardt, R., Canario, A.V., 2010. Gilthead sea bream (*Sparus auratus*) and European sea bass (*Dicentrarchus labrax*) expressed sequence tags: characterization, tissue-specific expression and gene markers. Mar. Genomics 3, 179–191.
Mackowski, M., Szymoniak, K., Szydlowski, M., Kamyczek, M., Eckert, R., Rozycki, M., Switonski, M., 2005. Missense mutations in exon 4 of the porcine LEPR gene encoding extracellular domain and their association with fatness traits. Anim. Genet. 36, 135–137.
Mammes, O., Betoulle, D., Aubert, R., Herbeth, B., Siest, G., Fumeron, F., 2000. Association of the G-2548A polymorphism in the 5′ region of the LEP gene with overweight. Ann. Hum. Genet. 64, 391–394.
Massault, C., Bovenhuis, H., Haley, C., de Koning, D.J., 2008. QTL mapping designs for aquaculture. Aquaculture 285, 23–29.
Massault, C., Hellemans, B., Louro, B., Batargias, C., Van Houdt, J.K., Canario, A., Volckaert, F.A., Bovenhuis, H., Haley, C., de Koning, D.J., 2010. QTL for body weight, morphometric traits and stress response in European sea bass *Dicentrarchus labrax*. Animal Genetics 41, 337–345.
McCormack, B.L., Chase Jr., C.C., Olson, T.A., Elsasser, T.H., Hammond, A.C., Welsh Jr., T.H., Jiang, H., Randel, R.D., Okamura, C.A., Lucy, M.C., 2009. A miniature condition in Brahman cattle is associated with a single nucleotide mutation within the growth hormone gene. Domest. Anim. Endocrinol. 37, 104–111.
McPherron, A.C., Lee, S.-J., 1997. Double muscling in cattle due to mutations in the myostatin gene. Proc. Natl. Acad. Sci. U. S. A. 94, 12457–12461.
Moghadam, H.K., Poissant, J., Fotherby, H., Haidle, L., Ferguson, M.M., Danzmann, R.G., 2007. *Quantitative trait loci* for body weight, condition factor and age at sexual maturation in Arctic charr (*Salvelinus alpinus*): comparative analysis with rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*). Mol. Gen. Genet. MGG 277, 647–661.
Moriya, Y., Itoh, M., Okuda, S., Yoshizawa, A.C., Kanehisa, M., 2007. KAAS: an automatic genome annotation and pathway reconstruction server. Nucleic Acids Res. 35, W182–W185.
Munoz, G., Ovilo, C., Silio, L., Tomas, A., Noguera, J.L., Rodriguez, M.C., 2009. Single- and joint-population analyses of two experimental pig crosses to confirm quantitative trait loci on *Sus scrofa* chromosome 6 and leptin receptor effects on fatness and growth traits. J. Anim. Sci. 87, 459–468.
O'Malley, K.G., Sakamoto, T., Danzmann, R.G., Ferguson, M.M., 2003. Quantitative trait loci for spawning date and body weight in rainbow trout: testing for conserved effects across ancestrally duplicated chromosomes. J. Hered. 94, 273–284.
Ødegård, J., Moen, T., Santi, N., Korsvoll, S.A., Kjøglum, S., Meuwissen, T.H.E., 2014. Genomic prediction in an admixed population of Atlantic salmon (*Salmo salar*). Front. Genet. 5, 402.
Ou, J.T., Tang, S.Q., Sun, D.X., Zhang, Y., 2009. Polymorphisms of three neuroendocrine-correlated genes associated with growth and reproductive traits in the chicken. Poult. Sci. 88, 722–727.
Pie, M.R., Alvares, L.E., 2006. Evolution of myostatin in vertebrates: is there evidence for positive selection? Mol. Phylogenet. Evol. 41, 730–734.
Power, D.M., Louro, B., Houston, R., Anjos, L., Cardoso, J.C.R., 2011. Genomic–Proteomic Research in Sparidae and Its Application to Genetic Improvement, Sparidae. Wiley-Blackwell, pp. 359–381.
Reid, D.P., Szanto, A., Glebe, B., Danzmann, R.G., Ferguson, M.M., 2005. QTL for body weight and condition factor in atlantic salmon (*Salmo salar*): comparative analysis with rainbow trout (*Oncorhynchus mykiss*) and Arctic charr (*Salvelinus alpinus*). Heredity 94, 166–172.
Rodgers, B.D., Garikipati, D.K., 2008. Clinical, agricultural, and evolutionary biology of myostatin: a comparative review. Endocr. Rev. 29, 513–534.
Rothschild, M.F., Hu, Z.L., Jiang, Z., 2007. Advances in QTL mapping in pigs. Int. J. Biol. Sci. 3, 192–197.
Rozen, S., Skaletsky, H., 2000. Primer3 on the WWW for general users and for biologist programmers. Methods Mol. Biol. 132, 365–386.
Saillant, E., Dupont-Nivet, M., Haffray, P., Chatain, B., 2006. Estimates of heritability and genotype–environment interactions for body weight in sea bass (*Dicentrarchus labrax* L.) raised under communal rearing conditions. Aquaculture 254, 139–147.
