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Analysis of genetic diversity in two different shell colors of the giant triton snail (*Charonia tritonis*) based on mitochondrial COI sequences

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The giant triton snail (*Charonia tritonis*) is widely distributed in tropical coral reefs in the Indo-West Pacific. Its distribution areas in China include the Penghu Islands, Hengchun Peninsula, and Xisha Islands. In this study, we use Multivariate Analysis of Variance (MANOVA) to compare the live weight and shell length between different shell colors of *C. tritonis*. In addition, we conducted sequence analysis of the mitochondrial cytochrome oxidase (COI) gene to assess the affinity of *C. tritonis* with two different shell colors based on the sample we obtained. Then, we constructed phylogenetic trees using the maximum likelihood (ML) and Bayesian Inference methods, and constructed haplotype network diagrams. In addition, we performed Tajima's D and Fu's neutrality tests. The results show that The Partial mitochondrial COI sequences of 28 *C. tritonis* were all 603 base pairs in length, and seven haplotypes were detected from the samples, besides, the gene flow was calculated to be 11.78, the genetic differentiation coefficient was 0.02078. Our results indicated that the population size of *C. tritonis* remained relatively stable. Besides, the genetic and size differentiation between the two different shell colors was small, and individuals of *C. tritonis* with different shell colors belong to the same genetic clade. In fact, the two morphotypes could not be distinguished by both genetic and morphometric data. The mitochondrial COI gene fragments of the two different shell colors were sequenced and analyzed to accumulate information about the population genetics of *C. tritonis* and to provide a scientific basis for the conservation of its species resources.The giant triton snail (*Charonia tritonis*) is widely distributed in tropical coral reefs in the Indo-West Pacific. Its distribution areas in China include the Penghu Islands, Hengchun Peninsula, and Xisha Islands. In this study, we use Multivariate Analysis of Variance (MANOVA) to compare the live weight and shell length between different shell colors of *C. tritonis*. In addition, we conducted sequence analysis of the mitochondrial cytochrome oxidase (COI) gene to

assess the affinity of *C. tritonis* with two different shell colors based on the sample we obtained. Then, we constructed phylogenetic trees using the maximum likelihood (ML) and Bayesian Inference methods, and constructed haplotype network diagrams. In addition, we performed Tajima's D and Fu's neutrality tests. The results show that The Partial mitochondrial COI sequences of 28 *C. tritonis* were all 603 base pairs in length, and seven haplotypes were detected from the samples, besides, the gene flow was calculated to be 11.78, the genetic differentiation coefficient was 0.02078. Our results indicated that the population size of *C. tritonis* remained relatively stable. Besides, the genetic and size differentiation between the two different shell colors was small, and individuals of *C. tritonis* with different shell colors belong to the same genetic clade. In fact, the two morphotypes could not be distinguished by both genetic and morphometric data. The mitochondrial COI gene fragments of the two different shell colors were sequenced and analyzed to accumulate information about the population genetics of *C. tritonis* and to provide a scientific basis for the conservation of its species resources.

KEYWORDS

***C. tritonis*, mitochondrial COI gene, haplotypes, genetic differentiation, shell color**

1 Introduction

The giant triton snail (*Charonia tritonis*) is one of the world's largest gastropod snails, and it is protected by a narrow shell that can reach half a meter in length (Hall et al., 2017). As a predator of echinoderms, this snail plays an important role in protecting coral reef ecosystems, especially from the crown-of-thorns starfish (COTS). Even though an adult *C. tritonis* only eats 1.5 COTS a week on average, its presence can change the behavior of COTS (Evan et al., 2005), which can effectively reduce the density of COTS and the severity of COTS outbreaks. However, overfishing, water pollution, and lack of understanding of the biology of *C. tritonis* have negatively impacted its habitat, resulting in a sharp decline in wild populations of this species (Bellwood et al., 2004; Feng et al., 2018), and *C. tritonis* is now as well listed as a national second-class protected animal in China (Ren et al., 2022). Therefore, studies are needed to better understand the basic biology and population structure of *C. tritonis*.

