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First linkage maps and a pilot QTL analysis for early growth performance in common dentex (*Dentex dentex*) and sharpsnout seabream (*Diplodus puntazzo*)

Stavroula Oikonomou [a,b,c], Alexandros Tsakogiannis [a], Christina Kriaridou [b], Theodoros Danis [a], Tereza Manousaki [a], Dimitris Chatziplis [c], Nikos Papandroulakis [a], Constantinos C. Mylonas [a], Alexandros Triantafyllidis [b], Costas S. Tsigenopoulos [a,*]

[a] *Institute of Marine Biology, Biotechnology and Aquaculture (IMBBC), Hellenic Centre for Marine Research (HCMR), Thalassocosmos, Ex-US Base at Gournes, Pediados, 715 00 Heraklion, Crete, Greece*

[b] *Department of Genetics, Development and Molecular Biology, Aristotle University of Thessaloniki, University Campus, 54124 Thessaloniki, Greece*

[c] *Laboratory of Agrobiotechnology and Inspection of Agricultural Products, Dept of Agricultural Technology, School of Geotechnical Sciences, International Hellenic University, Alexander Campus, P.O. Box 141, 57 400 Sindos, Thessaloniki, Greece*

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ABSTRACT

Two potential candidate species for the Mediterranean aquaculture–the common dentex (*Dentex dentex*) and the sharpsnout seabream (*Diplodus puntazzo*)-are used in the present study in order to depict their genetic architecture for the first time. We have constructed the first linkage maps for both species using SNP markers derived from ddRAD sequencing. The quality of the maps produced was verified from comparative analysis with the most studied and phylogenetically related sparid species, the gilthead sea bream (*Sparus aurata*). A high genetic similarity was detected, based on the high number of alignments of the two new species against the reference genome of the gilthead seabream. Furthermore, a pilot study for QTL analysis per species was performed using phenotypic measurements at the juvenile stage (approximately 2 g) and revealed putative genomic areas which affect juvenile growth performance. One of those areas was located close to a candidate gene (*ANGPTL6* gene) which affects growth performance. The present study improves our knowledge on the genetic architecture of those two species by presenting not only the first linkage maps, but also by providing some indications for growth performance QTL. The results can be used as a starting point to initiate further research for the genetic improvement of these two new species.

1. Introduction

The family Sparidae includes some of the leading marine aquaculture species, such as the gilthead sea bream *(Sparus aurata)* in the Mediterranean Sea, and the red seabream (*Pagrus major*) and snapper (*Chrysophrys auratus*) in East Asia and Oceania (Palaiokostas et al., 2016; Ashton et al., 2019; Parenti, 2019). The need to diversify the aquaculture production by adding new promising species, nevertheless, has lead the scientific and industrial community to investigate the potential of other sparids such as the red porgy (*Pagrus pagrus*), the common Pandora (*Pagellus erythrinus*), the sharpsnout seabream (*Diplodus puntazzo*) and the common dentex (*Dentex dentex*) (Pavlidis and Mylonas, 2011). From one point of view this diversification of aquaculture production could create an internal market competition between the "old" species (i.e., gilthead seabream and European seabass etc.) and the above named "new" species. However, from another point of view, these "new" species, that could potentially be reared with similar methods (and hence with limited extra investment in rearing practices), may address consumer preferences for high variability in seafood choices (Cardenete et al., 1997; Piedecausa et al., 2007; Papandroulakis and Divanach, 2014).

Common dentex is distributed mainly in the Mediterranean Sea (Viret et al., 2018) and is considered a high market value fish in Greece, as it is ranked very high among consumer preferences in fish restaurants. It is a gonochoristic species usually maturing after the second year of life and its spawning period starts in March and ends in July (Pavlidis et al.,

* Corresponding author.
E-mail address: tsigeno@hcmr.gr (C.S. Tsigenopoulos).

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2001, 2000). Common dentex grows fast in the first two years, and its growth rate is higher than that of the gilthead seabream (Morales-Nin and Morante, 1997; Rueda and Martínez, 2001), as well as that of other Mediterranean sparids. Apart from the fast growth, the management of reproduction is feasible under captivity using the available reproductive techniques, making the common dentex a good alternative solution for farmed fish in aquaculture (Rueda and Martínez, 2001).

Sharpsnout seabream is found in the Mediterranean and the Eastern Atlantic Ocean (Pajuelo et al., 2008) and it has been farmed for more than 25 years (Papandroulakis and Divanach, 2014). Farming of sharpsnout seabream could be expanded based on the positive performance of the fish under captivity (Sarà et al., 1999; Papandroulakis et al., 2004). Its growth performance is similar to gilthead seabream, but its smaller head and more round body results in a higher fillet yield than seabream of the same size. The fish reaches 45% of its maximum length during the first year of age (Domínguez-Seoane et al., 2006). Studies have focused on the reproduction and the sex determination of this rudimentary protandrous species (Pajuelo et al., 2008; Papadaki et al., 2008, 2018, 2020; Mouine et al., 2012; Manousaki et al., 2014).

