Received: 30 October 2018 | Revised: 9 January 2019 | Accepted: 23 January 2019

DOI: 10.1111/zsc.12343

ORIGINAL ARTICLE

WILEY Zoologica Scripta

Mitogenomic phylogeny of *Nassarius* (Gastropoda: Neogastropoda)

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Funding information
Fundamental Research Funds for the Central Universities, Grant/Award Number: 201762014; National Natural Science Foundation of China, Grant/Award Number: 31772843

Abstract

Nassariids (Family Nassariidae) are a group of marine snails that are distributed worldwide, with their maximum species diversity in tropical regions, particularly the Indo-Pacific. However, the traditional taxonomy of Nassariidae defined by shell or radula characters is usually inconsistent with little phylogenetic signal. In the present study, the complete mitochondrial (mt) genomes of nine *Nassarius* species were sequenced and compared with other eight nassariid species previously reported. All nassariid mt genomes showed the same gene order as in most caenogastropods and shared a very similar pattern with respect to genome size, nucleotide composition and AT contents. A deletion of three nucleotides in *nad6* gene was detected in *Nassarius jacksonianus* and *Nassarius acuticostus*, and this feature also provided implications for nassariid phylogeny. The genetic distance analysis and reconstructed phylogeny revealed a distant relationship between *N. jacksonianus* or *N. acuticostus* and other members in *Nassarius*. The mitogenomic phylogeny recovered the evolutionary relationships within *Nassarius* with high statistical support. In addition, a chronogram was reconstructed under an uncorrelated relaxed molecular clock, which dated the divergence among main lineages of *Nassarius* during ~31 MYA.

KEYWORDS

genetic distance, mitochondrial genomes, nassariidae, *Nassarius*, phylogeny

1 | INTRODUCTION

The mudsnail nassariids (Family Nassariidae, Iredale, 1916), which consist of more than 400 species (Galindo, Puillandre, Utge, Lozouet, & Bouchet, 2016), constitute a major component of the biodiversity of tropical, subtropical, temperate and even polar seas (Nekhaev, 2014). The maximum species diversity of nassariids is in tropical regions, particularly the Indo-Pacific (Cernohorsky, 1972). As scavengers, nassariids are found from deep waters of 1,000 m to the intertidal zone, associated with soft bottoms (e.g., sandy or muddy bottoms) and rocky shores to a lesser extent. The ecology (Gili & Martinell, 1994), physiology (Morton, 1990), phylogeography (Albaina, Olsen, Couceiro, Ruiz, & Barreiro, 2012) of nassariidas have been the subject of numerous studies. However, the use of scientific names in previously published papers, to a large extent, follows the Cernohorsky's (1984) systematic revision, within which plenty of names at a subfamily, genus or species level is recognized as invalid (Galindo et al., 2016).

The current classification of Nassariidae was largely established by Cossmann (1901) and modified slightly by Cernohorsky (1984), excluding Cossmann' Truncariinae. Based on morphological characters (e.g., teleoconch and protoconch, operculum, radula and egg-capsule), the recent species are traditionally divided into three subfamilies, 12 genera and 31 subgenera (Cernohorsky, 1984). However, the morphological-based taxonomy in snails has obvious shortcomings

(Haasl, 2000). At family-level, the accepted boundaries of Nassariidae ought to be revised, with the inclusion of several buccinid genera (e.g., *Antillophos*, *Engoniophos*, *Macron*, *Northia*, *Phos*, *Nassaria*) of the subfamilies Photinae or Pisaniinae (Haasl, 2000; Kantor, 2003; Landau, Harzhauser, Islamoglu, & Silva, 2013). Within Nassariidae, the taxonomic complexity appears even more apparent at genus or species level. Only about 60 extant species are classified in genera apart from *Nassarius*, which plays the role of a taxonomic wastebasket (Galindo et al., 2016). Moreover, the applications of subgenera within *Nassarius* are inconsistent and subjective (Allmon, 1990; Cernohorsky, 1984; Haasl, 2000).

Following a molecular phylogenetic analysis based on five gene fragments (three mitochondrial (mt) sequences COI, 12S and 16S, and two nuclear markers 28S and H3; Galindo et al., 2016), major revisions of nassariid systematics have been made: Several genera (e.g., *Antillophos*, *Engoniophos*, *Phos* and *Nassaria*) formerly considered within Buccinidae were removed into Nassariidae; *Nassarius* was defined as an exclusively Indo-West Pacific radiation; the Atlantic/Mediterranean nassariids, which were recovered in a distinct clade, were removed from genus *Nassarius* to *Tritia*; genus *Reticunassa* was elevated to genus level to include species of *Nassarius pauper*-complex. Galindo et al. (2016) also reject the use of shell and radula characters to define groups (genera) within Nassariidae in traditional taxonomy. Other molecular phylogenetic analyses mostly focused on the Indo-Pacific nassariids (*Nassarius*) along China seaside. Previously reported phylogenies of *Nassarius* were based on partial 16S or COI sequences (Chen & Zhang, 2012; Li, Lin, Fang, Zhu, & Gao, 2010; Zhang & You, 2009). However, these results were poorly supported and often contradicted each other, demonstrating that the partial mt genes were unable to resolve the phylogeny within *Nassarius*. To have a summary, the taxonomy and phylogeny of *Nassarius*, which is the largest group of Nassariidae, remain unresolved.

