Threadfin porgy (*Evynnis cardinalis*) haplotype pattern and genetic structure in Beibu Gulf, South China Sea

Lei Xu[1], Xuehui Wang[1], Liang Wang[1], Jiajia Ning[1], Yafang Li[1], Delian Huang[1], Shuangshuang Liu[1], and Feiyan Du[1]

[1]South China Sea Fisheries Research Institute

March 07, 2024

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1 South China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Guangzhou 510300, China

2 Guangdong Provincial Key Laboratory of Fishery Ecology and Environment, Guangzhou 510300, China

*Corresponding author: feiyanegg@163.com

Abstract

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a genetically homogeneous population structure in Beibu Gulf, which indicated a single panmictic stock of*E. cardinalis* in this area. The star-like haplotype network, unimodal mismatch distribution, and significantly negative Tajima's D and Fu's Fs values indicated recent population demographic expansion of*E. cardinalis* . The mismatch distribution and Bayesian skyline plot results indicated that *E. cardinalis* from Beibu Gulf experienced colonization and demographic expansion during the late Pleistocene due to sea level fluctuations.

KEYWORDS : Haplotype pattern, Genetic differentiation, Demographic expansion, COI, South China Sea

1 INTRODUCTION

Marine fish are generally assumed to have high dispersal potential because of their high dispersal capability at both larval and adult stages, and the absence of physical barriers to dispersal (Caley et al., 1996; Hellberg, 2009). Theoretically, the higher dispersal capability of a species, the lower its inter-population genetic structure. Therefore, in marine fish species, especially migratory species, the signal of population differentiation is weak and difficult to detect because of high levels of gene flow (Gandra, Assis, Martins, & Abecasis, 2020). Understanding the distribution of genetic diversity of important commercial species is vital to implementing protection policies and management regulations (Araki & Schmid, 2010). Genetic diversity (within and among populations) greatly influences species adaptive potential to environmental changes, and ultimately determines their long-term resilience to ecological disturbances (Pauls, Nowak, Bálint, & Pfenninger, 2013). Additionally, knowledge of genetic structure is useful for understanding fish spawning migration routes, areas, and seasons. Such information can help fisheries managers define the spatiotemporal scales over which they can implement effective stock management and conservation plans (Bradbury, Laurel, Snelgrove, Bentzen, & Campana, 2008). Therefore, from a resource management perspective, understanding the patterns of genetic diversity and levels of gene flow among populations is a fundamental issue. The effectiveness of marine protected areas depends on both their ability to self-recruit (reproductive potential) and the spillover of adults and export of larvae to nearby fished areas (Harrison et al., 2012; Le Port et al., 2017). Over the last 30 years, genetic studies have become an essential tool for stock management and conservation of estuarine, coral reef, and coastal populations because genetic studies are useful for estimating genetic diversity and the ability to survive anthropogenic activities (e.g., overfishing, habitat degradation, eutrophication, invasive species, and pollution) (Gaither, Toonen, Robertson, Planes, & Bowen, 2010; Gill & Kemp, 2002; Machado, da Silva Cortinhas, Proietti, & Haimovici, 2020; Ruzzante, Taggart, & Cook, 1998; Ryman, Utter, & Laikre, 1995). Mitochondrial DNA (mtDNA) markers are widely employed to detect population structure in marine species because they have large number of copies, high mutation rates, generally maternal inheritance, and almost nonexistent recombination. The primary advantages of mtDNA are the inheritance pattern and nonexistent recombination: clonal inheritance through the maternal line allows tracing of speciation events over multiple generations, and male dispersal does not homogenize the population (Prugnolle & de Meeus, 2002). These factors make mtDNA markers particularly appropriate indicators of population genetic structure in marine organisms such as zooplankton and migratory fishes, which generally have high gene flow (Machado et al., 2020; L. Wang et al., 2013; Xu, Li, Wang, & Du, 2019).

