# Construction of a microsatellite-based genetic linkage map for half-smooth tongue sole *Cynoglossus semilaevis*

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**Abstract** The half-smooth tongue sole *Cynoglossus semilaevis* is an important cultured marine fish and a promising model fish for the study of sex determination. Sex-specific genetic linkage maps of half-smooth tongue sole were developed with 567 markers (565 microsatellite markers and two SCAR markers). The parents and F1 progeny (92 individuals) were used as segregating populations. The female map was composed of 480 markers in 21 linkage groups, covering a total of 1388.1 cM, with an average interval 3.06 cM between markers. The male map consisted of 417 markers in 21 linkage groups, spanning 1480.9 cM, with an average interval of 3.75 cM. The female and male maps had 474 and 416 unique positions, respectively. The genome length of half-smooth tongue sole was estimated to be 1522.9 cM for females and 1649.1 cM for males. Based on estimations of map length, the female and male maps covered 91.1% and 89.8% of the genome, respectively. Furthermore, two female-specific SCAR markers, f-382 and f-783, were mapped on LG15f (linkage group 15 in female maps). The present study presents a mid-density genetic linkage map for half-smooth tongue sole. These improved genetic linkage maps may facilitate systematic genome searches to identify quantitative trait loci (QTL), such as disease resistance, growth and sex-related traits, and are very useful for marker-assisted selection breeding programs for economically important traits in half-smooth tongue sole [*Current Zoology* 59 (1): 99–108, 2013].

Keywords Half-smooth tongue sole, Cynoglossus semilaevis, Microsatellite, Genetic linkage map, MAS

Genetic linkage maps have become important tools in many areas of genetic research. To perform a linkage study, it is necessary to genotype and map a large number of the available genetic markers on mapping families. Microsatellites comprise an excellent choice for genomic mapping due to their abundance in most vertebrate genomes, genomic distribution, high polymorphism and ease of typing via PCR. Meanwhile, simple sequence repeat (SSR) alleles are typically co-dominant, and their polymorphisms can be scored in either a simple polyacrylamide gel separation format or with high-throughput capillary arrays. Genetic linkage maps based on microsatellite markers have been generated for economically important marine fishes such as salmon (Gilbey et al., 2004), tilapia (Lee et al., 2005), European sea bass (Chistiakov et al., 2005), rainbow trout (Guyomard et al., 2006), sea bream (Senger et al., 2006), barramundi (Wang et al., 2007), catfish (Wang et al., 2007), grass carp (Xia et al., 2010), Japanese flounder (Castaño-Sánchez et al., 2010) and Asian sea bass (Wang et al., 2011).

The half-smooth tongue sole Cynoglossus semilaevis is a commercially valuable flatfish widely distributed in Chinese coastal waters. Due to its commercial value, easy domestication and natural resource depletion, half-smooth tongue sole has been selected as a promising species for aquaculture. It is one of the most popular marine species in Chinese aquaculture. In addition, half-smooth tongue sole females grow two to three times larger and faster than males. Based on G-banding patterns analysis, it was confirmed that, in addition to having 20 euchromosome pairs, there is a pair of sex chromosomes (chromosome Z and W), and sex in this species is determined by a WZ/ZZ chromosomal system (Zhuang et al., 2006). These characteristics suggest that half-smooth tongue sole has great potential for the production of all-female stock, as well as for studying the mechanisms of both genome evolution and sex determination.

Half-smooth tongue sole breeding is still in its infancy. Like other aquaculture species, the production of half-smooth tongue sole is often affected by outbreaks

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of deadly infectious diseases caused by bacteria, viruses or protozoan pathogens. The traditional methods of genetic improvement of quantitative traits have relied mainly on phenotype (Falconer and Mackay, 1996), which are easily influenced by environmental factors. It is generally accepted that marker-assisted selection (MAS) accelerates genetic improvement in a relatively short period, especially when the target characteristics are disease-related and there is a sufficient amount of observed genetic variation in a given trait. Therefore, a genetic map constructed from a population segregated for a trait of interest is required for QTL identification. Information on genetic markers associated with QTL can be used in MAS breeding programs to identify and select individuals carrying desired traits. QTL for growth, disease resistance and stress response have been mapped in only a few species, such as rainbow trout (Ozaki et al., 2001), tilapia (Cnaani et al., 2003), salmon (Reid et al., 2004), Japanese flounder (Fuji et al., 2006), guppy (Tripathi et al., 2009) and European seabass (Massault et al., 2010).

Recently, a number of genetic studies in half-smooth tongue sole have been reported, including the development of microsatellite markers (Liao et al., 2009; Miao et al., 2011; Sha et al., 2011;) and female-specific DNA markers (Chen et al., 2007; Ma et al., 2009), construction of BAC libraries (Shao et al., 2010), molecular marker-assisted sex control (Chen et al., 2008), the characterization of certain sex-related genes (Deng et al., 2009) and artificial gynogenesis (Chen et al., 2009). A low-density genetic linkage map was constructed for half-smooth tongue sole (Liao et al., 2009); however, this map has provided very little information on the genomic organization of this important marine species. The half-smooth tongue sole breeding community lacks a detailed genetic linkage map to facilitate the breeding process. In the present study, we constructed a mid-density microsatellite genetic linkage map in half-smooth tongue sole, and identified sex-linked SCAR markers on linkage maps.

# 1 Materials and Methods

## 1.1 Mapping family

A full-sib family of half-smooth tongue sole was constructed and used for the development of a genetic linkage map. The male parent was selected from a group of fish derived from a wild population. The female parent was selected from a cultured population. Experimental crossing was conducted at the MingBo Aquaculture Company (Yantai, China). Induction of the maturation of broodstock and artificial fertilization of sperm and eggs were carried out as described previously (Chen et al., 2009). Ninety-two  $F_1$  offspring from the mapping family were collected, including forty-six females and forty-six males, and stored in absolute ethanol until DNA extraction. Genomic DNA of the two parents and progeny was extracted following phenol/chloroform procedures with RNase treatment (Sambrook and Russell, 2001).

