Austin Journal of Molecular and Cellular Biology

Open Access

Austin Publishing Group

Special Article - Molecular Breeding

Research Advances on Molecular Breeding of Groupers in China

Yu H[1]†, You XX[2]† and Shi Q[1,2,3]*

[1]BGI Education Center, University of Chinese Academy of Sciences, Shenzhen 518083, China

[2]Shenzhen Key Lab of Marine Genomics, Guangdong Provincial Key Lab of Molecular Breeding in Marine Economic Animals, BGI, China

[3]Laboratory of Aquatic Genomics, College of Life Sciences and Oceanography, Shenzhen University, China

†Equal contributors

***Corresponding author:** Qiong Shi, Bldg 11 Room 618, Beishan Industrial Zone, BGI, Shenzhen 518083, China

Received: July 26, 2016; **Accepted:** August 23, 2016; **Published:** August 24, 2016

Abstract

As a group of economically important fish in China, groupers (*Epinephelus* spp.) are facing with insufficiency of high-quality gametes and germ plasm resource degradation. This situation has severely restricted the healthy development of grouper aquaculture. It is an inevitable choice to breed new varieties or species of groupers to solve these problems. Traditional hybridization and recently developed molecular breeding are effective ways to generate novel high-quality species of groupers. So far, several hybrid groupers have been cultivated in China with significant economic values. The important areas of molecular breeding include Marker-Assisted Selection (MAS) and transgenic breeding. At present, the molecular breeding of groupers in China is focusing on MAS breeding. We have constructed genetic linkage maps and carried out Quantitative Trait Loci (QTL) analysis for a couple of groupers. Furthermore, we have cooperated with scientists at Sun Yat-Sen University to sequence the whole genome of orange-spotted grouper (*E. coioides*). All these works will provide a valuable resource for understanding the genetic regulation of growth and establishing the foundation for MAS breeding in groupers.

Keywords: Groupers; Hybrid; Molecular breeding; Marker-assisted selection

Abbreviations

ddRAD: Double Digest Restriction-Site Associated DNA; GH/IGF: Growth Hormone /Insulin-Like Growth Factor; GHRH: Growth Hormone-Releasing Hormone; GnRH: Gonadotropin-Releasing Hormone; MAS: Marker-Assisted Selection; MSG: Multiplexed Shotgun Genotyping; PRP-PACAP: PACAP-Related Peptide/Pituitary Adenylatecyclase Activating Polypeptide; QTL: Quantitative Trait Loci; SSR: Simple Sequence Repeat; SNP: Single-Nucleotide Polymorphism

Introduction

Groupers (*Epinephelus* spp.), a group of economically important marine fish species, are well-known for their rich nutrition, delicious taste and tender flesh [1,2]. Grouper industry has been developed rapidly in the past decades with wide cultivation of over 10 grouper species in China and South-East Asian countries [2]. Among them, *E. coioides*, *E. akaara*, *E. awoara*, and *E. malabaricus* are the main cultured species in China (Figure 1). In the last year, the yearly output of groupers in China is 100,006 tons, and the production of major cultured provinces, including Guangdong, Fujian and Hainan, are 42,601, 26,905, and 26,785 tons respectively [3]. At the same time, degeneration of germ plasm resources, decrease of genetic diversity and degradation of gamete quality have been threatening the development and progress of grouper industry. During the past decade, rapid development of genomic biotechnology and increasing focuses on more efficient selection programs have accelerated genetic improvement in groupers. The high throughput sequencing techniques have facilitated identification of efficient molecular markers and implementation of Marker-Assisted Selection (MAS) program.MAS breeding is going to become a prospective strategy for generation of ideal novel species of fish. Both construction of genetic linkage maps and identification of Quantitative Trait Loci (QTL) will lay the foundation for the development of MAS program in groupers. Meanwhile, traditional hybridization breeding has created several ideal grouper hybrids, which provide genetic resources for understanding the molecular mechanisms of growth superiority in the hybrid groupers.

