

RESEARCH ARTICLE

Phylogeography of the Rock Shell *Thais clavigera* (Mollusca): Evidence for Long-Distance Dispersal in the Northwestern Pacific

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Abstract

The present-day genetic structure of a species reflects both historical demography and patterns of contemporary gene flow among populations. To precisely understand how these factors shape current population structure of the northwestern (NW) Pacific marine gastropod, *Thais clavigera*, we determined the partial nucleotide sequences of the mitochondrial COI gene for 602 individuals sampled from 29 localities spanning almost the whole distribution of *T. clavigera* in the NW Pacific Ocean (~3,700 km). Results from population genetic and demographic analyses (AMOVA, Φ_{ST} -statistics, haplotype networks, Tajima's D , Fu's F_S , mismatch distribution, and Bayesian skyline plots) revealed a lack of genealogical branches or geographical clusters, and a high level of genetic (haplotype) diversity within each of studied population. Nevertheless, low but significant genetic structuring was detected among some geographical populations separated by the Changjiang River, suggesting the presence of geographical barriers to larval dispersal around this region. Several lines of evidence including significant negative Tajima's D and Fu's F_S statistics values, the unimodally shaped mismatch distribution, and Bayesian skyline plots suggest a population expansion at marine isotope stage 11 (MIS 11; 400 ka), the longest and warmest interglacial interval during the Pleistocene epoch. The lack of genetic structure among the great majority of the NW Pacific *T. clavigera* populations may be attributable to high gene flow by current-driven long-distance dispersal of prolonged planktonic larval phase of this species.

Introduction

The present-day genetic structure of a species reflects both historical demography (i.e., population history) and patterns of contemporary gene flow among populations [1]. An understanding of precisely how these factors shape current population structure has been one of the main issues in ecology and evolutionary biology. Historical demographic events might have a particularly large influence on the genetic structure if a species has not yet reached mutation-drift equilibrium owing to insufficient evolutionary time since a population expansion, which is often the case for marine organisms [2].

A series of marginal seas separates Asia from the Pacific, straddling the world's largest subduction zone of the Western Pacific [3]. During major glaciations, the large volume of accumulated ice reduced sea levels by approximately 120–140 m [

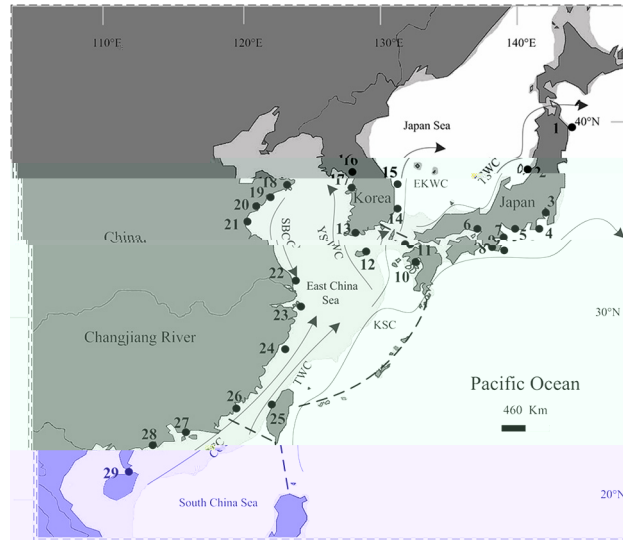


Fig 1. Map of East Asia showing the sampling sites of *Thais clavigera* and the summer ocean currents redrawn from [9]. Populations are labelled with numbers that correspond with those shown in Table 1. Shaded sea areas indicate regions 120 m in depth that would have been exposed during periods of low sea level. EKWC, East Korea Warm Current; TSWC, Tsushima Warm Current; KSC, Kuroshio Current; YSWC, Yellow Sea Warm Current; SBCC, Subei Coastal Current; CCC, China Coastal Current; TWC, Taiwan Warm Current. Dashed lines (—) represent a border of water bodies among South China Sea, East China Sea and Pacific Ocean.

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Most marine invertebrates remain in a pelagic larval stage for several days to months, and they may be able to disperse and metamorphose into sedentary adults [15]. It is generally thought that the propagule duration of marine species is significantly correlated with dispersal capacity [16]. Therefore, marine species with longer planktonic larval stages are expected to exhibit relatively low levels of population structure as a result of their increased opportunity for gene flow [11]. However, some surveys have shown that high levels of gene flow among populations are not always coupled with the duration of the pelagic larval stage. Small, but significant genetic structure has been observed in some marine invertebrates with high dispersal capability, including limpets [17], pen shells [18], and lobsters [19].

The rock shell *Thais clavigera* is the most common gastropod species in intertidal rocky shores of East Asia including China, Korea, Japan, and Taiwan. The life span of this species is a minimum of 7 years [20] and it is capable of adapting to eurythermic and euryhaline environments [21]. Although there is no precise estimate of the pelagic larval duration for *T. clavigera*, larvae of its congeneric species *T. haemastoma* [22] and *T. chocolata* [23] are known to last more than 60 days. Morphologically, *T. clavigera* exhibits a wide range of variation in shell sculpture, such as shape, the absence/presence of blotches on the nodules of shell surfaces, and shell apertures [24,25]. In a previous DNA barcoding analysis of Korean *Thais* species, a high degree of genetic divergence was discovered in some *T. clavigera* populations [26]. In addition, some genetic surveys of *T. clavigera* populations performed in certain NW Pacific localities have resulted in inconsistent conclusions [27–29]: Huang [29] revealed significant population structure along the China coast based on the partial sequence of mt COI; however, based on sequence variation in mt16S and COI gene fragments, Wang [27] concluded that there is no genetic differentiation between the ESC and SCS. Moreover, taxon sampling was limited to a few local areas in these studies (Taiwan [28], China Sea [27,29]), insufficient to understand genetic relationships among NW Pacific populations.