# 1.2 Microsatellite markers

A total of 2,276 half-smooth tongue sole microsatellite markers were tested for segregation across a set of eight progeny individuals. These microsatellite markers were recruited from three sources: (1) The first set of 1200 microsatellite markers was developed from genome sequencing. (2) The second set of 965 microsatellite markers was developed through the construction of microsatellite enriched libraries and EST libraries (Liao et al., 2009; Miao et al., 2011; Sha et al., 2011). (3) The remaining 111 markers were developed from public databases and the literature (Liu et al., 2007; Liu et al., 2008; Wang et al., 2008; Zhong et al., 2009).

# 1.3 SCAR markers

Two female-specific SCAR (sequence-characterized amplified region) markers were used from half-smooth tongue sole in the mapping family (Marker name: F-382, Forward primer: ATTCACTGACCCCTGAGAGC, Reverse primer: AACAACTCACACACACGACAAATG; F-783, Forward primer: TGTTCTTGTCTTCGCTCCCT, Reverse primer: AGGTGTAACCATCAACTTTTC). Detailed PCR amplification procedures are described by Chen et al. (2007) and Ma et al. (2009).

#### 1.4 Genotyping

Primers flanking the microsatellite regions were designed using Primer 3. All primers were designed for a 57.5°C annealing temperature, a total amplification product size of 150-300 bp. All microsatellite markers were used to genotype eight progeny for screening the segregation markers in the mapping population. The microsatellite markers that produced polymorphic fragments were used in subsequent genotyping of the parents and 92 progeny to construct linkage maps. Amplification reactions were carried out in a 15µl volume consisting of 10×Tag buffer, 0.5 U Tag polymerase, 0.6mM dNTP (+MgCl<sub>2</sub>), 0.6 µM of each primer and 10-30ng template DNA. The final volume was adjusted with sterile distilled water. Amplifications were performed in an ABI Veriti 96 well thermal cycler. The PCR amplifications were performed under the following conditions: 95°C for 5 min, followed by 32 cycles at 95°C for 30 s, 57.5 for 30 s and 72°C for 30 s, and the final extension was 72°C for 10 min. The PCR products were separated on 8% polyacrylamide gels (PAGE) and visualized by silver staining (Bassam et al., 1991).

## 1.5 Linkage analysis

Genetic marker data were scored according to the definition of JoinMap 4.0 (Van Ooijen, 2006). Linkage groups were constructed independently for males and females. Linkage groups with genetic markers on individual maps were merged to create an integrated map using the "Join-combine groups for map integration" command. All statistical analyses described below were made using the same software using a cross-pollinating (CP) type population, which handles F1 outbreeding population data containing various genotype configurations. Pairwise analyses were performed and markers were sorted in linkage groups at a minimum LOD score of 4.0. The "locus genotype frequency" function calculated the chi-square values for each marker to test for the expected Mendelian segregation ratio. The linkage distances were estimated for each LG assuming the Kosambi mapping function. All weak linkage markers were excluded to ensure a correct marker order. Although distorted segregation markers normally are excluded from linkage analysis, the use of the independent LOD score, one of the grouping parameters provided by JoinMap4.0, allows these markers to be included (You et al., 2010). This test for independence is not affected by segregation distortion and leads to a less spurious linkage.

### 1.6 Genome size and coverage

The estimated genome length (Ge) of the consensus female and male genome was estimated using two different methods. First, Genome Estimation size 1 (Ge1) was calculated by adding 2s to the length of each genetic linkage group to account for the chromosome ends, where s was the average spacing of the genetic linkage map. The first method estimates s on a genome scale (Fishman et al., 2001). Genome Estimation Size 2 (Ge2) was calculated by multiplying the length of each genetic linkage group by (m+1)/(m-1), where m was the number of loci in each genetic linkage group. The second method estimates the average spacing for each chromosome independently (Chakravarti et al., 1991). The estimated genome size (Ge) for each sex was taken as the average of the two estimates. Observed genome length was taken as the total length (Goa) considering all linkage groups, triplets and doublets (Cervera et al., 2001). The map coverage, Coa, was calculated as Goa/Ge (Liao et al., 2007).

# 2 Results

### 2.1 Genetic markers and segregation distortion

To obtain useful microsatellite markers for linkage analysis, we examined the segregation patterns of 2,276 markers in the mapping family. Amongst the 2,276 markers, 718 microsatellite markers (31.5%) were polymorphic in either the male or female of the family. Segregation distortion from that expected under Mendelian inheritance was found in 165 (23.0%) of 718 microsatellite markers.

#### 2.2 Sex-linked markers

Sex-specific molecular markers are a useful genetic resource for studying sex- determination mechanisms and controlling fish sex. We mapped two sex-linked SCAR markers (f-382 and f-783) to the female map LG15f (Fig. 1). The presence of sex-linked markers suggested the possibility of female heterogamety (ZZ male; WZ female) in half-smooth tongue sole, which is confirmed by the presence of a large heteromorphic sex chromosome in the females of this species. Therefore, LG15 should correspond to the sex chromosome.

#### 2.3 Linkage Analysis

A total of 720 demonstrably heterozygous markers were available for mapping. Among these 720 loci, 613 markers were used to construct the female map, and 567 markers were used to construct the male map. Thirty-two microsatellite markers did not exhibit any significant linkage to any other markers. When the total of 718 effective microsatellite markers and two SCAR markers were analyzed, 567 markers were found to be located on the linkage map containing 21 linkage groups (LGs) at a LOD threshold value of 4.0.

#### 2.4 Sex-specific maps

Significant linkages were identified for 720 genetic markers, including a total of 718 microsatellite loci and two SCAR markers. However, 154 microsatellite markers were unmapped in this analysis. Consequently, the mapping ratio of these markers is 78.6%. The female and male maps contained 480 and 417 markers, respectively, and both maps were found to have 21 linkage groups. The total length of the female map is 1,388.1 cM, with an average interval of 3.06 cM. Linkage group size ranged from 34.3 cM to 98.8 cM. The number of loci per genetic linkage group varied from 10 to 53. The male linkage map spanned a total genetic distance of 1480.9cM. The length of each linkage group varied from 41.5 to 90.8cM and contained 10-49 loci per group, with an average interval of 3.75 cM. Sex-specific genetic linkage maps are presented in Fig. 1 and Fig. 2.