The shells of mollusks are extremely colorful and diverse in morphology. They have been a fascinating subject throughout human history and are highly sought after by conchologists and shell enthusiasts (Liu et al., 2009). *C. tritonis* shells have a shiny surface, with a creamy white to yellowish-brown shell surface and half-moon or triangular dark brown markings and crescent-shaped markings (Qi, 1998). In the wild, *C. tritonis* with either white or flowery shells are widespread. Such color polymorphism usually has a simple genetic basis, which makes them an ideal system for studying the evolutionary mechanisms that maintain biodiversity (Estévez et al., 2020). In some marine gastropods, environmental factors (Creese and Underwood, 1976), genetic factors (Stolbova, 1996), and diet can impact shell coloration. The ability of a single genotype to alter its phenotype in a heterogeneous environment is an example of phenotypic plasticity (Houston and Mcnamara, 1992), and although environmentally induced alterations to shell color are usually not heritable, once formed they are stable and persistent (Liu et al., 2009). Accordingly, shell characters have historically played an important role in discrimination among species (Wilke and Falniowski, 2001).

The mitochondrial DNA genome is widely used in several research fields, including genetics, evolution, and species genetic diversity, due to its unique structural features and genetic characteristics (Brown et al., 1982; Wang et al., 2019; Zhang et al., 2021), and it has become a very important molecular genetics tool. The mitochondrial genome has been widely used in the study of genetic variation and phylogeny of gastropod populations (Grande et al., 2008; Groenenberg et al., 2012; Groenenberg et al., 2017). Mitochondrial cytochrome oxidase (COI) genes in mitochondrial DNA are very conserved and highly homologous and are often used to analyze and explore population phylogenetic relationships (Miya et al., 2001). The COI sequence, which encodes only about 650 nucleotides, contains an extremely rich phylogenetic signal that evolves at a moderate rate, allowing species to exhibit more pronounced intraspecific and interspecific variation (Kelly and Palumbi,

2009). Therefore, COI genes are considered to be the most desirable molecular markers for genetic structure studies, phylogenetic analysis, and species identification (Rydzanicz et al., 2011).

In the present study, the genetic diversity of two collected shell color populations (white shells and flower shells) of *C. tritonis* were studied by using partial sequences of mitochondrial COI. The purpose of this study is to provide comprehensive information about this genetic resource, clarify the genetic relationship between these two shell colors, and provide a scientific reference for the protection of *C. tritonis*.

2 Materials and methods

2.1 Sample collection

C. tritonis were collected from Tree Island, Xisha, South China Sea, Hainan Province, China in March 2020 by SCUBA diving. Since *C. tritonis* is a national second-class protected animal, it is precious and scarce in China, so we only obtained 28 individuals (6 with white shells and 22 with flower shells) in this study. It can be observed in Figure 1 that there are regular brown and black interlaced convex stripes on the surface of *C. tritonis* with flower shells, while the surface of *C. tritonis* with white shells is flat and without obvious stripes. The specimens were transported back to the breeding base and temporarily reared in water that was aerated and changed daily. Small pieces of the foot muscle of each specimen were excised and stored in 95% alcohol without harming the animal. Tissue samples were stored at 4°C in a freezer.

2.2 Mitochondrial sequencing

We isolated total DNA from the muscle tissue samples using the EasyPure® Marine Animal Genomic DNA Kit for marine organisms. The extracted DNA was subsequently examined by 1.2% agarose gel electrophoresis (Figure 2). The nucleic acid concentration and purity were evaluated using NanoDrop (Thermo Fisher Scientific, Waltham, MA, United States). Then, all samples were stored at 4°C.

FIGURE 2
Part of the electrophoresis results of the genomic DNA of *C. tritonis*.

The existing mitochondrial genome sequence (Query IDlcl|Query_276583) of *C. tritonis* was downloaded from NCBI (https://www.ncbi.nlm.nih.gov/), and subsequently the conserved regions were identified using BioEdit software comparison analysis. We designed the primers using Primer Premier 6.0, and the primers were synthesized by Beijing Liuhe Huada Gene Technology Co. (Beijing, China). PCR amplification of the synthesized primer pairs was carried out using DNA from the 28 *C. tritonis* specimens. The pairs of primers with single amplified bands and high efficiency were screened by agarose gel electrophoresis, and the specific primer sequences were:

COIa: 5′-GGTCAACAAATCATAAAGATATTGG-3′;

COIb: 5′- TAAACTTCAGGGTGACCAAAAAATCA-3′.