As far as the genetic improvement of fish broodstock is concerned, except for the classical selection methods for farmed species, breeding techniques utilizing Markers Assisted Selection (MAS) or Genomic Selection (GS) are used in order to improve the performance of the fish in intensive farm conditions. However, genomic selection approaches in fish are limited compared to the scale encountered in plants and livestock; the majority of the fish breeding programs perform family or mass selection. Even though due to advances in new Next Generation Sequence (NGS) techniques, and the implementation of reduced representation libraries sequencing methods such as RAD-seq (2b-RAD, ddRAD), molecular tools such as low or high density SNP panels, are available (Gjedrem and Robinson, 2014; Houston et al., 2020; Janssen et al., 2017; Peñaloza et al., 2020; Robledo et al., 2018). Nevertheless, important steps have been taken in order to moderate this situation. Even though in 2008 only two commercial breeding programs were utilizing Markers Assistance Selection (Atlantic salmon), fourteen commercial breeding programs were operating in 2016 utilizing MAS; the Atlantic salmon remains the leading species in Europe followed by the gilthead seabream (Chavanne et al., 2016).

Nowadays, the use of molecular tools in commercial aquaculture is becoming more and more popular, and many studies have been accomplished concerning the identification of Quantitative Trait Loci (QTL) in Mediterranean marine species such as the European sea bass (*Dicentrarchus labrax*) and the gilthead seabream, which affect not only the growth and disease resistance, but also stress indicators and sex determination (Chatziplis et al., 2007, 2020; Massault et al., 2009; Loukovitis et al., 2011, 2012; Louro et al., 2014; Griot et al., 2021). However, in commercial breeding programs growth remains the most important trait followed by morphology and disease resistance (Chavanne et al., 2016). The use of QTL analysis assists in the identification of any potential associations between genetic markers and phenotypes. Furthermore, those markers can be used as a first step for Marker Assisted Selection, thus candidates could be selected based on the genotypes of those markers (Lande and Thompson, 1990).

The aim of the present study was to develop genetic tools in order to obtain preliminary information for the basic genetic architecture of the sharpsnout seabream and common dentex. More specifically, our first objective was to construct the first linkage maps for both species, then to perform a pilot QTL analysis using the available phenotypes for early growth performance and finally, to conduct a genomic comparative analysis between the aforementioned species and the most studied sparid species, the gilthead seabream. Each of those steps could potentially assist future approaches for genetic improvement for sharpsnout seabream and common dentex.

2. Material and methods

2.1. Fish sampling and DNA extraction

All experiments were performed in accordance with the "Guidelines for the treatment of animals in behavioral research and teaching" (Animal Behavior Society 2001, see also Manousaki et al., 2016).

All fish came from the HCMR experimental aquaculture facilities. Broodstock fish for each species (already fin-clipped) were allowed to spawn spontaneously, and floating eggs were collected and reared using commercial larval rearing methods for sparid fish (Papandroulakis et al., 2004). Common dentex samples originated from a spawn on May 4th (hatched on May 6th) and sampled on July 31st, 2013, i.e., 75 Days post-hatching (DPH). Similarly, sharpsnout seabream eggs came from a spawn on October 26th (hatched on October 29th) and sampled on February 7th, 2014, i.e., 100 DPH. Family structure and descriptive statistics of phenotypic traits recorded are presented in Table 1.

For both species, juveniles were sampled at approximately 2 g size, and fish were weighed (body weight, ATOL:0000351 according to ATOLontology (http://archive.aquaexcel.eu)) and their standard length (ATOL: 0001659, the distance between the nose and the end of the last vertebrae) and greatest depth (body maximum height) were measured. Moreover, the total length (ATOL:0001660) the distance between the nose and the end of the longer lobe was measured only in common dentex (Table 3).

Muscle tissue samples were stored at −20 °C in absolute ethanol until DNA extraction which was based on a modified salt-extraction protocol using SSTNE extraction buffer (Miller et al., 1988) and treated with RNase to remove residual RNA. Total DNA was eluted in 5 mmol/L Tris (pH 8.5), quantified by spectrophotometry (Nanodrop 1000, ThermoFisher Scientific), quality-evaluated through electrophoresis on a 0.7% agarose gel, and stored at 4 °C until ddRAD library construction. Experiments were conducted in accordance with the "Guidelines for the treatment of animals in behavioral research and teaching" (2001) that were in force in 2013–2014.

2.2. Preparation of ddRAD libraries

We followed the ddRAD library preparation protocol initially described by Peterson et al. (2012) with modifications detailed in Manousaki et al. (2016). Briefly, 20 ng DNA per sample was simultaneously digested with two high fidelity restriction enzymes, *Sbf*I (CCTGCA = GG recognition site) and *Sph*I (GCATG = C recognition site) both sourced from New England Biolabs, (NEB) UK. One library was constructed per species, and in each one both parents were included three times (triplicates). For the sharpsnout seabream, we used a single full sib family composed of 129 offspring while for the common dentex, 131 offspring coming from two full sib families (the two full sib families constitute a half sib family with a common female parent and two male parents) were used (Table 1). The two libraries were eluted in 22 μL EB buffer. Finally, each ddRAD library was sequenced at the Hellenic Centre For Marine Research in Crete using three runs of an Illumina MiSeq (v2 chemistry, 300 cycles kit, 162 bp paired end reads) per library.

2.3. SNP discovery and genotyping

Raw data were analyzed using STACKS2.4 software (Catchen et al., 2013). More specifically, demultiplexing and filtering were performed using process_radtags command. When ustacks was used, the maximum distance between stacks was equal to 3 and the number of the minimum mismatches was set as 3. As there is no reference genome available for either species, the catalog was created using the parents of the fish utilized for each species, separately. Parents of all the families were sequenced three times in order to achieve higher quality of the reads. Moving on, the number of the minimum mismatches was set as 3 when

Table 1
Family structure and descriptive statistics per trait.