Hybrid Breeding

Traditional hybridization has been an effective approach to improve physiological properties [4]. The first Hybrid of *Epinephelus* species, reported in 2014, hasobvious advantage in growth comparing with its parents [5]. It has been improved to become popular because of rapid growth rates and better disease resistance in recent years [6]. The successful hybridization of groupers are mainly composed of *E.*

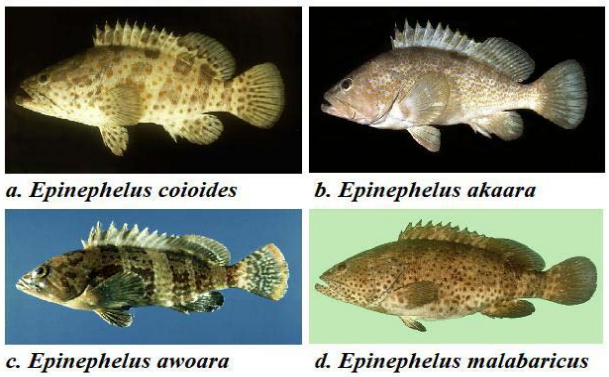

Figure 1: The main cultured groupers in China. The pictures are downloaded from http://www.worldfishcenter.org/databases.

Citation: Yu H, You XX and Shi Q. Research Advances on Molecular Breeding of Groupers in China. Austin J Mol & Cell Biol. 2016; 3(1): 1008.

Table 1: Summary of genetic linkage maps of groupers.

Grouper	Marker type	Marker number	Length of map (cM)	Average marker interval	References
White Grouper	microsatellite	Male map: 202	Male map: 886 Female map: 1053	Male: 5.0 Female: 5.8	[40]
		Female map: 203			
Kelp grouper	microsatellite	Male map: 161	Male map: 650.5 Female map: 944.4	Male: 5.0 Female: 6.7	[38,39]
		Female map: 173			
	SSR	Male map: 512	Male map: 1,370.39 Femalemap:1,475.95	Male: 4.0 Female: 4.1	
		Female map: 509			
Orange-spotted grouper	SNP	Sex-averaged map: 4,608	1,581.7	0.34	[41,42]
		Sex-averaged map: 3,029	1,231.98	0.41	

coioides♀×*E. lanceolatus*♂ [7,8], *E. fuscoguttatus*♀×*E. lanceolatus*♂ [9], *E. coioides*♀×*E. fuscoguttatus*♂ [10], *E. marginatus*♀×*E. aeneus*♂ [11], *E. costae*♀×*E. marginatus*♂ [12], and *E. amblycephalus*♀×*E. akaara*♂ [13]. Recently, researchers have paid more and more attention to the giant tiger groupers, Hulong (*E. fuscoguttatus*♀×*E. lanceolatus*♂) and Qinglong (*E. coioides*♀×*E. lanceolatus*♂) [14-16]. Using microsatellite markers, researchers analyzed the level of heterozygosis and genetic structure of these grouper hybrids [6]. We also revealed the molecular mechanism of growth superiority by conducting comparative transcriptome analyses between *E. fuscoguttatus* & *E. lanceolatus* and their hybrid Hulong. Differential gene expression was observed in the brain and liver GH/IGF (growth hormone/insulin-like growth factor) axis and the downstream signaling pathways (including protein and glycogen synthesis) [17]; further transcriptome sequencing of muscles (effector tissues) proved important contribution of glycolysis in addition with calcium signaling and up-regulated troponins to the growth superiority of Hulong (Sun Y. et al., our unpublished data). In addition, the complete mitochondrial genomes of Hulong and Qinglong hybrids had been reported [18,19].

Molecular Markers and Genetic Linkage Maps

Molecular markers are fundamental for construction of genetic linkage maps, which is the key step for MAS breeding. Molecular markers provide a potential resource to revolutionize the breeding methodology by improving germ plasm characterization and cost-efficient marker-assisted selection [20-22]. They can also be used to evaluate genetic resources with high accuracy. Construction of genetic linkage maps, based on marker development, could make significant contribution to chromosome assembly, genome mapping, positional cloning of genes, and functional genomic studies [23,24]. In many aquatic species [25-32], genetic linkage maps have been reported with different marker types, which determined the map density. Among the reported molecular markers, microsatellite (also termed as simple sequence repeat, SSR) was the most popular marker in many previous studies. However, with the rapid development of genomic sequencing in recent years, Single-Nucleotide Polymorphism (SNP) has become the most suitable marker type for construction of high-density genetic maps [33-35] because of its abundance and stability. In addition, maturity of sequencing techniques has made it possible to identify thousands of SNPs simultaneously and perform genotyping cost-efficiently [36,37].

