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Population structure of elongate ilisha *Ilisha elongata* along the Northwestern Pacific Coast revealed by mitochondrial control region sequences

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Abstract The elongate ilisha *Ilisha elongata* is an economically important species broadly distributed from the Western Pacific to the Java Sea off Sarawak. The fishery of elongate ilisha has been declining in recent years in terms of total catch, size of harvested fish, as well as absence of the fish from previously distributed areas. The knowledge about population structure and genetic diversity of elongate ilisha is important for managing sustainable fisheries. Only two studies have investigated the population genetics of elongate ilisha but both with limited sampling. In the present study, we sampled 16 sites from the coast of China, Korea and Japan. We sequenced the whole mitochondrial control region (D-loop), which showed a remarkably high polymorphism. The average haplotype diversity was 0.98 and the nucleotide diversity (π) was 0.006. Neutrality tests suggested past population expansions. Populations were weakly differentiated ($\Phi_{ST} = 0.0612$). This result, together with a lack of association between clades and locations ($R^2 = 0.0591$), indicated a weak population structure of the elongate ilisha. The only exception was from the population of Dandong in the West Korean Bay, which was different from all other populations in its D-loop sequence.

We did not find genetic differences between the fish with a bottom-sword caudal fin and the fish with a double-sword caudal fin.

Keywords Elongate ilisha · Genetic diversity · D-loop · Conservation · Cryptic species · Asymmetric tail

Introduction

The elongate ilisha *Ilisha elongata* (Clupeiformes: Pristigasteridae) is a marine species inhabiting both pelagic and inshore waters [1], broadly distributed from the Western Pacific [1, 2] to the Java Sea off Sarawak [3], including all of the coastal waters of China [4]. The elongate ilisha also was reported in the Indian Ocean and in Peter the Great Bay; however, the Indian record was based only on a single specimen [1] and the elongate ilisha has rarely been seen in Peter the Great Bay in recent years (Balanov, personal comm.).

The elongate ilisha lives in tropical, subtropical and temperate waters, even though it is sensitive to cold temperature, and does not reach the northern part of its range in cool years [1]. In breeding season, it enters river mouths and was found moving upstream as far as 15 km for spawning, where salinity varied from 12.0–23.7 ‰ [5], suggesting that it could tolerate low salinity [1, 6]. The elongate ilisha reproduces from April to June when water temperature rises above 19 °C [7]. Their breeding starts earlier in southern waters than in northern regions. Spawning activity occurs mostly at dusk or dawn [8]. Typically, a fish releases about 40,000–195,000 floating eggs [9]. The eggs hatch in 30 h when water temperature is between 23 and 26 °C [5]. After hatching, the fish spends its early life stage in river mouths [10]. The elongate ilisha preys on a broad list of

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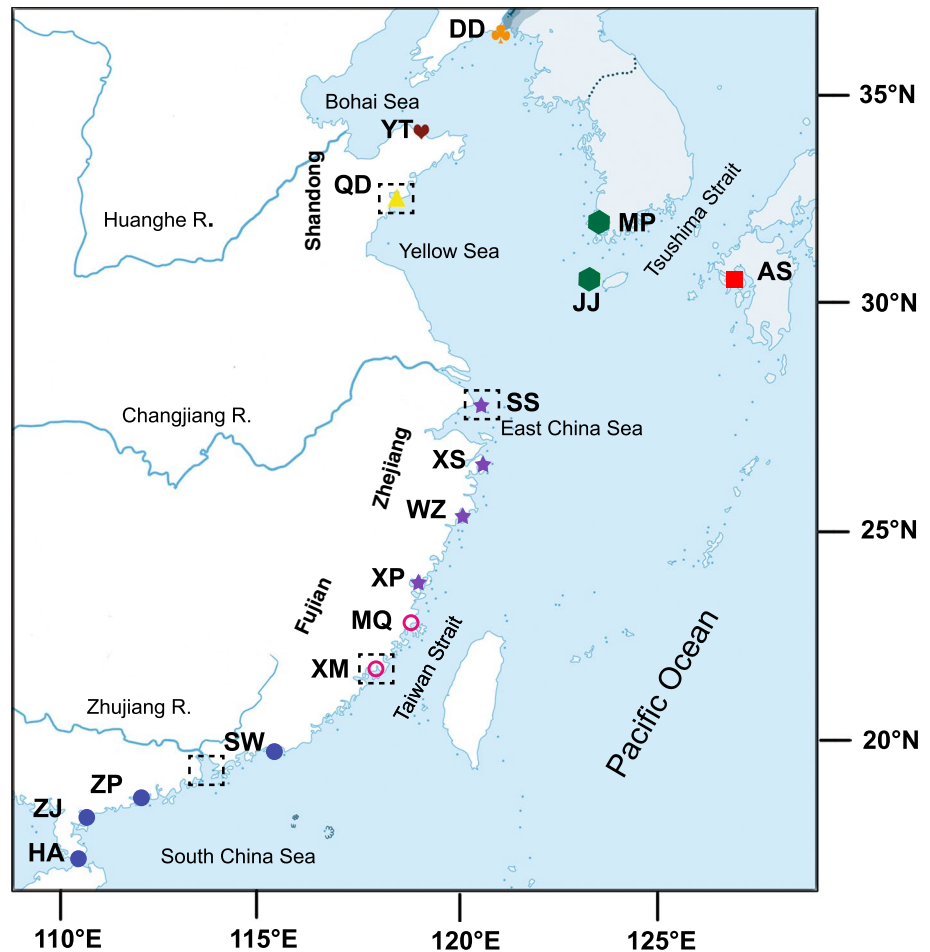
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Fig. 1 Sampling sites of the elongate ilisha. HA Haian, ZJ Zhanjiang, ZP Zhapo, SW Shanwei, XM Xiamen, MQ Mingqing, XP Xiapu, WZ Wenzhou, XS Xiangshan, SS Shengsi, QD Qingdao, YT Yantai, DD Dandong, MP Mokpo-si, Jeollanam-do, JJ Jeju-si, Jeju-do, AS Ariake Sound. Different colors and shapes denote graphical regions. Dots South China Sea; Circles Taiwan Strait; Stars East China Sea; Triangles Yellow Sea (QD); Hearts Yellow Sea (YT); Cloves Yellow Sea (DD); Hexagon Korea; and Squares Ariake Sound, Japan; Dashed rectangles indicate the sampling sites of previous studies [22, 23]



items. For example, more than 60 species were found in the stomach content of the elongate ilisha in the East China Sea, mostly planktonic crustaceans, fishes and cephalopods [11].

In the Yellow Sea and the East China Sea, the elongate ilisha were divided into three populations based on their difference in migration path and spawning grounds [12]. The first population spends the winter in the south Yellow Sea and spawns on the coast of Shandong ($36^{\circ}45' - 37^{\circ}35'N$, $119^{\circ}25' - 122^{\circ}22'E$). The second population occupies the southern areas of Jeju Island and spawns in the coastal areas near Zhejiang (west of $27^{\circ}N$, $125^{\circ}E$). The third population lives in the area north of Taiwan and moves to the coastal waters off Fujian for breeding. The name of Shandong, Zhejiang and Fujian provinces are labeled in Fig. 1. Zhang and Takita [13] studied growth traits of the elongate ilisha in Ariake Sound, Japan. They found that the Ariake population was different from the Shandong population, but the adults possessed similar growth traits as those of the southern China population, suggesting that it might share the same growth ground with the southern China population. Kim et al. [14] found that the marginal index of scales of the elongate ilisha was different between the samples

collected off the coast of the Korean peninsula and the samples from the East China Sea, suggesting that they might belong to different populations [6, 15].

