

# Exploring ecological specialization in pipefish using genomic, morphometric and ecological evidence

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## Abstract

**Aim:** Theory predicts that ecological specialization should be rare in marine ecosystems, given that dispersal barriers are less effective in the vastness of the sea compared with those in terrestrial settings. This paradigm, however, hardly fits with classical theories of local adaptation, raising the question of how marine diversity originates in a highly interconnected system. In the present study, we investigated how ecological specialization arises in a widely distributed marine species, the seaweed pipefish *Syngnathus schlegeli*.

**Location:** Chinese coastal waters.

**Methods:** We integrated morphological (geometric morphometry), genetic (RADseq) and ecological (species distribution models and *n*-dimensional hypervolumes) evidence to shed light on population structuring in *S. schlegeli*.

**Results:** We revealed the existence of a north-to-south phenotypic gradient in eye size among *S. schlegeli* populations. This morphological differentiation was paralleled by genetic divergence, with South China Sea populations emerging as relatively independent. We further investigated the ecological differentiation of *S. schlegeli* populations. We observed high niche differentiation among northern, central and southern populations, resulting from both niche expansion and niche shift processes. Projected habitat suitability onto the Last Glacial Maximum revealed the existence of historical barriers to dispersal between the South and East China seas.

Xin Wang and Zhixin Zhang are contributed equally to this work

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**Main conclusions:** We showed that the effect of this historical segregation, in concert with niche-driven ecological differentiation, might lead to the establishment of three distinct clades across the widely distributed marine pipefish. Ultimately, our study demonstrates that even the high connected sea environment maintains the potential for ecological specialization.

**KEYWORDS**

genetic divergence, Last Glacial Maximum, morphological variation, RADseq, species distribution model, *Syngnathus schlegeli*

## 1 | INTRODUCTION

Ecological specialization, the spatial and temporal differentiation of species in the use of habitat and resources (Devictor et al., 2010), is a pivotal eco-evolutionary paradigm to explain phylogeographical patterns, community assembly rules and the majority of adaptive radiations (Gillespie et al., 2020; Mammola et al., 2020; Todd Streelman & Danley, 2003; Wilson et al., 2020). Although marine fishes often show complex adaptations in spatially heterogeneous environments (e.g. Wainwright et al., 2004, 2015), ecological specialization is thought to be rare because of the weak geographic barriers in the vastness of seas (Wilson, Wegmann, Ahnesjö, & Goncalves, 2020). This may be especially true for marine species with planktonic larvae as they can maintain large population sizes with high gene flow (Palumbi, 1994). Therefore, marine organisms challenge the classical theories of local adaptation and speciation, dubbed as the “Marine Speciation Paradox” (Bierne, Bonhomme, & David, 2003).

Molecular phylogenetic reconstructions of some marine fishes suggest that ecological specialization has a great impact on their diversification (Rocha et al., 2005; Wainwright et al., 2004). This is especially true for geographically widespread species that may experience considerably different ecological conditions (Fox & Morrow, 1981; Loxdale et al., 2011; Shipley et al., 2009). The importance of natural selection in shaping adaptive trait differentiation among natural populations has long been recognized (Garant et al., 2005; Smith et al., 1997). Theory predicts that once populations are exposed to diverse ecological environments, rapid evolution of adaptive traits should occur (Hendry et al., 2000), and phylogeographical structuring along ecological gradients has been documented in many marine species (Liu et al., 2007; Ni et al., 2012; Wilson et al., 2020; Xu et al., 2009).

Divergent selection is an important element of natural selection and occurs when different environments favour different phenotypes, leading to increased differences between populations (Bolnick & Stutz, 2017). Theoretically, divergent selection should promote the evolution of traits in local populations, which provide an advantage under local environmental conditions (Kawecki & Ebert, 2004), and selection pressure may lead to genetic divergence and eventual speciation if the homogenizing effects of the gene flow are insufficient

to prevent it. The ecological processes, along with local adaptation, are most important responses of species to the changing environment, which both contribute to the specialization. Several recent studies on marine species have demonstrated population differentiation consistent with local adaptation and trophic partitioning in spatially heterogeneous environments (Rocha et al., 2005; Sanford & Kelly, 2011; Sanford & Worth, 2010; Wilson et al., 2020). In this regard, uncovering the differentiation among marine populations is of considerable importance for advancing our knowledge on the molecular mechanisms of adaptive evolution against a background of high-level gene flow.

The seaweed pipefish *Syngnathus schlegeli* (Kaup, 1856) is a well-known species showing exclusive paternal care of eggs (Watanabe & Watanabe, 2001). As common traits that shared by all syngnathids (Zhang et al., 2020), the offspring develop within the male brood pouch and are released as free-living juveniles. *Syngnathus schlegeli* is distributed along the coastlines of the north-western Pacific Ocean and generally inhabits sheltered areas in shallow waters; the geographic range of this species encompasses a wide variety of climatic conditions and nearshore microhabitats (Chen et al., 2017; Zhang, Capinha, et al., 2020). The vast region of the north-western Pacific is characterized by distinct tectonic and geographical features, with a series of marginal seas separating the eastern Asian continent from the Pacific Ocean (Tamaki & Honza, 1991). During Pleistocene glacial cycles, sea level fluctuations resulted in successive exposure and inundation of continental shelves, closure and opening of seaways, and separation and reunion of marginal seas (Wang, 1999). When the sea level fell, the seas were separated by land bridges extending between islands and the mainland, hindering the spread of most marine species (Ni et al., 2014; Wang, 1999). The historical influence of glaciation, which has been proposed as a dominant factor shaping present-day phylogeographical patterns (Hewitt, 2004), distinguishes this area from other marine realms to an extent that its biota would be distinctly different from those revealed in other regions (Xu et al., 2009).

In this study, we investigated the cranial morphology, genetic structure and ecological niche of *S. schlegeli* along Chinese seashores to reveal their phylogeographical patterns. First, we used geometric morphometrics to partition the variations in the cranial morphology among populations. Second, we employed the restriction

site-associated DNA sequencing (RADseq) technique to evaluate the level of genetic differentiation among seaweed pipefish populations based on the genome-wide genotypic and sequencing data. Finally, we compared the ecological niches of distinct *S. schlegeli* populations using *n*-dimensional hypervolumes and mapped habitat suitability of this species during present day and the Last Glacial Maximum (LGM) using species distribution model (SDM). By revisiting the phylogeographical history of the widespread pipefish using an integrative approach, our over-arching goal was to investigate the dynamics through which local specialization could arise in the apparent absence of dispersal barriers.

## 2 | METHODS

### 2.1 | Sample collection and DNA extraction

We sampled a total of 280 adult seaweed pipefish from nine sites along the coastal waters of China ranging from the Yellow Sea (YS) to the South China Sea (SCS) (Table 1, Appendix S1). We collected all samples using bottom trawling between 2015 and 2019 (from March to June, and October to December), covering a depth of 10–30 m. All individuals were verified morphologically and sequenced for mtDNA (cytochrome b) to confirm species identification. We derived morphological data from 163 seaweed pipefish specimens: 63 from the YS, 34 from the East China Sea (ECS) and 66 from the SCS. Bodies of the remaining 117 specimens were damaged, preventing quantification of reliable morphological measures. The total length of sampled specimens of *S. schlegeli* ranged 18.1–21.6 cm, and no significant differences in size existed among sampled localities (ANOVA, Duncan's multiple range tests,  $p > .05$ ). We extracted genomic DNA from muscle samples and then treated with RNase A. We checked the quality

and integrity of the DNA samples using agarose gel electrophoresis and DNA concentrations using a Qubit 2.0 fluorometer.

To get a preliminary understanding of genetic divergence of *S. schlegeli* populations, partial mitochondrial cytochrome b (*cytb*) and nuclear Sorting nexin 33 (*snx33*) from 270 pipefish individuals were amplified, and we obtained 248 and 265 valid *sh3* and *cytb* sequences after data correction (amplification products with double peaks were removed) (Table 1). Furthermore, genome-wide genetic differentiation and signatures of local adaptation were investigated using RAD sequencing of 69 *S. schlegeli* individuals (31 from the YS, 22 from the ECS and 16 from the SCS) (Table 1, Appendix S1). All experimental procedures performed in this study were in accordance with the relevant animal ethics guidelines and approved by the Ethical Committee of South China Sea Institute of Oceanology, Chinese Academy of Sciences.

### 2.2 | Comparative morphometric analysis of populations

To test for the presence of a north–south morphological differentiation among *S. schlegeli* populations, we examined the cranial morphology of 163 individuals (including the 69 individuals involved in the RADseq) using landmark-based geometric morphometry analysis. We acquired digital images of the right lateral side of each specimen using a Canon camera (EOS Rebel T4i; 18 mega pixels). We performed all morphometric analyses using thin-plate spline (tps) (available at <http://life.bio.sunysb.edu/morph/>). We sorted digital images according to the geographical population and converted to tps files using tpsUtil. For the head images, we digitized 26 landmarks using tpsDig2 (19 homologous landmarks and 7 semi-landmarks), which were modified from the geometric morphometric

**TABLE 1** Sampling information of *Syngnathus schlegeli*

Locations	Geographic Coordinates	Sample Number	Sample Number			
			Morphology	Mitochondrial gene	Nuclear gene	RADseq
YS						
QD1	E 121.977, N 36.611	15	15	14	14	15
QD2	E 121.839, N 36.573	25	18	25	25	8
HZW	E 119.386, N 34.927	48	30	43	47	8
ECS						
ZS1	E 122.476, N 30.848	15	11	15	14	6
ZS2	E 122.477, N 30.093	12	9	11	11	5
ZS3	E 122.175, N 29.544	25	14	23	19	11
SCS						
ZH	E 113.729, N 21.874	40	28	38	39	8
HK1	E 110.645, N 20.203	55	20	52	43	5
HK2	E 110.241, N 20.114	45	18	44	36	3
Total number		280	163	265	248	69

Abbreviation: Yellow Sea (YS), East China Sea (ECS), South China Sea (SCS), Qingdao (QD), Haizhouwan (HZW), Zhoushan, Zhuhai (ZH), Haikou

analysis conducted by Wilson (Wilson et al., 2020) and Leysen (Leysen et al., 2011)(Appendix S2), and the tps files with landmarks were then processed in tpsRelw to enable relative warp analysis and obtain X and Y coordinates for further analysis. ANOVA was performed on the relative warp scores of all *S. schlegeli* populations.