In the present study, to better understand contemporary genetic structure and historical demography of the NW Pacific *T. clavigera* populations, we sequenced a partial fragment of the mt COI gene from a total of 602 individuals sampled at 29 localities across the NW Pacific Ocean, including both its upstream (Taiwan and China mainland) and downstream (Korea and Japan) ranges. Specifically, we tested whether potential glacial refuges, ocean current systems, or freshwater outflows of the Changjiang River significantly influenced the current population genetic structure of *T. clavigera* in the NW Pacific.

Materials and Methods

Sample Collection and Sequencing

A total of 602 *T. clavigera* specimens were sampled from 29 localities across the NW Pacific Ocean spanning a distance of approximately 3,700 km in East Asia from September 2008 to June 2014 (Fig 1; Table 1). At least 20 individuals were collected and genetically analysed from each locality except two Japanese populations (Iwate and Kanagawa), from which only 10 and

Table 1. Sampling information, geographic coordinates, diversity indices, and neutrality tests for 29 *Thais clavigera* populations based on COI.

Locality	Coordinates	N	n	H	k	π	Tajima's D	Fu's Fs
1. Iwate Pref., Japan (IW)	39°38'N, 141°58'E	10	9	0.978	5.05	0.0077	-1.23	-3.62
2. Niigata Pref., Japan (NI)	37°55'N, 139°1'E	21	16	0.967	4.67	0.0071	-1.59	-8.35
3. Kanagawa Pref., Japan (KA)	35°16'N, 139°34'E	15	15	1	6.66	0.0101	-1.75	-10.11
4. Suzaki, Shizuoka Pref., Japan (SU)	34°37'N, 138°53'E	20	17	0.979	5.47	0.0083	-1.67	-9.84
5. Aichi Pref., Japan (AI)	34°42'N, 136°58'E	20	18	0.989	6.24	0.0095	-1.89	-11.04
6. Iwata river, Mie Pref., Japan (MI)	34°31'N, 136°42'E	20	17	0.979	5.26	0.008	-1.76	-10.17
7. Wakayama Pref., Japan (WA)	34°13'N, 135°9'E	20	17	0.984	5.36	0.0082	-1.72	-10
8. Shirahama, Wakayama Pref., Japan (SH)	33°41'N, 135°20'E	20	16	0.974	5.11	0.0078	-1.42	-8.34
9. Nishinomiya, Hyogo Pref., Japan (HY)	34°43'N, 135°20'E	21	19	0.991	5.65	0.0086	-1.48	-13.25
10. Nagasaki Pref., Japan (NA)	32°35'N, 129°45'E	20	16	0.963	5.61	0.0085	-1.76	-7.67
11. Fukuoka Pref., Japan (FU)	39°38'N, 130°12'E	20	18	0.984	6.26	0.0095	-1.77	-11
12. Seogwipo-si, Jeju-do, Korea (JJ)	33°13'N, 126°29'E	20	17	0.968	4.91	0.0075	-2.13	-10.76
13. Wando-gun, Jeollanam-do, Korea (JN)	34°11'N, 126°46'E	20	18	0.99	5.52	0.0084	-1.97	-12.11
14. Pohang-si, Gyeongsangbuk-do, Korea (PH)	35°59'N, 129°33'E	20	16	0.968	5.38	0.0082	-1.91	-7.96
15. Uljin-gun, Gyeongsangbuk-do, Korea (UJ)	37°06'N, 129°22'E	20	16	0.963	4.37	0.0066	-2.13	-9.58
16. Jung-gu, Incheon, Korea (IC)	37°32'N, 126°23'E	20	15	0.963	4.77	0.0072	-1.96	-7.04
17. Taeon-gun, Chungcheongnam-do, Korea (CN)	36°38'N, 126°17'E	20	18	0.99	6.77	0.0102	-1.52	-10.33
18. Wendeng, Shandong, China (WD)	36°54'N 122°03' E	30	22	0.97	4.49	0.0068	-1.77	-14.8
19. Qingdao, Shandong, China (QD)	36°06'N 120°34' E	25	21	0.983	4.8	0.0073	-1.97	-15.62
20. Rizhao, Shandong, China (RZ)	35°23'N 119°34' E	25	19	0.973	4.23	0.0064	-1.92	-12.49
21. Lianyungang, Jiangsu, China (LYG)	34°56'N 119°13' E	22	15	0.944	4.65	0.0071	-1.71	-6.19
22. Nantong, Jiangsu, China (NT)	32°08'N 121°32' E	20	15	0.963	4.38	0.0067	-1.99	-7.63
23. Zhoushan, Zhejiang, China (ZS)	30°04'N 122°16' E	24	20	0.975	4.88	0.0074	-1.8	-13.96
24. Wenzhou, Zhejiang, China (WZ)	27°36'N 121°09' E	23	19	0.98	6.36	0.0097	-1.6	-10.06
25. Changhua, Taiwan (CH)	24°01'N 120°21' E	20	13	0.947	5.8	0.0088	-0.75	-3.17
26. Dongshan, Fujian, China (DS)	23°35'N 117°26' E	22	19	0.983	4.97	0.0076	-2.02	-13.37
27. Shenzhen, Guangdong, China (SZ)	22°34'N 114°18' E	20	20	1	5.64	0.0086	-1.98	-18.31
28. Yangjiang, Guangdong, China (YJ)	21°46'N 112°04' E	24	20	0.975	5.38	0.0082	-1.74	-12.96
29. Haikou, Hainan, China (HK)	20°08'N 110°15' E	20	16	0.974	6.66	0.0101	-0.8	-6.48
Average		20.8	17	0.976	5.36	0.0081	-1.71	-10.21
Total		602	298	0.982	5.34	0.0081	-2.34	-663.21