The elongate ilisha is a commercially important species. In 2013, the catch in China alone was 84,033 ton [16]. However, a decrease of fisheries of the elongate ilisha has been reported from Japan [17], Korea [14], and different regions of the China coast [9, 18, 19]. The decline is not only seen in total catches, but is also shown as a smaller catching size of the adult fish [9] and the absence of elongate ilisha from the Bohai Sea, a previous distribution area with high abundance [20].

To maintain a sustainable fishery, understanding the population dynamics and the stock structure of the target species is often important and useful, e.g., Altukhov et al. [21]. Previous hypotheses about population structure of the elongate ilisha were mostly based on morphological characters, growth traits or their migration route and spawning grounds [12–14]; therefore, testing those hypotheses with genetic data is crucial [13, 14]. Nonetheless, only two genetic studies have been carried out on the elongate ilisha. Wu et al. [22] found that samples collected in Qingdao (the Yellow Sea) are different from samples collected in Zhoushan (the East China

Table 1 Sampling localities, number of samples from each site and sampling dates

Area	Sampling location	Sample ID	No. of samples	Latitude	Longitude	Sampling date
Yellow Sea	Yantai (YT)	CL1145_1-9 (bottom-sword)	9	37°27'49"	121°26'46"	Jun 2015
	Dandong (DD)	CL1133_1-4 (double-sword); CL1134_5-10 (bottom-sword)	10	40°00'40"	124°21'53"	Jun 2015
East China Sea	Qingdao (QD)	CL682_1-19	19	36°4'33"	120°21'58"	May 2014
	Wenzhou (WZ)	CL679_1-5	5	28°1'23"	120°41'2"	May 2014
	Xiangshan (XS)	CL683_1-5	5	29°28'30"	121°51'47"	May 2014
	Shengsi (SS)	CL700_1-10	10	30°43'48"	122°26'31"	May 2014
	Xiapu (XP)	CL1029_1-8	8	26°51'58"	119°58'44"	May 2014
Taiwan Strait	Minqing (MQ)	CL681_1-9	9	26°14'13"	118°52'37"	May 2014
	Xiamen (XM)	CL678_1-7	7	24°27'43"	118°4'19"	May 2014
South China Sea	Shanwei (SW)	CL674_1-7	7	22°46'19"	115°21'18"	May 2014
	Zhapo (ZP)	CL673_1-6	6	21°34'34"	111°50'9"	May 2014
	Zhanjiang (ZJ)	CL670-1	1	21°16'23"	110°21'29"	May 2014
	Haian (HA)	CL664_1-8	8	20°16'16"	110°13'23"	May 2014
Japan	Ariake Sound (AS)	CL1030_1-10	10	32°59'46"	130°21'1"	May 2014
Korea	Jejudo (JJ)	CL1031_1; CL1031_2-6	6	37°28'	126°37'	Jan 2015; Mar 2015
	Mokpo (MP)	CL963_1-5	5	34°48'29"	126°23'41"	Jun 2015

Sea), Xiamen (Taiwan Strait), and Guangzhou (the South China Sea; Fig. 1), whereas the latter three populations were not different from each other based on their mitochondrial control region (D-loop). Lü et al. [23] repeated the work of Wu et al. with the 16S rDNA gene. They found the same pattern among the four populations, and concluded that genetic diversity of elongate ilisha along Chinese coast was high. On the contrary, a second study using cytochrome c oxidase I (COX1) and D-loop loci found that genetic diversity of elongate ilisha in the East China Sea was low [24]. All of these studies surveyed a limited number of sampling sites that covered only a part of the distribution of the elongate ilisha; for example, the first study was based on fish collected from four sites (10–12 samples each site) [22, 23] and the second study used 16 individuals collected from only one site of the East China Sea [24]. The whole picture of population structure and genetic diversity of the elongate ilisha along the Western Pacific Coast is still lacking, which hinders effective fisheries management of the elongate ilisha in a sustainable way. To fill in this gap, we collected samples from 16 sites along the Western Pacific Coast, including the South China Sea (SCS), the Taiwan Strait (TWS), the East China Sea (ECS), the Yellow Sea (YS), Jejudo Island and Mokpo of Korea (KR) and the Ariake Sound of Japan (AS), to evaluate the genetic variability of the elongate ilisha and to understand its population structure using mitochondrial D-loop sequences.

During sampling, we found some specimens that had asymmetric caudal fin lobes; that is, the lower lobe of their caudal fin was obviously longer than the upper lobe of the caudal fin (bottom-sword tail). This morphotype also was

observed previously, but has not been reported (Wu, personal comm.). We examined whether there is a genetic difference between these abnormal elongate ilisha and the one with double-sword tail.

Materials and methods

Sample collection

A total of 125 samples were collected from 16 sites from May 2014 to Oct 2015 (Fig. 1; Table 1). In the South China Sea, four sites were sampled, including Haian (HA, $n = 8$), Zhanjiang (ZJ, $n = 1$), Zhapo (ZP, $n = 6$) and Shanwei (SW, $n = 7$); n refers to the number of individuals collected. Xiamen (XM, $n = 7$) and Minqing (MQ, $n = 9$) were two sites near the Taiwan Strait. Four sites in the East China Sea, Wenzhou (WZ, $n = 5$), Xiapu (XP, $n = 8$), Xiangshang (XS, $n = 5$) and Shengsi (SS, $n = 10$) were sampled. In the Yellow Sea, Qingdao (QD, $n = 19$), Yantai (YT, $n = 9$, all with a bottom-sword caudal fin) and Dandong (DD, $n = 10$, including 6 specimens with a bottom-sword caudal fin and four specimens with double-sword fin lobes) were sampled. We also sampled the Ariake Sound, Japan (AS, $n = 10$), and Jejudo Island (JJ, $n = 6$) and Mokpo (MP, $n = 5$) of Korea (KR). An African ilisha *Ilisha africana* was sampled as an outgroup. Muscle tissues were taken under the dorsal fin of the fish and preserved in 95 % ethanol and then stored at 4 °C until the DNA was extracted.

DNA extraction, PCR amplification and sequencing

DNA was extracted using an Ezup DNA extraction kit following the protocol of the manufacturer (Sangon Biotech, Shanghai, China). Twenty-five milligrams of tissue of each sample was used for DNA extraction. Whole mitochondrial sequences of *Pellona ditchela* and *Ilisha elongata* were downloaded from GenBank (National Center for Biotechnology Information; <http://www.ncbi.nlm.nih.gov/>, accessed Nov 11, 2015) and aligned in MEGA5 [25]. Two pairs of universal primers were designed on the alignment of *P. ditchela* and *I. elongata* using Primer3 [26], including the forward primers ilisha-L1 (CTCAAA-GCGCCGGTCTTGTAATC), ilisha-L3 (AGTAAGAAAC-CACCAACCAGT) and the reverse primers ilisha-R1 (CTGATAGTAAAGTCAGGACCAAGCCT) and ilisha-R3 (GCTTGCATGTGTAAATTGGGC). The primer pair 1 was used to amplify the whole D-loop region. The primer pair 3 was used as extra sequencing primers if the primer pair 1 did not work well.