Species	Common dentex					Sharpsnout seabream			
Trait	Weight (g)	Standard length (mm)	Total length (mm)	Greatest depth (mm)	Number of offspring	Weight (g)	Standard length (mm)	Greatest depth (mm)	Number of offspring
Average	1.82 ± 0.60	42.32 ± 4.7	50.40 ± 5.8	14.32 ± 1.9	131	1.92 ± 0.78	37.67 ± 5.1	16.72 ± 2.6	129
max	3.01	50.06	59.49	17.94		3.95	47.48	10.86	
min	0.62	31.15	36.87	9.53		0.68	26.39	22.07	
Number of families	1st family: 79 offspring 2nd family: 51 offspring					1 full sib family			

cstacks was used. Finally, using the gstacks command, a population map was created containing all the offspring and the parents while the number of threads was set as 20. The above process was performed in the computer cluster ZORBA of IMBBC (https://hpc.hcmr.gr/).

2.4. Construction of linkage map

All genotype datasets were filtered using the following criteria for quality control, more than 80% SNPs call rate, 0.05 minor allele frequency, 0.001 Mendelian errors and only offspring with more than 70% of the genotypic data were used. Plink software was used for the aforementioned process (Purcell et al., 2007). A linkage map was constructed using LepMap2 for both species (Rastas et al., 2016). More specifically, the LOD score was selected based on the number of linkage groups which was produced by the analysis to match the number of chromosomes in their karyotypes (Manousaki et al., 2016). Karyotype analysis of both species has previously revealed 24 chromosomes (Accioly and Molina, 2008; Vitturi et al., 1996), thus, the LOD scores were set 6.0 and 5.5 for sharpsnout seabream and common dentex, respectively. The linkage analysis was performed using the following thresholds, the Tolerance threshold was 0.001, the PolishWindow was set at 100, Filtered Window equal to 10, numThreads (parallel computation) were equal to 10, Kosambi linkage function was used and minError (genotyping errors) set equal to 0.01 and SexAverage = 1. Finally, the order analysis was repeated four times per linkage group and the order of the markers with the highest likelihood was selected per linkage group. MapChart (Supplementary figures) was used in order to illustrate the linkage maps (Voorrips, 2002).

2.5. QTL analysis

Body weight, depth, standard length and total length (the latter available only for common dentex) were combined with genotypic data, from the linkage maps produced herein, and analyzed separately per species in order to perform a QTL analysis per trait. The QTL analysis was performed using the qtl2/R software (Broman et al., 2019). Two models with and without polygenic components (using a kinship relationship matrix based on the genotypes) were performed per trait per species [kinship matrix was estimated using the qtl2/R software (Broman et al., 2019)]. Finally, no fixed effects existed in our data and hence no fixed effects were included in the analysis. Thresholds for the QTL analysis were estimated using a permutation test approach as described in the manual of qtl2/R and the number of the permutation was set at 1000 for each trait (Tables 4 and 5) (Broman et al., 2019).

2.6. Comparative analysis

Only alignments of the RAD *loci* containing the SNPs which were distributed in linkage groups were used in the genomic comparative analysis with seabream. The genome of the seabream (*Sparus aurata)* was from NCBI database (ID: 11609, https://www.ncbi.nlm.nih.gov/genome/?term=Sparus+aurata). The e-values thresholds for the similarity between reference genome of seabream and the *loci* from the two linkage maps were set at 10^{-5}. The best hit was selected and considered as homologous between the alignment (SNP) from the genetic map and the reference genome (Manousaki et al., 2016). The last step was to identify the synteny of the linkage groups from the linkage maps and the linkage groups of the reference genome of seabream. Moreover, the similarity between species and seabream was estimated based on the number of the *loci* which have been hit in the reference genome divided by the total number of all the available *loci* from the map. Finally, a Circos plot per species illustrated the results from the comparative analysis (Krzywinski et al., 2009). The above process was performed in the computer cluster of HCMR.

3. Results

From the ddRAD analysis for common dentex, 11,534 *loci* were detected; the mean length of *loci* was 231.83 bp while 4352 variants were identified. For sharpsnout seabream, 4761 *loci* were detected with mean length equal to 244.21 bp and 2736 variants were identified. After quality control, out of the total number of variants, 1718 and 1484 were used in the linkage analysis for common dentex and sharpsnout seabream, respectively. Finally, 1263 and 1207 SNPs were distributed in 24 linkage groups for common dentex and sharpsnout seabream, respectively (Tables 2 and 3). The average distance between markers was 1.28 and 1.29 cM while the total length per map was 1599.37 and 1550.66 cM for common dentex and sharpsnout seabream, respectively. The smallest linkage group included 8 markers and the largest 113 for common dentex (Table 2); in the sharpsnout seabream, the number of markers per linkage group ranged from 9 to 120 (Table 3). Furthermore, no gaps larger than 50 cM were found in any of the two linkage maps. Finally, the range of the total length of linkage groups was from 7.21 to 194 cM for common dentex, and from 7.37 to 227 cM for sharpsnout seabream map. Figs. 1 and 2 illustrate the linkage maps for sharpsnout seabream and common dentex using the modified results by MapChart [a more detailed representation of the linkage maps for both species can be found on Supplementary Figs. 1 and 2 (i.e., MapChart, A.1 and A.2 (Voorrips, 2002))].