The typical metazoan mt genome is a covalently circular molecule, encoding 37 genes: 13 protein-coding genes (PCGs), two ribosomal RNAs (rRNAs) and 22 transfer RNAs (tRNAs) (Boore, 1999). Thus far, plenty of studies utilizing complete mt genomes to address phylogenetic relationships within Gastropoda has been conducted and proved to enhance resolution and statistical confidence of inferred phylogenetic trees when compared with more traditional analyses based on partial mt genes (Cunha, Grande, & Zardoya, 2009; Osca, Templado, & Zardoya, 2014, 2015; Uribe, Colgan, Castro, Kano, & Zardoya, 2016; Uribe, Puillandre, & Zardoya, 2017; Uribe, Williams, Templado, Abalde, & Zardoya, 2017).

TABLE 1 Mitochondrial (mt) genomes analysed in this study

Species	Family	Length (bp)	GenBank acc. no.	Location
Nassarius succinctus	Nassariidae	15,329	KT768016	Qingdao, China
Nassarius nodifer	Nassariidae	15,337	KT818617	Zhanjiang, China
Nassarius conoidalis	Nassariidae	15,332	KT826694	Dongshan, China
Nassarius pullus	Nassariidae	15,278	KT900947	Beihai, China
Nassarius sinarus	Nassariidae	15,325	MH346208	Rizhao, China
Nassarius foveolatus	Nassariidae	15,343	MH346209	Beihai, China
Nassarius javanus	Nassariidae	15,325	MH346210	Zhanjiang, China
Nassarius jacksonianus	Nassariidae	15,234	MH346212	Beihai, China
Nassarius acuticostus	Nassariidae	15,240	MH346211	Zhanjiang, China

Species	Family	Length	GenBank acc. no.
Tritia obsoleta	Nassariidae	15,263	DQ238598
Tritia reticulatus	Nassariidae	15,271	EU827201
Nassarius variciferus	Nassariidae	15,269	KM603509
Reticunassa hiradoensis	Nassariidae	15,194	MG744569
Reticunassa fratercula	Nassariidae	15,174	KT826695
Reticunassa festiva-A	Nassariidae	15,195	KT735055
Reticunassa festiva-B	Nassariidae	15,194	MF148855
Reticunassa festiva-C	Nassariidae	15,172	MG744570
Buccinum pemphigus	Buccinidae	15,265	KT962044
Volutharpa perryi	Buccinidae	15,255	KT382829
Neptunea arthritica	Buccinidae	15,256	KU246047

In the present study, nine nassariid mt genomes were newly sequenced. For the first time, a mitogenomic phylogeny of Nassariidae including a total of 17 mt genomes was reconstructed. Our aims were (a) to reconstruct a robust phylogeny of *Nassarius* genus that could be used as a framework for further evolutionary studies; (b) to review the systematics of Nassariidae; and (c) to date main cladogenetic events within *Nassarius*.

2 | MATERIAL AND METHODS

2.1 | DNA extraction, PCR amplification and sequencing

All specimens of nine nassariidas were collected along the coast of China seas (Table 1). Samples were stored in 95% ethanol, and total genomic DNA was extracted from up to 100 mg of foot tissue following a modified CTAB method (Winnepenninckx, Backeljau, & Dewachter, 1993). Complete mt genomes (except that of *Nassarius jacksonianus*) were amplified through long PCR using different combinations of primers which were designed based on sequences of *Nassarius variciferus* and five *Reticunassa* species described in a previous study (Yang, Li, Kong, & Yu, 2018; Supporting Information Table S1). Long PCRs were carried out in a total volume of 25 μl with 0.5 μl of template DNA (approximately 100 ng), 2.5 μl of 10 × LA-buffer (Mg^{2+} plus), 0.5 μl dNTPs (10 mM), 1 μl of each primers (10 μM), 0.25 μl (1 U) LA-*Taq* DNA polymerase. The following PCR conditions were used as follows: a predenaturation at 94°C for 3 min; 35 cycles of denaturing at 94°C for 30 s, annealing at 56–60°C for 30 s and extension at 68°C for 60 s per kb; and a final extension step at 72°C for 10 min. The PCR products were confirmed by 1.5% agarose gel electrophoresis and stained with ethidium bromide, purified with EZ Spin Column PCR Product Purification Kit (Sangon). Purified products were sequenced using an ABI 3730 automatic sequencer (Applied Biosystems) at LiuHe HuaDa Biotechnology Company (Beijing, China), based on a primer-walking strategy. A total amount of 3 μg genomic DNA of *N. jacksonianus* was submitted to the Novogene Company (Beijing, China) for Illumina HiSeq PE150 sequencing.

2.2 | Genome assembly, gene annotation and sequence analysis

The Sanger sequencing data of each species were assembled using SeqMan (www.DNASTAR.com), respectively, and further adjusted manually in a few cases. The clean short-read DNA sequences from Illumina were assembled into a single sequence corresponding to a complete mt genome using CLC Genomics Workbench 11. PCGs were determined by ORF Finder (http://www.ncbi.nlm.nih.gov/orffinder) using the invertebrate mt code. The accurate boundaries of PCGs and rRNA genes were identified by comparing with those of *N. variciferus* and five *Reticunassa* species. tRNA genes were identified using ARWEN (Laslett & Canbäck, 2008). Codon usage and pairwise genetic distances (*p*-distance) of 13 PCGs, the A + T content values and nucleotide frequencies of mt genomes were estimated by MEGA 5 (Tamura et al., 2011).