Polymerase chain reaction (PCR) amplification was carried out in a reaction mix of 25 μ L (10 mM Tris-HCl, 50 mM KCl, 0.1 % TritonX-100, 2.5 mM $MgCl_2$, 0.1 mM of each dNTPs and 1 M of each primer) with one unit of *TaKaRa Ex Taq*[®] DNA polymerase and 50 ng genomic DNA. The thermal cycle followed: initial denaturation at 98 °C for 2 min, 16 cycles of denaturation at 95 °C for 30 s, primer annealing at 62 °C for 40 s and extension at 72 °C for 1 min, then 16 additional cycles but with 60 °C as the annealing temperature; the final extension was 5 min at 72 °C. The amplified fragments were checked on 1.5 % agarose gels. Successfully amplified products were sequenced at Sangon Biotech (Shanghai, China).

Sequence alignment and summary statistics

The forward and reverse sequences of each sample were assembled with DNAMAN software version 6.0. (Lynnon Biosoft, Canada). Chromatograms of the sequence data were viewed in MEGA5 [25] to aid visual inspection on the final assembled sequences. D-loop sequences of all samples were aligned using CLUSTALX [27]. The start and the end of each sequence were trimmed due to ambiguity shown in the chromatogram. Only the whole D-loop region of the sequences was kept. Number of haplotypes, haplotype diversity (h), average nucleotide difference (K), and nucleotide diversity (π) of each sampling site were calculated with Arlequin 3.5 [28].

Haplotype network and phylogenetic analysis

A median-joining network was created for all D-loop haplotypes in NETWORK 4.6.1.3 [29]. The resulting

haplotype network was adjusted in Photoshop CS6 for better viewing of the relationship among haplotypes. Phylogenetic relationships among haplotypes were reconstructed using RAxMLv7.5 [30, 31], under the GTRGAMMA model with 1000 bootstrap replicates to assess nodal support. The West African ilisha *Ilisha africana* was used as an outgroup. The best ML tree was visualized in Figtree v1.4.2 (<http://tree.bio.ed.ac.uk/software/figtree/>).

Genetic variation among populations

The genetic difference among populations (sampling sites) was measured as Φ_{ST} implemented in Arlequin 3.5 [28]. Ten thousand random replicates were used to estimate the significance of each pairwise comparison and a Bonferroni correction was applied for multiple tests [32]. The significance level after Bonferroni correction was 0.00037.

Hierarchical analysis of molecular variance (AMOVA) was performed in Arlequin 3.5. The sampling sites were grouped as different sea regions: the South China Sea (HA, ZP, ZJ, SW), the Taiwan Strait (MQ, XM), the East China Sea (XP, WZ, XS, SS), the Yellow Sea (QD, YT, DD), Korea (JJ, MP) and Japan (AS). Because preliminary analysis revealed that the DD population was a divergent group to the other populations, we carried another AMOVA excluding DD samples to evaluate the partition of genetic variance among/within groups without the DD population.

Genetic barriers and isolation by distance

In order to investigate geographical relationships among the groups, we used the method of Barrier [33] to identify the most robust spatial split on the genetic landscape of the elongate ilisha. We also examined the correlation between genetic distance (Φ_{ST}) and geographic distance (km) of each population with the Mantel test implemented on the IBD website [34]. The geographic distance was measured using the coordinates of the sampling sites (<http://map.baidu.com/>). Ten thousand permutations were applied to calculate the significance of the correlation.

Mismatch distribution and estimation of demographic parameters

Mismatch distribution tests implemented in Arlequin 3.5 were carried out for each sea region, except for DD samples being analyzed as an independent group because of their genetic distinctness from other Yellow Sea samples. The model of sudden population expansion was applied. Each deviation of the observed distribution from the sudden expansion model was evaluated using Harpending's

Table 2 Summary statistics of D-loop sequences in the 16 populations of the elongate ilisha

Population	No. of samples	No. of haplotypes	h^a	K (%) ^b	π^c
DD	10	9	0.98	6.1	0.006
YT	9	8	0.97	6.2	0.006
QD	19	11	0.91	5.3	0.005
SS	10	10	1.00	8.1	0.007
XS	5	5	1.00	6.8	0.006
WZ	5	4	0.90	8.0	0.007
XP	8	6	0.93	4.9	0.004
MQ	9	7	0.92	7.0	0.006
XM	7	7	1.00	7.3	0.007
SW	7	6	0.95	6.6	0.006
ZP	6	6	1.00	11.3	0.010
ZJ	1	1	–	–	–
HA	8	8	1.00	7.1	0.006
AS	10	4	0.53	1.5	0.001
JJ	6	5	0.93	3.2	0.003
MP	5	5	1.00	7.8	0.007
Total	125	82	0.98	10.3	0.006

For abbreviations of the populations, see Table 1

^a h , haplotype diversity

^b K , average nucleotide difference

^c π , nucleotide diversity

raggedness index (Hri , [35]) and the sum of squared deviations (SSD , [36]). Bootstrap testing with 10,000 replicates was used to test for significance and to obtain the demographic parameters of the mutational time scale (τ), and the expected pairwise differences before (θ_0) and after (θ_1) a rapid change in population size [36, 37]. Neutrality testing for each sampling site, Tajima's D [38] and Fu's F_S [39], were performed as implemented in Arlequin 3.5.

Results

Variation of D-loop sequences

Alignment of the D-loop region had 1145 bp. There were 82 haplotypes in the 125 individuals sequenced (accession numbers KX137942 - KX138066). The average haplotype diversity was 0.98, with AS having the lowest value, 0.53. In the population of SS and XS (East China Sea), XM (Taiwan Strait), ZP and HA (South China sea), and MP (Korea), the haplotype diversity reached 1.00, that is all sequenced fish had different haplotypes. The average nucleotide diversity (π) was 0.006. AS (Japan) had the lowest nucleotide diversity, 0.001, whereas ZP (South China Sea) had the highest nucleotide diversity, 0.010 (Table 2).

Haplotype network and phylogenetic tree

The haplotype network was color-coded for samples collected from different sea regions, except that the Yellow Sea samples were labeled with three different colors, orange for DD, brown for YT and yellow for QD (Fig. 2). The DD population was separated from other clades by 24 base substitutions. The other populations of the Yellow Sea, East China Sea, Taiwan Strait, South China Sea, AS (Japan) and JJ and MP (Korea) were mixed together, and no distinguishable clades could be identified, except that haplotypes from the South China Sea were almost absent from a “north clade” (Fig. 2). There were only 4 haplotypes in the 10 fish collected from AS (Japan), eight of which had haplotypes associated with the “north clade” and two fish related to the samples collected from Korea.