Threadfin porgy, *Evynnis cardinalis* (Lacepède 1802), occurs in the Indo-West Pacific from Japan, Korea, and China to Vietnam and Indonesia (Z. Chen & Qiu, 2005). This species is primarily distributed from 30–60 m depth but also can occur to 100 m depth (Iwatsuki & Carpenter, 2014). This species is found over a wide range of bottom types, but is more common close to reefs or on rough bottoms. Small individuals are very common at some localities in shallow, sheltered bays; larger fish often occur in deeper water (Eggleston, 1974).*Evynnis cardinalis* is one of the main commercial fishing targets in bottom trawl fisheries in the northern South China Sea and is considered to have three geographical stocks in the northern South China Sea: the Taiwan Strait, South China Sea, and Beibu Gulf stocks.*Evynnis cardinalis* is a migratory fish that is captured year-round with significant seasonal differences among fishing areas. In Beibu Gulf, *E. cardinalis* undergoes seasonal migration and is found toward the northeastern shallow area of the gulf in late autumn

and winter. Spawning takes place in northwest Weizhou Island in the spring, and the recruits disperse widely in the northern area of the gulf and begin to disperse south in late spring (Iwatsuki & Carpenter, 2014). However, this species exhibits life history characteristics, including late maturity and longevity, that predispose it to impacts from heavy exploitation. In the northern South China Sea, the Beibu Gulf stock of *E. cardinalis* declined 58% from 2001 to 2005, whereas the Taiwan Strait stock declined 62% from 1993 to 2008. Therefore, a recent IUCN Red List assessment reported *E. cardinalis* as endangered, and this species is mainly threatened by overexploitation (Iwatsuki & Carpenter, 2014). Previous studies on this species in Beibu Gulf have focused on feeding habits, growth and mortality, ecological distribution, phylogeny, and stock density (Cai, Chen, Xu, & Zhang, 2017; Z. Chen & Qiu, 2005; Z. Z. Chen & Qiu, 2003; D. Zhang, Shao, Su, & Jiang, 2007; K. Zhang et al., 2020; Y. Zhang, Dai, Yan, Yang, & Lu, 2014). However, no information is available to date on the genetic structure of *E. cardinalis* in the northern South China Sea, and this prevents reliable stock assessments and protection policy formulation. The objective of this study was to provide a population genetic analysis using a portion of the mitochondrial cytochrome c oxidase subunit I (COI) gene to assess the population genetic diversity pattern and historical demography, and estimate *E. cardinalis* expansion time in Beibu Gulf.

2 MATERIALS AND METHODS

2.1 Study area

Beibu Gulf (17°–22°N, 105°–110°E) is in the northwestern part of the South China Sea and has a long coastline that belongs to China and Vietnam. It is a semi-closed gulf of approximately 128,000 km^2 that ranges from Leizhou Peninsula, Qiongzhou Strait, and Hainan Island to Vietnam, and extends to the Guangxi coast in the north (Ma et al., 2010). The bottom of Beibu Gulf is flat and slopes from the northwest to the southeast, and the water depth in the gulf is typically less than 100 m (average depth, 42 m). The surrounding climate of this gulf is subtropical and monsoonal. Moreover, this gulf contains numerous estuaries from which rivers discharge nutrients. Beibu Gulf is a traditional fishing ground and main source of fishery products for coastal areas because of its high productivity and rich biological diversity, which benefit from its geographic location and climatic conditions (Z. Chen, Xu, Qiu, Lin, & Jia, 2009).

2.2 Sample collection

All fish specimens were collected from fishery surveys carried out by the South China Sea Fisheries Research Institute; these surveys were conducted by the commercial fishing vessel "Beiyu60011" in the northern South China Sea using bottom trawler nets in September 2018. Eleven sampling sites from Beibu Gulf were investigated in the study area, which covered over 45,000 km^2 (Fig. 1). In total, 11–16 specimens of each sampling site were used for the DNA analyses after morphological identification. The dorsal fin was removed from each specimen and preserved in absolute ethanol at -20degC.