2.3 | Phylogenetic analysis

A total of 20 taxa were selected for phylogenetic analysis, including the complete mt genomes of 17 nassariids. *Buccinum pemphigus* (KT962044), *Volutharpa perryi* (KT382829) and *Neptunea arthritica* (KU246047) from family Buccinidae (Neogastropoda: Buccinoidea) were used as out-groups.

The 13 PCGs were aligned by codons with Clustal W (Higgins et al., 1994) in MEGA 5 respectively, and further

TABLE 2 List of total size, AT content, AT skew and GC skew, for mitochondrial genomes of *Nassarius javanus* (*Nja*), *Nassarius sinarus* (*Nsi*), *Nassarius foveolatus* (*Nfo*), *Nassarius acuticostus* (*Nac*), *Nassarius jacksonianus* (*Njac*), *Nassarius nodifer* (*Nno*), *Nassarius succinctus* (*Nsu*), *Nassarius pullus* (*Npu*) and *Nassariusconoidalis* (*Nco*) with lengths of two tRNAs and potential origin of replication (POR)

	Nja	*Nsi*	*Nfo*	*Nac*	*Njac*	*Nno*	*Nsu*	*Npu*	*Nco*
Total size	15,325	15,325	15,343	15,240	15,234	15,337	15,329	15,278	15,332
%A + T	0.71	0.72	0.7	0.7	0.7	0.70	0.71	0.69	0.69
rrnS	964	966	968	954	955	960	964	969	964
rrnL	1,363	1,365	1,361	1,349	1,351	1,365	1,365	1,353	1,364
POR	56	57	58	57	65	57	57	58	57
AT skew mitogenome	−0.118	−0.112	−0.115	−0.122	−0.115	−0.114	−0.116	−0.118	−0.118
AT skew all PCGs	−0.168	−0.164	−0.167	−0.176	−0.164	−0.170	−0.167	−0.175	−0.172
AT skew rRNAs	0.044	0.057	0.051	0.026	0.035	0.068	0.043	0.059	0.058
GC skew mitogenome	0.063	0.063	0.049	0.046	0.030	0.047	0.059	0.048	0.048
GC skew all PCGs	0.054	0.049	0.034	0.029	0.003	0.040	0.045	0.034	0.028
GC skew rRNAs	0.175	0.183	0.168	0.186	0.171	0.152	0.189	0.164	0.162