LG1f	LG2f	LG2f LG3f LG4f LG5f		LG5f	LG6f	
0.0         scaffold1021_42142           0.2         scaffold2700_68530           1.0         scaffold2200_628530           scaffold2200_628530         scaffold888_37653           1.0         scaffold888_37653           1.0         scaffold888_37653           1.1.0         scaffold3009_71896           1.3.7         scaffold376_70735           1.3.7         scaffold376_70735           1.3.8         scaffold376_70735           1.3.9         scaffold3676_70735           1.3.9         scaffold3676_70735           1.3.9         scaffold6657_71709           1.0.8         scaffold6667_71709           1.0.8         scaffold6667_7140           1.0.8         scaffold6667_7140           1.0.8         scaffold6667_7140           1.0.8         scaffold6667_7145           1.0.8         scaffold2104_64144           50.3         scaffold2104_64144           50.3         scaffold2104_64144           50.3         scaffold2104_64144           50.3         scaffold2104_64144	0         cse72           7.1         scaffold2320_65962           13.2         scaffold2380_6664           15.7         vseaffold2380_6664           15.7         vseaffold2380_676664           15.7         vseaffold2380_67664           26.9         scaffold340_17565           28.9         scaffold340_17565           30.0         scaffold340_17563           31.2         scaffold340_1357           45.3         csou54           30.0         vse141           60.0         cyse14           45.3         csou54           35.2         scaffold369_26604           50.0         cyse241           55.9         csou30           62.9         scaffold1544_57049           LC88f         LC88f	0.0         csou51           4.0         scaffold767_34404           hncse27         scaffold767_31404           12.2         scaffold767_31404           15.4         scaffold753_11564           15.9         scaffold701_14617           17.7         cscaffold701_14617           17.7         cscaffold701_14617           23.5         cscaffold701_1621_62599           23.5         cscaffold5533_71563           27.4         csc988           28.6         cyse230           28.6         cyse20           29.1         cscaffold2161_61.64369           29.2         cscaffold2161_64369           29.4         scaffold2161_64369           20.2         hncse68           30.4         scaffold2161_64364371           30.8         cyse43	0.0         hncse229           7.8         scaffold1420_53607           13.2         scaffold11420_53607           13.2         scaffold11420_53607           13.2         scaffold11420_53607           19.8         scaffold11420_5349011           22.8         scaffold1258_49011           22.8         scaffold2005_6287           23.3         scaffold27_281           24.3         scaffold27_281           25.6         scaffold27_29518           47.3         gene177           53.1         scaffold33_25540           55.1         cs61           55.6         scaffold53_25474           58.6         hncse221           60.5         hncse106           62.7         scaffold312_e9947	0.0 scaffold1447_53897 5.3 hncyse119 5.3 scaffold209_35286 14.2 scaffold209_35286 5.3 scaffold2773_69031 24.3 scaffold2773_69031 26.6 scaffold2701_6783 26.6 scaffold2010_62849 35.5 scaffold2010_62849 35.5 scaffold2016_62849 35.5 scaffold2016_62849 35.5 scaffold2016_71810 42.4 scaffold2527_67825 34.8 scaffold2547_67825 35.8 scaffold261113 35.2 scaffold249_12377 49.5 scaffold249_12377 49.5 scaffold249_12377 49.5 scaffold249_12377 49.5 scaffold249_12377 49.5 scaffold543_25681	0.0 scafbid 129 59726 4.6 scafbid 129 59726 scafbid 1211_47503 10.9 scafbid 452_22891 11.6 scafbid 452_22891 11.6 scafbid 452_22891 15.6 scafbid 452_0284 41.7 scafbid 452_0284 11.6 scafbid 456_40257 24.3 nocyse97 13.3 scafbid 4234_20553 41.5 scafbid 4234_20553 41.5 scafbid 423_20553 41.5 scafbid 423_2055	
62.0 scaffold409_20762 64.3 hncyse28	0.0 4.5 scaffold1535_55453 scaffold3330_70410	33.6 scaffold961_40009 35.5 scaffold1067_43468 36.3 hncyse75	72.1 hncyse129 76.1 cyse267	LG11f 0.0 — — scaffold1118_45406	LG12f	
67.9 // hncyse136 71.2 // scaffold1719_58856 5743 777.3 // scaffold1298_51107	6.9 (cyse274 8.4 )hrcyse139 9.9 (cse86 10.4 )scaffold669_41193 15.8 /scaffold669_41193 15.8 /scaffold444_21127 8.3 /scaffold444_21127 8.3 /scaffold1345_52778 3.5 / (csaffold3865_71274	36.3         hncyse75           36.9         hsts-4           38.3         hncyse91           39.3         hncyse92           39.9         ST40           40.5         scaffold1385_53192           42.1         scaffold2996_69760           44.2         scaffold2585_68068           51.1         scaffold1639_57815	78.9 / scaffold686_31835 LG10f 0.0 scaffold1665_58305 1.8 scaffold1665_58308 5.6 hncse26 10.0 cyse13 12.9 scaffold2065_63431 14.0 cyse13	12.0 hncse64 14.7 scaffold94_5336 18.0 hncse170 20.9 scaffold56_26255 21.9 hncse174 22.8 hncse150 scaffold556_26262 27.1 cyse26 28.4 cyse66	0.0         cse74           2.5         scaffold672_31081           10.2         scaffold586_28587           22.1         cyse284           23.1         hncse45           27.2         scaffold379_19493           39.2         csou27	