2.3 DNA extraction, amplification, and sequencing

Genomic DNA was extracted from dorsal fin tissue using the TIANamp Marine Animals DNA Kit (TIANGEN, China) following the manufacturer's protocol. The concentration for use as a PCR template was adjusted to an A260 of approximately 0.05–0.2. Fragments of the mtDNA COI gene were amplified from total genomic DNA using the polymerase chain reaction. The primers FishF1 and FishR1 (Ward, Zemlak, Innes, Last, & Hebert, 2005) were used for COI PCR amplification. Each 50 μl PCR consisted of 31.25 μl ddH_2O, 5 μl PCR buffer, 5 μl CoralLoad concentrate, 4 μl of 25 μM $MgCl_2$, 1 μl of 10 μM dNTPs, 0.5 μl of 25 μM solution of each primer, 2.5 μl DNA template, and 0.25 μl TopTaq DNA polymerase (QIAGEN, Germany). The PCR conditions for amplification were: an initial step of 2 min at 95°C; 35 cycles of 0.5 min at 95°C (denaturation), 0.5 min at 54°C (annealing), and 1 min at 72°C (extension); followed by 7

min at 72°C (final extension) on a 2720 Thermal Cycler (Applied Biosystems, USA). PCR products were visualized on 1.5% agarose gels and the most intense products were selected for sequencing. Products were bidirectionally sequenced on an ABI 3730XL automated sequencer with both forward and reverse primers following manufacturer's instructions.

2.4 Genetic analysis

The sequences were assembled and edited in Bioedit (Hall, 1999) and aligned using the CLUSTALW multiple algorithm under default options. Ambiguous sequences were trimmed after alignment. The authenticity of our COI sequences was verified by a BLAST search in GenBank (BLASTn, megablast algorithm) and compared with the highest match (99%–100%). Molecular diversity from COI sequences was measured using DnaSP 5.0 (Librado & Rozas, 2009) with the following variables: number of haplotypes (H), polymorphic sites (S), haplotype diversity (h), and nucleotide diversity (π).

To visually represent the relationships among the mtDNA haplotypes of the sampled *E. cardinalis* individuals, we performed a network analysis using HAPLOVIEWER, which turns trees built from traditional phylogenetic methods into haplotype genealogies (Salzburger, Ewing, & Von Haeseler, 2011). The phylogenetic tree used for this graphical representation was obtained by employing a maximum likelihood approach in PhyML 3.0 (Guindon et al., 2010) using a GTR model with four gamma-distributed rate categories as the substitution model.

Population genetic differentiation was inferred by estimating pairwise Φ_{ST} values among 11 sampling sites using the Tamura–Nei model of nucleotide substitution in Arlequin 3.5 (Excoffier & Lischer, 2010). This model was assessed as most suitable for our data using jModeltest 2.1 (Darriba, Taboada, Doallo, & Posada, 2012). The significance of Φ_{ST} values was tested by 10,000 permutations. To estimate migration rates between sampling sites, we performed maximum likelihood analysis using the coalescence method in Migrate 3.2.1 (Beerli & Felsenstein, 1999). The estimated parameters were $?_{ij} = \vartheta_i M_{ij}$, where $?_{ij}$ is the number of effective migrants from i to j, ϑ is mutation-scaled population size, and M_{ij} is m_{ij}/μ (where m_{ij} is the immigration rate from population i into j, and μ is the mutation rate per generation).

2.5 Inferring the historical demography

Signatures of population demographic changes (bottlenecks or expansions) in *E. cardinalis* were first examined by Tajima' D and Fu's Fs (Fu, 1997; Tajima, 1989) statistics with 10,000 permutations in Arlequin 3.5 to determine whether *E. cardinalis* in the Beibu Gulf data conformed to or departed from neutral theory model expectations because of factors such as a population bottleneck or expansion. For neutral markers, significant negative Tajima's D and Fu's Fs values can be expected under population expansion.

Next, we calculated the mismatch distribution among haplotypes under the sudden expansion model. This measure quantifies the smoothness of the observed mismatch distribution. We employed parametric bootstrapping (1,000 replicates) as implemented in Arlequin 3.5 to test the goodness of fit of the observed mismatch distribution to that expected under the sudden expansion model using the sum of squared deviations and raggedness index (R index) (Excoffier, 2004; Ray, Currat, & Excoffier, 2003; Schneider & Excoffier, 1999). Populations that underwent substantial expansion are expected to exhibit unimodal mismatch distributions with a low R index.