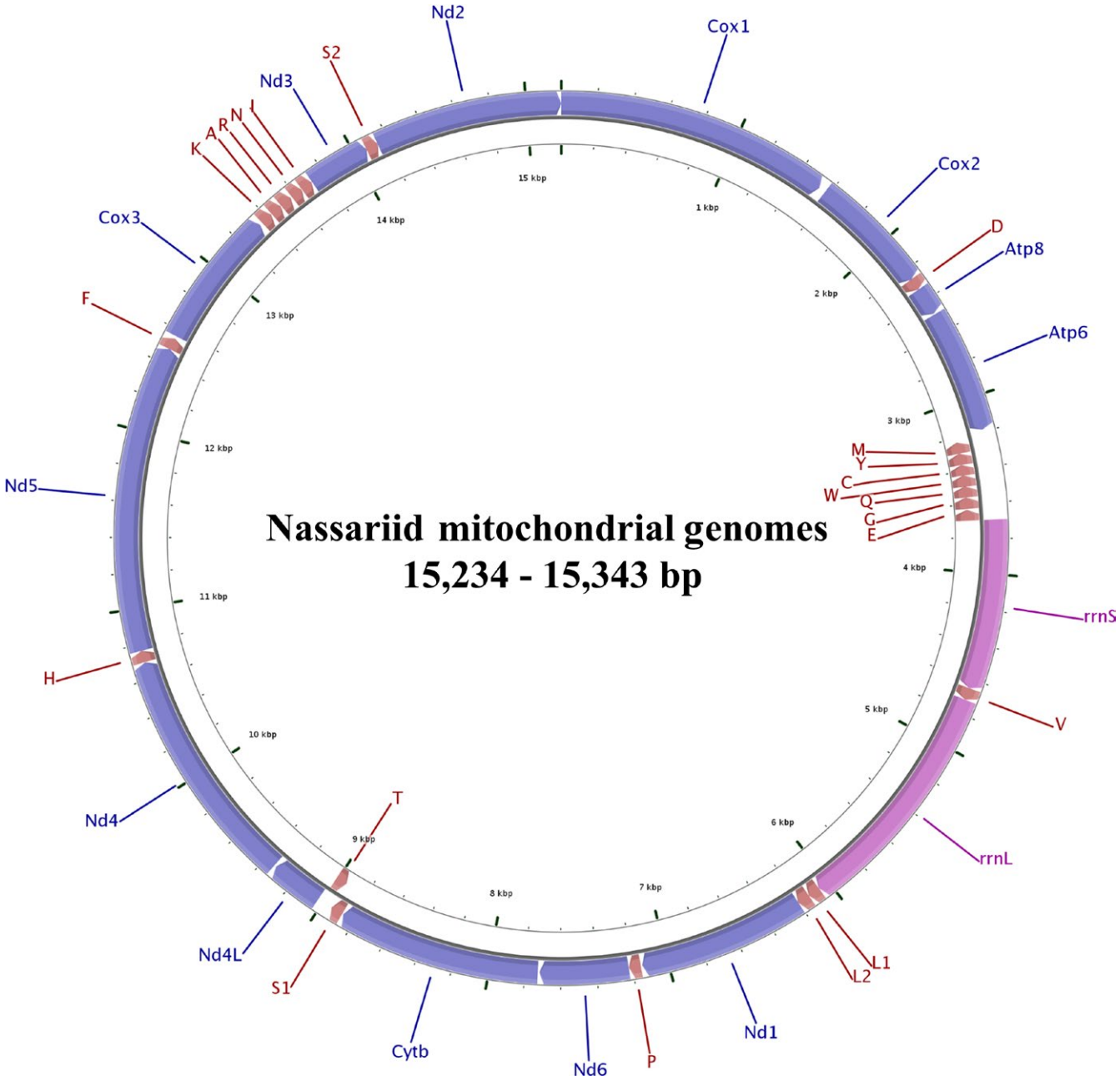

FIGURE 1 Gene map of the mt genomes of nine nassariids [Colour figure can be viewed at wileyonlinelibrary.com]

verified manually. GTR + I + G was selected as the best-fit nucleotide substitution model for each gene by jModelTest (Posada, 2008) based on the Akaike information criterion. The 3rd codons of all PCGs were discarded since a high saturation was detected on this position using Xia's test implemented in DAMBE5 (Xia, 2013). Phylogenetic analyses were conducted using maximum likelihood (ML) and Bayesian inference (BI). ML analyses were carried out using software RaxML v. 8.2.1 (Stamatakis, 2006) with the rapid hill-climbing algorithm and 10,000 bootstrap pseudoreplicates. BI analyses were performed with MrBayes v. 3.1.2 (Ronquist & Huelsenbeck, 2003), running four simultaneous Monte Carlo Markov chains for 10,000,000 generations, sampling every 1,000 generations and discarding the first 25% generations as burn-in. Parameter convergence was achieved within 10 million generations, and the standard deviation of split frequencies was <0.01. All parameters were checked with Tracer v. 1.6, and the effective sample size (ESS) values were above 200.

2.4 | Divergence time estimation

The divergence dates between nassariid clades were estimated using the 13 PCGs at the nucleotide level and an