The 50 µL reaction system for PCR amplification contained 25 µL of 2 × Es taq enzyme (TransGen Biotech,Guangzhou, China), 2 mM forward primer/reverse primer, 100 ng of DNA template, and ultra-pure water to bring the solution to 50 µL. The PCR procedure was as follows: pre-denaturation at 94°C for 2 min; denaturation at 94°C for 30 s; annealing at 55°C for 30 s; and extension at 72°C for 1 min, with 34 cycles of pre-denaturation, annealing, and extension, 1 cycle of denaturation, and final extension at 72°C for 5 min.

The PCR products were detected on a 1.2% agarose gel (Figure 3), and purification was performed using a gel recovery kit (Omega, Atlanta, GA, United States). The purified PCR products were sent to Guangzhou Tianyi Huiyuan Biotechnology Co., Ltd. (Guangzhou, China) for double-end sequencing using amplification primers.

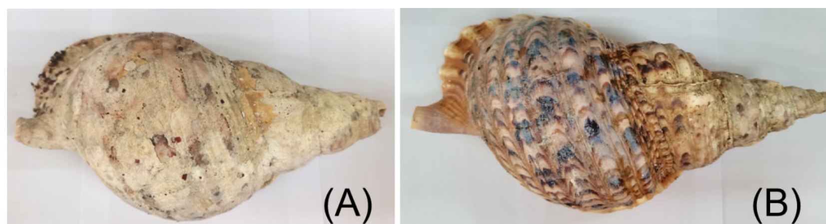

FIGURE 1
C tritonis with white shell **(A)**, *C tritonis* with flower shell **(B)**.

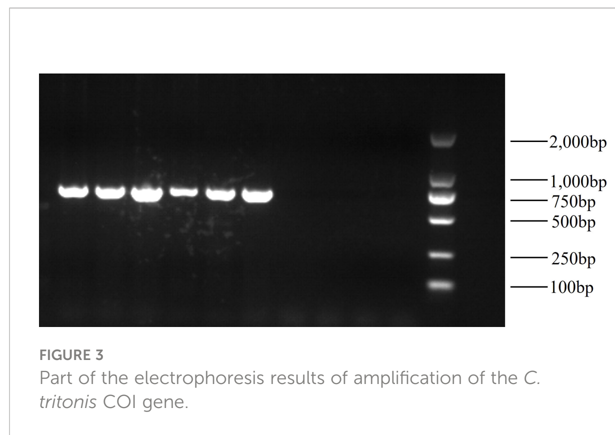

FIGURE 3
Part of the electrophoresis results of amplification of the *C. tritonis* COI gene.

The original image of Figures 2, 3 are in Supplementary Materials (Supplementary Figures 1, 2).

2.3 Sequence organization

We integrated and converted the 28 mitochondrial COI gene sequences into FASTA format and the 28 mitochondrial COI gene sequences of *C. tritonis* can be found here: NCBI, OP810573 to OP810600 (https://www.ncbi.nlm.nih.gov/nuccore/OP810600.1).

2.4 Sequence characterization

We used Mega6.0 software to analyze the base characteristics of the mitochondrial COI sequences and to determine the length of the COI sequences of the 28 *C. tritonis*. We then calculated the conserved sites, variable sites, parsimony sites, and singleton sites of the COI sequences, the proportion of bases A, T, C, and G in the sequences, the presence of base substitutions, and the ratio of reversal to conversion.

2.5 Building a phylogenetic tree

The COI sequence of *Ranella olearium*, a family of Cymatiidae, was downloaded by NCBI and used as an outer group. The ML phylogenetic tree was constructed by K2 model. The confidence of the nodes of the ML phylogenetic tree was estimated by Bootstrap value, and the reliability of the relationship tree was evaluated by 1,000 repeated sampling.

Bayesian evolutionary analysis sampling tree were constructed using MrBayes (Huelsenbeck and Ronquist, 2001), version 3.2.7. After using MrMTgui 1.01 to calculate the recommended model, results were produced from one chain with 30 million steps, sampled every 1,000 steps based on Markov Chain Monte Carlo (MCMC).