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15 samples were obtained, respectively. *T. clavigera* is not an endangered or protected species, and therefore all collections were made from public access area without specific permits.

Total genomic DNA was extracted using the E.Z.N.A. Mollusc DNA Kit (Omega Bio-Tek Inc., Norcross, GA, USA) following the instructions supplied by the manufacturer. A universal primer set (LCO1490: 5'-GGTCAACAAATCATAAAGATATTGG-3', HCO2198: 5'-TAAAC TTCAGGGTGACCAAAAAATCA-3' —

Table 2. Analysis of molecular variance (AMOVA) results of population structure. Significant *P*-values are indicated in bold.

Grouping	Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	Φ-Statistics	<i>P</i> value
Potential refugia (PC: 1, 3–11; ES (SJ): 2, 14,15; ECS: 12–26; SCS:27–29)	Among groups	3	27.1	0.04	1.61	Φ _{CT} = 0.016	< 0.001
	Among populations within groups	25	85.5	0.04	1.47	Φ _{SC} = 0.015	< 0.001
	Within populations	573	1490.9	2.60	96.92	Φ _{ST} = 0.031	< 0.001
Changjiang River (CJN: 18–22; CJS: 23–24, 26–29)	Among groups	1	20.5	0.14	5.14	Φ _{CT} = 0.051	0.003
	Among populations within groups	9	28.0	0.03	1.02	Φ _{SC} = 0.011	0.040
	Within populations	244	608.0	2.49	93.84	Φ _{ST} = 0.062	< 0.001
Ocean currents (KSC: 1–17, 25; CCSC: 18–24, 26–29)	Among groups	1	15.4	0.04	1.47	Φ _{CT} = 0.015	0.002
	Among populations within groups	27	97.3	0.05	1.80	Φ _{SC} = 0.018	< 0.001
	Within populations	573	1490.9	2.60	96.74	Φ _{ST} = 0.033	< 0.001

Potential refugia: PC, Pacific Ocean; ECS, East China Sea; SCS, South China Sea; ES, East Sea (SJ, Sea of Japan). Ocean currents: KSC, Kruoshio Current with the branch currents; CCSC, China Coastal Sea Current including China Coastal Current and Subei Coastal Current. Changjiang River: CJS, the South of Changjiang River; CJN, the North of Changjiang River.

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CSCC included 18–24 and 26–29. These AMOVA analyses partitioned the total molecular variance among groups (Φ_{CT}), among populations within groups (Φ_{SC}), and among populations whatever the groups (Φ_{ST}) and tested if those Φ-statistic values were statistically significant using 10,000 random permutations. The HKY model, which fit the data best according to jMODELTEST, cannot be implemented in ARLEQUIN, so the Tamura-Nei model was selected to correct for multiple substitutions.

Pairwise genetic differentiation between all 29 populations was further assessed with Φ-statistics in ARLEQUIN 3.5 [33]. The significance of each pairwise comparison was estimated using 10,000 permutations, and corrections for multiple tests were made following a sequential Bonferroni procedure [41]. To estimate isolation by distance (IBD), a nonparametric Mantel test was performed online to evaluate the association between matrices of pairwise comparisons among sampling locations of shortest geographical distances (estimated in km using Google Earth version 4.3) and genetic distances (log-transformed) with 10,000 randomizations of the data using Isolation By Distance Web Service 3.23 [42].

Demographic Analyses

Historical demographic analysis was conducted using two different methods. Tajima's *D* [43] and Fu's *F_s* [44] statistics were calculated to test for neutrality using DNASP. The significance levels of Tajima's *D* and Fu's *F_s* were evaluated under 10,000 permutations. The parameter τ obtained from the mismatch distribution was used to estimate the time elapsed since the sudden population expansion using the equation τ = 2μt, where μ is the mutation rate of the marker (per locus per generation) and t is the number of generations (Rogers & Harpending, 1992). In order to convert parameters into quantitative estimates of time, we used a mutation rate of 7.9 × 10⁻⁹ substitutions/site/year, the genus *Nucella*-specific average substitution rate

for COI [45], because *Nucella* is ever known the most closely relatives of *T. clavigera* (belongs to the same family Muricidae) in which fossil-based molecular clock calibration is thus far available. The generation time was assumed to be 1 year.