### 2.3 | Sequence quality and RADtag filtering

Selected samples were individually barcoded for genomic library construction utilizing the restriction digestion enzyme *EcoRI* (G/AATTC). We ligated individually barcoded Solexa P1 adapters onto the *EcoRI* cut site for each sample. After manually size-selecting 300–500 bp fragments by gel electrophoresis, we blunt-end-repaired libraries and added a 3' adenine overhang to each fragment. Then, we added a Solexa P2 adapter containing unique Illumina barcodes. Finally, we sequenced paired-end libraries with an insert size of 200–400 bp on the Illumina HiSeq PE150 platform. We filtered raw sequencing data through the removal of low-quality reads, reads with adapter contamination, reads with  $\geq 10\%$  unidentified nucleotides (N) and reads for which  $\geq 50\%$  of the length had a phred quality  $\leq 5$ .

### 2.4 | Single nucleotide polymorphism (SNP) calling

Genome-wide single nucleotide polymorphisms were detected for subsequent population genetic and selective sweep analyses. We mapped all quality-filtered reads to the *Syngnathus scovelli* reference genome using BWA-MEM with the parameters `aln -e 10 - t 4 - l 32 - i 15 - q 10` (Li & Durbin, 2009). We imported the alignment files to SAMtools for sorting and removing duplicated reads (Li, 2011). Sequencing coverage and depth for each sample were calculated. We detected SNPs using SAMtools and then filtered them to meet the following criteria: (1) quality score  $\geq 20$ ; (2) coverage depth  $\geq 5$  and  $\leq 1,000$ ; (3) SNPs with minor allele frequency (MAF)  $> 0.01$ ; (4) a missing ratio of samples  $< 50\%$  within each group (Zhang, Ravi, et al., 2020).

### 2.5 | Population genetics analyses based on the RADseq data

The genetic structure among *S. schlegeli* populations was conducted by genome-wide population genetics analyses. A neighbour-joining tree was conducted based on the pairwise genetic distance matrix data (P-distance among all 69 individuals) using TreeBest v1.9.2 (Vilella et al., 2009). We set the number of bootstrap replicates to 1,000 to assess the statistical support for nodes in the tree.

We performed principal components analysis (PCA) based on SNP markers using GCTA tools (Yang et al., 2011). We transformed the population genotypes into a matrix that included the numbers 0, 1 and 2, where 0 is a genotype that is homozygous for the reference allele; 1 is a genotype heterozygous for the reference allele; and 2 is

a genotype homozygous for the non-reference allele. We calculated the sample covariance of the matrix that contained the information for all individuals (with the numbers 0, 1 and 2). Finally, we calculated the eigenvector decomposition of the matrix and plotted the PCA using GCTA tools.

We used PLINK to generate the map files necessary for downstream analyses (Purcell et al., 2007). We determined population structure using the FRAPPE software (Tang et al., 2005). We increased the pre-defined parameter K from two to five to cover the maximum numbers of lineages that could be identified in the tree, representing the assumed groups of a simulated population in ancient times. Finally, we inferred population genetic structure and individual ancestry proportions using FRAPPE.

### 2.6 | Selective sweep analysis

Population-differentiation statistic ( $F_{ST}$ ) based on the mitochondrial *cytb* and nuclear *snx33* was estimated with Arlequin software version 3.5.2.2 (Excoffier et al., 2007). To characterize genome-wide patterns of genetic variation and population differentiation, we calculated nucleotide diversity ( $\pi$ ) and population-differentiation statistic using a sliding window approach (a 40-kb window sliding in 20-kb steps).  $\pi$  and  $F_{ST}$  were used to estimate the genomic diversity of populations and evaluate the strength of genomic differentiation between two populations, respectively.

We measured selection signatures of population-specific genomic regions between *S. schlegeli* populations (YS-ECS and SCS). We applied a sliding window approach (a 40-kb window sliding with a step size of 20 kb) to identify selected regions. We identified genomic regions with a significantly high  $F_{ST}$  (top 5%) and  $\theta\pi$  ratio ( $\log_2(\pi_1) - \log_2(\pi_2)$ , top 5%) values as selective sweep regions. We submitted genes to the Gene Ontology databases for annotation (Ashburner et al., 2000; Ogata et al., 1999). Gene ontology analyses were performed to annotate genes to functional ontology terms. Using these candidate genes, we performed a targeted population genetic analysis. We used a higher resolution sliding window analysis (10-kb window compared with a 100-kb window for the selection analysis) to calculate  $F_{ST}$  and  $\theta\pi$  between populations.

### 2.7 | Ecological niche analyses

We quantified the realized niches of different *S. schlegeli* populations in China via  $n$ -dimensional hypervolumes and predicted habitat suitability of this species during present-day and historical time periods using SDM. We collected 149 presence records of *S. schlegeli* from the Global Biodiversity Information Facility (GBIF, 2019), field investigations and published literature (Appendix S3). To avoid clustered records, we used only one presence record per 5 arcminutes grid cell (approximately  $10 \times 10$  km) to match the spatial resolution of marine environmental predictors. As a result, we retained 131 records for subsequent analyses (Appendix S4). We assigned presence records

**TABLE 2** Summary of relative warp analysis and ANOVA results of relative warps

RW	ANOVA			SV	PVE(%)	Description of the variation
	df	F	P			
RW1	2	0.811	0.446	0.36157	41.81	length of snout and gill cover
RW2	2	0.238	0.789	0.22652	16.41	angle of the snout
RW3	2	56.937	<0.001	0.15502	7.69	eye size

Abbreviation: RW, Relative warp; SV, singular values; PVE, per cent variance explained

of *S. schlegeli* along the coastal waters of mainland China to three distinct clades (YS, ECS and SCS) based on the genetic clustering results and previous phylogeographic evidence from related taxa in the same region (Dong et al., 2012; Liu, Gao, Wu, & Zhang, 2007; Ni et al., 2012; Xu et al., 2009).

We retrieved marine environmental predictors at a spatial resolution of 5 arcminutes from the MARSPEC database (<http://marspec.weebly.com>) (Sbrocco & Barber, 2013). We initially considered bathymetry (i.e. depth of the seafloor) (Appendix S1; Chen et al., 2017; Zhang, Capinha, et al., 2020) and 10 marine environmental predictors, including annual mean, range, variance and extreme values of sea surface temperature and sea surface salinity (Appendix S5). We calculated the pairwise Pearson's correlation coefficients ( $r$ ) among predictors and selected only one among highly correlated predictors (i.e.  $|r| > 0.7$ ) (Dormann et al., 2013). Accordingly, we retained five predictors for model development: bathymetry, mean annual sea surface salinity, annual range in sea surface salinity, mean annual sea surface temperature and annual range in sea surface temperature (Appendix S5).

We used  $n$ -dimensional hypervolumes (Blonder et al., 2014) (also known as Hutchinsonian niche; Hutchinson, 1957) to quantify realized niches and explore niche differentiation among the three clades of *S. schlegeli* along Chinese coastal waters. We delineated hypervolumes using the five selected marine predictors and a Gaussian kernel density estimator (Blonder et al., 2018). Prior to hypervolume construction, we normalized all predictors via z-score transformation, as recommended by Blonder et al., (2014). We measured niche overlap using both a similarity and a distance metric (Mammola, 2019), namely the hypervolumes' distance between centroids and pairwise overall differentiation ( $\beta$  diversity) (Carvalho & Cardoso, 2020; Mammola & Cardoso, 2020). The latter approach decomposes overall niche differentiation ( $\beta_{\text{total}}$ ) in two process: niche shifts ( $\beta_{\text{replacement}}$ ) and niche expansion/contraction ( $\beta_{\text{richness}}$ ) (Carvalho & Cardoso, 2020).  $\beta_{\text{total}}$  values range from 0 (when niches are identical) and 1 (fully dissimilar niches), holding true the equality  $\beta_{\text{total}} = \beta_{\text{replacement}} + \beta_{\text{richness}}$ .

Furthermore, we predicted habitat suitability of *S. schlegeli* during the present day and the LGM (about 21 kya) using SDM. SDMs represent a widely used tool to quantify a species' habitat suitability by exploring the relationships between distribution data and environmental predictors (Araujo et al., 2019; Thuiller et al., 2019). The MARSPEC database contains palaeo marine environmental layers (Sbrocco, 2014). For simplicity, Sbrocco (2014) assumed that sea level at the LGM was 120 m lower than today. Marine environmental

predictors at the LGM can be derived from general circulation models (GCMs). We considered palaeoclimate simulations from three GCMs (i.e. CCSM3, ECBilt-Clio and HadCM3M2) to reduce uncertainties derived from choices of GCMs (Thuiller et al., 2019).