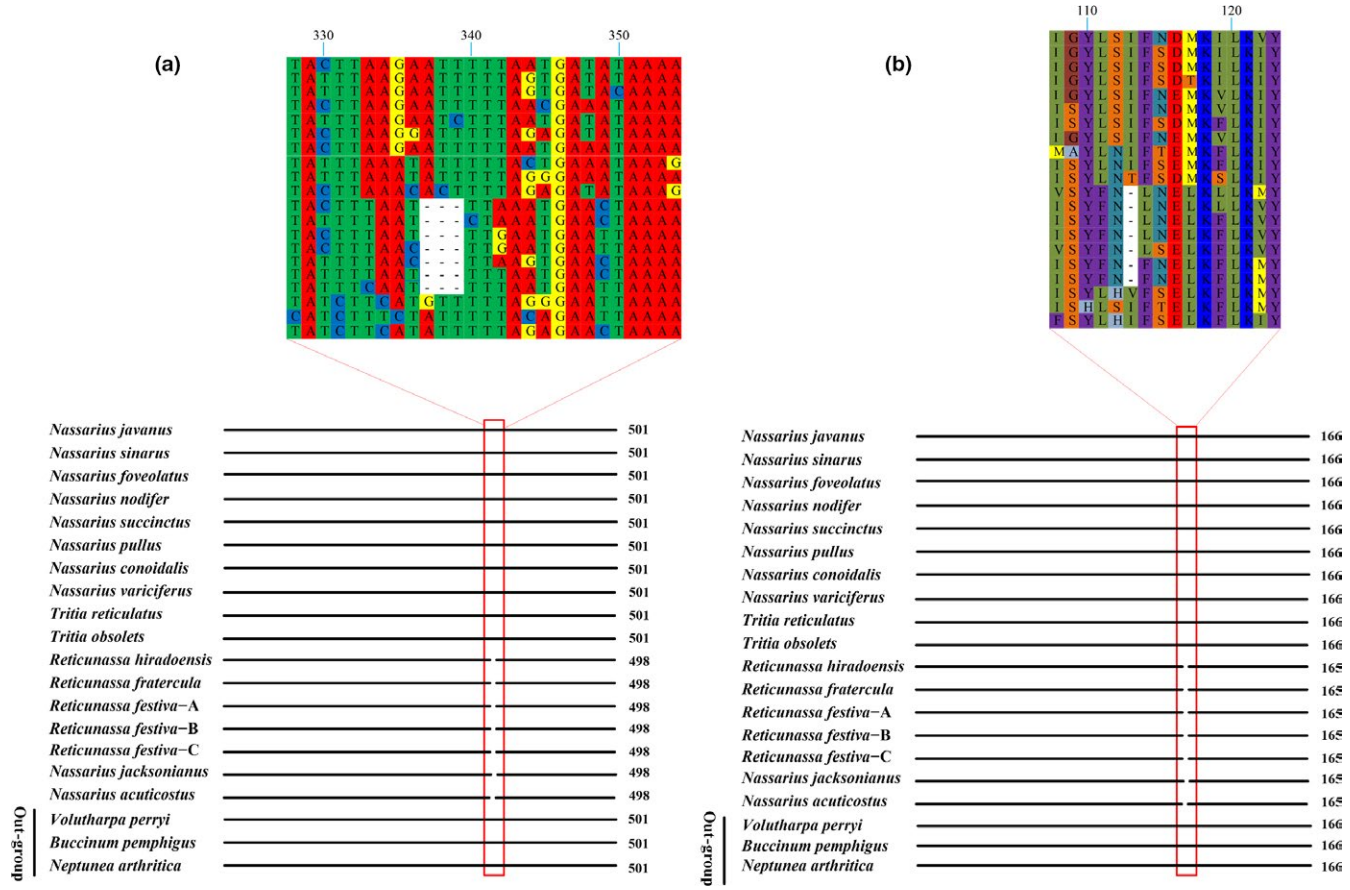

FIGURE 2 *Nad6* sequence differences in 17 nassariidas and three buccinids (out-groups). Nucleotide (a) and amino acid (b) alignments of a portion of *nad6* gene indicate that *Nassarius acuticostus*, *Nassarius jacksonianus* as well as *Reticunassa*, lack the three nucleotide deletion present in other nassariids and buccinids [Colour figure can be viewed at wileyonlinelibrary.com]

uncorrelated relaxed molecular clock model in BEAST 1.10.4 (Drummond, Suchard, Xie, & Rambaut, 2012). For the tree prior, a Yule process of speciation was employed. The best fitting evolutionary model GTR + I + G was applied. The final Markov chain was run twice for 100,000,000 generations, sampling every 20,000 generations. The first 10% samples were discarded as a burn-in, according to the convergence of chains checked with Tracer 1.6. The ESS of all the parameters was above 200. TreeAnnotator v1.10.4 software was used to generate the tree, and the divergence time was visualized using FigTree 1.4.2 software.

The posterior distribution of the estimated divergence times was obtained by specifying two calibration points as priors for divergence times of the corresponding splits. The first calibration point was set for the divergence of Nassariidae, based on the oldest known fossils of *Buccitriton* sp. (56–47.8 Ma) (Allmon, 1990; Galindo et al., 2016). A second calibration point was set at the divergence time of *Tritia*, since the oldest nassariids from Europe have been recognized in the Chattian (28–23 Ma) (Galindo et al., 2016; Lozouet, 1999).

3 | RESULTS AND DISCUSSION

3.1 | Mitochondrial genome organization and structural features

Compared with other nassariid mt genomes reported before (Cunha et al., 2009; Simison, Lindberg, & Boore, 2006; Yang et al., 2018), all newly sequenced mt genomes in the present study shared a very similar pattern with respect to genome size, nucleotide composition and AT contents. The size of the nine nassariid mitogenomes ranged from 15,234 (*N. jacksonianus*) to 15,343 bp (*Nassarius foveolatus*; Table 2). The nucleotide compositions were all strongly skewed away from C in favour of G (the GC-skews are from 0.030 to 0.063) and from A in favour of T (the AT skews are from −0.122 to −0.112). The AT contents of the nine mt genomes were from 69% to 72% (Table 2).

Like most metazoan mt genomes (Boore, 1999), nassariids encoded for 13 PCGs, 22 tRNAs and two rRNAs (Figure 1). Most of PCGs analysed in the present study used conventional initiation codons (108 genes use ATG, seven use ATA), but *nad4* employed alternative start codon in two species