For comparison with the mismatch distribution analysis, a Bayesian Skyline Plot (BSP) analysis was executed to examine changes in population size across time in Beast v1.7 software [46]. Three independent MCMC samplings were performed to assure the consistency of the results. Chains were run for 100 million generations and sampled every 1,000 generations, with the first 10% of generations discarded as “burn-in” under the HKY+G+I model determined by jMODELTEST v.0.1.1 with a constant skyline model and Bayesian skyline tree priors. All operators were optimized automatically. In all runs, the effective sample size yielded by MCMC chains for the parameters of interest was greater than 300. Finally, the results were visualized with the TRACER 1.6 software program [47].

Results

Genetic Diversity and Phylogenetic Relationships

A 658 bp sequence of the mt COI gene fragment was determined for 602 individuals of 29 *T. clavigera* populations sampled across the NW Pacific Ocean (Table 1; Fig 1). A total of 165 polymorphic sites were identified and 298 haplotypes were encountered. No deletion or insertion mutations were detected. Of these 165 variable sites, 44 were single-nucleotide polymorphic, and the remains were parsimony informative. The COI data in NW Pacific *T. clavigera* populations showed a high level of genetic diversity; haplotype diversity (h) for all studied populations was 0.982, ranging from 0.944 to 1.000. Nucleotide diversity (π) for all examined samples was 0.0081 and ranged from 0.0064 to 0.0102 for individual populations. The average number of nucleotide differences (k) between haplotypes was 5.34 (Table 1).

The phylogenetic trees for *T. clavigera* mtDNA COI sequence data inferred using NJ and BI methods were both unresolved, i.e., all nodes received low bootstrap support (all nodes $\leq 50\%$), suggesting that the 298 haplotypes sampled in all study populations lacked phylogeographic structure (S1 Fig). For three different groupings based on either potential glacial refuges, the Changjiang River, or the present-day ocean current systems, the centrality of the haplotype networks was occupied by the two most dominant haplotypes; however, these accounted for less than 20% of all sampled individuals (Fig 2). Furthermore, regardless of how we divided these populations into groups, there were no significant genealogical branches or geographic clusters detected in the three haplotype networks. For potential refuges, the two most dominant haplotypes accounted for 16.8% (101/602) of the total individuals (Fig 2A). The most dominant haplotype (9.5%, 57/602) occurred in all populations except Chungcheongnam-do (CN), Pohang-si (PH), Zhoushan (ZS), and Haikou (HK). We detected the

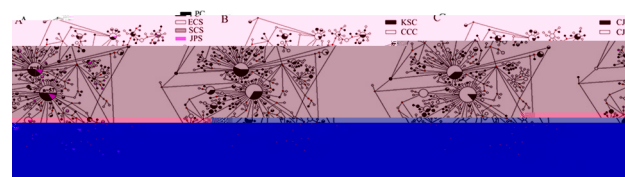


Fig 2. Network of *Thais clavigera* using COI data. The size of circles is proportional to haplotype frequency and median vectors are indicated with a red pie chart. The different shadings of the haplotypes refer to the respective locations in terms of the three population groupings: (a) potential refuges (PC, Pacific Ocean; ECS, East China Sea; SCS, South China Sea; ES, East Sea [SJ, Sea of Japan]); (b) ocean currents (KSC, Kuroshio Current with the branch currents; CCC, China Coastal Current and Subei Coastal Current); (c) Changjiang River (CJS, the South of Changjiang River; CJN, the North of Changjiang River).

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second most dominant haplotype (7.3%, 44/602) in 22 out of 29 populations. The great major-