The average body weight was 1.83 g and 1.92 g, while the average greatest depth was 14.32 mm and 16.72 mm for common dentex and sharpsnout seabream, respectively. Furthermore, the descriptive statistics per trait per species were calculated and illustrated in Table 1. Focusing on QTL analysis, no statistically significant QTL was detected in any species. However, test statistic peaks close to the threshold values were found in both species (Tables 4 and 5, Figs. 3 and 4). Even though, no QTL was detected, the alignments of the SNPs which were closer to the highest LOD score were used in the database of the NCBI (Standard Nucleotide BLAST, https://blast.ncbi.nlm.nih.gov/Blast.cgi) and the nearest gene was selected.

Finally, for the comparative analysis, 1032 SNPs and 1132 SNPs which were contained in RAD *loci* were hit in the genome of the gilthead seabream for common dentex and sharpsnout seabream, respectively; this implies that 82% of common dentex and 94% of sharpsnout seabream SNPs map successfully to the gilthead seabream genome.

For both species, it is notable that the majority of the SNPs per

Table 2
Linkage map of common dentex and the results from the comparative analysis with gilthead seabream.

LG	Number of markers	Total length (cM)	Average interval between markers (cM)	Max interval between markers (cM)	Number of alignments (SNPs) which had a homolog in the reference genome	Homologous LG of seabream	Number of alignments (SNPs) which are included in homologous LG seabream
1	113	194.144	1.73	23.29	92	11/19	58/34
2	101	161.355	1.61	27.411	80	16/7	54/26
3	73	86.777	1.21	6.215	57	23	52
4	69	55.343	0.81	5.306	57	6	53
5	67	67.827	1.03	11.814	52	15	48
6	64	53.052	0.84	8.779	56	2	54
7	62	60.412	0.99	10.885	53	8	51
8	61	96.096	1.6	16.467	54	13	52
9	62	86.617	1.42	8.093	54	4	50
10	60	63.659	1.08	7.327	51	5	49
11	59	89.625	1.55	15.344	48	9	44
12	52	59.394	1.16	10.256	43	17	42
13	51	74.433	1.49	13.73	44	18	38
14	47	68.671	1.49	13.64	39	3	32
15	45	61.506	1.4	7.906	36	21	34
16	44	53.282	1.24	9.648	33	22	30
17	44	32.956	0.77	4.518	34	1	30
18	40	71.326	1.83	25.755	33	20	27
19	40	38.953	1	9.171	30	14	28
20	38	35.205	0.95	8.977	34	12	30
21	33	32.047	1	6.113	24	10	20
22	18	40.386	2.38	9.755	13	24	12
23	12	11.976	1.09	3.373	9	24	9
24	8	7.21	1.03	3.615	6	1	5
Total	**1263**	**1599.37**	1.27		**1032**		

Table 3
Linkage map of sharpsnout seabream and the results from the comparative analysis with gilthead seabream.

LG	Number of markers	Total length (cM)	Average interval between markers (cM)	Max interval between markers (cM)	Number of alignments (SNPs) which had a homolog in the reference genome	Homologous LG of seabream	Number of alignments (SNPs) which are included in homologous LG seabream
1	120	227.98	1.92	27.739	111	5/12	57/54
2	71	70.14	1	9.231	66	9	60
3	65	95.26	1.49	32.42	60	15	58
4	63	84.33	1.36	15.087	60	4	54
5	62	62.97	1.03	11.562	58	17	50
6	61	66.62	1.11	18.604	60	6	53
7	60	53.45	0.91	9.693	57	22	55
8	59	67	1.16	7.715	55	11	49
9	57	68.03	1.21	7.125	52	19	47
10	55	65	1.2	9.659	52	18	50
11	51	58.1	1.16	8.762	50	16	47
12	50	51.71	1.06	6.222	44	21	40
13	47	52.32	1.14	8.932	44	8	43
14	47	45.47	0.99	13.474	44	12	41
15	47	69.33	1.51	15.822	44	1	38
16	43	70.28	1.67	11.086	40	13	37
17	40	80.35	2.06	15.507	38	7	32
18	40	55.01	1.41	11.285	37	10	36
19	39	42.67	1.12	7.157	37	2	35
20	38	34.58	0.93	13.404	37	23	36
21	30	16.34	0.56	4.834	29	14	27
22	27	51.79	1.99	28.799	25	24	22
23	26	54.55	2.18	11.035	25	20	21
24	9	7.38	0.92	5.856	7	14	7
Total	**1207**	**1550.66**	**1.29**		**1132**		

linkage group (LG) are also located on the same LG in gilthead seabream that we used as reference. More specifically, 955 of the 1032 SNPs (93%) of the common dentex were placed in the same LGs of the gilthead seabream while only 68 *loci* were placed in different linkage groups. For the sharpsnout seabream, 1035 of the 1132 SNPs (91%) were placed in the same LGs of the gilthead seabream and only 97 SNPs were placed in different linkage groups. There are 77 and 92 SNPs mapped on different LGs of the gilthead seabream with the majority of SNPs to show one-to-one correspondence for common dentex and sharpsnout seabream, respectively. In the case of common dentex, only the first two LGs correspond to two chromosomes of the gilthead seabream genome. More specifically, the LG1 included 92 SNPs with homologous sequences in the reference genome, of which 58 were found on chromosome 11 and the rest were hit in chromosome 19. For LG2, 54 SNPs were found in chromosome 16, while the rest were found in chromosome 7. Focusing on sharpsnout seabream, the SNPs of the LG1 were divided into two chromosomes: LG5 (57 SNPs) and LG12 (40 SNPs). Tables 2 and 3 illustrate the results per linkage group per species and the number of SNPs which matched the genome of gilthead seabream (A.3 and A.4, Figs. 5 and 6).