In addition, we used two methods to estimate the time of *E. cardinalis* population expansion in Beibu Gulf. First, the expansion time was directly estimated from the mismatch distribution with the statistic τ (tau) and translated into absolute time in years (t) using the equation $\tau = 2ut$, where u is the mutation rate of the sequence and is calculated as $u = 2\mu k$; k is the number of nucleotides in the sequence and μ is the mutation rate of the mtDNA gene per generation (Rogers & Harpending, 1992). Following Cantatore et al. (1994), mutation rates between 1% and 3% per million years were selected for our mitochondrial analysis.

Subsequently, we inferred historical demography from effective population size estimates over time using the Bayesian skyline plot (BSP) method in BEAST 1.8.0 (Alexei J. Drummond & Rambaut, 2007; A. J. Drummond, Rambaut, Shapiro, & Pybus, 2005). An HKY model with among-site rate heterogeneity across all branches that assumed a strict molecular clock was used for this calculation. Markov chains were run for 2.5×10^7 generations and sampled every 1,000 generations, with the first 2,500 samples discarded as burn-in. Three replicates were run and combined to separately analyze the COI datasets. Other parameters were set as default values. TRACER 1.5 was used to visualize the posterior probabilities of the Markov chain statistics and to calculate a statistical summary of the genetic parameters.

3 RESULTS

3.1 Genetic diversity

In total, 162 high-quality sequences were obtained after alignment and trimmed to 618 bp. The average base composition was A = 22.3%, C = 28.8%, G = 18.8%, T = 30.1%. In total, we found 44 different haplotypes in our 11 sampling sites (Table 1, Fig. 2). All haplotype sequences were deposited in GenBank (accession numbers MW881382–MW881425). Overall, the nucleotide diversity was low (average, 0.00232; range, 0.00185–0.00277), whereas the haplotype diversity was relatively high and heterogeneous (average, 0.788; range, 0.657–0.886) (Table 1).

The haplotype network analyses of the mtDNA from *E. cardinalis* in Beibu Gulf showed a typical star-like pattern, with the most frequent haplotype, H1, located in the central position of the haplotype network and surrounded by many low-frequency haplotypes that were divergent from H1 by only one or two mutations (Fig. 2). Most of the haplotypes were low-frequency, and 27 haplotypes were unique to only one sampling site. Only 11 out of the 44 haplotypes were observed at more than two sampling sites. However, several of the abundant haplotypes were found at multiple sampling sites. For example, the dominant widespread haplotype H1 was found in all 11 sampling sites (Fig. 3); the largest distance between two sampling sites harboring this haplotype was more than 300 km. H6 and H8 were distributed at eight and seven sampling sites, respectively. Moreover, between six and 10 haplotypes were found per sampling site, but there was no clear pattern of geographical variation (Fig. 3). The highest number of haplotypes (H = 10) was observed at sites 8 and 10 (Table 1).

The average genetic differentiation, Φ_{ST}, of *E. cardinalis* within Beibu Gulf was 0.031 (range, -0.0657 to 0.0671). All pairwise Φ_{ST} values among the sampling sites were small and not significant (Table 2). The effective number of migrants per generation among 11 sampling sites was from 5.98 to 45.76. The migration rates from S7 to S1 were the lowest, whereas those from S1 to S3 were the highest (Table 3).

3.2 Demographic history

The Tajima's D and Fu's Fs tests of *E. cardinalis* in Beibu Gulf were significantly negative (Tajima's D = -2.446, $P < 0.01$; Fu's Fs = -28.378, $P < 0.01$); this showed that *E. cardinalis* may have experienced population expansion (Table 4). The mismatch distribution appeared to be unimodal (Fig. 4), which was consistent with the expected distribution under a sudden expansion model (R index = 0.055, P = 0.26).

On the basis of the mutation rate of 1%–3% per million years for mitochondrial genes (Cantatore et al., 1994) and τ value of all data (1.531, 95% confidence intervals: 1.281–1.941), the expansion time for *E. cardinalis* in Beibu Gulf was estimated to have occurred from approximately 62–21 ka.