Table 3. Pairwise Φ_{ST} among *Thais clavigera* populations based on COI (below diagonal) and associated *P*-values (above diagonal) after the sequential Bonferroni correction.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	
	IW	NI	KA	SU	AI	HY	MI	SH	WA	NA	FU	JJ	JN	PH	UJ	IC	CN	WD	OD	RZ	LYG	NT	ZS	WZ	CH	DS	SZ	YJ	HK	
1.	*	0.091	0.807	0.756	0.366	0.835	0.376	0.387	0.406	0.233	0.663	0.659	0.833	0.383	0.255	0.054	0.188	0.038	0.101	0.128	0.194	0.033	0.001	0.058	0.025	0.041	0.001	0.022	0.022	0.01
2.	NI	0.038	*	0.421	0.109	0.418	0.068	0.72	0.094	0.433	0.483	0.221	0.265	0.415	0.002	0.277	0.297	0.119	0.137	0	0.002	0.047	0.134	0.025	0.324	0.16	0.29	0.109	0.344	0.204
3.	KA	-0.02	0	*	0.687	0.979	0.965	0.488	0.193	0.689	0.688	0.821	0.982	0.984	0.408	0.911	0.758	0.71	0.674	0.231	0.598	0.764	0.243	0.003	0.191	0.078	0.245	0.004	0.126	0.012
4.	SU	-0.018	0.022	-0.009	*	0.492	0.905	0.41	0.482	0.457	0.337	0.669	0.826	0.906	0.782	0.669	0.125	0.123	0.055	0.089	0.137	0.034	0.037	0	0.022	0.007	0.046	0.001	0.006	0.002
5.	AI	0.004	0	-0.024	-0.002	*	0.877	0.543	0.137	0.36	0.834	0.871	0.748	0.665	0.336	0.794	0.717	0.583	0.916	0.191	0.506	0.585	0.181	0.003	0.165	0.153	0.254	0.006	0.133	0.033
6.	HY	-0.025	0.026	-0.025	-0.018	-0.016	*	0.351	0.2	0.293	0.391	0.912	0.934	0.96	0.613	0.795	0.383	0.167	0.16	0.321	0.625	0.437	0.1	0.001	0.039	0.031	0.077	0	0.017	0.002
7.	MI	0.003	-0.012	-0.003	-0.001	-0.004	0.003	*	0.433	0.783	0.477	0.289	0.549	0.66	0.012	0.274	0.161	0.181	0.164	0.002	0.005	0.07	0.266	0.013	0.409	0.238	0.294	0.047	0.222	0.114
8.	SH	0.001	0.027	0.012	-0.004	0.016	0.011	-0.002	*	0.664	0.215	0.205	0.222	0.275	0.085	0.168	0.014	0.014	0.003	0.001	0.002	0.006	0.023	0	0.035	0.009	0.035	0.001	0.008	0.008
9.	WA	-0.001	-0.001	-0.001	-0.003	0.002	0.005	-0.014	-0.011	*	0.263	0.282	0.439	0.658	0.065	0.275	0.057	0.043	0.05	0.001	0.005	0.031	0.085	0.002	0.134	0.08	0.096	0.006	0.039	0.017
10.	NA	0.015	-0.004	-0.009	0.004	-0.015	0.002	-0.003	0.013	0.008	*	0.719	0.683	0.441	0.044	0.784	0.534	0.397	0.329	0.041	0.15	0.154	0.167	0.001	0.352	0.135	0.398	0.014	0.173	0.167
11.	FU	-0.011	0.011	-0.014	-0.007	-0.015	-0.017	0.005	0.011	0.006	-0.01	*	0.646	0.657	0.37	0.857	0.264	0.739	0.411	0.244	0.675	0.558	0.061	0	0.069	0.075	0.091	0.001	0.029	0.018
12.	JJ	-0.012	0.007	-0.024	-0.014	-0.01	-0.019	-0.005	0.01	-0.001	-0.008	-0.006	*	0.962	0.139	0.939	0.732	0.083	0.234	0.046	0.188	0.257	0.269	0.001	0.059	0.031	0.21	0.001	0.054	0.002
13.	JN	-0.022	0	-0.027	-0.018	-0.007	-0.021	-0.009	0.006	-0.009	-0.001	-0.006	-0.019	*	0.31	0.793	0.438	0.215	0.088	0.038	0.205	0.162	0.227	0.002	0.104	0.038	0.238	0.002	0.09	0.008
14.	PH	0.002	0.066	0.001	-0.012	0.004	-0.007	0.046	0.024	0.027	0.03	0.002	0.016	0.004	*	0.191	0.022	0.054	0.011	0.379	0.575	0.026	0.001	0	0.001	0.002	0	0	0	0
15.	UJ	0.011	0.009	-0.018	-0.009	-0.012	-0.012	0.006	0.015	0.007	-0.015	-0.014	-0.011	0.011	*	0.608	0.372	0.373	0.272	0.432	0.416	0.202	0.002	0.117	0.075	0.457	0.001	0.107	0.013	
16.	IC	0.037	0.006	-0.011	0.015	-0.009	0.001	0.012	0.048	0.026	-0.003	0.006	-0.009	-0.001	0.038	-0.006	*	0.227	0.558	0.303	0.303	0.78	0.566	0.01	0.089	0.059	0.472	0.012	0.196	0.011
17.	CN	0.019	0.019	-0.01	0.018	-0.005	0.013	0.013	0.045	0.031	0.001	-0.01	0.019	0.01	0.026	0.002	0.009	*	0.458	0.178	0.335	0.571	0.045	0	0.081	0.081	0.112	0.002	0.1	0.03
18.	WD	0.046	0.014	-0.009	0.025	-0.016	0.012	0.013	0.059	0.027	0.003	0.001	0.007	0.018	0.043	0.001	-0.005	-0.001	*	0.043	0.193	0.595	0.065	0.002	0.063	0.045	0.101	0.004	0.04	0.003
19.	OD	0.026	0.072	0.009	0.019	0.01	0.004	0.057	0.062	0.063	0.026	0.007	0.024	0.025	0.002	0.006	0.022	0.011	0.026	*	0.902	0.216	0.003	0	0	0.003	0	0	0	0
20.	RZ	0.023	0.056	-0.006	0.013	-0.002	-0.007	0.048	0.06	0.047	0.013	-0.007	0.01	0.009	-0.005	0	0.005	0.004	0.01	-0.014	*	0.6	0.004	0	0.001	0.002	0.007	0	0.001	0
21.	LYG	0.019	0.028	-0.013	0.029	-0.005	-0.001	0.023	0.059	0.035	0.014	-0.005	0.006	0.011	0.038	0	-0.013	-0.005	-0.006	0.009	-0.006	*	0.158	0.004	0.034	0.029	0.206	0.006	0.068	0.002
22.	NT	0.052	0.015	0.008	0.035	0.009	0.02	0.007	0.048	0.024	0.013	0.024	0.004	0.007	0.077	0.008	-0.006	0.029	0.022	0.064	0.057	0.013	*	0.177	0.153	0.122	0.984	0.045	0.694	0.019
23.	ZS	0.115	0.034	0.061	0.102	0.056	0.085	0.042	0.107	0.066	0.07	0.076	0.068	0.064	0.153	0.07	0.045	0.077	0.062	0.139	0.128	0.06	0.012	*	0.055	0.061	0.149	0.663	0.301	0.032
24.	WZ	0.049	0.005	0.014	0.043	0.015	0.033	0	0.043	0.019	0.003	0.027	0.028	0.022	0.085	0.024	0.022	0.025	0.024	0.083	0.072	0.033	0.016	0.024	*	0.529	0.587	0.394	0.519	0.535
25.	CH	0.072	0.02	0.03	0.058	0.018	0.041	0.011	0.065	0.03	0.024	0.028	0.038	0.037	0.096	0.032	0.032	0.027	0.032	0.089	0.076	0.043	0.023	0.028	-0.006	*	0.228	0.08	0.328	0.242
26.	DS	0.045	0.007	0.007	0.029	0.006	0.021	0.005	0.036	0.02	0.002	0.019	0.007	0.007	0.068	-0.002	-0.003	0.019	0.016	0.057	0.047	0.008	-0.02	0.013	-0.008	0.012	*	0.109	0.98	0.125
27.	SZ	0.112	0.022	0.056	0.1	0.049	0.086	0.031	0.098	0.058	0.05	0.074	0.071	0.071	0.155	0.08	0.046	0.064	0.054	0.143	0.134	0.059	0.029	-0.007	0	0.028	0.018	*	0.362	0.249
28.	YJ	0.065	0.002	0.018	0.055	0.016	0.044	0.009	0.055	0.032	0.016	0.034	0.026	0.022	0.097	0.022	0.011	0.021	0.027	0.086	0.074	0.024	-0.01	0.004	-0.004	0.004	-0.023	0.002	*	0.271
29.	HK	0.095	0.016	0.06	0.083	0.045	0.084	0.024	0.071	0.054	0.021	0.054	0.076	0.063	0.13	0.067	0.056	0.046	0.066	0.127	0.118	0.079	0.052	0.039	-0.008	0.013	0.023	0.01	0.007	*