The phylogenetic reconstruction corroborated the patterns revealed in the haplotype network. The DD population diverged earlier from the rest of the populations with a high bootstrap support (99 %). Samples collected from the other populations were mixed together and most of the nodes had support values less than 70 % (Fig. 3a, b). The “north clade” identified in the haplotype network containing only one fish from the South China Sea was also found in the reconstructed tree. There were two morphotypes in the DD population, one with a bottom-sword tail (six fish, CL_1134_1–6) and the other with a double-sword tail (four fish, CL_1133_1–4); however, the fish were not grouped together according to their morphotypes in the phylogenetic tree (Fig. 3a). Similarly, samples collected from YT (nine fish, CL_1145_1–9), which all had a bottom-sword tail were scattered in the tree and grouped with fish having a double-sword tail instead of grouped together by themselves (Fig. 3a, b).

Genetic divergence among populations

Pairwise Φ_{ST} values were high (>0.5) and significant between the DD and other populations, but the difference between the two morphotypes of the DD population was not significant ($\Phi_{ST} = 0.08$ and $P > 0.05$; Table 3). The next divergent population was AS, which had significant Φ_{ST} values ranging from 0.099 to 0.9 compared with other populations. The rest of pairwise Φ_{ST} comparisons excluding the DD and AS populations were low and not significant with a few exceptions, such as the Φ_{ST} value between JJ and ZJ ($\Phi_{ST} = 0.6836$ and $P < 0.01$; Table 3).

The AMOVA showed that 65.72 % of the genetic variation was within populations, 6.58 % was among population within groups, and 27.69 % was among groups (Table 4). The AMOVA excluding the DD population resulted in –0.46 % of variation among groups, 8.43 % among

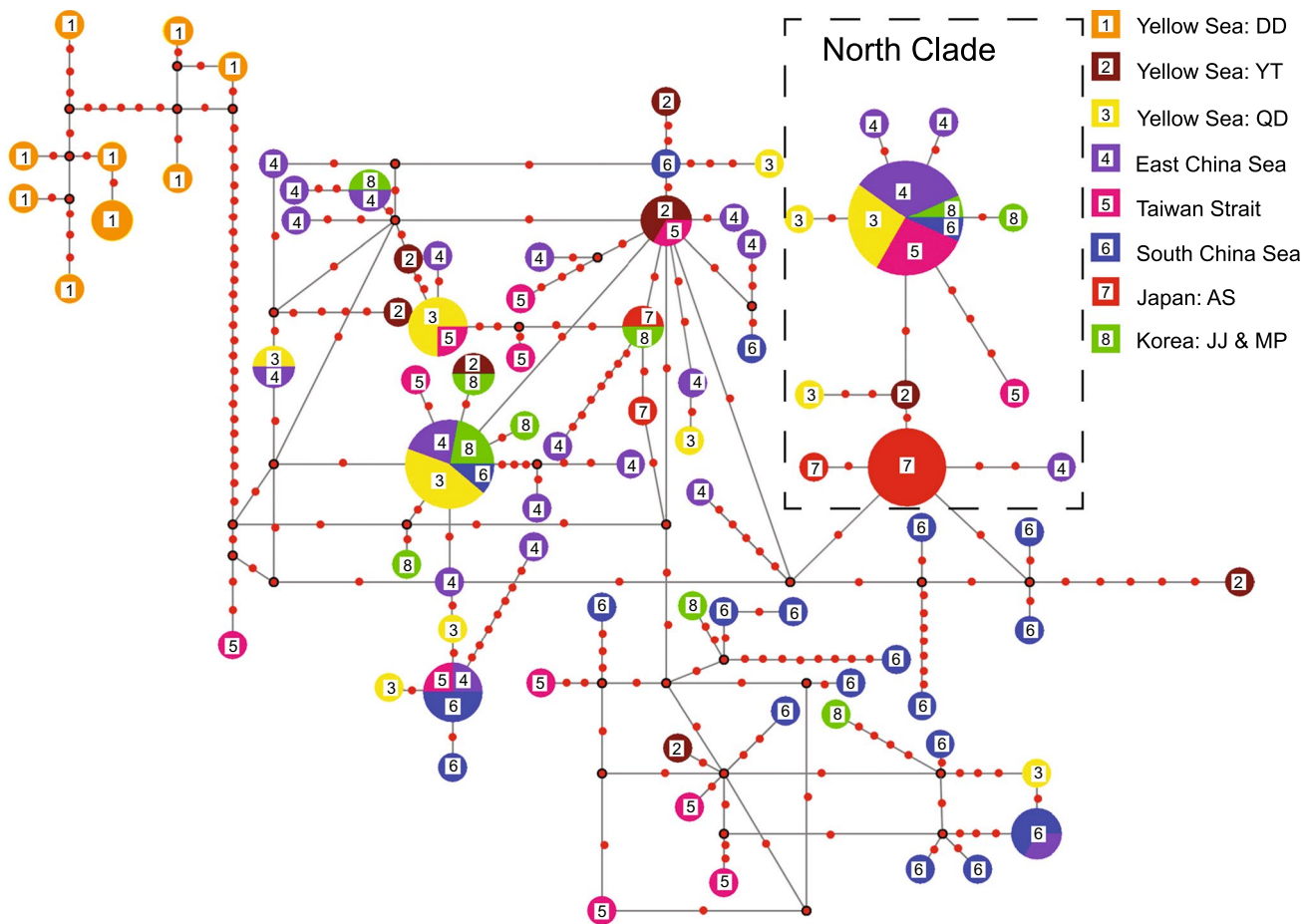


Fig. 2 Haplotype network of elongate ilisha based on the mtDNA D-loop region (82 haplotypes). The dots between the nodes indicate the number of mutations. The area of each circle is proportional to the corresponding haplotypes

populations within groups, and 92.03 % within populations (Table 4).

Barrier and IBD analyses

Barrier analysis predicted the most possible division among the 16 sampling sites between the DD population and the others (Fig. 4). The other two probable barriers could be between the SW and ZP populations, or between the ZP and ZJ/HA populations. The isolation by distance (IBD) test showed a significant correlation between the genetic distance and the geographical distance among populations ($P < 0.01$), but the R^2 value was small, only 0.0591 (Fig. 5).

Demographic history

The mismatch analysis of the 16 populations showed different shapes of frequency distribution, reflecting a diversity of demographic histories. The first type had a unimodal frequency of the pairwise difference distribution, providing evidence for a sudden population expansion, which

occurred in DD and SCS populations (Fig. 6a, e). The second type of distribution showed one prominent peak around 5–9 conforming a sudden population expansion hypothesis, but also had a minor peak around 50, which was seen in YS, ECS, TWS, KR populations (Fig. 6b, c, d, f). Type three, found in the AS population, deviated from the non-hypothesis of a sudden population expansion model (Fig. 6g). The sum of squared deviation (SSD) was highest in the AS population (0.372; $P < 0.01$), while the SCS population had the lowest SSD value (0.003; Table 5). Except for the AS population, all other populations did not significantly deviate from the sudden population expansion hypothesis ($P > 0.05$). H_{ri} was not significant in all populations. The estimated current effective female population size was higher than before the expansion. The highest θ_1 was 99,999 in the AS population, and the next two high θ_1 values were 422 in the KR population and 130 in the SCS population (Table 5).