Genetic linkage maps of groupers have been reported, including

Figure 2: Distribution of SNPs on the chromosomes of orange-spotted grouper. SNP markers on the scaffolds (dark green) were aligned onto the corresponding chromosomes (chr; bright green). The length unit of the upper axis (bright green, for the chromosomes) is centimorgan (cM) and the lower axis (dark green, for the sacaffolds) is millinbase (Mb)s. These data are summarized from our previous report [42] and unpublished results.

Table 2: The growth-related QTLs and genes identified in the orange-spotted grouper [42].

QTL	Trait	LOD*	$R^{2\#}$ (%)	Annotation
qLG1	BW	3.3	19.6	fasciculation and elongation protein zeta-2-like (*fez2*)
qLG2	BL	3.3	21	Dol-P-Man: Man(5) GlcNAc(2)-PP-Dol alpha-1,3-mannosyltransferase (*alg3*)
				endothelin converting enzyme 2 (*ece2*)
qLG5_1	BW	4.7	24.3	armadillo repeat gene deleted in velocardiofacial syndrome (*arvcf*)
qLG5_2	BW	3.1	17.1	
qLG5_3	BW	2.8	16.5	solute carrier family 27 (fatty acid transporter), member 4 (*slc27a4*)
				tyrosine-protein kinase SgK223 (*sgk223*)
qLG5_4	BW	2.9	15.8	calcium/calmodulin-dependent protein kinase 2 (*camk2*)
qLG5_5	BW	3.2	19.1	proline-rich coiled-coil 2B (*prrc2b*)
				melanin-concentrating hormone receptor 1 (*mchr1*)
qLG5_6	BW	4.2	24.6	
qLG5_7	BW	3.5	20.8	
qLG5_8	BW	3.4	20	pregnancy-associated plasma protein- A (*papp-a*)
qLG5_9	BW	2.6	21.5	spleen tyrosine kinase (*syk*)
qLG5_10	BW	2.8	24.5	
qLG5_11	BW	2.8	28.3	telomerase reverse transcriptase (*tert*)
qLG5_12	BW	2.7	14.6	WD repeat-containing protein 91-like (*wdrcp91*)
				ftz transcription factor 1 (*ftz-f1*)
qLG5_13	BW	3	29.1	
qLG5_14	BW	3.2	17.6	
qLG5_15	BW	2.9	15.6	
qLG5_16	BL	4.4	23.3	
qLG5_17	BL	2.6	14.5	
qLG5_18	BL	3.8	20.5	
qLG5_19	BL	3.4	20.3	sarcosine dehydrogenase (*sardh*)
qLG5_20	BL	3.8	22.2	
qLG5_21	BL	3.5	20.8	
qLG7_1	BW	2.8	16.8	multidrug and toxin extrusion protein 1-like (*mate1*)
qLG7_2	BL	2.6	15.1	multidrug and toxin extrusion protein 1-like (*mate1*)
qLG21	BL	2.9	15.5	neurogenic locus notch homolog protein 1-like (*notch1*)
qLG24	BW	2.6	14.4	