The BSP analysis indicated that the *E. cardinalis* haplotypes in Beibu Gulf coalesced approximately 40 ka when a mutation rate of 2% per million years was used for analysis. However, the BSP pattern revealed a coalescence time of 30–70 ka when using a mutation rate of 1%–3% per million years (Fig. 5). Despite

these different coalescence times, the BSP patterns showed similar tendencies and indicated that a steady population expansion took place between 5–30 ka.

4 DISCUSSION

Studies of population connectivity with mitochondrial markers provide critical information on gene flow and genetic relationships between neighboring populations (García, Vergara, & Gutiérrez, 2008; Turner, McPhee, Campbell, & Winemiller, 2004). Many studies showed that mitochondrial markers are highly effective for revealing marine fish genetic diversity and population connectivity (T. Gao et al., 2019; Lavergne et al., 2014; Machado et al., 2020). In this study on*E. cardinalis* , mtDNA sequence analysis of specimens from Beibu Gulf revealed no significant genetic differentiation among sampling sites, with low Φ_{ST}values indicating genetic homogeneity.

In contrast to freshwater species, marine fish are usually expected to show low genetic differentiation across their distribution. This is mainly attributed to genetic exchange being maintained by adult mobility throughout Beibu Gulf during reproduction, and through the passive dispersal of eggs and larvae due to the lack of noticeable physical barriers in "open" oceans (Grant & Bowen, 1998; Hellberg, 2009; Machado et al., 2020). The dominant widespread haplotype H1 was found in all 11 sampling sites, which also indicated high dispersal potential of planktonic egg, larval, or adult stages of *E. cardinalis* in Beibu Gulf.

Previous studies suggested that *E. cardinalis* breed once a year in Beibu Gulf (Z. Z. Chen & Qiu, 2003; Hou, Feng, Lu, & Zhu, 2008).*Evynnis cardinalis* gonads begin to develop in November and spawning occurs from December to February. The population is concentrated in the northern Beibu Gulf during spawning. In the early spring, spawned fish mainly occur in the northeast of the gulf, and juveniles concentrate in shallow nearshore of this area in the late spring. Then, juveniles gradually migrate southwest and widely disperse in deep waters of Beibu Gulf in summer or early autumn (K. Zhang et al., 2020).

In addition, the dispersal pattern of *E. cardinalis* was also impacted by circulation in Beibu Gulf. In spring, the density gradient and monsoon wind drive the ocean current from northeast to southwest in the gulf. The surface current velocity reaches 30 cm/s, and the current in the middle layer is approximately 5–10 cm/s (J. Gao, Wu, & Ya, 2017). The direction of the spring currents roughly coincides with the migration of *E. cardinalis* . Therefore, the seasonal migration and ocean current may be responsible for gene exchange among different locations, and therefore why *E. cardinalis* shows low levels of genetic differentiation in Beibu Gulf. If we refer to the biological description of a stock as given by Ihssen et al. (1981), "a stock is an intraspecific group of randomly mating individuals with temporal and spatial integrity," then the lack of distinct spatial boundaries and genetic substructure (low Φ_{ST} values) revealed by genetic analyses indicated that *E. cardinalis* in Beibu Gulf belong to a single stock.

The presence of a single stock in Beibu Gulf indicates that geographical isolation might block gene exchange between the Beibu Gulf stock and the other two *E. cardinalis* stocks, the Taiwan Strait and South China Sea stocks. In Beibu Gulf, the circulation, Hainan Island, and Leizhou Peninsula could act as barriers that impede free dispersal of *E. cardinalis* into this gulf from other areas of the South China Sea (J. Gao et al., 2017). The findings from our study and similar investigations conducted elsewhere demonstrated that marine fish that inhabit coastal waters usually constitute a single panmictic stock. For example, Rodrigues et al. (2008) revealed that *Cynoscion acoupa*from northern Brazil represents a single stock, even though it occupies at least 1260 km of coastline. T. Gao et al. (2019) reported a high level of genetic homogeneity in the *Pholis fangi* population around Bohai Sea and Yellow Sea, and suggested it should be considered as a single panmictic stock. Hoolihan, Anandh, and van Herwerden (2006) also reported a homogeneous distribution of Spanish mackerel (*Scomberomorus commerson*) throughout the Arabian Gulf, Gulf of Oman, and Arabian Sea on the basis of mtDNA analyses.