Estimates of Tajima's D of all the populations were negative, but only the ECS population had a significant Tajima's D ($P < 0.05$). Summary statistics for testing

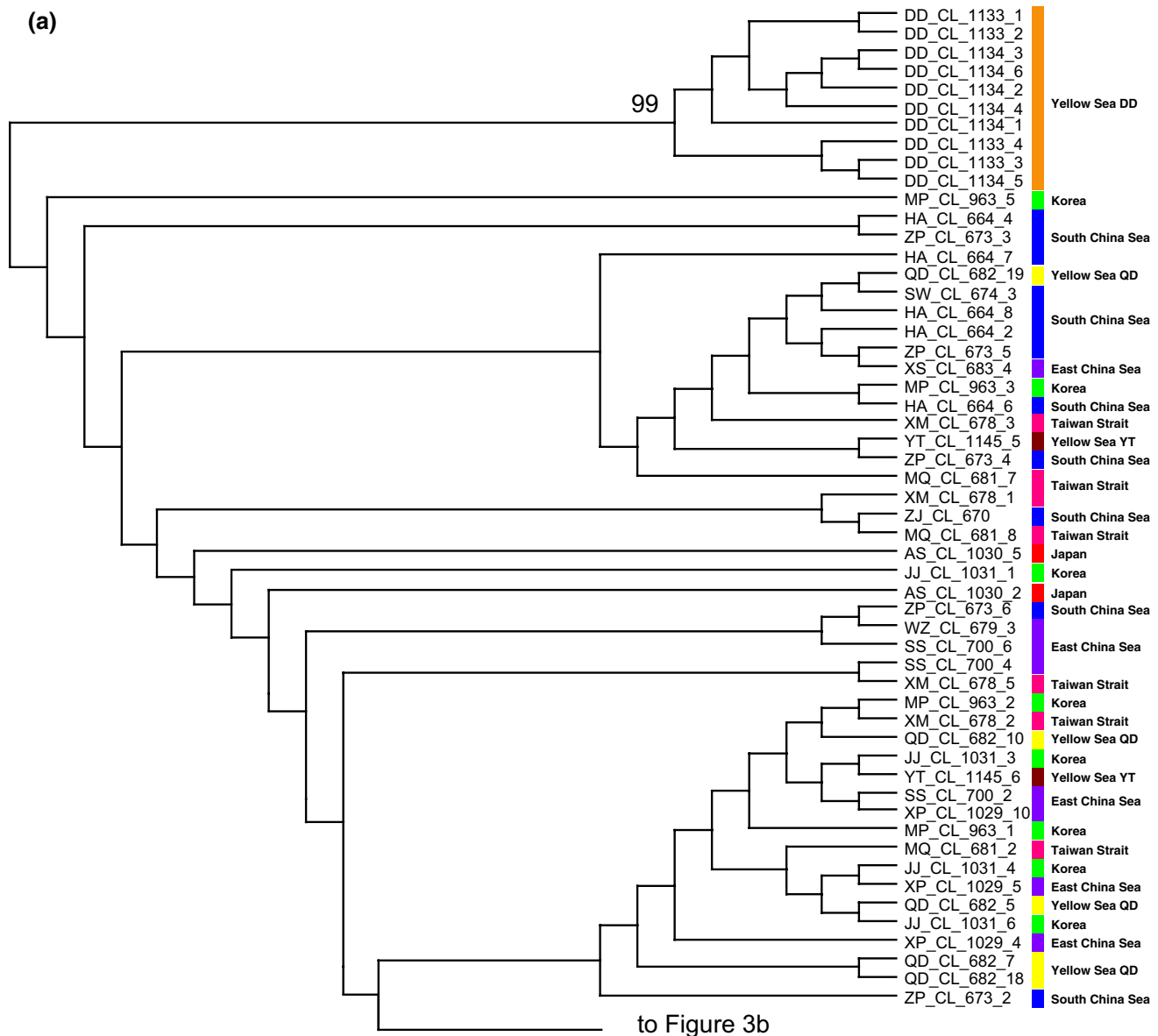


Fig. 3 **a** The ML tree reconstructed using RAxML on 82 haplotypes identified in 125 individuals of elongate ilisha. The West African ilisha was used as an outgroup (not shown). **b** Continued from **a**

drift-mutation equilibrium Fu's F_S showed that nearly all values were negative, but only the SCS population had a significant value ($P < 0.01$; Table 5).

Discussion

Genetic diversity of the elongate ilisha along the Western Pacific

We found 82 D-loop haplotypes in the 125 individuals of elongate ilisha collected from 16 sites along the Western Pacific Coast. The average haplotype diversity was 0.98

(0.53–1.00). All populations surveyed in our study had haplotype diversity values greater than 0.90, except that for the AS population was only 0.53 (Table 2). The high haplotype diversity of elongate ilisha was comparable to previous studies with limited sampling sites (either 0.964–1 [22] or 0.983 [24]), and to the values found in other marine and migratory clupeiforms, such as Pacific herring *Clupea pallasii* (0.986 using D-loop) [40], the hilsa shad *Tenualosa ilisha* (0.882–0.960 using RFLP) [41], the gold-spotted grenadier anchovy *Coilia dussumieri* (0.7421–0.9368 in ATPase 6/8) [42], the European anchovy *Engraulis encrasicolus* (0.79 in ND5/6) [43] and the Japanese anchovy *Engraulis japonicus* (0.4706–0.9935 in cyt b) [44]. The average nucleotide