*LOD: Logarithm of Odds

#a Parameter for phenotypic variation explained

microsatellite-based genetic maps of kelp grouper (*E. bruneus*) and white grouper (*E. aeneus*), and SNP-based genetic maps of orange-spotted grouper (*E. coioides*) (see more details in Table 1). One sex-specific genetic linkage map of kelp grouper was constructed using 222 microsatellite markers [38]. The reported male map was 650.5cM in length while the total length of the female map reached 944.4cM; the average inter-marker distances were 5.0 cM and 6.7 cM, respectively. SSR markers (714) were employed to construct another genetic map of kelp grouper [39]. It was reported that, in male and female maps, the mean intervals were 4.0 cM and 4.1 cM respectively. With development of 222 microsatellites, a genetic linkage study of white grouper was conducted [40]. The constructed female and male maps spanned 1,053 cM and 886 cM respectively, with the average intervals of 5.8 cM and 5.0 cM. In several recent studies, we constructed two SNP-based genetic linkage maps of orange-spotted grouper [41,42]. The first one contained 4,608 SNPs, with the total length of 1,581.7 cM; the average marker interval was 0.34 cM [41]. The second one consisted of 3,029 SNPs, spanned 1,231.98 cM, with the mean distance of 0.41 cM [42]. However, the genotyping technology was different. The former was sequenced with Multiplexed Shotgun Genotyping (MSG) [41], while the latter was sequenced with recently developed Double Digest Restriction-Site Associated DNA (ddRAD) method [42]. Furthermore, we constructed a novel linkage map (Figure 2), based on 3,029 SNPs [42] corresponding to the scaffolds of the

reference genome (Zhang Y. et al., our unpublished data) of orange-spotted grouper.

QTL Mapping and Candidate Gene Identification

Genetic linkage map is essential for QTL mapping.QTL mapping can be used for qualitative and quantitative traits (such as growth related or disease resistant) that are measured quantitatively [43]. In fishes, QTL for these two important traits have been reported [44,45]. In the examination of groupers, 3 significant QTLs affecting both body weight and total length were identified and confirmed [39] in the kelp grouper. In a recent report of orange-spotted grouper [42], we identified 27 significant growth-related QTLs and determined 17 genes corresponding to these QTLs (Table 2). Interestingly, we supposed the leptin gene to be an important candidate for controlling growth-related traits. In previous studies of leptin gene of orange-spotted grouper [46,47], researchers detected 6 and 1 growth-related SNPs in leptin-a and leptin-b respectively. Moreover, Growth Hormone-Releasing Hormone (GHRH), its receptor (GHRHR) and PACAP-Related Peptide/Pituitary Adenylatecyclase Activating Polypeptide (PRP-PACAP) were verified because of their association with growth in orange-spotted grouper [48]. These genetic linkage maps, growth-related QTLs and candidate genes could be applied together for further MAS breeding and may promote the research on genetic regulation of growth-related traits in groupers.

Conclusion

Both traditional hybridization and novel MAS breeding are effective ways to generate new groupers with good properties, such as high growth rates, efficiency of food conversion and disease resistance. However, the hybridization has some disadvantages, for example, it is difficult to overcome the barrier of distant cross-incompatibility and the breeding cycle is too long. The MAS not only gets over the difficulty and shortens the breeding cycle, but also can enhance the accuracy of breeding schemes. Despite MAS is at the early development stage, it is clear that the development of sequencing techniques, genetic linkage maps, QTL analysis and availability of whole genome sequences of groupers are creating good opportunities for applications of the MAS breeding strategy. Furthermore, analyses of the hybrid grouper transcriptomes and candidate genes for growth will play an important role to facilitate the progress of MAS breeding in groupers. Meanwhile, with the rapid development of genomic sequencing and high-throughput genotyping technologies, MAS is going to be more and more cost-efficient, which supports MAS breeding to become an effective strategy for generation of novel grouper varieties or species.

Acknowledgement

This work was financially supported by Natural Science Foundation of China (No. 31370047& 31372526), Special Project on the Integration of Industry, Education and Research of Guangdong Province (No. 2013B090800017), Shenzhen Special Program for Future Industrial Development (No. JSGG20141020113728803), Special Fund for State Oceanic Administration Scientific Research in the Public Interest (No. 201305018), and Shenzhen Dapeng Special Program for Industrial Development (KY20160102).