Significant pairwise Φ_{ST} and *P* values are bolded (*P* < 0.05). See Table 1 for detailed information on site abbreviation.

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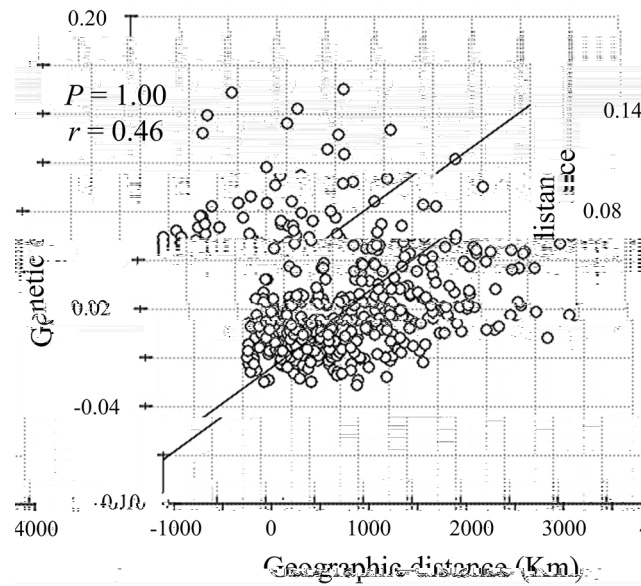


Fig 3. Isolation by distance plot for all *Thais clavigera* populations from the northwestern Pacific Ocean.

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variation among the NW Pacific populations was generally low, perhaps owing to a combination of high contemporary gene flow and recent common ancestry of haplotypes. Nevertheless, both AMOVA and pairwise Φ_{ST} analyses indicated weak, but significant genetic structure across the Changjiang River (Tables 2 & 3), suggesting the presence of geographical barriers to continuous larval dispersal at this locality; however, it should be noted that no signal of IBD was detected, nor did the haplotype networks show distinct genealogical branches or geographic clusters (Figs 2 & 3). These results support the hypothesis that the populations of *T. clavigera* that were examined have a high level of gene flow throughout the NW Pacific Ocean.

Although there are some exceptions [e.g., the marine clam genus *Lasaea* [48]], marine invertebrate species with a long-lived planktotrophic larval stage are generally capable of long-distance dispersal, and their offspring are spread several hundred to thousand kilometres away from their origin by the prevailing surface flow of the ocean current [16,49,50]; hence, the

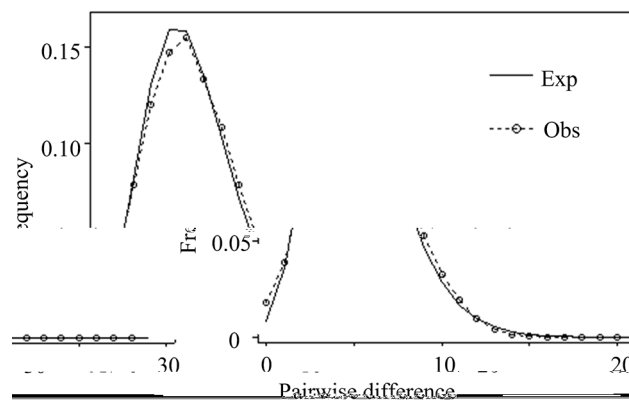


Fig 4. Mismatch distribution of *Thais clavigera* based on COI. The dotted line with circles represents the observed distribution, whereas the solid line shows the expected value under the sudden demographic expansion model.