In addition, mtDNA sequence regions are particularly appropriate to infer historical processes that might be responsible for the contemporary geographic distribution of marine species because they are more prone

to genetic drift than nuclear markers and have a smaller effective population size (Avise, 1994; Slatkin & Hudson, 1991). In our study, the haplotype network of *E. cardinalis* from Beibu Gulf exhibited a star-like and unstructured pattern with a predominance of scattered. The dominant haplotype (carried by 45% of the specimens) was in the central position of the haplotype network and surrounded by many haplotypes that diverged from the dominant haplotype by only few mutations. Most surrounding haplotypes were unique to each sampling site and showed few differences between them (Fig. 2). Similar star-like haplotype networks have been observed for other species in different coastal areas: *Terapon jarbua* , which consists of a panmictic stock from the Socotra Archipelago to the Hadhramout Coast along the wider Gulf of Aden (Lavergne et al., 2014); and *Pogonias courbina* , which did not display distinct structure along the coast of the southwestern Atlantic Ocean (Machado et al., 2020).

A star-like haplotype network pattern, high haplotype diversity, and low nucleotide diversity are often considered consequences of recent population expansion linked to the Pleistocene environmental changes (Craig, Eble, Bowen, & Robertson, 2007; Liu et al., 2011; Pereira, Márquez, Marin, & Marin, 2009). The recent demographic expansion of*E. cardinalis* from Beibu Gulf is also supported by the unimodal mismatch distribution and significantly negative Tajima's D and Fu's Fs values. The population expansion of *E. cardinalis* in Beibu Gulf, which was directly estimated from the mismatch distribution, started 62–21 ka before present, which was during the late Pleistocene. BSP analysis indicated steady population expansion that started around 30 ka. Both two methods of estimated period of population expansion are consistent with the environment changes of the northern South China Sea in the Pleistocene.

Evynnis cardinalis is primarily distributed from 30–60 m depth, and spawns in coastal habitats and shallow shorelines. Therefore, the*E. cardinalis* distribution is closely correlated with fluctuating sea levels. When sea levels fell 120 m below the present level during the last glacial maximum of the Pleistocene, the northern South China Sea included Beibu Gulf, which was part of the South China continent, Hainan Island and Taiwan Island were connected to mainland China, and the entire South China Sea was separated from the Indian Ocean to form a semi-closed basin (Voris, 2000; P. Wang & Sun, 1994). Similar to other terrestrial species, *E. cardinalis* may have moved and survived in a potential glacial refuge during this period, such as the semi-closed South China Sea (Hewitt, 1999). In the late Pleistocene, the sea level was still 30 m below the present level, but the glaciation began to disappear and the sea water gradually poured into Beibu Gulf via the Qiongzhou Strait (Lu, Huang, Li, & Zhang, 2003). An initial population of *E. cardinalis* may have immigrated to Beibu Gulf from neighboring areas after it was filled with sea water and sufficiently deep. This initial panmictic stock quickly colonized the empty novel environment under the founder and priority effects, and experienced rapid population expansion when favorable conditions occurred (Boileau, Hebert, & Schwartz, 1992; Shulman et al., 1983).

5 CONCLUSION

A homogeneous population structure with low genetic diversity and star-like haplotype pattern was revealed for *E. cardinalis* in Beibu Gulf. Furthermore, pairwise comparisons of Φ_{ST} and the effective number of migrants between all sampling sites in Beibu Gulf indicated a single panmictic stock of *E. cardinalis* . This is mainly attributed to the free genetic exchange of *E. cardinalis*during reproduction. However, *E. cardinalis* from the eastern South China Sea cannot disperse into Beibu Gulf because of the circulation patterns and ocean geographical features such as the semi-closed sea of Beibu Gulf. This molecular evidence revealed that*E. cardinalis* from Beibu Gulf experienced colonization and population expansion during the late Pleistocene due to sea level fluctuations.