References

1. Guo CY, Huang YH, Wei SN, Ouyang ZL, Yan Y, Huang XH, et al. Establishment of a new cell line from the heart of giant grouper, *Epinephelus lanceolatus* (Bloch), and its application in toxicology and virus susceptibility. Journal of Fish Diseases. 2015; 38: 175-186.
2. Pierre S, Gaillard S, Prévot-D'Alvise N, Aubert J, Rostaing-Capaillon O, Leung-Tack D, et al. Grouper aquaculture: Asian success and Mediterranean trials. Aquatic Conservation Marine & Freshwater Ecosystems. 2008; 18: 297-308.
3. Bureau of Fisheries, Ministry of Agriculture of China. China Fishery Statistical Yearbook. 2016: 32.
4. Shull GH. The composition of a field of maize. Journal of Heredity. 1908; 296-301.
5. Huang W, Liu Q, Xie J, Wang W, Xiao J, Li S, et al. Characterization of triploid hybrid groupers from interspecies hybridization (*Epinephelus coioides*♀×*Epinephelus lanceolatus*♂). Aquaculture Research. 2014; 47: 2195-2204.
6. Huang YS, Li ZB, Ning YF, Shangguan JB, Yuan Y, Mao XQ, et al. Isolation and characterization of microsatellite loci in hybrid giant tiger grouper. Genetics and Molecular Research. 2015; 14: 14706-14710.
7. Chu KIC, Shaleh SRM, Akazawa N, Oota Y, Senoo S. Egg and Larval Development of a New Hybrid Orange-Spotted Grouper *Epinephelus coioides*×Giant Grouper *E. lanceolatus*. Aquaculture Science. 2010; 58: 1-10.
8. Kiriyakit A, Gallardo WG, Bart AN. Successful hybridization of groupers (*Epinephelus coioides* x *Epinephelus lanceolatus*) using cryopreserved sperm. Aquaculture. 2011; 320: 106-112.
9. Ch'ng CL, Senoo S. Egg and Larval Development of a New Hybrid Grouper, Tiger Grouper *Epinephelus fuscoguttatus* xGiant Grouper *E. lanceolatus*. Aquaculture Science. 2008; 56: 505-512.
10. Chu KIC, Shaleh SRM, Senoo S. Egg and Larval Development of a New Hybrid Orange-spotted Grouper *Epinephelus coioides*xTiger Grouper *E. fuscoguttatus*. Aquaculture Science. 2008; 56: 441-451.
11. Glamuzina B, Kozul V, Tutman P, Skaramuca B. Hybridization of Mediterranean groupers: *Epinephelus marginatus*♀x *E. aeneus*♂and early development. Aquaculture Research.1999; 30: 625-628.
12. Glamuzina B, Glavić N, Skaramuca B, Kožul V, Tutman P. Early development of the hybrid *Epinephelus costae*♀x *E. marginatus* ♂. Aquaculture. 2001; 198: 55-61.
13. Tseng WY, Poon CT. Hybridization of Epinephelus species. Aquaculture. 1983; 34: 177-182.
14. Luin M, Chingfui F, Senoo S. Sexual maturation and gonad development in tiger grouper (*Epinephelus fuscoguttatus*)× giant grouper (*E. lanceolatus*) hybrid. Journal of Aquaculture Research & Development. 2014.
15. Sutthinon P, Thongprajukaew K, Saekhow S, Ketmanee R. Juvenile hybrid grouper (*Epinephelus coioides* × *E. lanceolatus*) are euryhaline and can grow in a wide range of salinities. Aquaculture International. 2014; 23: 671-682.
16. Lim LM, Senoo S, Siddiquee S, Rodrigues KF. Molecular markers for parentage analysis in the grouper F1 hybrid *Epinephelus coioides*×*Epinephelus lanceolatus* (Actinopterygii: Perciformes: Serranidae). Acta Ichthyologica Et Piscatoria. 2014; 44: 59-64.
17. Sun Y, Guo CY, Wang DD, Li XF, Xiao L, Zhang X, et al. Transcriptome analysis reveals the molecular mechanisms underlying growth superiority in a novel grouper hybrid (*Epinephelus fuscogutatus*♀ x *E. lanceolatus*♂). BMC Genetics. 2016; 17: 1-10.
18. Zhu K, Zhang D, Wei J, Huang G, Guo Y, Jiang S, et al. The complete mitochondrial genome of the hybrid grouper *Epinephelus fuscoguttatus*(♀) x*Epinephelus lanceolatus*(♂). Mitochondrial DNA Part A. 2016; 27: 1968-1969.
19. Wang Q, Chen H, Xu W, He J, Xie Z, Tang L, et al. The complete mitochondrial genome of the hybrid grouper *Epinephelus coioides*♀ x *Epinephelus lanceolatus*♂. Mitochondrial DNA. 2015: 1-2.