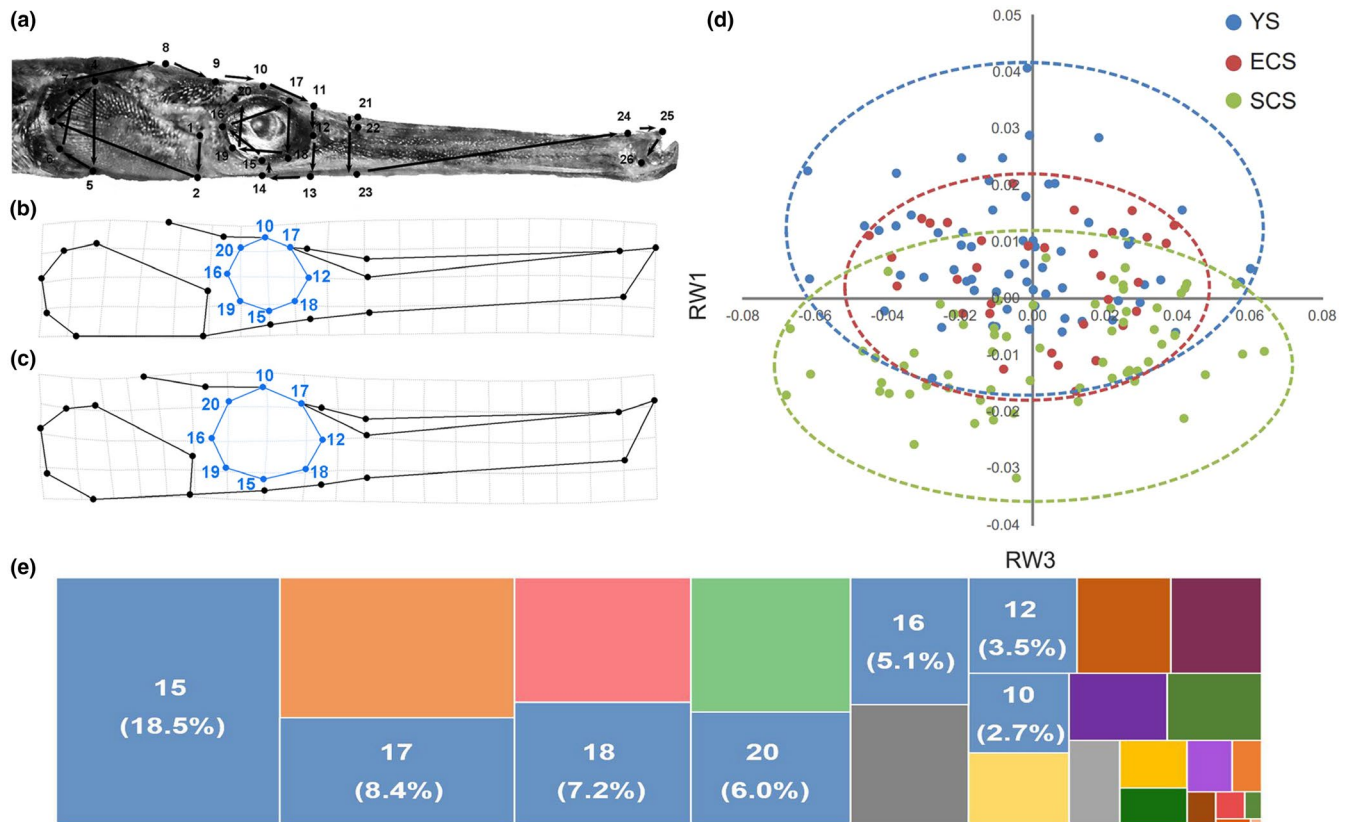
We developed SDM using all distribution data (i.e. 131 records) and the five non-collinear predictors. To reduce uncertainties associated with SDM algorithm choices, we used an ensemble modelling approach (Thuiller et al., 2019). We fitted and tested the performance of nine algorithms available in *biomod2* R package (Appendix S5), using default settings (Thuiller et al., 2020). Note that we did not consider random forest algorithm in *biomod2* R package because it cannot handle imbalanced data (Barbet-Massin et al., 2012). In addition to species presence data, we randomly generated 10,000 background points within the study area as pseudo-absences. We evaluated the predictive abilities of the nine algorithms using a five-fold cross-validation approach with ten replicates. True skill statistic (TSS) and the area under the receiver operating characteristic curve (AUC) were used to measure SDM predictive performance: algorithms with TSS >0.75 and AUC >0.9 were considered to exhibit excellent predictive capacity (Zhang et al., 2020). The selected algorithms were used to predict the habitat suitability of *S. schlegeli* under present-day and LGM climate conditions. Predictions of single algorithms were ensembled using a committee averaging strategy (Thuiller et al., 2020; Thuiller et al., 2019). The continuous habitat suitability results (ranging from 0 to 1) were transformed into binary suitable/unsuitable maps by using the 10th percentile presence probability threshold (Zhang et al., 2021).

### 3 | RESULTS

#### 3.1 | Analysis of morphological variation

All specimen collected in this study were identified with the morphological characteristics of *S. schlegeli* described by published literature (Dawson, 1985), especially on the key identification characteristics of trunk rings (18–20, usually 19), dorsal-fin rays (30–47) and pectoral-fin rays (11–15, usually 12–13).

The shape of the specimens was described using relative warp analysis (Appendix S6). The relative warp 1 showed the length of the snout and gill cover, and the relative warp 2 explained the angle of the snout (Table 2). The first three relative warps explained 65.91% of the variance in relative warps, and the ANOVA results



**FIGURE 1** Geometric morphometric analysis of different pipefish populations. (a) The 26 landmarks on pipefish used for the geometric morphometry analysis. (b) Head shape at the negative axis of relative warp 3. (c) Head shape at the positive axis of relative warp 3. (d) Relative warp scatter plot analysis based on two principal components. (e) Treemap of eye size-associated landmarks and their contribution to the relative warp analysis

showed significant differences between populations in terms of relative warp 3, which represents eye size (Table 2). Specimens plotted on the positive axis of relative warp 3 tended to have a larger eye, whereas those on the negative axis had smaller eyes (Figure 1b,c).

The morphological difference in different pipefish populations shown by the relative warp scatter plot revealed that the YS population generally had a higher relative warp 3, which indicated that the fish in this population had larger eyes. In comparison, individuals in the SCS population had lower relative warp 3 values than those in the other two populations, with the ECS population having intermediate relative warp 3 values (Figure 1d). We found that seven of the total 26 landmarks were related to morphological variation in the eye size, which accounted for 51.4% of the variation in relative warp analysis (Figure 1e).

### 3.2 | RADseq and population structure analysis

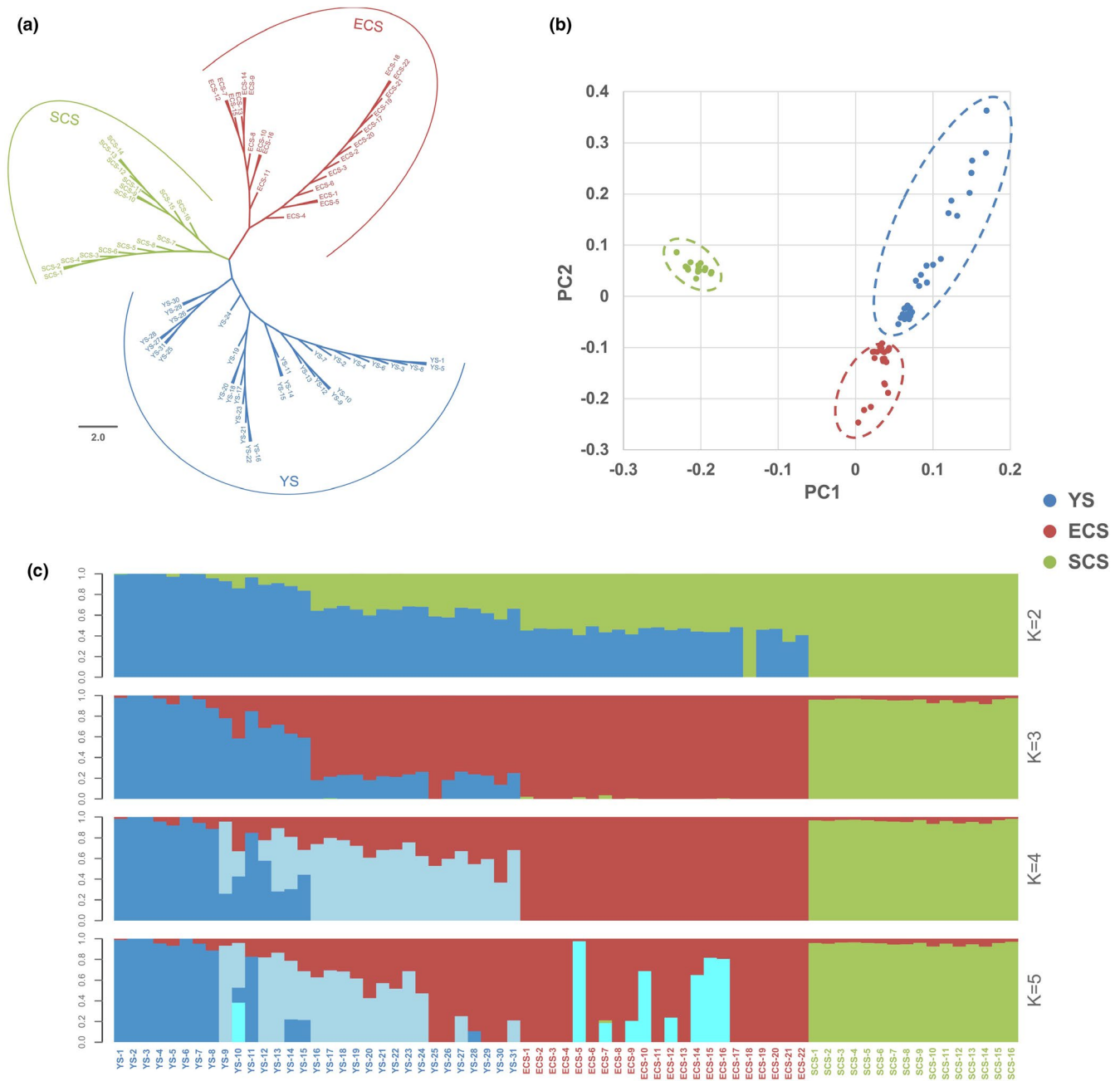
We performed RADseq for 69 *S. schlegeli* individuals, obtaining a total of 120.08 Gb raw sequencing data, 118.13 Gb of which were clean data remained after filtering (Appendix S7). The sequencing reads were aligned to the reference genome (gulf pipefish *Syngnathus scovelli*) to evaluate the mapping rate, sequencing depth and coverage. Results showed that the average mapping rate of the sequencing reads was 85.28%, the average sequencing

depth was 10-fold, and the average coverage of the data was 20.70% (at least 1x) and 10.82% (at least 4x) (Appendix S8). We identified a total of 1,607,529 SNPs from *S. schlegeli* populations and retained 93,845 high-quality SNP markers after filtering (Appendix S9).