3.3         scaffold775_34737           15.4         scaffold775_34737           18.3         hncyse124           24.8         scaffold73335_71053           29.2         rcyse160           31.0         scaffold73335_71053           32.1         rcse190           36.7         cse190           36.7         cse1190           36.7         rcse110	42.7         scaffold1528_55393           45.3         cyse210           45.4         hncyse85           49.5         cyse107           50.2         scaffold305_24900           51.9         scaffold305_24900           56.4         scaffold346_17345	LG9f 0.0 3.7 14.6 17.7 19.9 23.0 25.6 25.5 25	19.8         scaffold86_3568           21.2         cyse111           24.9         scaffold0355_70439           27.7         scaffold1032_42172           32.1         scaffold2632_68357           37.9         scaffold2633_68357           53.3         scaffold2063_63713	33.7         hsts-J           36.5         scaffold72_2796           36.5         hcse10d1677_58093           40.0         hcse13           41.7         hcse236           53.3         hcse3           63.7         scaffold1797_59428           LG19f	40.4 scaffold2223.6565 41.0 scaffold228.44234 42.9 scaffold286.44234 42.9 scaffold282.65517 43.6 ST10 43.9 ST10 43.9 scaffold252_5172 51.6 scaffold252_10803 58.6 scaffold282_27999	
46.8 cyse148 49.7 scaffold7735_71828		27.0 scaffold2079_63850 28.0 hsts-k	61.5 scaffold2495_67575	0.0 ST181 11.7 St215 14.6 ST173	LG20f	
52.8 ST213 60.1 cyse213 61.1 scaffold2366_737 62.3 scaffold1932_62165 67.6 A73.56 71.3 hnose181 72.0 scaffold2778_859	LG14f 0.0 scaffold1526_55284 11.3 scaffold316_70379 13.6 pene116 22.8 gene116 26.4 scaffold399_19427 29.6 scaffold399_19427 29.6 scaffold399_19427 22.8 scaffold1644_57993 31.3 scaffold1645_27838 39.1 scaffold1777_59215 40.7 scaffold1777_59216 43.3 hncse27.3 scaffold1777_59216	28.8 scaffold2755_69081 30.4 scaffold784_59253 31.0 scaffold784_59253 32.7 scaffold782_35153 scaffold782_35153 scaffold782_35153 scaffold782_6850 cyse286 46.4 scaffold51_1364 cyse278 59.7 scaffold1784_59258	LG17f 0.0 0.3 9.3 12.4 13.0 15.1 16.2 17.9 1.9 1.9 1.9 1.9 1.9 1.9 1.9 1	14.6         511/3           26.3         ST219           27.4         ST195           28.7         S233-1           30.3         ST151           31.1         ST166           32.6 f         ST219           33.8         ST169           34.4         ST204           35.8         ST154           36.8         ST196           36.8         ST154           36.8         ST154           36.8         ST154           36.8         ST209           37.4         ST157           37.9         ST193	0.0 hncyse150 cse257 hncyse102 hncyse99 13.7 scaffold4604_71363 5719 9.4 scaffold368_18530 20.3 scaffold1337_51158 22.1 scaffold1865_58230 hncse231 cyse292 30.2 scaffold180103_71897 cse153 37.1 scaffold1901_61969	
LG13f	44.6 scaffold1227_48418 46.9 scaffold1363_51755 51.2 cse105	LG16f	18.7 hncse130 19.8 scaffold822_35662 20.8 hncse182	38.3 V ST214 38.5 ST228 ST170 ST186	38.3 // hncse176 44.2 // cs006	
0.0 scaffold2230_65448 2.9 scaffold950_39778 3.0 scaffold950_39778 3.6 scaffold1940_62137 5.8 cyse243 7.8 hsts-3 10.1 \_cyse243_64406	52.7 hncyse47 57.6 scaffold1788_60032 58.9 csaffold1788_60033 63.0 cyse223 67.2 cse225	0.0 scaffold216_10757 13.4 gene072 17.9 cyse57 20.0 scaffold1628_57677 22.4 cyse79 24.1 cse73	24.5 / Cse55 27.3 / Scaffold255_12500 29.8 / Cse155 31.3 / Scaffold637_29532 34.3 / Scaffold637_29530	38.7 ST177 ST155 39.2 ST222 39.5 ST175 39.8 St27 40.6 ST210 41.1 ST153 43.1 C ST232	54.5 / scaffold90_4658 64.6 / scaffold117_6029 66.3 / hncse43 75.5 / hncse22	
16.8	LG15f HXTS31 10.3 19.2 22.1 23.3 24.3 25.4 25.4 25.4 26.7 27.8 27.8 26.7 27.8 27.	29.5         hncse82           36.6         scaffold2329_66082           40.3         scaffold1329_66082           40.3         scaffold1486_55203           49.9         scaffold1420_5484           57.0         cyse150           65.5         scaffold1219_71183           66.6         scaffold1333_51187           66.6         scaffold1646_6005           66.6         scaffold165_53179           78.7         scaffold376_18254           scaffold176_153179         scaffold414_2_71074           88.5         scaffold74_3002	LG18f 0.0 scaffold198_9634 17.4 scaffold1291_50712 52.9 scaffold7297_51163 scaffold7291_50712 scaffold1291_50712 scaffold1291_50712 scaffold1291_50712 scaffold14291_50712 scaffold1445_1100 scaffold144_35282 30.9 scaffold14_35282 37.7 hncse127 38.6 cyse61 40.9 ST26 cyse20 41.8 cyse61 hncse100_1 scaffold1240_49281	44.5         ST176           48.3         hncyse107           48.3         scaffold1934           50.7         scaffold1934           53.0         scaffold1934           53.0         scaffold1934           53.0         scaffold1934           53.0         scaffold1934           62.1         scaffold1823           63.3         cse248           67.8         hncse258           69.5         scaffold681           73.0         ST50           73.4         scaffold682           92.3         hncse72           73.4         scaffold682           92.3         hncse98           84.2         cse148           83.3         scaffold6808           92.9         hncse101           92.9         hncse104           98.8         hncse104	LG21f 0.0 csou15 21.1 hncse148 24.9 HXTS25 26.2 HXTS21 27.6 scaffold3045_69830 31.6 scaffold3045_69830 38.5 scaffold486_Z3323 38.5 scaffold486_Z3323 38.5 scaffold1060_43080 51.2 cyse270 56.9 cyse207	