Sanchez-Molano, E., Cerna, A., Toro, M.A., Bouza, C., Hermida, M., Pardo, B.G., Cabaleiro, S., Fernandez, J., Martinez, P., 2011. Detection of growth-related QTL in turbot (*Scophthalmus maximus*). BMC Genomics 12, 473.
Sarropoulou, E., Nousdili, D., Magoulas, A., Kotoulas, G., 2008. Linking the genomes of nonmodel teleosts through comparative genomics. Mar. Biotechnol. 10, 227–233.
Sarropoulou, E., Franch, R., Louro, B., Power, D.M., Bargelloni, L., Magoulas, A., Senger, F., Tsalavouta, M., Patarnello, T., Galibert, F., Kotoulas, G., Geisler, R., 2007. A gene-based radiation hybrid map of the gilthead sea bream *Sparus aurata* refines and exploits conserved synteny with *Tetraodon nigroviridis*. BMC Genomics 8, 44.
Sawatari, E., Seki, R., Adachi, T., Hashimoto, H., Uji, S., Wakamatsu, Y., Nakata, T., Kinoshita, M., 2010. Overexpression of the dominant-negative form of myostatin results in doubling of muscle-fiber number in transgenic medaka (*Oryzias latipes*). Comp. Biochem. Physiol. A Mol. Integr. Phys. 155, 183–189.
Sellick, G.S., Pitchford, W.S., Morris, C.A., Cullen, N.G., Crawford, A.M., Raadsma, H.W., Bottema, C.D., 2007. Effect of myostatin F94L on carcass yield in cattle. Anim. Genet. 38, 440–446.
Shimizu, K.K., Purugganan, M.D., 2005. Evolutionary and ecological genomics of *Arabidopsis*. Plant Physiology 138, 578–584.
Souche, E.L., Hellemans, B., Van Houdt, J.K., Volckaert, F.A., 2012. Characterisation and validation of single-nucleotide polymorphism markers in expressed sequence tags of European sea bass. Mol. Ecol. Res. in press.
Szklarczyk, D., Franceschini, A., Kuhn, M., Simonovic, M., Roth, A., Minguez, P., Doerks, T., Stark, M., Muller, J., Bork, P., Jensen, L.J., von Mering, C., 2011. The STRING database in 2011: functional interaction networks of proteins, globally integrated and scored. Nucleic Acids Res. 39, D561–D568.
Tine, M., Kuhl, H., Gagnaire, P.-A., Louro, B., Desmarais, E., Martins, R.S.T., Hecht, J., Knaust, F., Belkhir, K., Klages, S., Dieterich, R., Stueber, K., Piferrer, F., Guinand, B., Bierne, N., Volckaert, F.A.M., Bargelloni, L., Power, D.M., Bonhomme, F., Canario, A.V.M., Reinhardt, R., 2014. European sea bass genome and its variation provide insights into adaptation to euryhalinity and speciation. Nat. Commun. 5, 5770.
Van Laere, A.S., Nguyen, M., Braunschweig, M., Nezer, C., Collette, C., Moreau, L., Archibald, A.L., Haley, C.S., Buys, N., Tally, M., Andersson, G., Georges, M., Andersson, L., 2003. A regulatory mutation in IGF2 causes a major QTL effect on muscle growth in the pig. Nature 425, 832–836.
Vandeputte, M., Dupont Nivet, M., Haffray, P., Chavanne, H., Cenadelli, S., Parati, K., Vidal, M.-O., Vergnet, A., Chatain, B., 2009. Response to domestication and selection for growth in the European sea bass (*Dicentrarchus labrax*) in separate and mixed tanks. Aquaculture 286, 20–27.
Visscher, P.M., Hopper, J.L., 2001. Power of regression and maximum likelihood methods to map QTL from sib-pair and DZ twin data. Ann. Hum. Genet. 65, 583–601.
Volckaert, F., Hellemans, B., Batargias, C., Louro, B., Massault, C., Van Houdt, J., Haley, C., de Koning, D., Canario, A., 2012. Heritability of morphometric traits and cortisol response to confinement stress in European sea bass *Dicentrarchus labrax*. Gen. Select. Evol. 44.
Voorrips, R.E., 2002. MapChart: software for the graphical presentation of linkage maps and QTLs. J. Hered. 93, 77–78.
Walling, G.A., Visscher, P.M., Wilson, A.D., McTeir, B.L., Simm, G., Bishop, S.C., 2004. Mapping of quantitative trait loci for growth and carcass traits in commercial sheep populations. J. Anim. Sci. 82, 2234–2245.
Wiener, P., Smith, J.A., Lewis, A.M., Woolliams, J.A., Williams, J.L., 2002. Muscle-related traits in cattle: the role of the myostatin gene in the South Devon breed. Genet. Sel. Evol. 34, 221–232.
Wringe, B.F., Devlin, R.H., Ferguson, M.M., Moghadam, H.K., Sakhrani, D., Danzmann, R.G., 2010. Growth-related quantitative trait loci in domestic and wild rainbow trout (*Oncorhynchus mykiss*). BMC Genet. 11, 63.
Xie, C., Mao, X., Huang, J., Ding, Y., Wu, J., Dong, S., Kong, L., Gao, G., Li, C.Y., Wei, L., 2011. KOBAS 2.0: a web server for annotation and identification of enriched pathways and diseases. Nucleic Acids Res. 39, W316–W322.