2.6 Data analysis

The live weight and shell length of all samples were measured, and these data were averaged and analyzed by MANOVA. MANOVA was conducted by SPSS25.0 with the shell color of *C. tritonis* as the independent variable and the body length and live weight of *C. tritonis* as the dependent variables.

The mitochondrial COI gene sequence of *C. tritonis* was transformed into FASTA format and then homology matched them using Clustal W in Mega6.0 software. After that, sequences with obvious differences were selected for reverse complementation, followed by secondary homology matching by Clustal W. Redundant sequences at both ends caused by sequencing technology limitations were removed by manual splicing to generate the COI gene sequence set. They were exported into FASTA format and used for subsequent data analysis.

Then, we used DnaSP 6.0 software to analyze the genetic diversity of the COI gene sequences and to calculate values for the following: number of polymorphic sites, number of haplotypes, haplotype diversity, nucleotide diversity, mean nucleotide differences, gene flow, Tajima's D and Fu's neutrality tests. The molecular variance analysis (AMOVA) method in Arlequin 3.1 software was used to estimate the distribution of genetic variation between and within populations, and the genetic differentiation coefficient (Fst) and its P value among populations were calculated. We then constructed a parsimonious haplotype network using PopART to analyze the evolutionary relationships among haplotypes.

3 Results

3.1 Morphological variation analysis

All samples were measured and the average shell length was 28.7 cm, with mean live weight 2.57 kg. We can observe from the Table 1 that in the test results for shell color of *C. tritonis*, the significance of shell length was 0.314 and the significance of body weight was 0.253, both of which were greater than 0.05, indicating that there were no significant differences in shell length and body weight levels of *C. tritonis* between different shell colors.

3.2 Sequence analysis of the mitochondrial COI gene fragment of *C. tritonis*

In this study, we were able to stably amplify the mitochondrial COI sequences of the 28 *C. tritonis* samples, and the PCR amplification products showed bright and clear bands 600–700 bp in size. After the amplification products were purified and sequenced, the mitochondrial COI sequences were

TABLE 1 Results of the multivariate analysis of variance (MANOVA) showing the difference of live weight and shell length of *C. Tritonis* with different shell colors.

Source	Dependent Variable	df	Mean Square	F	Sig.	Partial Eta Squared
shell color	Shell length	1	14.538	1.052	0.314	0.039
	Live weight	1	0.392	1.368	0.253	0.050
Total	Shell length	28				
	Live weight	28				

analyzed using Mega6.0 software, which revealed 588 conserved sites and 14 variable sites. Among the 14 variant sites, 9 were parsimony informative sites and the rest were single variant sites (Table 2). Table 3 shows the proportions of bases A, T, C, and G in the sequences of the fascias of the two different shell colors. The A +T content was higher than the G+C content, which was consistent with the characteristics of the mitochondrial base composition.

3.3 Genetic diversity of *C. tritonis* based on mitochondrial COI gene sequence information

Analysis of the codon distribution of COI gene sequences showed that the effective codon number was 52.715. The ratio of transitions to reversals was 2.8, and a value > 2 indicated that the mitochondrial COI gene sequences of *C. tritonis* were not yet saturated with mutations. Therefore, the results obtained from the subsequent genetic diversity analysis of COI genes were reliable.

Table 4 shows the results of the genetic diversity analysis based on mitochondrial COI gene sequence information. *C. tritonis* haplotype diversity reached 0.728 ± 0.067, which indicated high genetic diversity (Grant and Bowen, 1998).

The gene flow was 11.78, which is greater than 1, indicating that flow between the two types of *C. tritonis* was high, which could reduce the genetic differentiation between populations generated by genetic drift to some extent. The analysis of variance (AMOVA) results for the two populations of *C. tritonis* are shown in Table 5, which shows that the genetic

TABLE 2 Variable sites of the mitochondrial COI gene of *C. tritonis*.

	Variable site									
Haplotype		1	3	3	3	4	4	6		
	2	3	2	3	5	7	9	1	White shell	Flower shell
	4	0	1	3	1	6	2	5		
Hap_1	A	G	G	C	C	G	G	C	3	10
Hap_2	G	*	*	*	*	*	*	*	2	5
Hap_3	*	*	A	*	*	*	A	*	1	0
Hap_4	*	*	*	T	*	A	*	T	0	3
Hap_5	*	*	*	T	*	*	*	*	0	2
Hap_6	*	*	*	*	T	*	*	*	0	1
Hap_7	*	A	*	*	*	*	*	*	0	1

The "*" indicates that the nucleotide at this site is the same as the nucleotide at this site for Hap_1. For example, the second "*" of Hap_2 indicates that this haplotype has the same nucleotide at 130bp as Hap_1 at G.