Fig. 1 Linkage maps of the female-specific map for Cynoglossus semilaevis

The female-specific genetic map comprises 480 markers assigned to 21 linkage groups (LG1f-LG21f), and spans a total map length of 1388.1cM. Genetic distances in Kosambi centimorgans are listed on the left side of the linkage groups, and markers are listed on the right side of the linkage groups.

The female and male maps display 474 and 417 unique positions, respectively. Estimated genome lengths, based on the two methods, were 1,516.8 cM (Ge1) and 1,529.0 cM (Ge2) for the female, and 1,638.4 cM (Ge1)

and 1,659.9 cM (Ge2) for the male. The average of these two values was taken as the expected genome length, namely 1,522.9 cM for the female and 1,649.1 cM for the male. Based on recent estimations of map

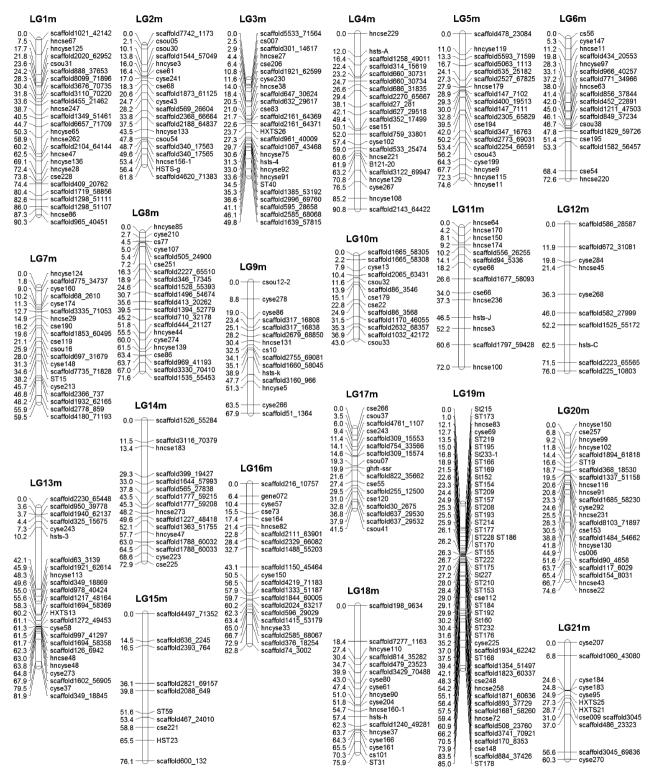


Fig. 2 Linkage maps of the male-specific map for *Cynoglossus semilaevis* 

The male-specific genetic map comprises 417 markers assigned to 21 linkage groups (LG1m-LG21m), and spans a total map length of 1480.9cM. Genetic distances in Kosambi centimorgans are listed on the left side of the linkage groups, and markers are listed on the right side of the linkage groups.

length, the genomic coverage of the female and male maps was 91.1% and 89.8%, respectively. The characterization of genetic linkage maps of half-smooth tongue sole is presented in Table 1 and Table 2.

#### 2.5 Integrated maps

Either bridge markers or homologous loci were used to identify co-linear regions in the female and male maps. The integrated map was composed of 565

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LG	Female maps			Male maps			Integrated maps		
LU	No. of markers	Length (cM)	cM/marker	No. of markers	Length (cM)	cM/marker	No. of markers	Length (cM)	cM/marker
LG1	29	77.3	2.67	26	90.3	3.74	38	102.2	2.69
LG2	19	65.9	3.47	20	61.8	3.09	25	77.7	3.11
LG3	37/35 ª	51.1	1.38/1.46 <sup>b</sup>	26	49.8	1.92	38/37 <sup>a</sup>	66.1	1.74/1.79 <sup>b</sup>
LG4	26	78.9	3.03	22	90.8	4.13	31/30 <sup>a</sup>	88.9	2.87/2.96 <sup>b</sup>
LG5	20	54	2.70	20	74.6	3.73	28	66.9	2.39
LG6	22	70.1	3.51	18	72.6	4.03	27	87.3	3.23
LG7	22	72	3.27	20	59.5	2.98	26	74.8	2.88
LG8	24	56.4	2.35	21	71.6	3.41	24	69.6	2.90
LG9	21	59.7	2.84	15	67.9	4.53	22	60	2.73
LG10	14	61.5	4.39	13	43	3.31	18	78.1	4.34
LG11	17	63.7	3.75	14	72	5.14	19	79.6	4.19
LG12	16	62.5	3.91	10	76	7.6	16	67.2	4.20
LG13	30	63.9	2.13	26	81.9	3.15	30	75	2.50
LG14	20	67.2	3.36	16	72.9	4.56	20	52.8	2.64
LG15	18/17 <sup>a</sup>	70.6	3.92/4.15 <sup>b</sup>	10	76.1	7.61	26/23 <sup>a</sup>	75.1	2.89/3.27 <sup>b</sup>
LG16	23	88.5	3.85	21	82.8	3.94	27	87.5	3.24
LG17	18	34.3	1.91	17	41.5	2.44	23	39.7	1.73
LG18	19	59.3	3.12	18	75.9	4.22	24	56.6	2.36
LG19	53/50 <sup>a</sup>	98.8	1.86/1.98 <sup>b</sup>	49	85	1.73	64/63 <sup>a</sup>	97.5	1.52/1.55 <sup>b</sup>
LG20	22	75.5	3.43	23	74.6	3.24	26	76.1	2.93
LG21	10	56.9	5.69	12/11 <sup>a</sup>	60.3	$5.02/5.48^{b}$	15	58.7	3.91
Total	$480/474^{a}$	1388.1	2.89/2.93 <sup>b</sup>	417/416 <sup>a</sup>	1480.9	$3.55/3.56^{b}$	567/561 <sup>a</sup>	1537.4	2.71/2.74 <sup>b</sup>

 Table 1
 Number of markers and genetic length for each linkage group

<sup>a</sup> Data are shown as number of markers mapped/unique locations. <sup>b</sup> Data are shown as centimorgans/marker and centimorgans/unique marker loca-

#### Table 2 Summary of genetic linkage maps

	Female maps	Male maps	Integrated maps
Number of markers scored	613	568	720
Number of markers mapped	480	417	567
Average number of markers per group	23	20	27
Average marker spacing (cM)	3.06	3.75	2.85
Observed genome length (cM)	1388.1	1480.9	1537.4
Estimate genome length (cM)			
Gel	1516.8	1638.4	1657.0
Ge2	1529.0	1659.9	1664.4
Ge	1522.9	1649.1	1660.7
Genome coverage (%)	91.1	89.8	92.6

microsatellite markers and two SCAR markers in 21 linkage groups (Fig. 3), covering a total of 1,537.4 cM with an average interval of 2.85cM. The genome length of half-smooth tongue sole was estimated to be 1660.7cM, and coverage of 92.6% was observed. The

linkage group length varied from 39.7 cM to 102.2 cM, and the number of markers on the linkage group varied from 15 to 64 (Table 1 and Table 2).