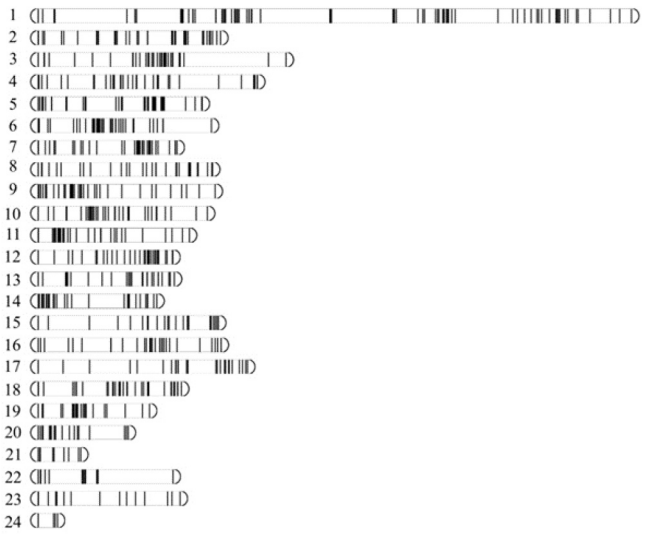

Fig. 1. De novo linkage map for sharpsnout seabream.

Fig. 2. De novo linkage map for common dentex.

4. Discussion

This is the first time we obtain genomic information for common dentex and sharpsnout seabream, two species with potential future use in the aquaculture research. Specifically, a linkage map per species was constructed using SNP markers. Furthermore, a great similarity of the genome organization was detected using two comparative genome analyses with gilthead seabream for each species. Finally, no major QTL was identified significantly affecting early growth performance in both species.

4.1. Linkage analysis

In the present study, two linkage maps were constructed for two sparids species, the common dentex and sharpsnout seabream. The family Sparidae includes the emblematic species for the Mediterranean aquaculture, the gilthead seabream (*Sparus aurata*) for which Franch et al. (2006) presented the first-generation linkage map using 204 microsatellites, distributed into 26 linkage groups and with a total map length equal to 1241.9 cM. Moreover, the second generation genetic map included 232 microsatellites, 85 ESTSSRs and 4 SNPs distributed into 27 linkage groups and a total length equal to 1769.7 cM (Tsigenopoulos et al., 2014).

Apart from gilthead seabream, more species have been genetically studied such as the snapper (*Chrysophrys auratus*). Snapper's linkage map had a total length equal to 1363.0 cM and an average interval between genetic markers equal to 0.129 cM (Ashton et al., 2019). Moreover, a linkage map with 24 LGs was constructed for the common pandora (*Pagellus erythrinus*) using 917 SNPs markers from ddRAD methodology; the total length was 2201.78 cM and the interval average between markers was 3.98 cM for the above map (Manousaki et al., 2016).

In the present study, the total length of the maps for the common dentex and sharpsnout seabream are in the middle of the range of the aforementioned linkage maps with 1599.37 and 1550.66 cM and the interval is equal to 1.27 and 1.29 cM, for common dentex and sharpsnout seabream, respectively (Tables 2 and 3). Moreover, the maximum of the average interval between SNP was 2.18 cM in LG23 in sharpsnout seabream, while 2.38 cM in LG22 for common dentex, but the max interval was 28.79 cM (LG22) and 27.41 cM (LG2) for sharpsnout seabream and common dentex, respectively. As far as the smallest linkage group is concerned, i.e., LG24, it included only 8 and 9 SNPs in both species (Tables 2 and 3).

4.2. QTL analysis

In aquaculture, the commercial traits such as growth and morphology are considered to be influenced by multiple genomic regions with a small additive effect each. This, polygenic architecture is making the detection of QTL affecting them difficult (Houston et al., 2020). However, many studies in sparids have reported QTL affecting growth such as Loukovitis et al., (2011, 2012, 2013), who found a QTL affecting the body weight at harvest, length and depth. Ashton et al. (2019), identified QTL affecting not only growth rates but also length, when they studied snapper (*Chrysophrys auratus)*. However, in our study no statistically significant QTL affecting early growth performance was detected, although some indications for putative QTL were found (Tables 4 and 5). Nevertheless, our results seem to confirm a polygenic nature of growth in the two species studied herein. However, the high LOD scores, near the significance threshold value, in some SNP markers (Table 4) might indicate putative QTL that were difficult to detect due to their effect size (i.e., no major effect), the stage of growth (i.e., very early

Table 4
Highest LOD scores from QTL analysis for sharpsnout seabream.

LG	Position (cM)	Marker	QTL			QTL and polygenic effect		
			Body weight	Standard length	Greatest depth	Body weight	Standard length	Greatest depth
2	9.757	2773_244	2.72	2.69	2.19	1.85	1.81	1.33
2	30.234	4998_263	**3.08**	2.70	2.91	2.64	2.30	2.50
5	44.102	2352_37	2.54	**3.01**	2.40	2.54	3.12	2.36
6	32.628	4829_114	**4.14**	**4.02**	**3.73**	**4.05**	**3.95**	**3.68**
6	45.816	4856_123	2.87	**3.25**	2.99	3.06	**3.50**	**3.19**
12	42.034	1290_33	2.66	2.39	2.67	2.30	1.95	2.27
12	51.712	3756_215	2.40	2.59	2.40	2.09	2.20	2.00
Threshold from permutations			4.22	4.22	4.19	4.25	4.14	4.31

Table 5
Highest LOD scores from QTL analysis for common dentex.