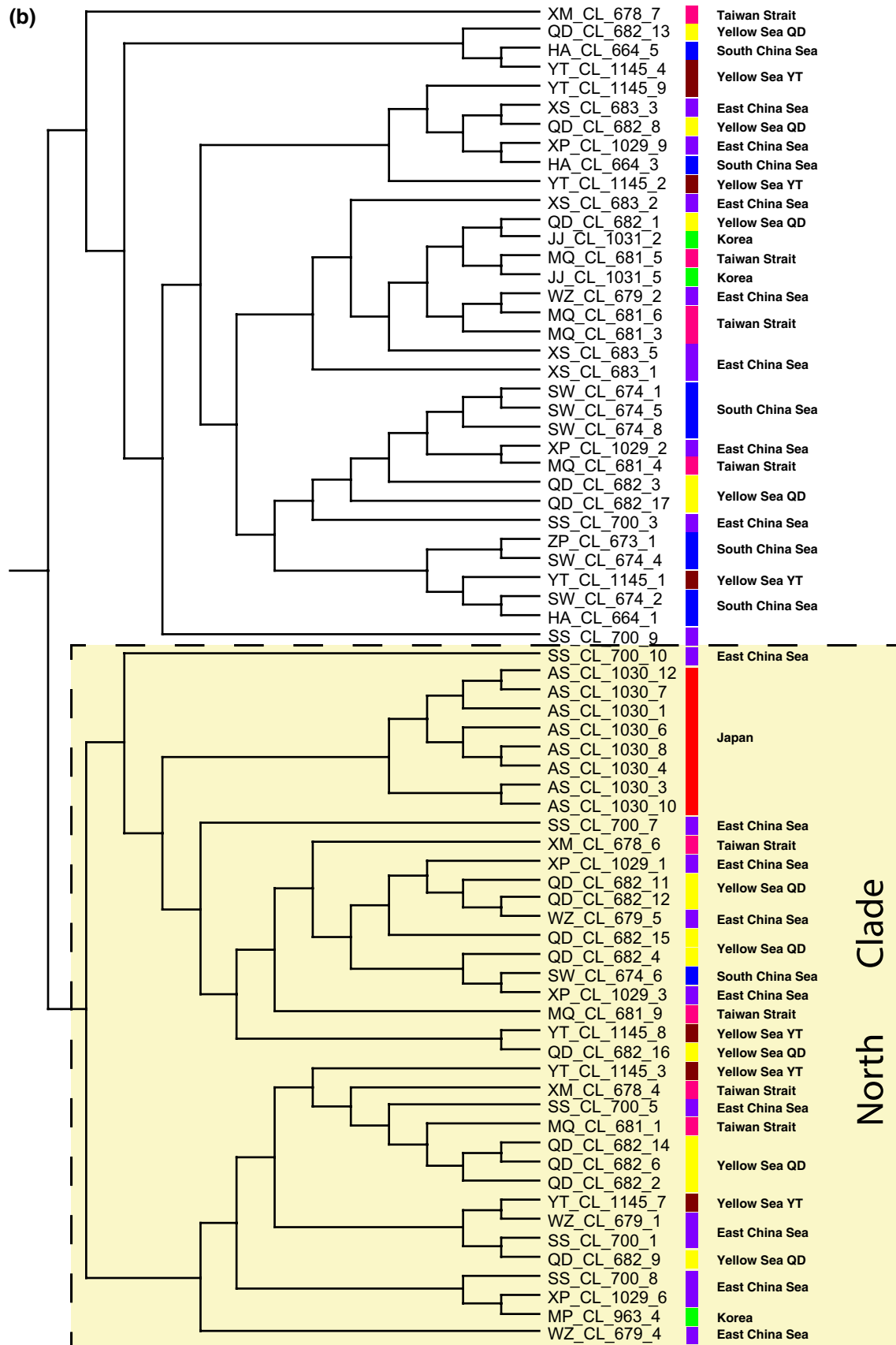


Fig. 3 continued

Table 3 Φ_{ST} among populations

	YT	DDs	DDa	QD	WZ	XS	SS	XP	MQ	XM	SW	ZP	ZJ	HA	AS	JJ
DDs	0.6427*															
DDa	0.6987*	0.0819														
QD	0.0372	0.7870*	0.8122*													
WZ	-0.0408	0.7268*	0.7826*	-0.0205												
XS	-0.0033	0.7723	0.8162*	-0.0196	-0.0545											
SS	0.0005	0.5264*	0.5927*	0.1948	0.0598	0.1035*										
XP	-0.0002	0.8058*	0.8372*	-0.0180	0.0054	0.0504	0.1215									
MQ	-0.0667	0.6283*	0.6861*	0.0107	-0.0486	-0.0711	0.0115	-0.01121								
XM	-0.0229	0.7544*	0.7980*	0.0486	0.0353	0.0275	0.1060	0.0329	-0.0205							
SW	0.0204	0.7647	0.8098	0.0468	0.0282	0.0757	0.1235	0.0534	0.0151	0.0714						
ZP	0.0323	0.6920*	0.7527*	0.1771*	0.0862	0.0894	0.1255	0.1536	0.0425	0.0060	0.1001					
ZJ	0.0390	0.7778	0.8508*	0.4861	0.2973	0.4127	-0.0702	0.5607	-0.0207	0.2719	0.4382	0.0595				
HA	0.0260	0.7442*	0.7873*	0.1570*	0.1151	0.0940	0.1498	0.1633*	0.0424	-0.0169	0.1380	-0.0585	0.2220			
AS	0.0990	0.9013*	0.9145*	0.1708	0.2477*	0.3387*	0.2012*	0.2531*	0.1133*	0.2211	0.1959	0.3047*	0.8642*	0.3324*		
JJ	-0.0205	0.8375	0.8650*	-0.0512	-0.0009	0.0072	0.1036	-0.0759	-0.0425	0.0328	0.0899	0.1644	0.6836*	0.1877*	0.3409*	
MP	0.0308	0.5514	0.6384*	0.1717*	0.0328	0.0418	0.0980	0.0906	0.0378	0.0073	0.0832	-0.0083	-0.2935	0.0201	0.2678*	0.0790

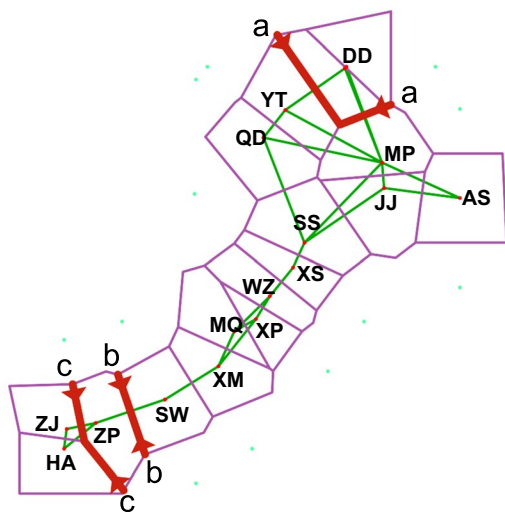
For other abbreviations of population names, see Table 1

DDs Dandong samples with a double-sword tail, DDa Dandong samples with a bottom-sword tail

* Indicates significant *P* values

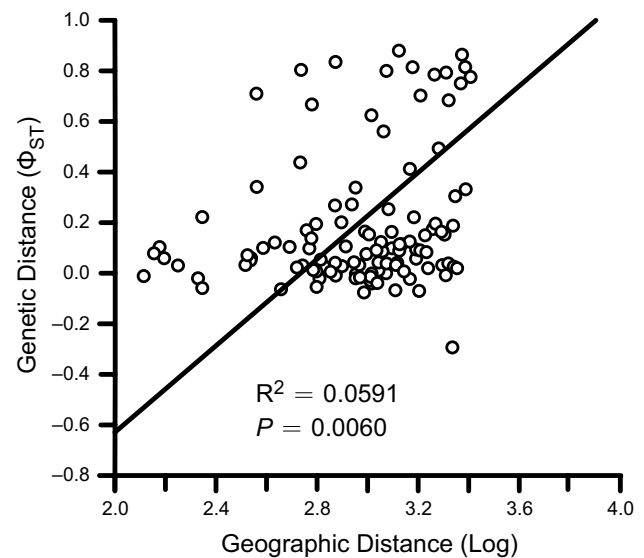
Table 4 Analysis of molecular variance (AMOVA) of D-loop sequences among the elongate ilisha samples

Source of variation	Degrees of freedom	Variance components	Percentage of variation	<i>P</i> value
I (including all samples)				
Among groups	6	2.13	27.69	0.07820
Among populations within groups	9	0.51	6.59	0.02737*
Within populations	109	5.06	65.72	0.00000*
II (excluding the DD samples)				
Among groups	5	−0.026	−0.46	0.49951
Among populations within groups	9	0.48	8.43	0.01857*
Within populations	100	5.24	92.03	0.01075*

* Significant $P < 0.05$ **Fig. 4** Voronoi tessellation graph computed with Barrier 2.2 [33]. Samples are depicted as dots labeled with location codes showing sampling localities: DD Dandong, YT Yantai, QD Qingdao, SS Shengsi, XS Xiangshan, WZ Wenzhou, XP Xiapu, MQ Minging, XM Xiamen, SW Shanwei, ZP Zhapo, ZJ Zhanjiang, HA Haian, MP Mokpo, JJ Jejudo, AS Ariake Sound. The outer lines are Voronoi tessellation, and lines connecting sampling sites are the Delaunay triangulation. The thick arrowed lines indicate the most possible barriers

diversity of elongata ilisha was 0.006 (0.001–0.010), with the lowest diversity in the AS population of Japan and the highest in the ZP population of the South China Sea. The nucleotide diversity found in other clupeiforms was also low, such as 0.0012–0.0025 (ATPase 6/8) in the gold-spotted grenadier anchovy [42], and 0.0014–0.0090 (cyt b) in Japanese anchovy [44], except that Pacific herring had a high nucleotide diversity (0.020 in D-loop) [40].