#### 2.6 Recombination rate

The availability of SSR markers in the male and female maps allowed an evaluation of the respective meiotic recombination rates. The recombination rates obtained from 21 linkage groups were on average 0.0306 in females and 0.0375 in males. Therefore, the relative recombination ratio (female-to-male; F/M) in these pairs was 1:1.2, slightly higher in males than females. The average recombination rate in integrated maps is approximately 0.029 in half-smooth tongue sole.

# **3** Discussion

Linkage analysis and map construction using molecular markers is more complicated in full-sib families of out-breeding species than in progenies derived from homozygous parents. For example, markers may vary in the number of segregating alleles, one or both parents

LG1	LG2	LG3	LG4	LG5	LG6	LG7
0.0         HXTS12           0.0         cyce112           0.1         cyce112           0.2         carloi1021_42142           0.3         carloi1021_621           13.4         carloi1021_623           19.2         hncpe13           19.2         hncse13           19.2         carloid2020_6292           24.3         carloid309_71895           25.7         hncse13           53.4         carloid309_71895           54.4         carloid305_71402           53.3         carloid305_71402           54.4         carloid357_1402           57.2         hncse24           hncse24         hncse24           57.2         hncse24           57.2         hncse247           57.3         hncse240           57.4         scarloid1202_02782           57.6         scarloid1128_05111           57.6         scarloid1128_05111           5	0.7         seaffidd1873_61125           25.6         cyse43           29.0         scaffold569_26604           30.6         cyse71           37.0         scaffold569_26604           41.7         hncyse133           46.8         csou54           51.0         cyse71           37.0         scaffold367_31557           41.7         hncyse133           46.8         csou54           51.0         cyse104           51.0         cyse104           52.0         scaffold340_17563           53.0         scaffold320_16562           54.0         scaffold2320_65664           53.4         scaffold2388_66864           77.7         cse72	0.0 5.8 5.4 5.4 5.4 5.4 5.4 5.4 5.4 5.4	0.0 hncse229 6.0 scaffold142_53607 11.4 hts.A caffold114_4624 hts.A caffold114_4624 hts.A caffold114_4624 hts.A caffold270_65667 23.9 hncse178 23.9 hncse178 24.8 scaffold270_2518 46.4 gene177 25.1 scaffold250_25518 46.4 gene177 25.2 scaffold250_25518 25.2 scaffold352_1749 55.2 scaffold352_25474 51.2 scaffold352_25474 51.2 scaffold352_269647 58.1 scaffold312_69647 58.1 scaffold312_69647 58.1 scaffold312_69647 58.1 scaffold312_69647 58.1 scaffold312_69647 58.1 scaffold312_69647 hncyse108 58.9 scaffold312_69647 hncyse108 58.9 scaffold312_69647 58.1 scaffold312_69647 58.2 scaffold312_69647 58.1 scaffold312_69647 58.2 scaffold312_69647 58.2 scaffold312_69647 58.1 scaffold312_69647 58.2 scaffold312_69647 58.1 scaffold312_69647 58.1 scaffold312_69647 58.2 scaffold312_69647 58.2 scaffold312_69647 58.1 scaffold312_69647 58.1 scaffold312_69647 58.1 scaffold312_69647 58.1 scaffold312_69647 58.1 scaffold312_69647 58.1 scaffold312_69647 58.1 scaffold312_69647 58.1 scaffold312_69647 58.2 scaffold312_69447 58.2 scaffold312_69447 58.2 scaffold312_6947 58.2 scaffold3185 58.2 scaffold32_6747 58.2 scaffold32_6747 58.2 scaffold32_	0.0 hncyse11 2.3 hncyse11 6.2 scaffold447_53997 7.2 hncyse19 10.7 cyse199 14.6 scaffold809_35286 18.6 scaffold254_66591 23.5 csou43 32.0 scaffold2010_62849 33.2 scaffold2010_62849 33.2 scaffold2010_62849 scaffold2010_62849 scaffold2010_62849 scaffold201_6783 scaffold230_65829 scaffold230_65829 scaffold230_65829 scaffold730_789031 44.8 yscaffold47_7101 53.4 scaffold53_25182 52.6 scaffold730_71810 53.4 scaffold53_25182 58.4 csaffold53_25183 58.4 csaffold53_25183 59.5 csaffold53_25183 59.5 csaffold53_25183 59.5 csaffold53_25183 59.5 csaffold53_25183 59.5 csaffold53_25183 59.5 csaffold53_25183 59.5 csaffold53_25183 50.5 csaffold53_251	LG13	0.0 scaffold2250_65597 3.6 scaffold2250_65588 15.7 scaffold2250_65588 15.7 scaffold2250_65588 15.7 scaffold2250_75_34737 17.5 hncyse124 24.7 cyse160 25.7 scaffold325_71053 26.8 cyse174 30.0 scaffold375_71053 26.9 cyse174 30.0 scaffold373_71053 36.7 kstse 45.9 cyse148 50.1 scaffold373_571828 51.2 scaffold373_571828 51.2 scaffold373_571828 51.2 scaffold373_571828 51.2 scaffold373_571828 52.2 scaffold373_571828 51.2 scaffold373_571828 52.2 scaffold373_5718 53.2 scaffold373_5718 53.2 scaffold373_571828 53.2 scaffold373_5718 53.2 scaffold373
LG8	LG9	LG10	LG11	LG12	LG13	LG14
0.0         scafbid444_21127           3.7         hncyse44           6.3         cyse274           8.0         hncyse139           10.2         scafbid330_70410           13.8         scafbid330_70410           17.8         scafbid330_70410           17.8         scafbid1330_70410           17.8         scafbid134_52779           30.9         scafbid1394_52779           34.5         scafbid1394_52779           39.6         scafbid1346_17345           51.3         scafbid13605_71276           53.8         scafbid13605_71276           64.3         cyse1707           64.3         cyse1707           65.1         csc771           63.6         csc7107           63.6         hncyse85	14.7 hncyse5 21.8 cyse86 23.3 scatfold1816_50732 24.8 scatfold1816_50732 25.6 scatfold1822_35133 26.1 hst-k 77.1 scatfold275_50801 30.6 scatfold725_50805 31.8 csatfold725_50805 31.8 scatfold725_68805 31.4 hncs131 37.0 