LG	Position (cM)	Marker	QTL				QTL and polygenic effect			
			Body weight	Standard length	Total length	Greatest depth	Body weight	Standard length	Total length	Greatest depth
1	149.97	**7440_212**	2.06	1.44	1.48	**3.02**	1.75	1.30	1.24	2.45
3	40	Linked to 3669_100, 268_75, 4090_197, 5064_67	**2.97**	2.93	**3.10**	2.91	2.22	2.46	2.39	2.12
3	75.61	**4401_209**	**3.44**	2.32	3.06	**3.75**	**3.21**	2.50	3.15	**3.03**
3	75	Linked to 4401_209	2.77	**3.76**	**3.45**	1.97	2.78	**3.62**	**3.36**	1.86
7	50	Linked to 6359_264	**3.34**	**3.61**	**3.81**	2.98	2.26	2.59	2.46	1.89
7	59.15	6359_264	2.39	2.00	2.56	**3.05**	1.58	1.61	1.93	1.78
8	45	Linked to 1013_105, 2177_206, 3990_204,4735_204, 5799_229, 5952_255, 4469_210	1.72	**3.12**	2.49	1.36	2.21	**3.37**	2.93	2.01
12	0	6540_66	1.63	0.94	1.47	**3.27**	1.34	0.98	1.45	2.57
Threshold from permutations			4.15	4.17	4.15	**4.21**	4.19	4.19	4.21	4.20

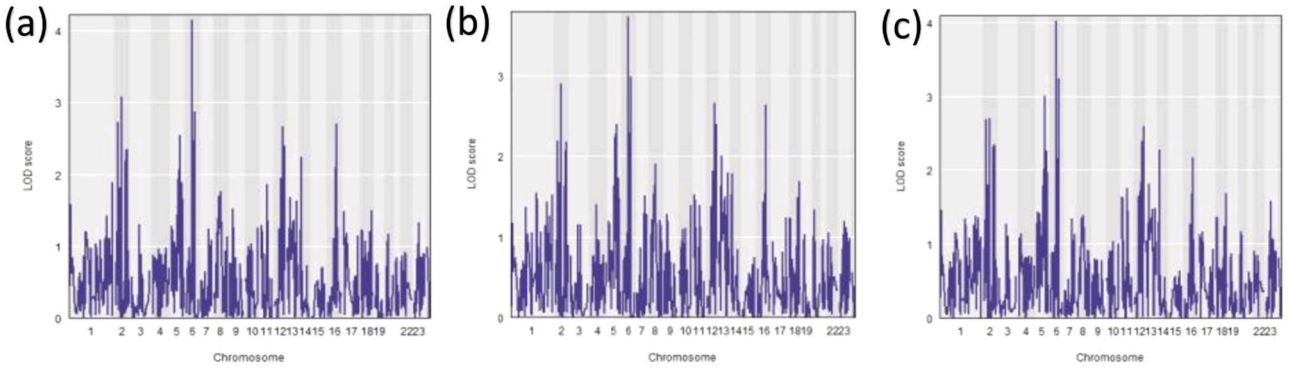

Fig. 3. QTL analyses without the polygenic component for sharpsnout seabream, the first plot illustrates the results for body weight (a), the second illustrates the results for greatest depth (b) and the last one the results for standard length (c).

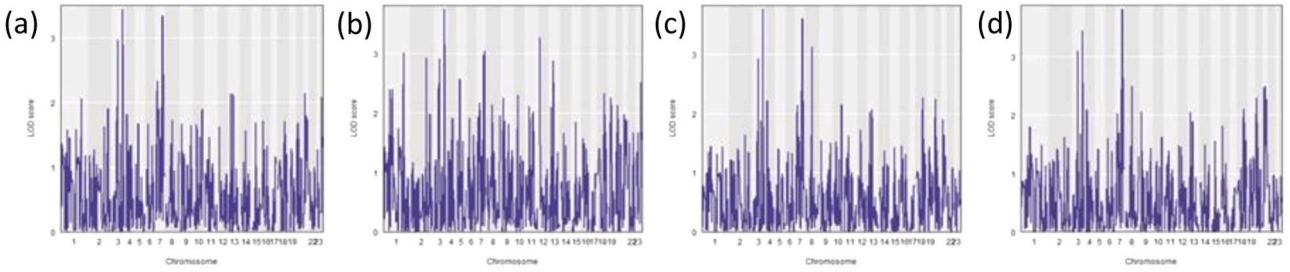

Fig. 4. QTL analyses without the polygenic component for common dentex, the first plot illustrates the results for body weight (a), the second illustrates the results for greatest depth (b) and the last two illustrate the results for standard (c) and total lengths (d).

growth stages), the small sample size and the limited number of families participating in the study.

The QTL analysis for the sharpsnout seabream showed that a potentially putative QTL (marker name 4829_114) affects the body-weight at early growth stages. More specifically, LOD score for the body weight and standard length was very high (4.14 and 4.02 for body weight and standard length, respectively) and very close to the threshold value (4.22 for both traits) even though only 129 offspring have been used in the present study (Table 4). It is important to mention that the LOD scores remain high (4.05 and 3.95 for body weight and standard length) even under analysis utilizing a mixed model (QTL and polygenic component, threshold 4.25 and 4.14, Table 4). Further investigation is required using larger sample size and multiple families in order to clarify if this putative QTL affects the body weight and the length of the fish.