Grant and Bowen [45] classified the genetic pattern of marine fishes into four categories based on their haplotype

**Fig. 5** Correlation between geographic distance (Log) and genetic distance (Φ_{ST}) among different populations of the elongate ilisha sampled along the northwestern Pacific Coast (samples from Dandong were excluded from analysis due to their abnormal genetic distance from the other populations)

and nucleotide diversities of mtDNA. The genetic patterns of most elongate ilisha populations (Table 2) and most of the other clupeiforms reported previously fell into Grant and Bowen's category two, that is high haplotype diversity (h) and low nucleotide diversity (π), which was attributed to expansion after a period of low effective population size [45]. The population of AS had a different pattern, low values in both h (0.53) and π (0.001; Table 2), which may represent a recent recolonization event [45].

The population expansion was also evident in mismatch distribution tests and demographic parameters estimated (Table 5; Fig. 6). Tjima's D and Fu's F_S tests were all negative, suggesting population expansion (Table 5); however, other factors, such as gene flow, directional selection and balancing selection, could also have caused the observed

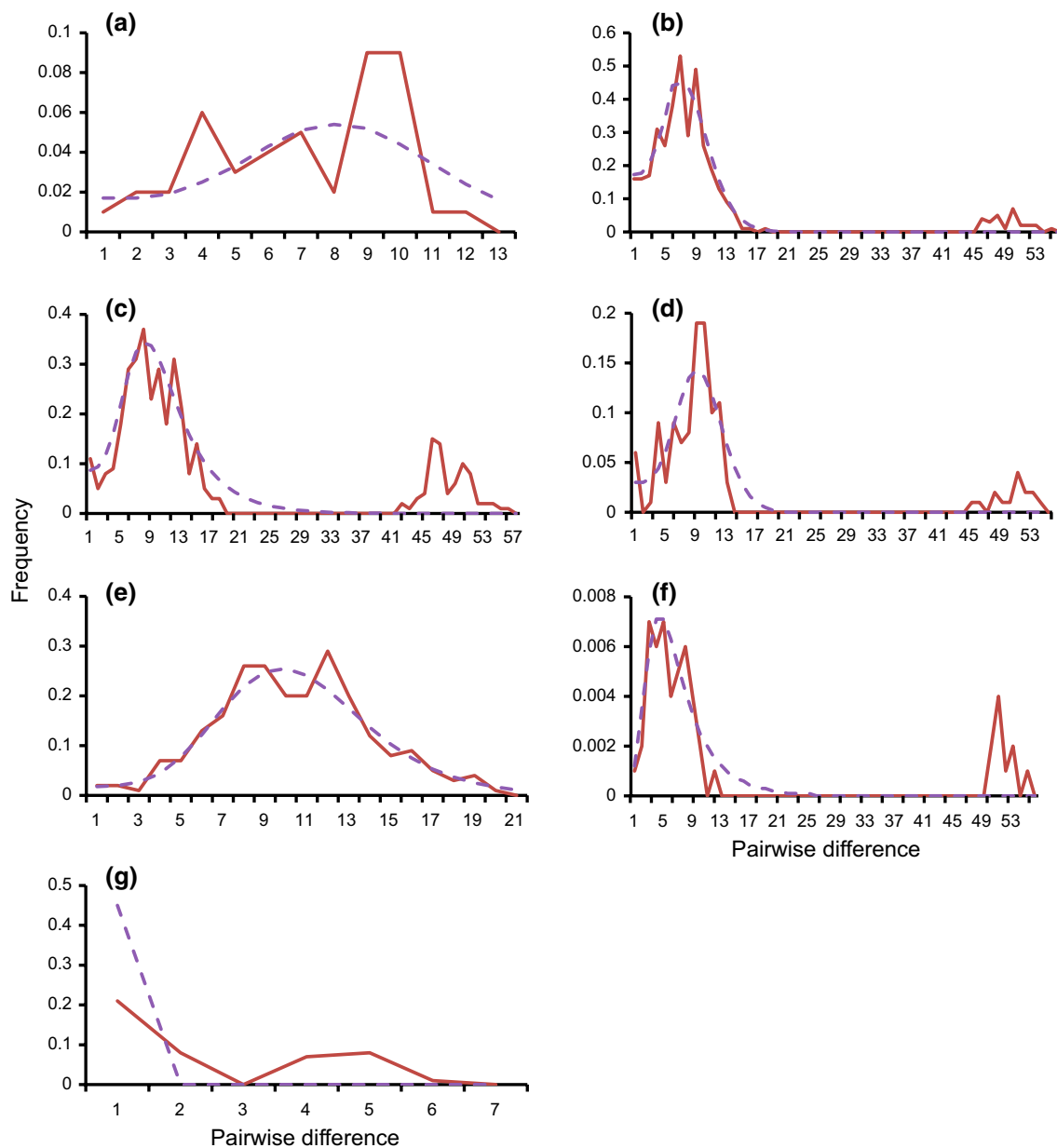


Fig. 6 Mismatch distribution of populations of elongate ilisha from different sea regions: **a** Dandong, **b** Yellow Sea, **c** East China Sea, **d** Taiwan Strait, **e** South China Sea, **f** Korea, **g** Ariake Sound, Japan. The *dashed lines* are simulated distribution, while the *solid lines* are observed data

pattern. Nonetheless, the model of sudden population expansion also was not rejected for elongate ilisha of all regions except for the AS population in Japan in the mismatch test. The mismatch test of the AS population corroborated the pattern revealed in its h and π ; that is, the AS population probably was colonized by other populations. The observed pairwise differences in other populations generally confirmed simulated results from the null expansion models. However, we noticed that there was an extra small peak of high pairwise nucleotide difference in the fish of the Yellow Sea, the East China Sea, the Taiwan

Strait and Korea, suggesting that there may exist additional minor ancestral lineages in those regions (Fig. 6). Interestingly, this small peak was not observed in the DD population and the South China Sea.