We reconstructed phylogenetic relationships among the 69 sequenced *S. schlegeli* individuals based on the SNPs data using the neighbour-joining method. Results showed three distinct clusters that represent three geographical lineages (Figure 2a). PCA recovered the same clusters revealed by the phylogenetic analysis (Figure 2b), showing that the YS population was closely related to the ECS population, whereas the SCS population formed a separate cluster. Multi-level ( $K = 2, 3, 4$  and  $5$ ) population structure showed that SCS pipefish was a relatively stable and independent group, with a change in  $K$  value from 2 to 5, indicating that this group had a low genetic exchange with other populations. In contrast, the YS and ECS populations were well connected with appreciable gene exchange (Figure 2c).

### 3.3 | Screening for selective sweeps

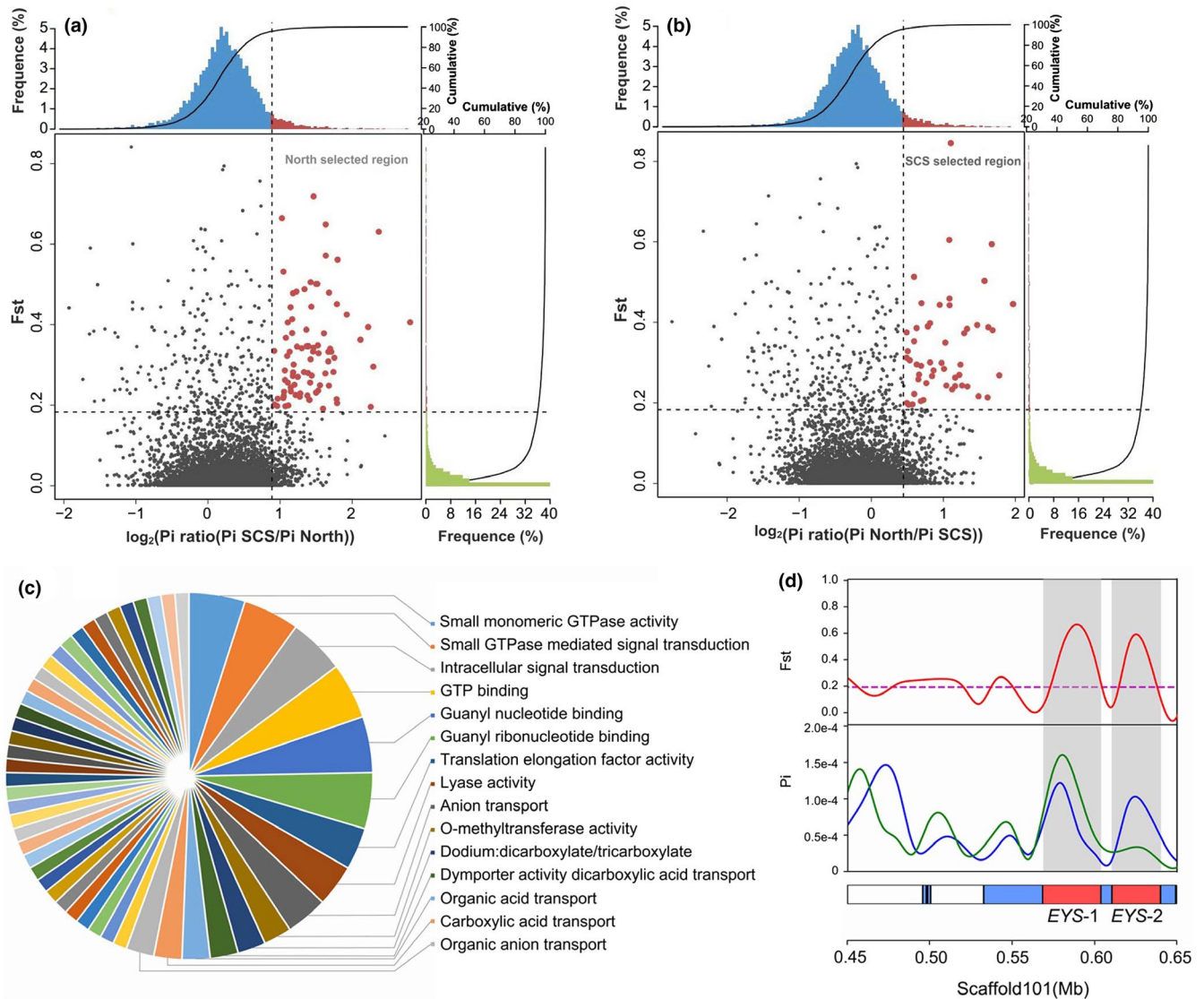
$F_{st}$  values between 0.05 and 0.25 are commonly identified as moderate differentiation at the population level (Yao et al., 2014). In this



**FIGURE 2** Genetic relationships and population structure of 69 sequenced pipefish based on SNPs. (a) Neighbour-joining tree of the 69 pipefish. The scale bar represents the proportional similarity (p distance). (b) Principal component analysis plots based on the first two principal components (PC). (c) Genetic structure and individual ancestry derived from FRAPPE. The length of each coloured segment represents the proportion of the individual's genome from  $K = 2$ –5 ancestral populations

study, the pairwise  $F_{ST}$  of *snx33* gene indicated significant genetic differentiation among different *S. schlegeli* populations (SCS versus YS,  $F_{ST} = 0.057$ ,  $p < .001$ ; SCS versus ECS,  $F_{ST} = 0.058$ ,  $p < .05$ ) (Appendix S10). Then, we calculated  $\pi$  and  $F_{ST}$  for each population using a 50-kb sliding window with a 10-kb step size based on the RADseq data. Selected genomic regions invariably show specific patterns of variation, such as high  $F_{ST}$  and lower levels of  $\pi$  (Ellegren & Sheldon, 2008; Sabeti et al., 2006; Wang et al., 2015). We calculated the distribution of  $\pi$  ratio and  $F_{ST}$  values to identify the selected regions for the north (YS-ECS) and SCS populations, and we

inferred the selected genomic regions from high  $\pi$  log-ratios and an extreme divergence of allele frequencies (Figure 3a,b). Most of the genomic regions showed a low level of differentiation (Figure 3b), and the differentiated genome regions harboured 143 annotated protein-coding genes, including cold shock domain-containing protein E1 (*csde1*), paired box protein 7 (*pax7*), eyes shut homolog gene, retinal structural protein (*rx3*) and retinal guanylate cyclase (*retgc-2*) (Appendix S11), which we assumed to be subject to selection. Gene Ontology annotations of these genes were conducted to further understand their regulatory functions (Figure 3c). The



**FIGURE 3** Selective sweep analysis of the populations from different regions. (a, b) Distribution of  $\text{Pi}$  ratios (north/SCS, SCS/north) and  $F_{ST}$  values calculated using the sliding window approach. Data points located to the right of the vertical dashed line (corresponding to the 5% right tails of the empirical  $\text{Pi}$  ratio distribution, where the  $\theta\pi$  ratios are 0.887 and 0.446, respectively) and above the horizontal dashed line (upper 5% tail of the  $F_{ST}$  distribution, where  $F_{ST}$  is 0.183) were identified as selected regions for north populations and SCS populations (red points), respectively. (c) The distribution of selected genes (north versus SCS) related to Gene Ontology terms. (d)  $\text{Pi}$  ratios and  $F_{ST}$  values plot using a 10-kb sliding window of the genes (north versus SCS) with strong selective sweep signals; *EYS* regions are marked in red

selection sweep analysis of the candidate gene *eys* revealed high  $\pi$  value difference and an extreme divergence of allele frequencies. (Figure 3d).