Focusing on QTL analysis for common dentex, no statistically significant associations were detected. However, there were also high LOD scores close to the threshold value, more specifically, using the non-polygenic component model, for the body weight. There were two test statistic peaks on LG3 and LG7 and they were equal to 3.44 and 3.34, but when polygenic component was used, the first peak only drops at 3.21 on marker 4401_209 (Table 5). When analyzing the standard length, a possible putative QTL linked to the marker 4401_209 could be affecting the aforementioned trait because the LOD score for the model without polygenic component was equal to 3.76 but using the polygenic component in the model, it decreases to 3.36. In the same position, a potential QTL affecting the total length was noted (LOD score 3.45 without polygenic component, 3.36 with polygenic component, Table 5). It seems that marker 4401_209 and linked genomic regions may be affecting the growth performance but the small sample size of the study did not help to reveal any significant effect of this marker. There too, further research is required in order to investigate the effect of this marker on growth. Moreover, apart from the marker 4401_209, indications of possible putative QTL linked to close markers 1013_105, 2177_206, 3990_204, 4735_204, 5799_229, 5952_255 and 4469_210

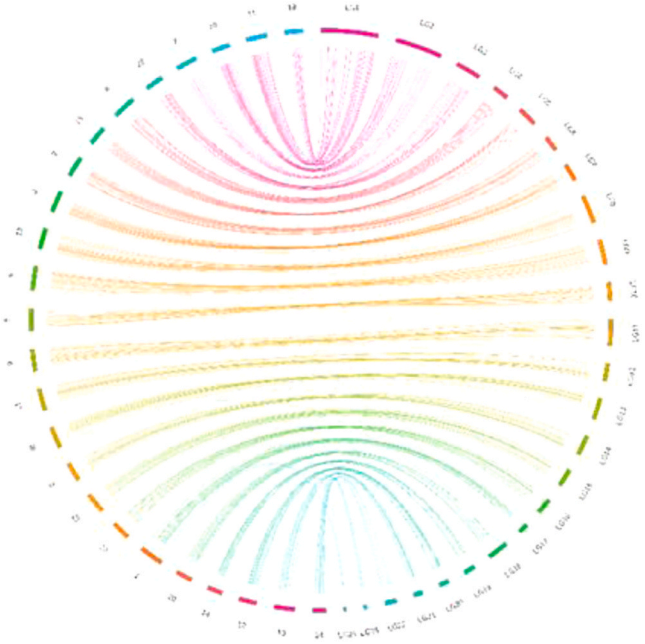

Fig. 5. Circos plot between gilthead seabream (*Sparus aurata*) on the left side and common dentex (*Dentex dentex*) on the right side.

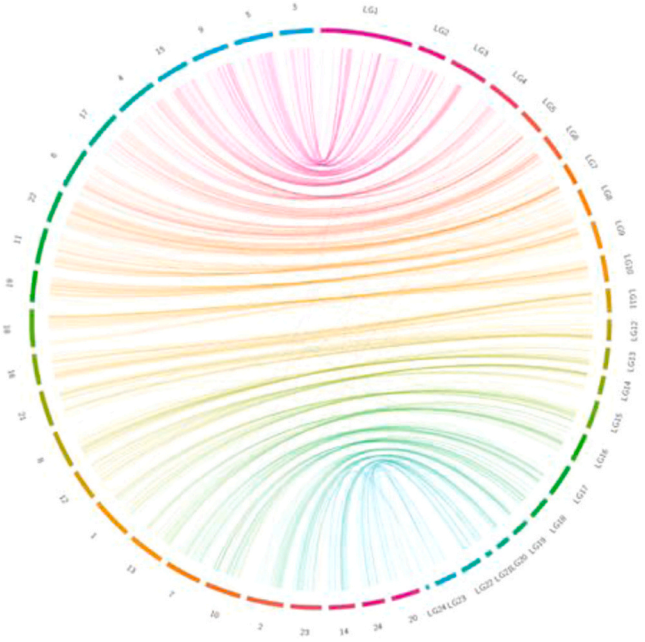

Fig. 6. Circos plot between gilthead seabream (*Sparus aurata*) on the left side and the sharpsnout seabream (*Diplodus puntazzo*) on the right side.

was found to affect standard length (LOD score 3.12–3.37 depending on the model used, Table 5). The SNP 4401_209 is located near the ANGPTL6 gene which affects the body weight after the age of 12 weeks, when the wild type and the *Angpt16* $^{-/-}$ mice are compared, based on Oike et al. (2005). Consequently, this SNP constitutes a valuable candidate gene for a future larger and more statistically powerful study of early or other growth stages on those two species.

4.3. Comparative analysis

The comparative analysis using the genome of the gilthead seabream helped us to validate and investigate the quality of the map. The selection of the gilthead seabream as the reference genome was based on the fact that all candidates belong to the same family of Sparidae (Parenti, 2019). Only the first and second linkage groups for common dentex, and only the first linkage group are split into 2 different chromosomes for sharpsnout seabream (Tables 2 and 3). When Manousaki et al. (2016) compared the linkage map of the common pandora (*Pagellus erythrinus)* with the reference genome of the European sea bass (*Dicentrarchus labrax*) and medaka (*Oryzias latipes*), the same pattern was noted. There were two LGs (3 & 16) in common pandora whose alignments were placed in two different LG in European sea bass. Moreover, the SNPs of the LG16 were also split into 4 different chromosomes in medaka. However, when the same linkage map was compared also with Nile tilapia *(Oreochromis niloticus)* and stickleback (*Gasterosteus aculeatus*), the above observation was not noticed.