20. Gupta PK, Varshney RK, Sharma PC, Ramesh B. Molecular markers and their applications in wheat breeding. Plant Breeding. 1999; 118: 369-390.

21. Landjeva S, Korzun V, Börner A. Molecular markers: actual and potential contributions to wheat genome characterization and breeding. Euphytica. 2007; 156: 271-296.

22. Varshney RK, Langridge P, Graner A. Application of genomics to molecular breeding of wheat and barley. Advances in Genetics. 2007; 58: 121-155.

23. Harushima Y, Yano M, Shomura A, Sato M, Shimano T, Kuboki Y, et al. A high-density rice genetic linkage map with 2275 markers using a single F2 population. Genetics. 1998; 148: 479-494.

24. Hemleben V. The Handbook of Plant Genome Mapping. Genetic and Physical Mapping. Journal of Plant Physiology. Journal of Plant Physiology. 2007; 164: 813-814.

25. Eshel O, Shirak A, Weller JI, Hulata G, Ron M. Linkage and Physical Mapping of Sex Region on LG23 of Nile Tilapia (*Oreochromis niloticus*). G3 (Bethesda, Md). 2012; 2: 35-42.

26. Geng X, Sha J, Liu S, Bao L, Zhang J, Wang R, et al. A genome-wide association study in catfish reveals the presence of functional hubs of related genes within QTLs for columnaris disease resistance. BMC Genomics. 2015; 16: 196.

27. Liu S, Vallejo RL, Gao G, Palti Y, Weber GM, Hernandez A, et al. Identification of single-nucleotide polymorphism markers associated with cortisol response to crowding in rainbow trout. Marine Biotechnology (New York, NY). 2015; 17: 328-337.

28. Tsai HY, Hamilton A, Guy DR, Tinch AE, Bishop SC, Houston RD. The genetic architecture of growth and fillet traits in farmed Atlantic salmon (*Salmo salar*). BMC Genetics. 2015; 16: 51.

29. Xiao T, Lu C, Xu Y, Li C, Zheng X, Cao D, et al. Screening of SSR markers associated with scale cover pattern and mapped to a genetic linkage map of common carp (*Cyprinus carpio L.*). Journal of Applied Genetics. 2015; 56: 261-269.

30. Kudo Y, Nikaido M, Kondo A, Suzuki H, Yoshida K, Kikuchi K, et al. A microsatellite-based genetic linkage map and putative sex-determining genomic regions in Lake Victoria cichlids. Gene. 2015; 560: 156-164.

31. Sandal N, Krusell L, Radutoiu S, Olbryt M, Pedrosa A, Stracke S, et al. A genetic linkage map of the model legume Lotus japonicus and strategies for fast mapping of new loci. Genetics. 2002; 161: 1673-1683.

32. Hayashi M, Miyahara A, Sato S, Kato T, Yoshikawa M, Taketa M, et al. Construction of a genetic linkage map of the model legume Lotus japonicus using an intraspecific F2 population. DNA research: An International Journal for Rapid Publication of Reports on Genes and Genomes. 2001; 8: 301-310.

33. Ting NC, Jansen J, Mayes S, Massawe F, Sambanthamurthi R, Ooi LC, et al. High density SNP and SSR-based genetic maps of two independent oil palm hybrids. BMC Genomics. 2014; 15: 309.

34. Stolting KN, Nipper R, Lindtke D, Caseys C, Waeber S, Castiglione S, et al. Genomic scan for single nucleotide polymorphisms reveals patterns of divergence and gene flow between ecologically divergent species. Molecular Ecology. 2013; 22: 842-855.

35. Wang J, Luo MC, Chen Z, You FM, Wei Y, Zheng Y, et al. *Aegilops tauschii* single nucleotide polymorphisms shed light on the origins of wheat D-genome genetic diversity and pinpoint the geographic origin of hexaploid wheat. The New Phytologist. 2013; 198: 925-937.