scatfold727_68850 34.4 hncs131 37.0 scatfold727_68850 38.7 scatfold727_68850 38.7 scatfold727_68850 39.7 scatfold717_68080 39.7 scatfold717_68080 30.8 scatfold718_0080 30.8 scatfold718_0080 30.8 scatfold718_0080 30.8 scatfo	0.0         scaffold1170_46055           16.3         scaffold1665_58308           21.0         scaffold1665_58308           21.0         hncse28           24.0         scaffold1665_58308           25.0         yseaffold86_5868           26.3         scaffold86_5568           35.9         cyse111           37.1         scaffold2832_570439           46.7         scaffold2832_68357           48.6         scaffold2832_68357           49.5         scaffold2032_42172           51.0         cse22           54.6         csou33           69.7         scaffold2063_63713           78.1         scaffold24495_67575	0.0 scaffold1118_45406 10.8 hncse64 15.1 hncse170 20.9 scaffold252255 20.9 scaffold252255 20.9 scaffold252276 27.8 scaffold72_2796 36.2 scaffold72_2796 36.2 scaffold77_58093 42.9 scaffold77_58093 45.7 hncse136 54.7 hncse3 59.1 hncse3 68.2 scaffold179_59428 79.6 hncse100	0.0 scaffold586_28587 7.8 cse74 10.7 scaffold672_31081 18.9 cyse284 20.3 hncse45 27.1 scaffold379_19493 33.9 cyse288 40.5 csou27 42.6 scaffold3260_689312 43.3 scaffold525_55172 43.8 scaffold1085_44234 47.2 ST10458_227999 57.6 hsts-C 63.3 scaffold222_165565 67.2 scaffold225_10803	3.4 scaffold950_39778 3.5 scaffold926_3275 3.6 scaffold256_5675 3.6 scaffold2168_64365 22.5 scaffold2168_64365 22.5 scaffold2168_64365 25.0 scaffold2168_4313 9.6 scaffold2313 9.6 scaffold23139 9.6 scaffold33139 9.6 scaffold349_1826 9.6 scaffold349_1826 9.6 scaffold349_1826 9.6 scaffold36_3139 9.6 scaffold349_1826 9.6 scaffold36_3139 9.6 scaffold349_1826 9.6 scaffold36_3139 9.6 scaffold36	2.9 gene116 5.2 scaffold39_19427 8.5 scaffold39_19427 8.5 scaffold364_57933 14.5 hncse85 16.1 scaffold1765_27338 14.5 scaffold177_59208 21.1 scaffold177_59208 21.1 scaffold177_59208 21.1 scaffold178_15208 21.1 scaffold178_16_70379 31.7 hncyse47 33.6 cse105 37.2 cse225 38.1 scaffold1788_60033 39.4 scaffold1788_60032 43.0 cyse223 52.8 scaffold1526_55284
LG15	LG16	LG17	LG18 LG	G19[1] LG19[2]	LG20	LG21
0.0 HXTS31 4.4 scaffold497_T1352 9.5 scaffold3636_2245 13.4 scaffold2670_815 cc54 24.1 ST100 cc54 25.3 ST100 cc54 26.7 Vise scaffold2719_837 scaffold2710_837 scaffold2710_837 scaffold2710_837 scaffold2088_649 1.783 31.7 is scaffold2088_649 1.322 1.782 3.64 scaffold2088_649 1.322 1.783 3.64 scaffold2088_649 1.322 1.783 3.64 scaffold2088_649 1.325 1.783 3.64 scaffold2088_649 1.325 1.783 3.64 scaffold2088_649 1.325 1.783 3.64 scaffold2088_649 1.325 1.783 3.64 scaffold2088_649 1.325 1.783 3.64 scaffold2088_649 1.325 1.68 scaffold2088	11.1         gene072           15.8         cyse77           17.8         scaffold1628_57677           19.9         cse164           20.2         cyse79           21.6         cse73           25.2         scaffold12329_60082           33.8         scaffold1323_960082           38.1         scaffold1348_55203           48.2         scaffold133_5118_45424           cyse150         scaffold133_5118_45424           54.0         scaffold144_6006_7103           56.2         scaffold144_6008_7103           57.1         scaffold155_5179           58.3         scaffold15_5179           59.9         scaffold15_5179           51.6         scaffold76_858_6007           52.3         scaffold72_753           scaffold735_68207         scaffold741	3.9         scaladorf (1,16)         110           8.8         cse243         15           10.3         scaffold309_15553         20           13.5         ysaffold309_15553         20           14.8         cse134         3368           15.1         scaffold305_15574         21           14.8         cse134         23           15.1         scaffold310_2         55           16.1         scaffold31_3577         21           18.1         scaffold31_3577         21           19.1         scaffold31_3577         21           20.9         scaffold31_255         20           21.8         hncse182         33           22.9         scaffold255         12500           23.4         cse55         54           24.5         cse150         51           31.1         scaffold325_252         56	3 scaffold1291_50712 scaffold4251_100 2 scaffold7321_1165 3 scaffold7321_1165 3 scaffold7321_1165 3 scaffold7321_1165 3 scaffold7323_12632 4 scaffold742_3232 4 scaffold742_3232 5 scaffold742_3232 4 cyse60 5 scaffold742_3232 5 scaffold724_3232 5 scaffold724_323 5 scaffold724_3232 5 scaffold724_3232 5 scaffold724_323 5 scaffold724_33 5 scaffold724_33 5 scaffold724_33 5 scaffold724_33 5 scaffold724	no.1	10.1         // hncyse102           12.3         // scaffold604_71           15.8         // scaffold605_165           17.8         // scaffold605_165           17.8         // scaffold605_165           17.8         // scaffold605_165           19.9         scaffold605_165           20.6         scaffold605_165           21.8         mncse01           21.8         scaffold605_62           25.7         cysc292           26.7         scaffold1605_62           22.5         scaffold1605_62           23.6         scaffold1604_64           36.6         scaffold16164_64           40.0         scaffold1645_62           42.6         hncse176           42.6         hncse176           42.6         hncse176           45.1         cs060           45.7         scaffold164_64_56           55.5         scaffold90_4658	18 23.4 23.7 23.7 23.7 25
	87.5 Scaffold74_3002		47.4	scaffoxt1354_51497 ST168 97.5 ST18	76.1 hncse22	