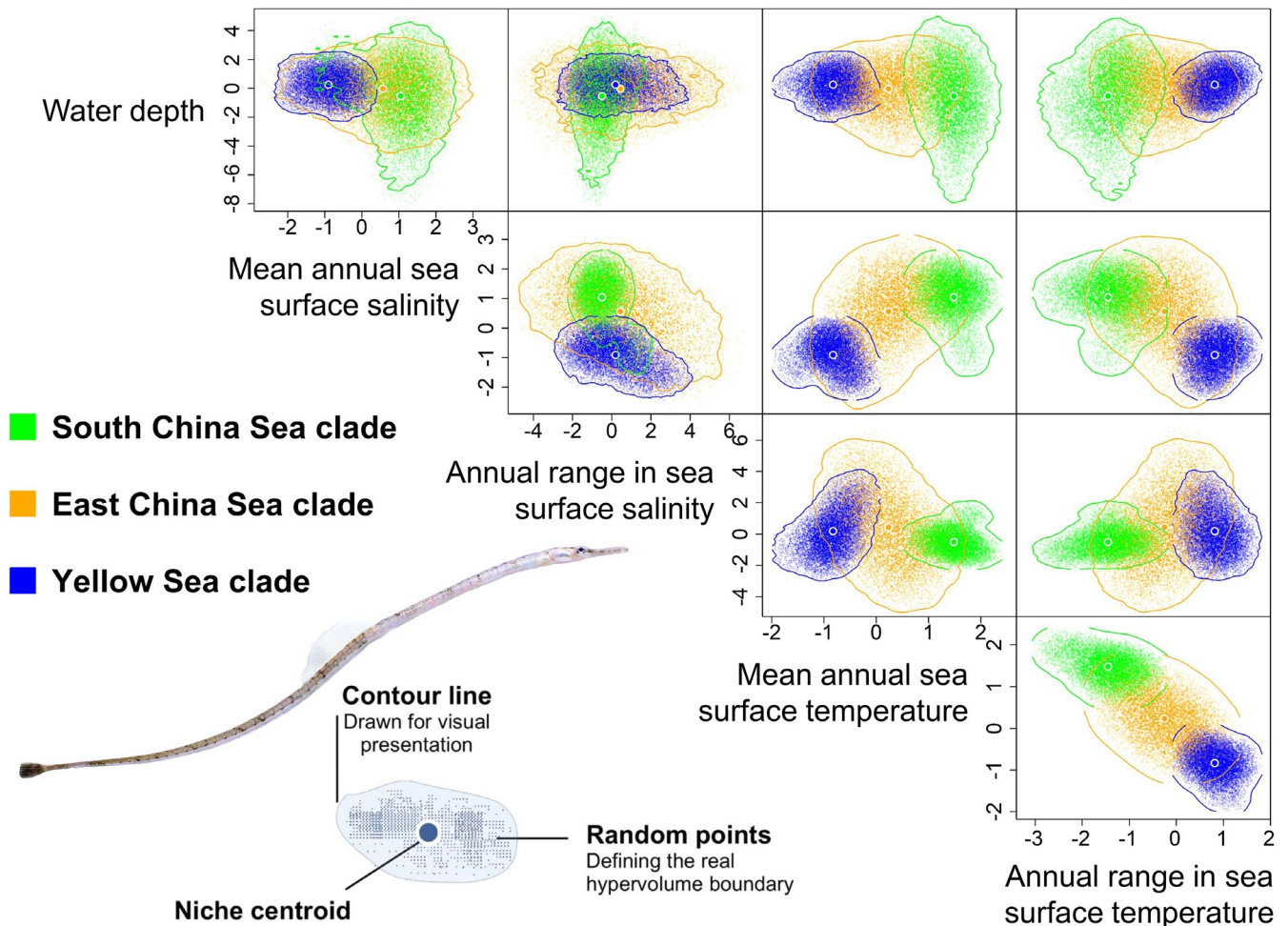
### 3.4 | Ecological niche analyses results

Ecological niche characterization with hypervolumes indicated that the three clades diverge in their realized niches across their distribution range (Figure 4). Clade YS had a relatively narrower ecological niche than clades ECS and SCS. The greater niche differentiation occurred between clade YS and SCS (Table 3), and this was due to two simultaneous niche-based operations. We observed both a niche shift, with clade SCS experiencing a wider breadth of depths

and climatic conditions, and parallel niche expansion of SCS (or a contraction of clade YS). The niche of clade ECS, although disjunct, was more similar to that of the other two clades, as emphasized by a smaller distance between centroids. The differentiation of clade ECS with respect to clades YS and SCS was only due to niche expansion/contraction processes (Table 3).

TSS and AUC scores revealed that all algorithms but surface range envelop showed excellent performance (Appendix S12); thus, eight algorithms were used to develop the ensemble models. The ensemble SDMs had high predictive capacity with the mean ( $\pm$  standard error) TSS = 0.891 ( $\pm$  0.004) and the mean AUC = 0.974 ( $\pm$  0.001). Projections of ensemble SDM suggested that a large part of Chinese, Japanese and Korean coastal areas were suitable for *S. schlegeli*, which generally matched the current known distribution





**FIGURE 4** Pairplots representing the estimated realized niches for the three genetic lineages of pipefish. For each clade, coloured points are 10,000 points stochastically sampled from each hypervolume, representing the actual shape and boundaries

**TABLE 3** Pairwise niche differentiation among hypervolumes, as estimated through a distance metric (lower panel; distance between centroids) and an overlap metrics (upper panel;  $\beta_{\text{total}} = \beta_{\text{replacement}} + \beta_{\text{richness}}$ ). The diagonal of the matrix is the volume of each hypervolume

	Clade YS	Clade ECS	Clade SCS
Clade YS	46.50 [v]	0.97 = 0.04 + 0.93 [ $\beta$ ]	1.00 = 0.60 + 0.40 [ $\beta$ ]
Clade ECS	2.13 [dc]	885.82 [v]	0.94 = 0.12 + 0.82 [ $\beta$ ]
Clade SCS	3.92 [dc]	2.10 [dc]	109.34 [v]

Abbreviation:  $\beta$  = Beta diversity; dc = distance between hypervolume centroid; v = volume

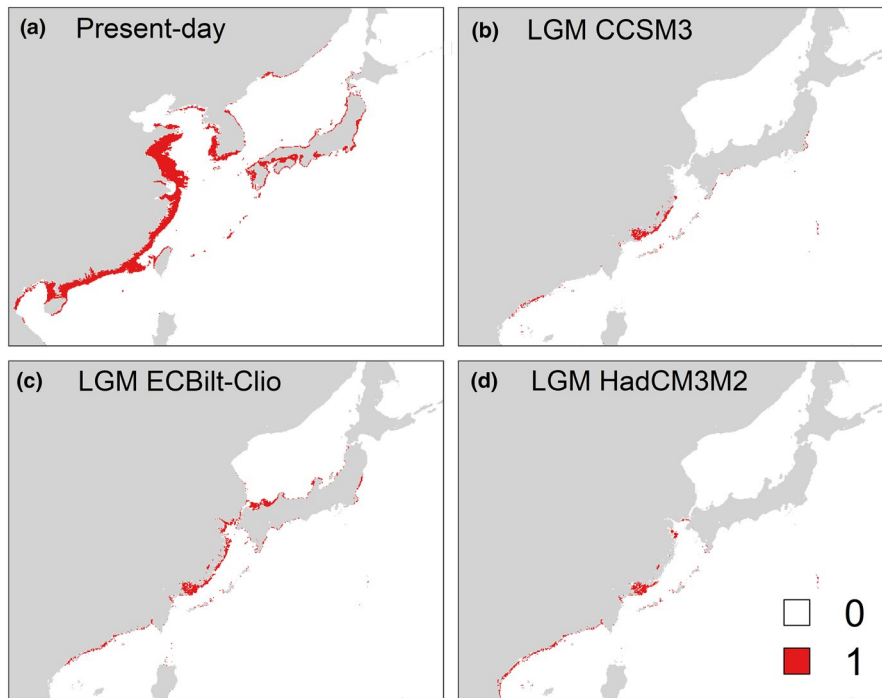
range of this species (Figure 5a, Appendix S13a). Habitat suitability projections for this species derived from all three GCMs showed a clear geographical separation between south and north populations during the LGM (Figure 5b-d, Appendix S13b-d). In addition, SDM projections suggested that the suitable habitat for *S. schlegeli* was greatly reduced during the LGM: the projected distribution area

during the LGM was 10%–20% compared with the current one, depending on the different GCMs.

## 4 | DISCUSSION

Here, we investigated the cranial morphology, genetic structure and ecological niche of *S. schlegeli* along Chinese seashores to reveal the phylogeographical patterns resulting from ecological specialization. We delimited three independent clades of *S. schlegeli* using genomic data and observed that this differentiation was mirrored in the morphological divergence of local populations. The integration of genetic, morphological and ecological data supports the hypothesis that even the barrier-free sea environment maintains the potential for ecological specialization. A similar phylogeographical pattern has been documented in the north-western Pacific using fishes, molluscs and crustaceans (Liu et al., 2007; Ni et al., 2012; Wilson et al., 2020; Xu et al., 2009).

In marine environments, worldwide glaciation is the most efficient way to generate intraspecific genetic splits (Hewitt, 2000), as



**FIGURE 5** Binary predictions of habitat suitability for pipefish *Syngnathus schlegeli* under the present-day (a) and Last Glacial Maximum (b, c, d) climatic conditions. Grey colour represents the land, white colour (0) indicates unsuitable, and red colour (1) indicates a suitable marine range. The threshold to convert continuous projections into binary results was set at 0.875. CCSM3, ECBilt-Clio and HadCM3M2 represent different general circulation models used to simulate palaeoclimates

well as shape the present-day phylogeographical pattern of marine species (Dong et al., 2012; Wilson et al., 2020). Examples of this have been observed in different biogeographic realms, from the Indo-West Pacific to the Atlantic and Mediterranean basins (Cunha et al., 2008; Ni et al., 2014). Pleistocene sea level fluctuation resulted in the isolation of marginal seas; for instance, the sea level during the LGM decreased in the marginal seas of north-western Pacific (approximately 100–120 m drop in the SCS, and 130–150 m drop in the ECS) (Wang & Sun, 1994). Therefore, along with drop in the sea level, the ECS was reduced to an elongated trough, the Okinawa Trough, while the SCS became a semi-enclosed sac-shaped gulf connected with the Pacific mainly through the Bashi Strait (Wang & Sun, 1994). These sea basins served as separate refuges for different geographic populations of local marine species. The present genetic data suggest a genetic split between the ECS-YS and SCS lineages, which has also been reported in other marine fishes (Liu et al., 2007). A few genomic regions in *S. schlegeli* showed high differentiation while the rest of the genome was very similar, as the  $F_{ST}$  analysis between north and SCS populations indicated. Such a heterogeneous genome divergence has been commonly described in situations where differentiated genomes have experienced differential introgression following secondary contact (Xu et al., 2009). Postglacial range expansions of marine species may cause secondary contact of the formerly isolated populations at suture zones between seas including ECS/SCS (Ni et al., 2014). Palaeo habitat suitability projections showed a clear geographical separation between southern and northern populations during the LGM, which provided ecological support for the observed differentiation of ECS-YS and SCS lineages of *S. schlegeli*. In light of these, we hypothesized that Taiwan Strait land bridge may have served as a barrier to the dispersal of coastal

marine species during the glacial periods, favouring inter-population genetic divergences.