36. Rafalski A. Applications of single nucleotide polymorphisms in crop genetics. Current Opinion in Plant Biology. 2002; 5: 94-100.

37. Davey JW, Hohenlohe PA, Etter PD, Boone JQ, Catchen JM, Blaxter ML. Genome-wide genetic marker discovery and genotyping using next-generation sequencing. Nature Reviews Genetics. 2011; 12: 499-510.

38. Liu Q, Sakamoto T, Kubota S, Okamoto N, Yamashita H, Takagi M, et al. A genetic linkage map of kelp grouper (*Epinephelus bruneus*) based on microsatellite markers. Aquaculture. 2013; 414–415: 63-81.

39. Kessuwan K, Kubota S, Liu Q, Sano M, Okamoto N, Sakamoto T, et al. Detection of Growth-Related Quantitative Trait Loci and High-Resolution Genetic Linkage Maps Using Simple Sequence Repeat Markers in the Kelp Grouper (*Epinephelus bruneus*). Marine Biotechnology (New York, NY). 2016; 18: 57-84.

40. Dor L, Shirak A, Gorshkov S, Band MR, Korol A, Ronin Y, et al. Construction of a microsatellites-based linkage map for the white grouper (*Epinephelus aeneus*). G3 (Bethesda, Md). 2014; 4: 1455-1464.

41. You X, Shu L, Li S, Chen J, Luo J, Lu J, et al. Construction of high-density genetic linkage maps for orange-spotted grouper *Epinephelus coioides* using multiplexed shotgun genotyping. BMC Genetics. 2013; 14: 113.

42. Yu H, You X, Li J, Liu H, Meng Z, Xiao L, et al. Genome-Wide Mapping of Growth-Related Quantitative Trait Loci in Orange-Spotted Grouper (*Epinephelus coioides*) Using Double Digest Restriction-Site Associated DNA Sequencing (ddRADseq). International Journal of Molecular Sciences. 2016; 17.

43. Kumar J, Choudhary AK, Solanki RK, Pratap A. Towards marker-assisted selection in pulses: a review. Plant Breeding. 2011; 130: 297-313.

44. Wang L, Wan ZY, Bai B, Huang SQ, Chua E, Lee M, et al. Construction of a high-density linkage map and fine mapping of QTL for growth in Asian seabass. Scientific Reports. 2015; 5: 16358.

45. Shao C, Niu Y, Rastas P, Liu Y, Xie Z, Li H, et al. Genome-wide SNP identification for the construction of a high-resolution genetic map of Japanese flounder (*Paralichthys olivaceus*): applications to QTL mapping of Vibrio anguillarum disease resistance and comparative genomic analysis. DNA research: An International Journal for Rapid Publication of Reports on Genes and Genomes. 2015; 22: 161-170.

46. Wei Y, Huang H, Meng Z, Zhang Y, Luo J, Chen G, et al. Single nucleotide polymorphisms in the leptin-a gene and associations with growth traits in the orange-spotted grouper (*Epinephelus coioides*). International Journal of Molecular Sciences. 2013; 14: 8625-8637.

47. Huang H, Wei Y, Meng Z, Zhang Y, Liu X, Guo L, et al. Polymorphisms of leptin-b gene associated with growth traits in orange-spotted grouper (*Epinephelus coioides*). International Journal of Molecular Sciences. 2014; 15: 11996-12006.

48. Guo L, Xia J, Yang S, Li M, You X, Meng Z, et al. GHRH, PRP-PACAP and GHRHR Target Sequencing via an Ion Torrent Personal Genome Machine Reveals an Association with Growth in Orange-Spotted Grouper (*Epinephelus coioides*). International Journal of Molecular Sciences. 2015; 16: 26137-26150.

Austin J Mol & Cell Biol - Volume 3 Issue 1 - 2016
Submit your Manuscript | www.austinpublishinggroup.com

Citation: Yu H, You XX and Shi Q. Research Advances on Molecular Breeding of Groupers in China. Austin J Mol & Cell Biol. 2016; 3(1): 1008.