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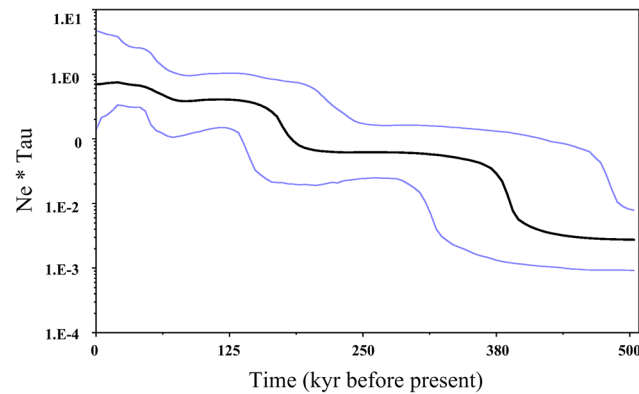


Fig 5. Demographic history of *Thais clavigera* estimated using Bayesian skyline plots from COI data. The black line represents the median population estimates, while the blue lines are the upper and lower bounds of the 95% confidence interval.

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long-distance dispersal associated with a prolonged pelagic larval stage and the present-day oceanic current have been regarded as the most influential factors contributing to continuous gene flow over a wide geographic scale in many marine invertebrates, including molluscan species [11–13,18]. A reproductive ontogenetic study has reported that *T. clavigera* undergoes indirect development with a planktonic veliger larval stage [20], and its pelagic larval stage lasts up to approximately 2 months. Additionally, *T. clavigera* is perennial with an average lifespan of 7 years. The species can reach sexual maturity during their second year, and accordingly have a reproductive lifespan of approximately 6 years [20]. It should also be noted that in the NW Pacific, there are two influential current systems in surface water circulation (Fig 1), namely, the KSC (Kuroshio Current) and the CSCC (China Sea Coastal Current), with the KSC flowing northward year-round [51,52], and the CCC (China Coastal Current) entering the ECS (East China Sea) from the SCS (South China Sea) in the summer [7]. These prevailing currents transport a great number of warm-water marine species from their tropical centre to the north and expand their ranges [53]. The long planktonic larval stage in *T. clavigera* may facilitates gene flow by current-driven dispersal of pelagic larvae and consequently decreases genetic structure among distant populations spanning over 3,700 km in the NW Pacific coastline. Aside from long-distance dispersal ability and ocean currents, we hypothesize that its utilization of a wide range of habitats is a key factor for successful colonization in a new environments. *T. clavigera* is abundant in the intertidal zone over a wide range of environmental conditions including different temperatures and salinities [21]; thus its ability to inhabit a wide range of eurythermic and euryhaline environments may also indicate its potential to colonize new environments.

In this study, the AMOVA analysis and pairwise Φ_{ST} values (Tables 2 & 3) suggest that the Changjiang River poses a weak but significant barrier to gene flow among some *T. clavigera* populations, indicating that the larval pool is not well mixed geographically across this area despite the long planktonic larval stage. Nonetheless, it is evident that there is generally a low level of genetic structure among populations, and we did not detect geographic clusters in the haplotype networks, consistent with previous results in marine invertebrates with long-lived pelagic larvae [18,19]. In addition to potential glacial refuges in the NW Pacific region, the Changjiang River and ocean circulation systems are two potentially important geographical barriers shaping current population structure [5,14]. We observed genetic divergence ($\Phi_{CT} = 0.051, P = 0.003$) between the northern and southern populations of the Changjiang River in our AMOVA analysis (Table 2). As documented earlier, freshwater outflows from the

Changjiang River may act as physical barriers that limit northward dispersal of planktotrophic larvae from southern populations [5]. Genetic subdivision may also be attributed to the habitat of *T. clavigera* near the mouth of the Changjiang River. *T. clavigera* is most commonly found in shadowy crevices in intertidal rocky shores. There are relatively well developed mudflat areas formed by the deposition of sediments near the mouth of the Changjiang River. These conditions provide a relatively insufficient rocky shore substratum, and are consequentially unsuitable for the settlement of *T. clavigera* larvae. When specimens were sampled near the northern mouth of the Changjiang River (e.g., Nantong), only a few *T. clavigera* individuals were found; by contrast, near the southern mouth, *T. clavigera* were very abundant in the rocky seashore of the Zhoushan archipelago. This habitat discontinuity may have reduced effective gene flow between the northern and southern populations of the Changjiang River.