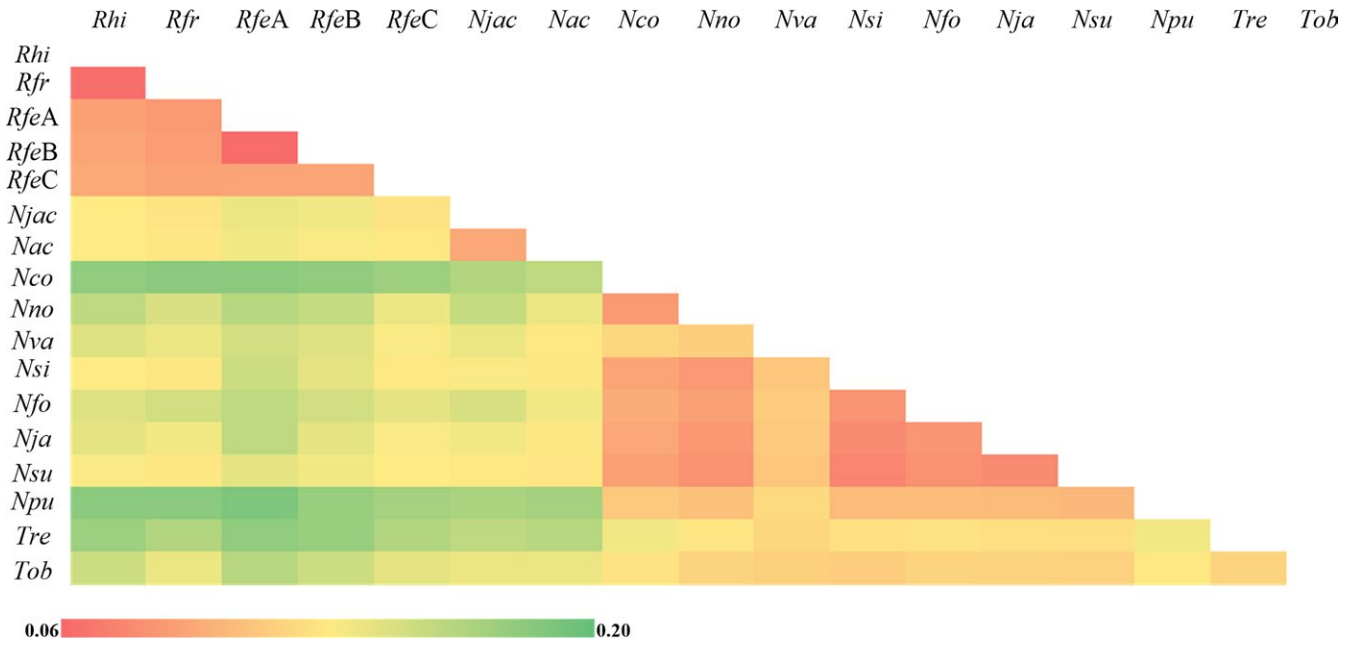

FIGURE 3 Heatmap of pairwise genetic distance of 13 PCGs of *Reticunassa hiradoensis* (*Rhi*), *Reticunassa fratercula* (*Rfr*), *Reticunassa festiva-A* (*Rfe*A), *Reticunassa festiva-B* (*Rfe*B), *Reticunassa festiva-C* (*Rfe*C), *Nassarius variciferus* (*Nva*), *Nassarius javanus* (*Nja*), *Nassarius sinarus* (*Nsi*), *Nassarius foveolatus* (*Nfo*), *Nassarius acuticostus* (*Nac*), *Nassarius jacksonianus* (*Njac*), *Nassarius nodifer* (*Nno*), *Nassarius succinctus* (*Nsu*), *Nassarius pullus* (*Npu*), *Nassariusconoidalis* (*Nco*), *Tritia reticulatus* (*Tre*) and *Tritia obsolets* (*Tob*) [Colour figure can be viewed at wileyonlinelibrary.com]

(*N. jacksonianus* and *Nassarius acuticostus*: CTT). The complete termination codons TAG ($N = 34$) and TAA ($N = 80$) were used in most PCGs, except for *nad2* genes of *N. jacksonianus*, *N. acuticostus* and *Nassarius pullus*, ending with the incomplete stop codon TA (Supporting Information Table S2). The incomplete stop codon might be modified to the TAA termini by polyadenylation of the transcribed messenger RNAs (Ojala, Montoya, & Attardi, 1981). The largest non-coding region was found in all mt genomes between *trnF* and *cox3* (56–65 bp), in a position that has been assumed as candidate to contain the control region in other caenogastropod mt genomes (Cunha et al., 2009; Osca, Templado, & Zardoya, 2015).

When the *nad6* genes of eight nassariid species were aligned, a deletion of three continuous nucleotides, which only led to the deletion of one amino acid and did not bring other changes in the deduced amino acid sequences, was discovered in all five *Reticunassa* mt genomes (Yang et al., 2018). In the present study, the same deletion was also detected in the *nad6* genes of *N. acuticostus* and *N. jacksonianus* mt genomes (Figure 2), and this deletion might reflect unusual constraints on the protein in these taxa (Sevigny et al., 2015). The result (Figure 2) also revealed that same deletions were not found in the three buccinid (out-groups) mitogenomes, confirming that this character was a synapomorphy of the clade formed by *N. acuticostus* + *N. jacksonianus* and *Reticunassa*.

Pairwise genetic distances of 13 PCGs between species in genus *Nassarius*, *Reticunassa* and *Tritia* are from 0.083–0.180, 0.063–0.113, and 0.148, respectively (Figure 3; Supporting Information Table S3). Most high genetic distance values (within genus) are detected in *Nassarius*, especially between *N. jacksonianus* or *N. acuticostus* and any other species in this genus, and the pairwise genetic distance values are from 0.083 to 0.152 in *Nassarius* if *N. jacksonianus* and *N. acuticostus* are not considered. Furthermore, the genetic distance values between *N. jacksonianus* or *N. acuticostus* and *Reticunassa* or *Tritia* range from 0.161 (*N. jacksonianus* and *Reticunassa fratercula*) to 0.177 (*N. acuticostus* and *Tritia reticulatus*), similar to those between *N. jacksonianus* or *N. acuticostus* and other species in *Nassarius* (values ranging from 0.162–0.180). Pairwise genetic distance analysis has been proved as a useful tool for species identification in Nassariidae (Yang et al., 2018). In the present study, a relatively distant relationship between *N. jacksonianus* + *N. acuticostus* and other *Nassarius* species revealed by pairwise genetic distance analysis, may also provide implications for nassariid taxonomy at genus level. However, the point that *N. jacksonianus* and *N. acuticostus* should be removed from *Nassarius* has still to be verified by nuclear loci and morphological evidence (e.g., the character of protoconch).

3.2 | Phylogenetic relationships of *Nassariidae*

Analyses using different inferences generated almost identical topologies, only differing in the internal relationships