Furthermore, gilthead seabream and sharpsnout seabream belong to the subfamily Sparinae and common dentex belongs to the subfamily Denticinae (Parenti, 2019) and Natsidis et al. (2019) reported based on a phylogenomic analysis that gilthead seabream and sharpsnout seabream are related while the common dentex is more closely related to the common pandora and the red porgy. Thus, the higher genetic similarity from the comparative analysis between sharpsnout seabream and gilthead seabream genome compared to common dentex and the gilthead seabream genome agreed with the above findings.

The similarity ranged from 43% (European sea bass) to 10% (medaka), between common pandora and the aforementioned species (Manousaki et al., 2016). Chistiakov et al. (2008) also compared the European sea bass with green spotted pufferfish, fugu, medaka, three-spined stickleback and zebrafish using microsatellites, and the similarity ranged between 3.1% (zebrafish) and 33.6% (stickleback). When gilthead seabream was compared with green spotted puffer (*Tetraodon nigroviridis*) the similarity was 70% (301 of the 428 gene-based markers were found in green spotted puffer) (Sarropoulou et al., 2007). However, in the present study, the similarity was much higher at 82% for common dentex and 94% for sharpsnout seabream with the same family species the gilthead seabream (*Sparus aurata*).

Although there were a limited number of families available with few offspring and a stochastic genotyping method (i.e., ddRAD) was utilized for the genotyping of SNP markers, the results of the comparative analysis verify the construction of a good quality linkage maps for both species in this study. Furthermore, there is an important similarity between the studied species and gilthead seabream as far as marker-phenotype association is concerned.

The above high genetic similarity can inform us for further possible genomic areas including QTLs of interest. Kyriakis et al. (2019), using SNP markers produced with ddRAD methodology found genomic areas which affect the body weight at different growth stages in chromosomes 1, 2, 6, 13, 16 and 22 of gilthead seabream, using a GWAS analysis. In common dentex, the comparative analysis revealed that those chromosomes are considered as homologous for LG1, 17, 6, 8, 2 and 16. From the QTL analysis of this species indicative evidence was found only in LG8 to affect standard length, but not body weight. Regarding the sharpsnout seabream, the comparative analysis reveals that for the above chromosomes, the corresponding LGs are 6, 7, 11, 15, 17 and 19. From the QTL analysis of this species, a putative QTL that may affect all traits analyzed was revealed on LG6 which corresponds to chromosome 6 in the Seabream genome. Although the comparative analysis among the three different species, revealed a genetic similarity between the two new species and the gilthead seabream, further research utilizing more powerful experimental designs, with larger fish growth period and higher density of markers (especially in LG8 and LG6 for common dentex and sharpsnout seabream, respectively) is needed in order to make any powerful inferences concerning the results of our QTL analysis and the GWAS analysis in gilthead seabream by Kyriakis et al. (2019).

The pilot QTL analysis for the two species is statistically underpowered due to the sample size availability and the limited family

structure. However, it presents a first application of the available linkage maps for those species. It is notable that, even with a small sample size and a small number of families included in the analysis, indicative evidence for possible putative QTL can be found in both species. Nevertheless, it was the first genetic approach for the creation of genetic linkage maps and QTL analyses in those species and the results of the study could be a good starting point for further research and the spark for future breeding programs for those two species. Further research utilizing more statistically powerful experiments should be done in order to clarify any effect of those markers on growth performance. Given the high genomic similarity (82 & 94%) of the results of this study with the gilthead seabream, the utilization of information from other studies (e.g., 1,51–53, and any new upcoming QTL research results on gilthead seabream) in such experiments could be of assistance in targeting genomic areas something that potentially could reduce the experimentation cost for commercial aquaculture companies applications. In any case, any verification of such marker effects could be directly utilized for preselection of broodstock and the establishment of a nucleus in a commercial breeding program.

5. Conclusions

A linkage map per species-common dentex and sharpsnout seabream-was constructed using SNP markers from ddRAD methods. The linkage maps were validated using comparative analysis with gilthead seabream and a great similarity was detected for each species. Finally, evidence of putative QTLs effect on early growth performance in both species.

CRediT authorship contribution statement

Stavroula Oikonomou: Formal analysis, Investigation, Methodology, Software, Visualization, Writing – original draft, Writing – review & editing. **Alexandros Tsakogiannis:** Investigation, Methodology. **Christina Kriaridou:** Formal analysis, Software. **Theodoros Danis:** Formal analysis, Software. **Tereza Manousaki:** Data curation, Investigation, Methodology, Resources, Validation, Writing – review & editing. **Dimitris Chatziplis:** Conceptualization, Data curation, Investigation, Methodology, Software, Validation, Writing – review & editing. **Nikos Papandroulakis:** Resources, Writing – original draft. **Constantinos C. Mylonas:** Resources, Writing – original draft. **Alexandros Triantafyllidis:** Investigation, Writing – original draft. **Costas S. Tsigenopoulos:** Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Writing – review & editing.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Fig A.1, De novo linkage map for sharpsnout seabream.

Fig A.2, De novo linkage map for common dentex.

Table A.3, De novo linkage map for sharpsnout seabream, comparative analysis between sharpsnout seabream and gilthead seabream and markers sequencing are illustrated.

Table A.4, De novo linkage map for common dentex, comparative analysis between sharpsnout seabream and gilthead seabream and markers sequencing are illustrated.

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.aqrep.2021.100855.

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