TABLE 3 Base composition of the COI gene in *C. tritonis*.

Shell color	Base content/%					
	A	G	C	T	A+T	G+C
White	33.90	20.78	20.61	24.70	58.60	41.40
Flowery	33.89	20.78	20.56	24.77	58.66	41.34

TABLE 4 Genetic diversity based on COI gene sequences of *C. tritonis*.

	Samples	Number of haplotypes	Haplotype diversity	Nucleotide diversity	Polymorphism sites	Average number of nucleotide differences
C. tritonis	28	7	0.728 ± 0.067	0.00235 ± 0.00351	8	1.376

variation in the two *C. tritonis* populations occurred mainly within the population, accounting for 98.96%, while the among populations genetic variation accounted for 1.04%. Also, according to Table 5, the Fixation Index (Fst) is 0.01040 and the statistical results were not significant ($p > 0.05$), indicating that the two types of *C. tritonis* were closely related.

The Tajima's D neutrality test value for the *C. tritonis* population in this study was –1.37771 and $P > 0.05$, and the Fu's Fs statistic neutrality test value for the *C. tritonis* population in this study was –3.180 and $P > 0.05$ (Table 6), indicating that the population may not have experienced expansion and continuous growth patterns, and the population size remained relatively stable.

3.4 Phylogenetic relationships of *C. tritonis*

Taking the COI sequence of the triton *Ranella olearium* as the outgroup, we used the ML and Bayesian inference methods to construct phylogenetic trees and analyze the phylogenetic relationship among *Ranella olearium* and *C. tritonis* with different shell colors. Among them, the COI sequences of *C. tritonis* with white shells are represented by W1 to W6, while those of the other 22 *C. tritonis* with flower shells are represented by NW1 to NW22 (Figures 4, 5). The phylogenetic trees constructed by the two methods showed a consistent topology, with individuals of different shell colors of *C. tritonis* clustering together without any obvious group clustering first.

Figure 6 is a haplotype network diagram that shows the presence of seven haplotypes. Hap_1 includes the largest number of samples, and only Hap_1 and Hap_2 is common to both shell colors of *C. tritonis*. The other haplotypes that are unique to each shell color indicated that the different shell colors had their own genetic advantages.

4 Discussion

Mollusks are often known for their different shell forms, carvings and colors, and shell characteristics have historically played an important role in distinguishing species (Wilke and Falniowski, 2001). The Multivariate Analysis of Variance (MANOVA) indeed suggests that different shell length and live weight have no significant effect on the shell colors of *C. tritonis*. Similar results were observed in *Heleobia opachensis*, *Cyclonaias infucata* and *Echinolittorina malaccana* (Gonzalo et al., 2016; Lopes-Lima et al., 2019; Marshall et al., 2021), and this result corresponds to that reflected by the phylogenetic tree.

Genetic diversity of species is an important component of biodiversity and an important parameter in the assessment of the status of biological resources, while rich genetic diversity helps to enhance the adaptability of species to the environment (Wu et al., 2022). *C. tritonis* with different shell colors showed almost no genetic differences and exhibited high haplotype diversity, indicating high genetic diversity and abundant genetic resources, in addition *C. tritonis* exhibited low nucleotide diversity, which can result from large population sizes and different environments (Gollner et al., 2016), and we speculated that the high haplotype diversity of the *C. tritonis* population our study was most likely due to variable environmental factors. What we need to mention here is the special life history of the snail, The mating to spawning period for *C. tritonis* is about 130 days (Strathmann, 1987), and subsequently *C. tritonis* can maintain its floating life state for about 10 months without metamorphosis (Nugranad et al., 2000), therefore, when marine organisms are classified by ecological type, *C. tritonis* is a meroplankton, a fact that is consistent with the larger gene flow of the phasmid found in our study (Dunphy and Hamrick, 2005). Genetic data confirm that historic events have had a significant impact on the distribution of populations (Athanasios et al., 2006; Nakano and Ozawa,

TABLE 5 AMOVA analysis of two *C. tritonis* populations base on COI gene sequences.