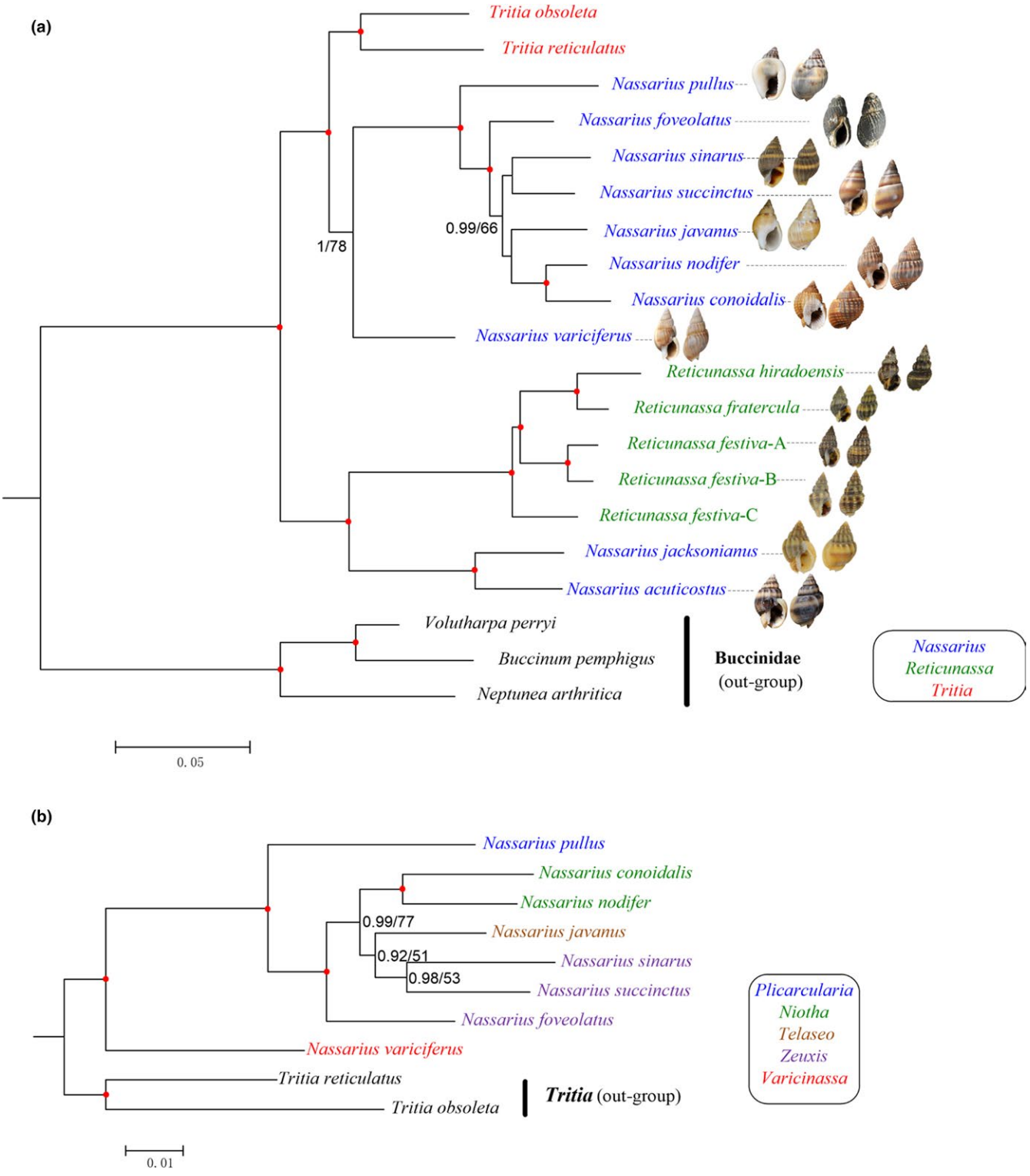

FIGURE 4 Phylogenetic trees of Nassariidae (a) and *Nassarius* (b) derived from maximum likelihood (ML) and Bayesian inference analyses based on nucleotide sequences of 13 mitochondrial protein-coding genes. The first number at each node is Bayesian posterior probability, and the second number is bootstrap probability of ML analyses. Solid red circles represent nodes with posterior probabilities ≥0.95 and bootstrap proportions ≥90 [Colour figure can be viewed at wileyonlinelibrary.com]

within *Nassarius* (Figure 4a). Within Nassariidae, the reconstructed phylogeny recovered *Reticunassa* + (*N. jacksonianus* + *N. acuticostus*) as sister group to *Tritia* + the remaining *Nassarius* (Figure 4a). As a result, the monophyly of *Nassarius* was not supported. Based on shell and radula characters, *N. jacksonianus* and *N. acuticostus* were attributed to subgenus *Niotha* (*Nassarius*) (Cernohorsky, 1984). However, the conflict between morphological classification