ACKNOWLEDGMENTS

We thank all colleagues and students for their help with sampling. This study was funded by Science and Technology Basic Resources Investigation Program of China (2018FY100105) and Fund of Guangdong Provincial Key Laboratory of Fishery Ecology and Environment (FEEL-2019-9). We thank Mallory Eckstut, PhD, from Liwen Bianji (Edanz) (https://www.liwenbianji.cn), for editing the language of a draft of this manuscript.

DISCLOSURE STATEMENT

None of the co-authors has any conflict of interest to declare. The authors alone are responsible for the content and writing of the paper.

AUTHOR CONTRIBUTIONS

Lei Xu : Conceptualization, Methodology, Formal analysis, Data curation, Writing - original draft, Writing - review & editing.**Kay Van Damme** : Writing - original draft.

Lianggen Wang , **Jiajia Ning** , **Delian Huang** ,**Yafang Li** , **Shuangshuang Liu** and **Hong Li** : Methodology, Formal analysis. **Feiyan Du** : Funding acquisition, Project administration, Resources.

DATA ACCESSIBILITY

DNA sequences: GenBank accessions: MW881382–MW881425.

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Table 1 . Descriptive statistics of genetic diversity of*Evynnis cardinalis* sampled from Beibu Gulf based on COI sequence data.

Sampling sites	N	H	S	h	π
S1	15	8	9	0.79±0.105	0.00231±0.0006
S2	15	9	9	0.886±0.069	0.0025±0.0005
S3	15	9	10	0.886±0.069	0.00253±0.0005
S4	15	6	7	0.705±0.114	0.00185±0.0005
S5	15	8	7	0.79±0.105	0.00203±0.0004
S6	15	7	7	0.657±0.138	0.00188±0.0005
S7	15	8	9	0.79±0.105	0.0025±0.0006
S8	15	10	12	0.857±0.09	0.00277±0.0006
S9	15	7	9	0.724±0.121	0.00231±0.0007
S10	16	10	11	0.825±0.098	0.00222±0.0005
S11	11	7	7	0.873±0.089	0.00277±0.0006
Overall	162	44	44	0.788±0.033	0.00232±0.0002

N , sample size; H , number of haplotypes; S , number of segregating sites; h , haplotype diversity (±S.D.); π , nucleotide diversity (±S.D.)

Table 2 . Pairwise differentiation Φ_{ST} of*Evynnis cardinalis* sampled from Beibu Gulf based on COI sequence

data.

	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11
S1	0										
S2	-0.0597	0									
S3	-0.0203	-0.0456	0								
S4	-0.0213	-0.0364	-0.0322	0							
S5	0.0041	-0.039	-0.0386	-0.0397	0						
S6	-0.0204	-0.0442	-0.0421	-0.0424	-0.0567	0					
S7	-0.033	-0.0403	-0.0276	-0.0246	-0.0079	-0.0509	0				
S8	-0.0283	-0.0275	-0.0197	-0.0505	-0.0113	-0.0239	-0.0323	0			
S9	-0.008	-0.0312	-0.0295	-0.0212	-0.0228	-0.0588	-0.0657	-0.0266	0		
S10	-0.0274	-0.0289	-0.0205	-0.0443	-0.0337	0.006	-0.0098	-0.0249	0.0014	0	
S11	-0.0476	-0.0141	0.0107	0.0281	0.0367	0.0671	0.0335	0.0302	0.0598	0.0037	0

Table 3 . Migration rates (number of migrants per generation) among *Evynnis cardinalis* sampling sites from Beibu Gulf (receives/exports).