Sources of variation	df	Sum of square	Variance component	Variance proportion (%)
Among populations	1	0.753	0.00720 Va	1.04
Within populations	26	17.818	0.68531 Vb	98.96
Total	27	18.571	0.69252	
Fixation index	Fst: 0.01040 (P>0.05)			

TABLE 6 Tajima's D and Fu's F statistic among *C. tritonis*.

	Samples	Tajima's D P	Fu's Fs statistic P
C. tritonis	28	-1.37771	-3.180
		P > 0.05	P > 0.05

2007). According to the theory of population genetics, a longer planktonic larval stage will lead to increased gene flow between geographically distant populations and, therefore, their level of genetic differentiation will decrease (Kelly and Palumbi, 2009). There are many examples of maintaining population connectivity through the dispersal of larvae, such as the main currents flowing along the Atlantic coast of Iberia could have been responsible for maintaining connectivity of populations of Patella rustica through occasional larval transport across a considerable stretch of inhospitable habitat (Ribeiro et al., 2010); Two economically important spiny lobsters, *Panulirus homarus* and *Panulirus ornatus* show no genetic differences from the western Indian Ocean to the Indo-Malay archipelago, because larval stage of lobster species had to go through a long floating state (Permana et al., 2019). Contrary to species with long planktonic period, species with short planktonic larval period can show strong population structure, such as the clam *Macridiscus multifarius*, which planktonic larval stage is about

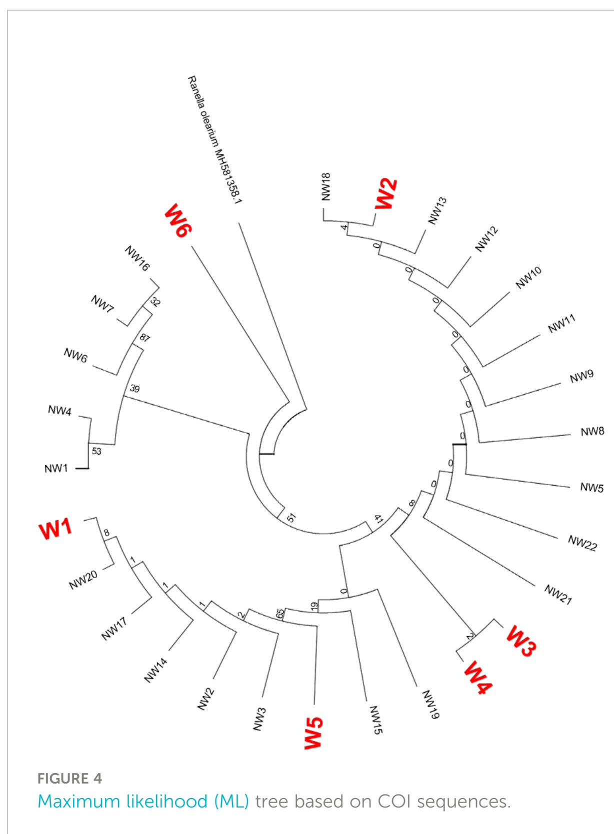

FIGURE 4
Maximum likelihood (ML) tree based on COI sequences.

10 days, high level and significant FST values were obtained among the different localities of *M. multifarius* on the basis of either COI or 12S rRNA gene, indicating a high degree of genetic differentiation among the populations (Ye et al., 2015). The two shell colors of *C. tritonis* in this study had high haplotype diversity as well as high gene flow and high genetic similarity, so we concluded that the different shell colors produced by *C. tritonis* in this study were not caused by genetic differences.

According to the standard put forward by Balloux et al. (Balloux and Lugon-Moulin, 2002), Fst values of 0-0.05, 0.05-0.15, and 0.15-0.25 indicate low, medium, and high levels of genetic differentiation between populations, respectively. In this study, the ANOVA results showed that the coefficient of genetic differentiation (FST) between populations was 0.01040, indicating that the genetic differences between the two populations of *C. tritonis* were not significant and there was no obvious genetic structure of the population. In addition, the results of AMOVA analysis showed that the variation of individuals within the population was the main source of genetic variation and that different shell color *C. tritonis* did not form a separate genetic structure, so we could not judge the shell color of *C. tritonis* by COI gene.