It has been reported that oceanographic patterns play an important role in maintaining genetic and phenotypic differentiation in the acorn barnacle *Tetraclita japonica* in the NW Pacific [14]. Moreover, in southern Australia, the major ocean currents influence the phylogeography and population structure of the intertidal barnacle *Catomerus polymerus* [54]; however, in the present study of *T. clavigera*, we found a lack of genetic structure across major ocean current systems, we found very low, but statistically significant genetic structure between the two major ocean circulation systems (CSCC and KSC) by AMOVA ($\Phi_{CT} = 0.015$, $P = 0.002$), but Φ_{SC} (i.e., structure among populations within groups) was equivalent and statistically significant ($\Phi_{SC} = 0.018$, $P < 0.001$). The lack of genetic structure between the two current systems may be attributable to high gene flow owing to the long spawning time and prolonged planktonic larval phase of *T. clavigera*. Our findings that the most common two haplotypes occur at almost every site support this high gene flow hypothesis. Some additional observations suggest that although no water mass from a sub-branch of the KSC reaches the SCS in the summer, in some years this does occur [55]. Moreover, in other seasons, a south-westward current from Kuroshio flowing into the SCS has been observed [55]. In southern China and Taiwan, *T. clavigera* spawning could occur from spring to summer (February to August) [20,28]; furthermore, its pelagic larval duration lasts up to approximately 2 months. Therefore, *T. clavigera* larvae are likely to enter and mix into the CSCC system from the Taiwan coastline, which may increase gene flow between populations in the two circulation systems to some extent, therefore resulting in a lack of genetic differentiation.

From haplotype network analysis for COI data, we found a complicated network pattern that suggested that *T. clavigera* populations underwent a demographic expansion (Fig 2). Also, the observed pattern of mtDNA variation in *T. clavigera* further supports the hypothesis of non-equilibrium historical processes such as population range expansion. We observed very high COI haplotype diversity due to an excess of singleton variants (76% of the 298 detected haplotypes) coupled with relatively low nucleotide diversity. The retention of a surplus of rare COI variants may indicate a recent population expansion of *T. clavigera*; otherwise, these rare variants are predicted to be eliminated by genetic drift [56]. This suggests that mutation-drift equilibrium has not yet been attained in *T. clavigera* in the NW Pacific [57], an interpretation consistent with the significantly negative neutrality test statistics, a clear unimodal mismatch distribution, and BSP analysis. Furthermore, both mismatch distribution and BSP revealed an MIS 11 population expansion approximately 400 ka (Figs 4 & 5). This stage is the longest and the warmest during the Pleistocene epoch [12] and has been described as a super-interglacial period because of its long duration of 25–30 kyr [58]. The sequence of land mollusc species fossils in the Chinese loess-soil shows that the summer monsoon was particularly strengthened during MIS 11, which is typical of warmer climates [59]. Paleontological and palaeoecological estimates of MIS 11 deposits from Japan, Hawaii, Bermuda, and the Bahamas suggest a global

sea level had risen during this stage [60–62]. Such climatic conditions are necessary to allow warm-water species to reach the northern Pacific and expand their range.

The Pleistocene glacial age, and particularly the last glacial maximum (LGM) approximately 20,000 years ago, had an important influence on the evolution and genetic structure of marine organisms. Many species in various marine realms appeared to arise at the beginning of LGM [2,63]; however, population expansion of *T. clavigera* is assumed to have occurred pre-LGM. These results differ from the traditional view of demographic expansion that it occurs during the period of LGM. Nevertheless, population expansion that occurred pre-LGM has been reported in species such as the cold-water barnacle *Chthamalus challengerii* [13] and the marine snail *Concholepas concholepas* [12]. In the NW Pacific, Ni *et al.* [7] also estimated a period of 120–140 ka that corresponded with dramatic population expansion of various species, including molluscs [13,64], fishes [65,66], and crustaceans [67,68]. These earlier reports indicate the importance of pre-LGM events in determining the demography of marine populations and should be considered in the future. Nonetheless, it is still unclear why the glacial events did not significantly impact the current population structure and demographic history of these species.

Conclusions

To better understand contemporary genetic structure and historical demography of the NW Pacific *T. clavigera* populations, we determined the partial sequence of the mt COI gene from 602 individuals sampled from 29 localities across the NW Pacific Ocean. We observed a high level of genetic diversity within each of sampled populations, and no significant genealogical branches or geographic clusters, suggesting high levels of gene flow among populations throughout the NW Pacific. Nevertheless, we detected low, but significant genetic differentiation that corresponds to habitat conditions and freshwater discharge from the Changjiang River. Since we used only a single mtDNA marker, further studies of *T. clavigera* using multiple nuclear markers are required to validate the observed genetic structure. Also, population genetic studies of other marine species with a planktonic larval phase in the region will provide additional insight into the phylogeographic patterns of NW Pacific organisms.

Supporting Information

S1 Fig. Neighbour-joining (NJ) tree of COI haplotypes for populations of *Thais clavigera* from the northwestern Pacific. Both NJ and Bayesian inference (BI) analyses yield the same topology. Bootstrap values for NJ (the former number) and the posterior probabilities for BI (the latter number) analyses are indicated at the nodes. (TIF)

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Author Contributions

Conceived and designed the experiments: JKP QL. Performed the experiments: XG DZ. Analyzed the data: XG DZ. Contributed reagents/materials/analysis tools: JKP QL. Wrote the

paper: XG JKP. Interpreted the data and edited the manuscript: HJL. Collected the samples: JKP DZ DJ SK CP LFK TN GN AM.

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