Gene exchange among YS and ECS populations of *S. schlegeli* is relatively frequent, as revealed by the genetic ancestry analyses, although ECS and YS populations are separated based on the PCA and phylogenetic tree clustering. During glacial periods, the northern regions of the YS were covered with land, and the ECS populations of *S. schlegeli* may have experienced an expansion concomitant with the rise in the sea level during interglacial periods, leading to the genetic exchange between the ECS and YS populations (Chen et al., 2006; Yu & Zhou, 2001). In parallel, genetic breaks may be attributed to freshwater outflow from rivers, as demonstrated, for example, in the Amazon basin (Rocha et al., 2002). As the third-largest river worldwide, Yangtze (Changjiang) River pours into the ECS with an average annual discharge of 924 billion  $m^3$  (Ni et al., 2012). While the barrier effect of the vast freshwater outflow on the coastal species gene flow is still a controversial issue, we suggest that the freshwater outflow may have affected the gene change among marine species in more recently, following potential changes in the estuary and variable river discharge. As freshwater outflow affects mostly intertidal species with a strong habitat specificity (Dong et al., 2012), and SDM projections suggest that a large part of Chinese, Japanese and Korean coastal areas are suitable for *S. schlegeli*, we believe that the phylogeographical pattern of *S. schlegeli* can largely be explained by the historical glaciation.

It is worth noting that pipefish are unique among fishes as the males have a brood pouch and are ovoviviparous. Moreover, they are slow swimmers. These life history traits render them sensitive to environmental changes (Wilson et al., 2003; Zhang, Capinha, et al., 2020). Compared with other marine fishes, environmental heterogeneity is

more likely to affect the *S. schlegeli* population structure due to local adaptation and limited gene flow between different geographical populations. Hypervolume analysis indicated that *S. schlegeli* from different geographic regions diverged in their realized niches, which may have contributed considerably to the genetic divergence of *S. schlegeli* populations in their evolutionary history. A recent study on *Syngnathus typhle* based on geometric morphometrics, prey availability and dietary composition demonstrated a pronounced variation in snout morphology across their distribution area, which may contribute to their adaptation in novel environments (Wilson et al., 2020).

In the present study, a north-to-south variation of cranial morphology was detected in different *S. schlegeli* populations, which indicated that the individuals in the south of the distributional range (SCS) had smaller eyes, and opposite patterns were observed in the north (ECS and YS). In marine fishes, visual acuity is an important part of environmental adaptation, as it plays an important role in numerous behaviours, including predation, predator-avoidance and locating breeding grounds. Morphological differences in seaweed pipefish populations may be associated with the phenotypic plasticity in response to various environmental conditions, such as sea water turbidity and food type. Plastic phenotypic changes induced by environmental shifts can be regarded as the first phase of adaptation, whereas the second phase is characterized by genetic changes driven by selection. Thus, selection favouring these particular phenotypes will result in genetic changes that mimic the plastic response (Price et al., 2003). Today, it is widely accepted in ecological developmental biology that phenotypic plasticity is an important mechanism promoting diversification and speciation (Pfennig et al., 2010; Rundle & Nosil, 2005). However, heritability of the morphological variation observed in *S. schlegeli* populations has yet to be confirmed because of the challenge of artificial breeding of this species under common garden conditions. Further studies are needed to prove the findings in other relative species (e.g. *Syngnathus typhle*, *Hippocampus erectus*) that can be easily maintained in the standard laboratory conditions.

Divergent selection resulting from diverse environmental conditions constitutes barriers to gene flow (Tobler et al., 2008), and identification of loci or genes under natural selection is important to determine the genetic basis of local adaptation affecting fitness traits (Zardi et al., 2013). A total of 143 genes were annotated from the selected genomic regions, revealed using genome scan method, among which many key genes are involved in growth (*pax7*) (Akolkar et al., 2016), cold adaptation (*csde1*) (Yang et al., 2012) and eye development (*eys*, *rx3*) (Loosli et al., 2003; Yu et al., 2016). Accordingly, local populations of *S. schlegeli* may benefit from the adaptive variation to improve the adaptation of this species to local environments.

Although marine fish generally show high genetic diversity and shallow population structure (Takahashi et al., 2015; Utter & Seeb, 2010), the results of this study confirmed that *S. schlegeli* has an obvious geographical population structure along with its distribution range in the north-western Pacific. The divergence among the different geographical populations might be related to geological

events, as well as adaptation to habitat heterogeneity across latitudinal gradients.

## 5 | CONCLUSION

We disentangled the morphological, molecular and ecological mechanisms underlying adaptive evolution against a background of high-level gene flow in a seemingly barrier-free environment. We showed that historical processes during the LGM, in concert with niche-driven ecological differentiation, have led to the establishment of three distinct clades across a widely distributed marine pipefish. Ultimately, our study shows that even interconnected sea environments maintain a high potential for ecological specialization.

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## CONFLICT INTERESTS

The authors declare that there are no conflicts of interest.

## PEER REVIEW

The peer review history for this article is available at <https://publons.com/publon/10.1111/ddi.13286>.

## DATA AVAILABILITY STATEMENT

All mitochondrial and nuclear gene sequences were deposited into the GenBank database under accession numbers MT467667–MT468179. The sequencing reads of RADseq in the study have been deposited to the CNGB (China National Genebank) with the accession number CNP0000953 (<https://db.cngb.org/cnsa/>).

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## REFERENCES

- Akolkar, K. S., Asaduzzaman, M., Kinoshita, S., Asakawa, S., & Watabe, S. (2016). Characterization of Pax3 and Pax7 genes and their expression patterns during different development and growth stages of