The shells of mollusks are produced by the outer folds of the mantle, and shell growth and pigmentation are under the control of neurosecretion (Boettiger et al., 2009; Budd et al., 2014). The shell pigmentation is caused by uroporphyrin I and that the amount of pigment produced is not genetically controlled but is

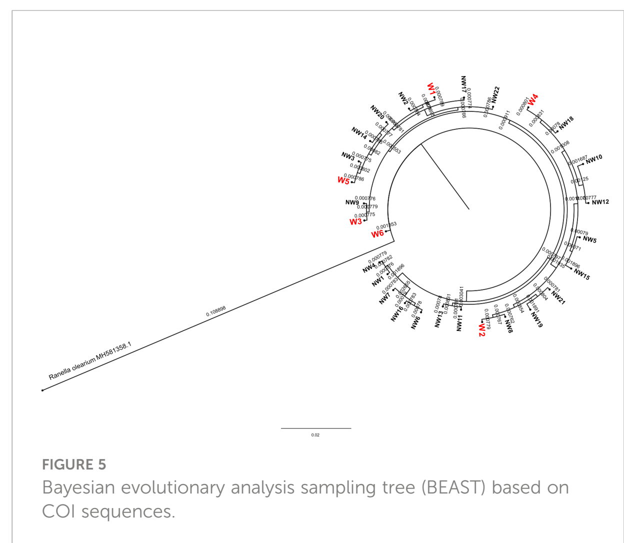

FIGURE 5
Bayesian evolutionary analysis sampling tree (BEAST) based on COI sequences.

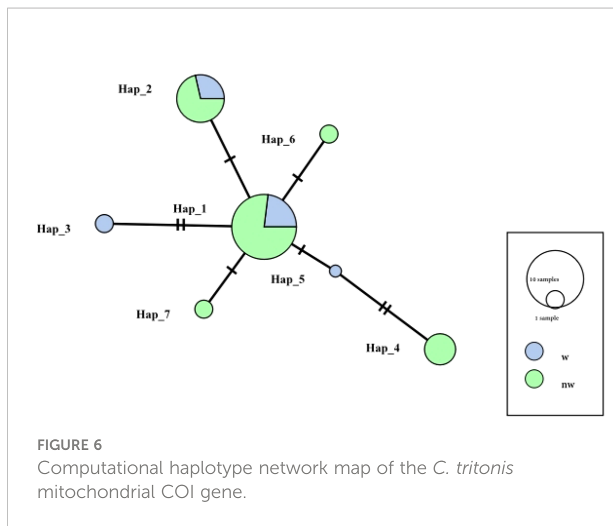

FIGURE 6
Computational haplotype network map of the *C. tritonis* mitochondrial COI gene.

influenced by environmental factors (Creese and Underwood, 1976), in addition, Comfort suggests that the porphyrins found in gastropod shells may be derived from the animal's diet (Comfort, 1950). According to Manriquez et al. The shell color pattern of the abalone *Cocholepas concholepas* during early development depends on the color of the more abundant prey consumed, and they even change their shell color to adapt to different prey. Etter (Etter, 1988) found that the whelk *Nucella lapillus* showed considerable shell color variation both within and between populations. They reported that populations from high wave energy shores tended to be highly polymorphic and predominantly brown, while those located in more secluded locations showed less polymorphism and are predominantly white, which was in fact a selective gradient response to physiological stress. The frequency of the snail *Littorina fabalis* shell coloration and sexual competence were significantly correlated, showing a negative frequency-dependent selection pattern (i.e., high asymmetry in color mating) (Estévez et al., 2020). *L. fabalis* also exhibited a preference for rare or novel color patterns in mate selection. Due to the high dispersal of *C. tritonis* larvae, and *C. tritonis* feeds mainly on sea cucumbers, sea stars, and mother-of-pearl mussels. Thus, local adaptation to one prey species was impossible for *C. tritonis*, and natural selection drove the evolution of corresponding adaptations and flexibility, such as color plasticity. Different shell colors resulting from phenotypic plasticity due to environmental factors are indistinguishable by genetic information. It was found that three genetic species of *Mactra chinensis* could not be determined reliably based on analysis of the shell coloration due to the plasticity of these morphological characters (Reunov et al., 2021); in *Corbicula* population, morphotypes defined by internal shell color were indistinguishable in genetic background (Li et al., 2022), and there are also examples of different species with the same shell color (Williams et al., 2016), different species of marine snails *Clanculus pharaonius* and *Clanculus margaritarius* were examined that contained different pigments but produced similar colors. This fact shows that there may be some adaptive value for these colors and patterns despite differences in their habitats. The above examples show that the difference of shell color of *C. tritonis* may be the result of phenotypic plasticity due to adaptation to the environment, but this difference cannot be distinguished by COI gene, and it also shows that shell color may be a bad taxonomic feature for *C. tritonis*.