Population structure and spatial patterns

The haplotype network contained a distinct clade of fish from the DD population, which was separated from the other haplotypes by 24 substitutions (Fig. 2). The average distance between the DD clade and the other samples was

2.7 %. McCune and Lovejoy [46] reported that interspecific divergences of Cytb sequences in allopatric fish species ranged from 2.0 to 6.3 %. Because the D-loop often evolves faster than Cytb and our observed divergence between the DD samples and other samples was in the low range of interspecific divergence observed by McCune and Lovejoy, we hesitate to designate the DD samples as a new species. Nonetheless, the DD samples formed a monophyletic clade in both the haplotype network (Fig. 2) and the phylogenetic tree (Fig. 3). DD sampling site belongs to the West Korea Bay, a north part of the Yellow Sea. Samples collected from two other sites of the Yellow Sea, YT and QD, were more closely related to samples of the East China Sea and South China Sea than to the DD samples (Figs. 2, 3). Wu et al. [22] found that the genetic distance of D-loop sequence between samples of elongate ilisha collected in Qingdao (the Yellow Sea) and samples collected in Zhoushan (the East China Sea), Xiamen (Taiwan Strait), and Guangzhou (the South China Sea) was 3.4 %, which is comparable to the genetic distance (2.7 %) between the DD samples and other samples found in this study. Therefore, we suspect that the sample they collected from the Yellow Sea should be associated with our DD samples. It is unfortunate that their sequence data is not accessible now. A biogeography study of Eel Gobies (Gobioidei: *Odontamblyopus*) also sampled the West Korea Bay, and other sites in the Yellow Sea, Bohai, and East China Sea [47], but they did not find genetic distinctness of the sample collected from the West Korea Bay. More samples from the coast of the Yellow Sea in China and North Korea should be collected to further investigate the range and dynamics of the DD clade as compared to other elongate ilisha populations in the future.

The pairwise Φ_{ST} was highest between the DD population and other populations, due to the high sequence divergence between them (Table 3). The next high Φ_{ST} value was between the AS population and other populations, which probably was caused by the allele frequency change in the AS population as a consequence of bottleneck events. There were a few more significant Φ_{ST} values scattered in Table 3, which were mostly between populations from the South China Sea and the Yellow Sea and JJ of Korea, suggesting that the populations of the South China Sea are more divergent from the others. The distinctiveness of South China Sea populations also was revealed in the haplotype network and the phylogenetic tree. There was a more “derived” north clade that was composed of the samples from the Yellow Sea, the East China Sea, the Taiwan Strait, Japan and Korea, but only one sample from the South China Sea (Figs. 2, 3), suggesting that the South China Sea might hold ancestral populations of the elongate ilisha.

The AMOVA showed that the most variation was within populations (65.72 %), and the among-group variation

comprised about 28 % of the total variation (Table 4), which, however, became -0.46 % if we excluded the DD samples from the analysis (Table 4). Thus, we can infer that samples from different sea regions are not different from each other when excluding samples of DD. A variance of 6.58–8.43 % was assigned among populations within groups, suggesting that there were noticeable substructures within sea regions. The barrier analysis also detected a distinct separation between the DD population and other sites and further marginal barriers among sites within the South China Sea (Fig. 4). A low, but significant, correlation between the genetic distance and geographic distance confirmed that there are subtle population structures within sea regions (Fig. 5).

Zhang and Takita [13] suggested that the adults of elongate ilisha of Ariake Sound might share the same growth ground with the southern China population, which was confirmed partially by our results. We found that the haplotypes closely related to the AS haplotypes were from the East China Sea (Figs. 2, 3b), and the AS population had the smallest Φ_{ST} value as compared to the MQ population (East China Sea), but AS also shared haplotypes with the JJ population of Korea, suggesting that there might have been multiple colonization events occurring within the AS population.

Kim et al. [14] found differences in the marginal index of scales between the elongate ilisha collected off the coast of the Korean peninsula and the samples from Jeju, suggesting that they might belong to different populations [6, 15]. We did not find differences in D-loop sequences between the JJ and MP (coast of the Korean peninsula) samples, because their haplotypes were mixed in the reconstructed phylogeny (Fig. 3) and their Φ_{ST} value was small (0.0790; $P > 0.05$). Therefore, the growth difference between the JJ and MP populations may be due to differences in environmental condition such as prey abundance.

The morphotype with bottom-sword caudal fin

We found two morphotypes in the elongate ilisha, one with a double-sword caudal fin, and the other with a longer lower lobe of the caudal fin. The bottom-sword morphotype was found in the DD and YT populations. The average ratio of the upper lobe to lower lobe of the caudal fin ranged from 0.763 to 0.808 in different populations, but all significantly deviated from 1. The morphotypes were not grouped together in the phylogenetic tree (Fig. 3), such that the bottom-sword morphotypes of the DD population were clustered with the double-sword samples of DD instead of being grouped with bottom-sword samples from YT. Pairwise Φ_{ST} values also suggested that the bottom-sword samples of DD were not different from the double-sword samples of DD (Table 3). In bony fishes, the asymmetric

Table 5 Demographic parameters of the elongate ilisha and results of neutrality tests in different sea regions

	DD	YS	ECS	TWS	SCS	AS	KR
Demographic parameters							
<i>SSD</i> (<i>P</i>)	0.034 (0.290)	0.005 (0.740)	0.009 (0.330)	0.0168 (0.300)	0.003 (0.730)	0.372 (0.000)	0.016 (0.540)
<i>H_{ri}</i> (<i>P</i>)	0.075 (0.410)	0.016 (0.620)	0.011 (0.600)	0.033 (0.380)	0.009 (0.830)	0.164 (1.000)	0.025 (0.800)
θ_0	0.000	0.989	4.078	0.000	1.984	0.000	3.445
θ_1	25.703	21.155	43.047	39.453	129.688	99.999	421.875
τ	7.863	6.398	5.932	8.775	7.953	0.000	2.473
Tjima's <i>D</i>	-0.438 (0.327)	-1.264 (0.082)	-1.889* (0.017)	-1.245 (0.094)	-1.332 (0.083)	-0.682 (0.284)	-1.269 (0.092)
Fu's <i>F_s</i>	-2.961 (0.047)	-4.240 (0.074)	-5.338 (0.042)	-1.813 (0.194)	-9.543* (0.002)	-0.077 (0.447)	-1.536 (0.155)

DD Dandong, YS the Yellow Sea, ECS the East China Sea, TWS the Taiwan Strait, SCS the South China Sea, AS the Ariake Sound, Japan, KR Jeju and Mokpo, Korea

caudal fin type is mostly found in ancient and primitive fishes, such as sturgeon that retain a larger upper lobe of the caudal fin. In addition, some flying fish possess a larger lower lobe of the caudal fin, which provides thrust for them to become airborne and avoid predators [48, 49]. The bottom-sword morphotype of the elongate ilisha has not been reported previously (Wu, personal comm.); therefore, broader surveys as well as studies related to its function and development are needed. Furthermore, we tested the genetic difference between the double-sword and bottom-sword forms only with mtDNA, so more nuclear loci should be included in future studies.

Conclusion and implications in fisheries management

We identified a distinct population of elongate ilisha in the West Korea Bay, a northern region of the Yellow Sea, based on both haplotype difference and population divergence. We only had samples from one site of this region, so further sampling and investigation should be carried out to understand the current distribution and dynamics of this population, which is vital for making policy related to management and conservation of this population in the future. Except for the Ariake Sound population, all other populations showed evidence of recent expansion from smaller ancestral population size, and no genetic signal of overfishing was detected. However, the population expansion inferred from the genetic variation in D-loop sequences might be a reflection of historical post-Pleistocene events, but not the more recent population fluctuations; thus, fast-evolving markers such as microsatellite loci need to be added in the future to fully understand current population dynamics. In addition, the absence of elongate ilisha from the Bohai Sea warned us that action must be taken to closely monitor the habitat changes of the elongate ilisha.

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