- Japanese pufferfish *Takifugu rubripes*. *Gene*, 575(1), 21–28. <https://doi.org/10.1016/j.gene.2015.08.031>
- Araujo, M. B., Anderson, R. P., Barbosa, A. M., Dormann, C. F., Early, R., Garcia, R. A., & Rahbek, C. (2019). Standards for distribution models in biodiversity assessments. *Science Advances*, 5(1), eaat4858. <https://doi.org/10.1126/sciadv.aat4858>
- Ashburner, M., Ball, C., Blake, J. A., Botstein, D., Butler, H., Cherry, J., Davis, A., Harris, M. (2000). Gene ontology: Tool for the unification of biology. *The Gene Ontology Consortium. Nature Genetics*, 25(1), 25–29. <https://doi.org/10.1038/75556>
- Barbet-Massin, M., Jiguet, F., Albert, C. H., & Thuiller, W. (2012). Selecting pseudo-absences for species distribution models: How, where and how many? *Methods in Ecology and Evolution*, 3, 327–338. <https://doi.org/10.1111/j.2041-210X.2011.00172.x>
- Bierne, N., Bonhomme, F., & David, P. (2003). Habitat preference and the marine-speciation paradox. *Proceedings of the Royal Society B - Biological Sciences*, 270(1522), 1399–1406. <https://doi.org/10.1098/rspb.2003.2404>
- Blonder, B., Lamanna, C., Violle, C., & Enquist, B. J. (2014). The  $n$ -dimensional hypervolume. *Global Ecology and Biogeography*, 23(5), 595–609. <https://doi.org/10.1111/geb.12146>
- Blonder, B., Morrow, C. B., Maitner, B., & Harris, D. J. (2018). New approaches for delineating  $n$ -dimensional hypervolumes. *Methods in Ecology and Evolution*, 9(2), 305–319. <https://doi.org/10.1111/2041-210X.12865>
- Bolnick, D. I., & Stutz, W. E. (2017). Frequency dependence limits divergent evolution by favouring rare immigrants over residents. *Nature*, 546(7657), 285–288. <https://doi.org/10.1038/nature22351>
- Carvalho, J. C., & Cardoso, P. (2020). Decomposing the causes for niche differentiation between species using hypervolumes. *Frontiers in Ecology and Evolution*, 8, 243. <https://doi.org/10.3389/fevo.2020.00243>
- Chen, Y., Chen, Y., He, D., & Sui, X. (2006). Current Status and Perspective of Biogeography Study in Taiwan Strait China. *Chinese Journal of Zoology*, 41(1), 118–122. [https://doi.org/10.1016/S1004-4132\(06\)60023-6](https://doi.org/10.1016/S1004-4132(06)60023-6)
- Chen, Z., Zhang, Y., Han, Z. Q., Song, N., & Gao, T. X. (2017). Morphological characters and DNA barcoding of *Syngnathus schlegelii* in the coastal waters of China. *Journal of Oceanology and Limnology*, 36(3), 537–547. <https://doi.org/10.1007/s00343-017-6206-2>
- Cunha, R. L., Tenorio, M. J., Afonso, C., Castilho, R., & Zardoya, R. (2008). Replaying the tape: Recurring biogeographical patterns in Cape Verde Conus after 12 million years. *Molecular Ecology*, 17(3), 885–901. <https://doi.org/10.1111/j.1365-294X.2007.03618.x>
- Dawson, C. E. (1985). *Indo-Pacific pipefishes (Red Sea to the Americas)*. Gulf Coast Research Laboratory.
- Devictor, V., Clavel, J., Julliard, R., Lavergne, S., Mouillot, D., Thuiller, W., & Mouquet, N. (2010). Defining and measuring ecological specialization. *Journal of Applied Ecology*, 47(1), 15–25. <https://doi.org/10.1111/j.1365-2664.2009.01744.x>
- Dong, Y. W., Wang, H. S., Han, G. D., Ke, C. H., Zhan, X., Nakano, T., & Williams, G. A. (2012). The impact of Yangtze River discharge, ocean currents and historical events on the biogeographic pattern of *Cellana toreuma* along the China coast. *PLoS One*, 7(4), e36178. <https://doi.org/10.1371/journal.pone.0036178>
- Dormann, C. F., Elith, J., Bacher, S., Buchmann, C., Carl, G., Carré, G., Marquéz, J. R. G., Gruber, B., Lafourcade, B., Leitão, P. J., Münkemüller, T., McClean, C., Osborne, P. E., Reineking, B., Schröder, B., Skidmore, A. K., Zurell, D., & Lautenbach, S. (2013). Collinearity: A review of methods to deal with it and a simulation study evaluating their performance. *Ecography*, 36(1), 27–46. <https://doi.org/10.1111/jbi.13362>
- Ellegren, H., & Sheldon, B. C. (2008). Genetic basis of fitness differences in natural populations. *Nature*, 452(7184), 169–175. <https://doi.org/10.1038/nature06737>
- Excoffier, L., Laval, G., & Schneider, S. (2007). Arlequin (version 3.0): An integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online*, 1, 47–50. <https://doi.org/10.1143/JJAP.34.L418>
- Fox, L. R., & Morrow, P. A. (1981). Specialization: Species Property or Local Phenomenon? *Science*, 211(4485), 887–893. <https://doi.org/10.1126/science.211.4485.887>
- Garant, D., Kruuk, L. E., Wilkin, T. A., Mcleery, R. H., & Sheldon, B. C. (2005). Evolution driven by differential dispersal within a wild bird population. *Nature*, 433(7021), 60–65. <https://doi.org/10.1038/nature03051>
- Gillespie, R. G., Bennett, G. M., De Meester, L., Feder, J. L., Fleischer, R. C., Harmon, L. J., & Wogan, G. O. U. (2020). Comparing Adaptive Radiations Across Space, Time, and Taxa. *Journal of Heredity*, 111(1), 1–20. <https://doi.org/10.1093/jhered/esz064>
- Hendry, A. P., Wenburg, J. K., Bentzen, P., Volk, E. C., & Quinn, T. P. (2000). Rapid evolution of reproductive isolation in the wild: Evidence from introduced salmon. *Science*, 290(5491), 516–519. <https://doi.org/10.1126/science.290.5491.516>
- Hewitt, G. (2000). The genetic legacy of the Quaternary ice ages. *Nature*, 405(6789), 907–913. <https://doi.org/10.1038/35016000>
- Hewitt, G. M. (2004). Genetic Consequences of Climatic Oscillations in the Quaternary. *Philosophical Transactions of the Royal Society of London Series B: Biological Sciences*, 359(1442), 183–195. <https://doi.org/10.1098/rstb.2003.1388>
- Hutchinson, G. (1957). Concluding remarks. *Cold Spring Harbor Symposia on Quantitative Biology*, 22, 415–427. <https://doi.org/10.1101/SQB.1957.022.01.039>
- Kawecki, T., & Ebert, D. (2004). Conceptual issues in local adaptation. *Ecology Letters*, 7(12), 1225–1241. <https://doi.org/10.1111/j.1461-0248.2004.00684.x>
- Leysen, H., Roos, G., & Adriaens, D. (2011). Morphological variation in head shape of pipefishes and seahorses in relation to snout length and developmental growth. *Journal of Morphology*, 272(10), 1259–1270. <https://doi.org/10.1002/jmor.10982>
- Li, H. (2011). A statistical framework for SNP calling, mutation discovery, association mapping and population genetical parameter estimation from sequencing data. *Bioinformatics*, 27(21), 2987–2993. <https://doi.org/10.1093/bioinformatics/btr509>
- Li, H., & Durbin, R. (2009). *Fast and accurate short read alignment with Burrows-Wheeler transform*. Oxford University Press.
- Liu, J. X., Gao, T. X., Wu, S. F., & Zhang, Y. P. (2007). Pleistocene isolation in the Northwestern Pacific marginal seas and limited dispersal in a marine fish, *Chelon haematocheilus* (Temminck & Schlegel, 1845). *Molecular Ecology*, 16(2), 275–288. <https://doi.org/10.1111/j.1365-294X.2006.03140.x>
- Loosli, F., Staub, W., Finger-Baier, K. C., Ober, E. A., Verkade, H., Wittbrodt, J., & Baier, H. (2003). Loss of eyes in zebrafish caused by mutation of *chokh / rx3*. *EMBO Reports*, 4(9), 894–899. <https://doi.org/10.1038/sj.embor.embor919>
- Loxdale, H. D., Lushai, G., & Harvey, J. A. (2011). The evolutionary improbability of 'generalism' in nature, with special reference to insects. *Biological Journal of the Linnean Society*, 103(1), 1–18. <https://doi.org/10.1111/j.1095-8312.2011.01627.x>
- Mammola, S. (2019). Assessing similarity of  $n$ -dimensional hypervolumes: Which metric to use? *Journal of Biogeography*, 46(9), 2012–2023. <https://doi.org/10.1111/jbi.13618>
- Mammola, S., Arnedo, M. A., Fišer, C., Cardoso, P., Dejanaz, A. J., Isaia, M., & Heleno, R. (2020). Environmental filtering and convergent evolution determine the ecological specialization of subterranean spiders. *Functional Ecology*, 34(5), 1064–1077. <https://doi.org/10.1111/1365-2435.13527>
- Mammola, S., & Cardoso, P. (2020). Functional diversity metrics using kernel density  $n$ -dimensional hypervolumes. *Methods in Ecology & Evolution*, 11(8), 986–995. <https://doi.org/10.1111/2041-210X.13424>
- Ni, G., Li, Q., Kong, L., & Yu, H. (2014). Comparative phylogeography in marginal seas of the northwestern Pacific. *Molecular Ecology*, 23(3), 534–548. <https://doi.org/10.1111/mec.12620>