Both seaweeds and barnacles are similar to *C. tritonis* in that currents have a certain influence on their distribution. However, their evolutionary driving forces are different. Dietary specialization on particular prey types was crucial in the evolution of gastropods (Wagele, 2004; Goodheart et al., 2017), *Conus geographus* that belong to the same gastropods as *C. tritonis*, in order to catch as many fish as possible, *Conus geographus* evolved special adaptive ability, which made it possible for snails to catch a whole group of fish by net fishing strategy (Olivera et al., 2015). The maritime environment helps drive plant community homogenization (Gurgel et al., 2014), many seaweed species float on the sea surface and go with the currents after leaving their benthic habitats (Macaya et al., 2016). Different ecological niches will have different evolutionary driving forces, unlike *C. tritonis* which is a predator, seaweed as a primary producer in the ocean, are influenced by a variety of factors, including abiotic stress (Seapy and Littler, 1982), nutritional availability (Bracken and Nielsen, 2004; Kraufvelin et al., 2010) and herbivore (Nielsen, 2003; Altieri et al., 2009). In addition, the barnacles are sessile crustaceans with planktonic larval stages (Maruzzo et al., 2012), similar to *C. tritonis*, which is influenced by ocean currents. However, adult barnacles live permanently in a parasitic state attached to a wide range of substrates and other organisms (Power et al., 2010). Different lifestyles stimulate different driving forces for evolution, unlike *C. tritonis*, differentiation of barnacles is considered to be related to their hosts (Via, 2001). Invasion and subsequent adaptation to new hosts will promote reproductive isolation by selecting unsuitable hybrids, leading to genetic differentiation and eventually species differentiation (Schluter, 2001; Turelli et al., 2001). A high degree of morphological and genetic variation can be exhibited in barnacle populations from different coral hosts, such as *Savignium dentatum* (Mokady et al., 1999). In contrast, some coral barnacles, such as Cantellius species, can appear on a wide range of hosts and invade different kinds of corals and these barnacles were similar to the *C. tritonis* observed in this study, showing obvious phenotypic differences but lack of genetic differentiation (Mokady et al., 1999). Seaweeds and barnacles have similar planktonic stages to *C. tritonis*, but different lifestyles lead to different evolutionary drivers, with seaweeds evolving for fuller photosynthesis, barnacles evolving for host

adaptation, and *C. tritonis* evolving for dietary specialization, according to Goodheart and Wagele's theory (Wagele, 2004; Goodheart et al., 2017). Perhaps *C. tritonis* of different shell colors differ in their food preferences, but due to the lack of geographic isolation, they can only show distinct phenotypic differences and cannot produce differences at the genetic level.

In summary, in this study we used morphological data and DNA sequences of the COI gene to analyze differences between *C. tritonis* with two different shell colors. It was found that different shell lengths and live weight had no significant effect on the shell coloration of *C. tritonis*, and we hardly detected any genetic differences between the two shell colors. This fact suggests that the variation of shell color of *C. tritonis* may be the result of phenotypic plasticity caused by environmental factors or genetic differences, independent of body length and body weight, also, without forming independent genetic structures. Our research will provide essential information for the conservation of *C. tritonis*.

Data availability statement

The data presented in the study are deposited in the NCBI repository, accession number is from OP810573 to OP810600.

Author contributions

CX and GZ: Experiment, writing, and editing. MH: Reviewing.and supervision. WL: Project administration and approval. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2022.1066750/full#supplementary-material

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