Fig. 3 Linkage maps of the integrated map for Cynoglossus semilaevis

The integrated map comprises 567 markers assigned to 21 linkage groups (LG1m-LG21m), and spans a total map length of 1537.4cM. Genetic distances in Kosambi centimorgans are listed on the left side of the linkage groups, and markers are listed on the right side of the linkage groups.

may be heterozygous, markers may be dominant or co-dominant, and usually the linkage phases of marker pairs are unknown. Given these differences, marker pairs provide different amounts of information for the estimation of recombination frequencies and the linkage phases of the markers in the two parents, and usually these have to be estimated simultaneously (Maliepaard et al., 1997). Therefore, the maps are constructed independently for maternal and paternal meiosis.

Genetic maps provide important genomic information and allow the exploration of QTL, which can be used to maximize the selection efficiency of target traits. The availability of a large number of genetic markers is essential for constructing a useful mid-density linkage map and for QTL mapping of genetic traits of interest. In this study, we constructed a mid-density microsatellite genetic linkage map using 567 markers in half-smooth tongue sole. Both female and male maps were found to have 21 linkage groups, which is in agreement with the karyotype of 2n=42. There were no small linkage groups (doublet or triplet), indicating that this linkage map is complete. Only 32 of the 720 markers studied remained unlinked to any other marker. This degree of completeness supports the utility of the genetic map as a reference tool for future genetic analysis in this species. In the mapping family, the male parent was derived from a wild population and the female selected from a cultured population, but the number of segregation markers in males (568) is less than that in females (613). This is possibly because the recombination ratio was higher in males than females or the number of genetic markers is restricted.

The marked sexual dimorphism of the growing half-smooth tongue sole has led to suggestions that the efficiency of the culture systems could be improved by setting up a production system focused on the faster-growing sex. In a previous study, a femalespecific DNA marker f-382 was located on the linkage map of half-smooth tongue sole and this was the first report on the mapping of a sex-linked marker on a genetic linkage map in teleosts (Liao et al., 2009). We were able to map another SCAR marker f-783 onto the LG15f region in which female-specific SCAR marker f-382 was assigned. Both the male and female maps share the homologous region of LG15f containing the same microsatellite markers (scaffold4497 71352, scaffold2821 69157, scaffold467 24010), which imply that the LG15 segment is homologous in females and males, and is an indication of a pseudoautosomal region of the sex chromosome. The mapping of a sex-linked marker in a general population of half-smooth tongue sole is vital for the further development of mono-sex culture in this species. This is especially important in half-smooth tongue sole because of a large difference in the growth rate between males and females. The identified female-specific SCAR markers of f-783 and f-382 can be used for the molecular identification of genetic sex, and also provide an important tool for screening and isolation of the sex-determining locus and sex manipulation in half-smooth tongue sole.

The average interval between markers was slightly less for the female map (3.06 cM) than the male map (3.75 cM), suggesting that the recombination rate was slightly higher in males than in females. The recombi-

nation ratio between the male and female parents of half-smooth tongue sole was 1.2:1. Differences in map length can result from a variation in the number of recombination events in the two parents as well as variations in the number and location of the mapped loci. Despite this being a common phenomenon, the mechanism responsible for the different recombination rates between the genders is not well understood. Some studies have shown that recombination rate differences are associated with QTL (Kai et al., 2005). Selection using linked markers is more efficient when recombination does not occur between the markers and the OTL loci. It is common to find a difference in the recombination ratio between the two sexes in most aquatic species, but in these species, the recombination rate was higher in females than in males. For instance, the male/female recombination ratios are 1:3.25 in rainbow trout (Sakamoto et al., 2000), 1:8.26 in Atlantic salmon (Moen et al., 2004), 1:2 in halibut (Reid et al., 2007) and 1:1.43 in Japanese flounder (Castaño-Sánchez et al., 2010). The average recombination rate across all of linkage groups is approximately 0.029 in this study, which is higher than that in zebrafish (Shimoda et al., 1999), tilapia (Lee et al., 2005), catfish (Wang et al., 2007) and grass carp (Xia et al., 2010), and lower than rainbow trout (Rexroad et al., 2008), Asian sea bass (Wang et al., 2011) and Japanese flounder (Castaño-Sánchez et al., 2010).

In this mapping family, segregation distortion was observed for 165 markers and the distortion rate was approximately 23.0%, which is lower than the ratio of 33% reported by Liao et al. (2009). This suggests that a high ratio of segregation distortion may be a common phenomenon in half-smooth tongue sole. The distortion rate is 16% in channel catfish (Liu et al., 2003) and 16.3% in common carp (Cheng et al., 2009). The reasons for the distortion of the segregation ratios may be due to sampling errors (Plomion et al., 1995), scoring errors (Nikaido et al., 1999), the progeny population size and amplification of a single-sized fragment derived from several different genomic regions (Faris et al., 1998). Additionally, lethal effects caused by a recessive homozygote in the juvenile period may affect distorted segregation (Hubert et al., 2004).

In conclusion, the second generation of the linkage map of half-smooth tongue sole containing 565 microsatellites and two SCARs has been constructed. With higher marker density (3.06 cM and 3.75 cM in the female and male maps, respectively), the new map is presently the densest flatfish linkage map. The map will not only facilitate selective breeding and mapping of QTL, but also provide new data for comparative genomic studies.

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