	S1	S2	S3	S4	S5	S6	S7	S8	S9
S1	0								
S2	19.34/12.78	0							
S3	17.37/45.76	13.73/43.34	0						
S4	20.67/8.33	25.16/16.59	25.61/10.87	0					
S5	17.26/12.19	11.62/10.72	38.16/7.41	9.08/14.31	0				
S6	15.36/18.03	23.55/19.19	42.58/18.06	17.02/22.18	6.01/16.28	0			
S7	5.98/30.87	17.41/45.36	21.88/30.68	12.38/22.22	12.13/37.6	18.83/30.66	0		
S8	15.67/39.67	20.4/36.59	14.36/36.54	7.69/35.23	11.57/18.65	22.88/33.07	24.08/9.91	0	
S9	20.77/22.94	25.72/9.85	31.27/19.78	20.88/15.8	14.99/33.7	34.08/14.1	41.58/36.78	37.02/29.12	0
S10	23.62/40.04	7.89/30.56	41.27/28.81	8.65/38.6	6.98/36.74	20.98/26.3	22.18/28.17	31.06/24.21	22.9
S11	9.6/45.44	23.9/32.68	37.6/38.86	11.78/30.71	10.46/36.42	13.41/18.61	28.33/33.01	16.41/40.41	15.9

Table 4 Mismatch distribution, Tajima's D and Fu's Fs statistics for

Evynnis cardinalis

sites	Tajima's D	Tajima's D	Fu's Fs	Fu's Fs	Mismatch distribution	Mismatch distribution	Mismatch distribut
	D	p	Fs	p	τ (95% CI)	R index	R index p
S1	-1.471	0.05	-6.441	0	2.719 (0.758, 3.865)	0.151	0.27
S2	-1.591	0.04	-8.46	0	2.379 (0.84, 3.629)	0.221	0.08
S3	-1.844	0.015	-8.333	0	2.455 (0.955, 3.832)	0.235	0.05
S4	-1.39	0.044	-3.927	0.001	2.607 (0.852, 4.15)	0.178	0.4
S5	-1.359	0.102	-7.415	0	2.234 (0.932, 3.813)	0.352	0.07
S6	-0.863	0.248	-5.146	0	2.658 (1.123, 4.184)	0.15	0.33
S7	-1.219	0.132	-6.157	0.001	3.006 (1.061, 4.217)	0.115	0.41
S8	-1.796	0.014	-9.165	0	2.893 (0.963, 4.479)	0.163	0.23
S9	-1.319	0.12	-4.663	0	2.945 (0.459, 4.707)	0.068	0.74
S10	-1.944	0.003	-10.011	0	2.428 (0.781, 3.863)	0.275	0.05
S11	-0.73	0.26	-4.09	0.004	2.617 (1.654, 4.047)	0.154	0.37

sites	Tajima's D	Tajima's D	Fu's Fs	Fu's Fs	Mismatch distribution	Mismatch distribution	Mismatch distribut
total	-2.446	0	-28.378	0	1.531 (1.281, 1.941)	0.055	0.26

SSD = sum of squared deviation; R index = Raggedness index;

Figure Captions

Fig. 1. Map showing study area and 11 sampling locations in Beibu Gulf, South China Sea.

Fig. 2. Haplotype network for *Evynnis cardinalis* on the basis of nucleotide variation at the cytochrome c oxidase subunit I (COI) gene. Each circle represents a unique haplotype, and its size is proportional to the number of individuals sharing that specific haplotype. For each branch with more than one mutational step each mutational step is labelled.

Fig. 3. Pie diagrams showing the relative abundance of different haplotypes across the eleven sampling sites (total number of different haplotypes = 44). The size of each pie fraction is proportional to the fraction of individuals with a given haplotype.

Fig. 4. Observed mismatch distributions (bars) and expected mismatch distributions under sudden expansion model (line) of COI for*Evynnis cardinalis* from Beibu Gulf

Fig. 5. Bayesian skyline plot revealing the demographic trends of*Evynnis cardinalis* in Beibu Gulf. The dark solid line is median estimate under the assumption of per site mutation rate of 2% per million years. The dark gray solid line shows the median obtained under the assumption of per site mutation rate of 1% per million years, while the light gray bottom line shows the median obtained under the assumption of per site mutation rate of 3% per million years, and the dashed lines show the 95% highest posterior density (HPD) limits.