- Ni, G., Li, Q., Kong, L., Zheng, X., & Crandall, K. A. (2012). Phylogeography of Bivalve *Cyclina sinensis*: Testing the Historical Glaciations and Changjiang River Outflow Hypotheses in Northwestern Pacific. *PLoS One*, 7(11), e49487. <https://doi.org/10.1371/journal.pone.0049487>
- Ogata, H., Goto, S., Sato, K., Fujibuchi, W., Bono, H., & Kanehisa, M. (1999). KEGG: Kyoto Encyclopedia of Genes and Genomes. *Nucleic Acids Research*, 27(1), 29–34. <https://doi.org/10.1093/nar/27.1.29>
- Palumbi, S. R. (1994). Genetic Divergence, Reproductive Isolation, and Marine Speciation. *Annual Review of Ecology and Systematics*, 25(1), 547–572. <https://doi.org/10.1146/annurev.es.25.110194.002555>
- Pfennig, D. W., Wund, M. A., Snell-Rood, E. C., Cruickshank, T., Schlichting, C. D., & Moczek, A. P. (2010). Phenotypic plasticity's impacts on diversification and speciation. *Trends in Ecology and Evolution*, 25(8), 459–467. <https://doi.org/10.1016/j.tree.2010.05.006>
- Price, T. D., Qvarnstrom, A., & Irwin, D. E. (2003). The role of phenotypic plasticity in driving genetic evolution. *Proceedings of the Royal Society B - Biological Science*, 270(1523), 1433–1440. <https://doi.org/10.1098/rspb.2003.2372>
- Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M. A., Bender, D., & Daly, M. J. (2007). PLINK: A tool set for whole-genome association and population-based linkage analyses. *American Journal of Human Genetics*, 81(3), 559–575. <https://doi.org/10.1086/519795>
- Rocha, L. A., Bass, A. L., Robertson, D. R., & Bowen, B. W. (2002). Adult habitat preferences, larval dispersal, and the comparative phylogeography of three Atlantic surgeonfishes (Teleostei: Acanthuridae). *Molecular Ecology*, 11(2), 243–251. <https://doi.org/10.1046/j.0962-1083.2001.01431.x>
- Rocha, L. A., Robertson, D. R., Roman, J., & Bowen, B. W. (2005). Ecological speciation in tropical reef fishes. *Proceedings of the Royal Society B - Biological Sciences*, 272(1563), 573–579. <https://doi.org/10.1098/2004.3005>
- Rundle, H. D., & Nosil, P. (2005). Ecological speciation. *Ecology Letters*, 8(3), 336–352. <https://doi.org/10.1111/j.1461-0248.2004.00715.x>
- Sabeti, P. C., Schaffner, S. F., Fry, B., Lohmueller, J., Varilly, P., Shamovsky, O., & Lander, E. S. (2006). Positive natural selection in the human lineage. *Science*, 312(5780), 1614–1620. <https://doi.org/10.1126/science.1124309>
- Sanford, E., & Kelly, M. W. (2011). Local adaptation in marine invertebrates. *Annual Review of Marine Science*, 3, 509–535. <https://doi.org/10.1146/annurev-marine-120709-142756>
- Sanford, E., & Worth, D. J. (2010). Local adaptation along a continuous coastline: Prey recruitment drives differentiation in a predatory snail. *Ecology*, 91(3), 891–901. <https://doi.org/10.1890/09-0536.1>
- Sbrocco, E. J. (2014). Paleo-MARSPEC: Gridded ocean climate layers for the mid-Holocene and Last Glacial Maximum. *Ecology*, 95(6), 1710. <https://doi.org/10.1890/14-0443.1>
- Sbrocco, E. J., & Barber, P. H. (2013). MARSPEC: Ocean climate layers for marine spatial ecology. *Ecology*, 84(4), 979. <https://doi.org/10.1890/12-1358.1>
- Shiple, L. A., Forbey, J. S., & Moore, B. D. (2009). Revisiting the dietary niche: When is a mammalian herbivore a specialist? *Integrative & Comparative Biology*, 49(3), 274–290. <https://doi.org/10.1093/icb/icmp051>
- Smith, T. B., Wayne, R. K., Girman, D. J., & Bruford, M. W. (1997). A Role for Ecotones in Generating Rainforest Biodiversity. *Science*, 276(5320), 1855–1857. <https://doi.org/10.1126/science.276.5320.1855>
- Takahashi, H., Takeshita, N., Tanoue, H., Ueda, S., Takeshima, H., Komatsu, T., & Nishida, M. (2015). Severely depleted genetic diversity and population structure of a large predatory marine fish (*Lates japonicus*) endemic to Japan. *Conservation Genetics*, 16(5), 1155–1165. <https://doi.org/10.1007/s10592-015-0729-x>
- Tamaki, K., & Honza, E. (1991). Global tectonics and formation of marginal basins: Role of the Western Pacific. *Translated World Seismology*, 14(3), 224–230.
- Tang, H., Peng, J., Wang, P., & Risch, N. J. (2005). Estimation of individual admixture: Analytical and study design considerations. *Genetic Epidemiology*, 28(4), 289–301. <https://doi.org/10.1002/gepi.20064>
- Thuiller, W., Georges, D., Engler, R., & Breiner, F. (2020). *biomod2: Ensemble Platform for Species Distribution Modeling*. R package version 3.4.6. <https://CRAN.R-project.org/package=biomod2>
- Thuiller, W., Guéguen, M., Renaud, J., & Karger, Dirk, N. (2019). Uncertainty in ensembles of global biodiversity scenarios. *Nature Communications*, 10(1), 1446. <https://doi.org/10.1038/s41467-019-09519-w>
- Tobler, M., Dewitt, T. J., Schlupp, I., Fj, G. D. L., Herrmann, R., Feulner, P. G., & Plath, M. (2008). Toxic hydrogen sulfide and dark caves: Phenotypic and genetic divergence across two abiotic environmental gradients in *Poecilia mexicana*. *Evolution*, 62(10), 2643–2659. <https://doi.org/10.1111/j.1558-5646.2008.00466.x>
- Todd Strelman, J., & Danley, P. D. (2003). The stages of vertebrate evolutionary radiation. *Trends in Ecology & Evolution*, 18(3), 126–131. [https://doi.org/10.1016/s0169-5347\(02\)00036-8](https://doi.org/10.1016/s0169-5347(02)00036-8)
- Utter, F., & Seeb, J. (2010). A perspective on positive relationships between genetic diversity and abundance in fishes. *Molecular Ecology*, 19(22), 4831–4833. <https://doi.org/10.1111/j.1365-294X.2010.04823.x>
- Vilella, A., Severin, J. V. A., Heng, L., Durbin, R., & Birney, E. (2009). EnsemblCompara GeneTrees: Complete, duplication-aware phylogenetic trees in vertebrates. *Genome Research*, 19(2), 327–335. <https://doi.org/10.1101/gr.073585.107>
- Wainwright, P. C., Bellwood, D. R., Westneat, M. W., Grubich, J. R., & Hoey, A. S. (2004). A functional morphospace for the skull of labrid fishes: Patterns of diversity in a complex biomechanical system. *Biological Journal of the Linnean Society*, 82(1), 1–25. <https://doi.org/10.1111/j.1095-8312.2004.00313.x>
- Wainwright, P. C., McGee, M. D., Longo, S. J., & Hernandez, L. P. (2015). Origins, innovations, and diversification of suction feeding in vertebrates. *Integrative and Comparative Biology*, 55(1), 134–145. <https://doi.org/10.1093/icb/icv026>
- Wang, M. S., Li, Y., Peng, M. S., Zhong, L., Wang, Z. J., Li, Q. Y., & Wang, L. (2015). Genomic analyses reveal potential independent adaptation to high altitude in Tibetan chickens. *Molecular Biology & Evolution*, 32(7), 1880–1889. <https://doi.org/10.1093/molbev/msv071>
- Wang, P. (1999). Response of Western Pacific marginal seas to glacial cycles: Paleooceanographic and sedimentological features. *Marine Geology*, 156(1–4), 5–39. [https://doi.org/10.1016/S0025-3227\(98\)00172-8](https://doi.org/10.1016/S0025-3227(98)00172-8)
- Wang, P. X., & Sun, X. J. (1994). Last glacial maximum in China: Comparison between land and sea. *Catena*, 23(3–4), 341–353. [https://doi.org/10.1016/0341-8162\(94\)90077-9](https://doi.org/10.1016/0341-8162(94)90077-9)
- Watanabe, S., & Watanabe, Y. (2001). Brooding season, sex ratio, and brood pouch development in the seaweed pipefish, *Syngnathus schlegelii*, in Otsuchi Bay, Japan. *Ichthyological Research*, 48(2), 155–160. <https://doi.org/10.1007/s10228-001-8130-9>
- Wilson, A. B., Ahnesjö, I., Vincent, A. C. J., & Meyer, A. (2003). The dynamics of male brooding, mating patterns, and sex roles in pipefishes and seahorses (family Syngnathidae). *Evolution*, 57(6), 1374–1386. <https://doi.org/10.1111/j.0014-3820.2003.tb00345.x>
- Wilson, A. B., Wegmann, A., Ahnesjö, I., & Gonçalves, J. M. S. (2020). The evolution of ecological specialization across the range of a broadly distributed marine species. *Evolution*, 74(3), 629–643. <https://doi.org/10.1111/evo.13930>
- Xu, J., Chan, T. Y., Tsang, L. M., & Chu, K. H. (2009). Phylogeography of the mitten crab *Eriocheir sensu stricto* in East Asia: Pleistocene isolation, population expansion and secondary contact. *Molecular Phylogenetics and Evolution*, 52(1), 45–56. <https://doi.org/10.1016/j.ympev.2009.02.007>
- Yang, C., Wang, L., Siva, V. S., Shi, X., Jiang, Q., Wang, J., & Uversky, V. N. (2012). A Novel Cold-Regulated Cold Shock Domain Containing Protein from Scallop *Chlamys farreri* with Nucleic Acid-Binding

- Activity. *PLoS One*, 7(2), e32012. <https://doi.org/10.1371/journal.pone.0032012>
- Yang, J., Lee, S. H., Goddard, M. E., & Visscher, P. M. (2011). GCTA: A Tool for Genome-wide Complex Trait Analysis. *American Journal of Human Genetics*, 88(1), 76–82. <https://doi.org/10.1016/j.ajhg.2010.11.011>
- Yao, Y. F., Dai, Q. X., Li, J., Ni, Q. Y., Zhang, M. W., & Xu, H. L. (2014). Genetic diversity and differentiation of the rhesus macaque (*Macaca mulatta*) population in western Sichuan, China, based on the second exon of the major histocompatibility complex class II DQB (*MhcMamu-DQB1*) alleles. *BMC Evolutionary Biology*, 14(1), 130. <https://doi.org/10.1186/1471-2148-14-130>
- Yu, H. X., & Zhou, Y. W. (2001). Geomorphology and geological features of the continental shelf in northern and western Taiwan. *China Science in China (Series D)*, 31(6), 486–495.
- Yu, M., Liu, Y., Li, J., Natale, B. N., Cao, S., Wang, D., & Hu, H. (2016). Eyes shut homolog is required for maintaining the ciliary pocket and survival of photoreceptors in zebrafish. *Biology Open*, 5(11), 1662–1673. <https://doi.org/10.1242/bio.021584>
- Zardi, G. I., Nicastro, K. R., Costa, J. F., Serrão, E. A., & Pearson, G. A. (2013). Broad scale agreement between intertidal habitats and adaptive traits on a basis of contrasting population genetic structure. *Estuarine Coastal & Shelf Science*, 131(6), 140–148. <https://doi.org/10.1016/j.ecss.2013.08.016>
- Zhang, B. D., Xue, D. X., Wang, J., Li, Y. L., Liu, B. J., & Liu, J. X. (2016). Development and preliminary evaluation of a genomewide single nucleotide polymorphisms resource generated by RAD-seq for the small yellow croaker (*Larimichthys polyactis*). *Molecular Ecology Resources*, 16(3), 755–768. <https://doi.org/10.1111/1755-0998.12476>
- Zhang, Y. H., Qin, G., Zhang, H. X., Wang, X., & Lin, Q. (2017). DNA barcoding reflects the diversity and variety of brooding traits of fish species in the family Syngnathidae along China's coast. *Fisheries Research*, 185, 137–144. <https://doi.org/10.1016/j.fishres.2016.09.015>
- Zhang, Y. H., Ravi, V., Qin, G., Dai, H., Zhang, H. X., Han, F. M., & Lin, Q. (2020). Comparative genomics reveal shared genomic changes in syngnathid fishes and signatures of genetic convergence with placental mammals. *National Science Review*, 7(2), 964–977. <https://doi.org/10.1093/nsr/nwaa002>
- Zhang, Z. X., Capinha, C., Karger, D. N., Turon, X., Maclsaac, H. J., & Zhan, A. B. (2020). Impacts of climate change on geographical distributions of invasive ascidians. *Marine Environmental Research*, 159, 104993. <https://doi.org/10.1016/j.marenvres.2020.104993>
- Zhang, Z. X., Kass, J. M., Mammola, S., Koizumi, I., Li, X., Tanaka, K., & Usio, N. (2021). Lineage-level distribution models lead to more realistic climate change predictions for a threatened crayfish. *Diversity and Distributions*, 27(4), 684–695. <https://doi.org/10.1111/ddi.13225>

#### BIOSKETCH

The research team mainly focus on the research of marine biodiversity with a particular focus on the male-pregnant syngnathid fishes. Dr. Wang Xin's research interests centre on the marine biogeography and molecular ecology. Dr. Zhang Zhixin mainly focuses on biogeography, invasive biology and conservation biology.

#### SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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