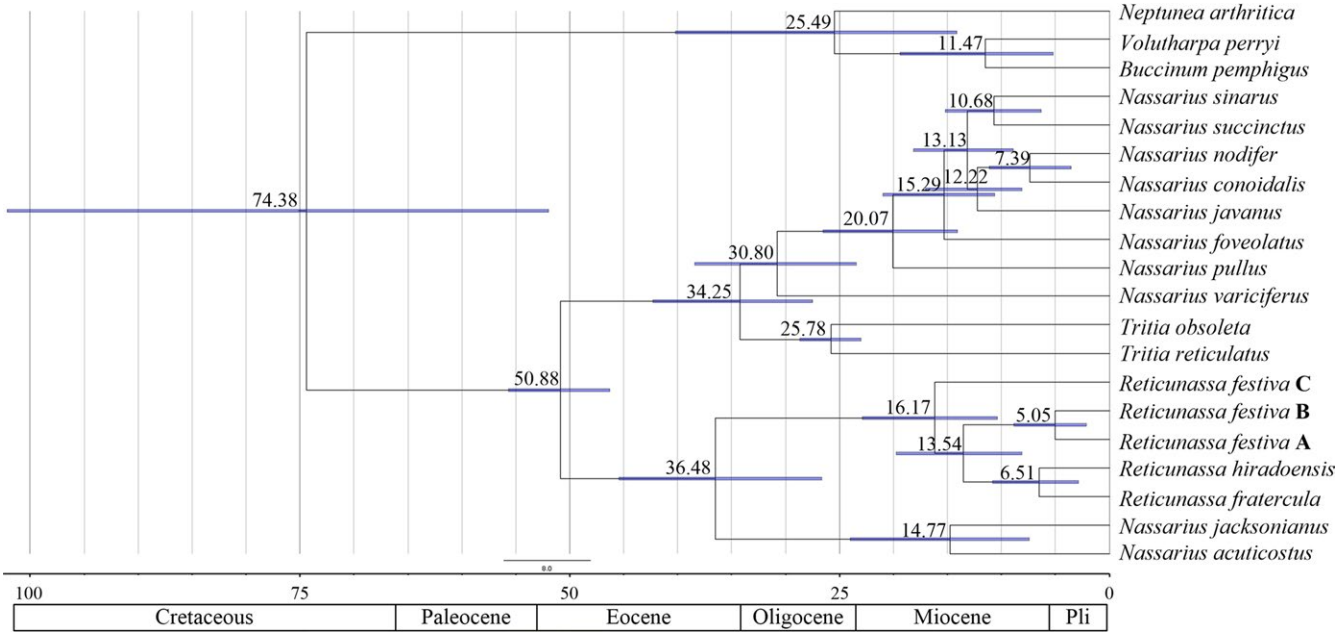

FIGURE 5 Divergences time estimations for the Nassariidae using Bayesian relaxed dating methods (BEAST). Dates (and credibility intervals) are in millions of years, and horizontal bars represent 95% credibility intervals of relevant nodes [Colour figure can be viewed at wileyonlinelibrary.com]

and mitogenomic phylogeny in the present study may be the result of convergence during nassariid evolution (Galindo et al., 2016). The close affinity of *Reticunassa* to *N. jacksonianus* + *N. acuticostus* is also supported by comparative mitogenomic analysis (the nucleotide deletion in *nad6* genes). *Reticunassa* is a group including *N. pauper*-complex, which was originally considered as morphological variants of a single species (Cernohorsky, 1984) and then recognized as different species (Kool & Dekker, 2006, 2007). Members in *Reticunassa* share several synapomorphies, such as a complex and ornamented planktotrophic protoconch, a small adult size (usually less than 8 mm), and the secondary spiral sculpture on the shell (Galindo et al., 2016; Kool & Dekker, 2006, 2007). Although *N. acuticostus* and *N. jacksonianus* group with *Reticunassa*, they do not accord with the morphological characters of *Reticunassa* and thus are not supposed to be classified to this genus. The molecular phylogeny conducted by Galindo et al. (2016) revealed that nassariids from Indo-Pacific (*Nassarius*) were recovered in an independent clade from the Atlantic/Mediterranean clade (namely *Tritia*) and *Reticunassa*. Nevertheless, the present phylogeny challenges that systematics due to the relative positions of *N. jacksonianus* and *N. acuticostus*, with high support values, indicating that the Indo-Pacific nassariids (*Nassarius*) are not an exclusively radiation. The phylogenetic analysis and comparative mitogenomic analysis, in which *N. jacksonianus* and *N. acuticostus* are first included, support the erection of a new genus. However, more data on other species are needed to further delineate this group in the future.

The phylogenetic analyses of genus *Nassarius* based on combined PCGs using ML and BI arrived identical tree topologies, with most nodes highly supported (Figure 4b). Species in genus *Tritia* (*T. reticulatus* and *T. obsoleta*) were used as out-group. The reconstructed phylogeny recovered *N. variciferus* as sister group of the remaining, which were organized into five clades (Figure 4b). Within *Nassarius*, the use of subgenus is quite common in taxonomic description. However, previous molecular analyses did not find support to maintain the current use of subgenera due to the homoplastic of shell or radula characters, which are usually used to define subgenera (Galindo et al., 2016; Pu et al., 2017). In the present study, the monophyly of subgenus *Zeuxis*, which was represented by three species, was not supported, corresponding with previous molecular phylogenies using short gene fragments, including COI, 16S and/or ITS (Chen & Zhang, 2012; Li et al., 2010; Pu et al., 2017; Zou, Li, & Kong, 2012). As the most species-rich group in *Nassarius*, *Zeuxis* is not well-defined. For example, the presence of accessory lateral plates, which were used to define *Zeuxis*, was not consistent within this group (Cernohorsky, 1984). Consequently, both morphological and molecular evidence indicated that *Zeuxis* was not phylogenetically valid and needed further revision. In addition, the monophyly of subgenus *Niotha*, represented by *N. conoidalis* and *N. nodifer*, was highly supported in both ML and BI analyses (Figure 4b). Nevertheless, the monophyletic group formed by *N. conoidalis* and *N. nodifer* has never been revealed in previous studies (Chen & Zhang, 2012; Pu et al., 2017; Zou et al., 2012). All these phylogenies, each based on short gene

fragments, were poorly supported, often contradicting each other. The reconstructed phylogeny in the present study is statistically robust and may serve as a framework for nassariid taxonomy at subgenus level. The present study also emphasizes that complete mt genomes are a very promising tool for achieving important levels of resolution within *Nassarius*.

3.3 | Divergence times

The reconstructed time tree using a relaxed molecular clock model is shown in Figure 5. The confidence intervals for divergence dates are relatively narrow for the terminal clades but increase when deeper nodes are considered. According to the chronogram, the divergence between the Atlantic/Mediterranean lineage *Tritia* and the Indo-Pacific clade *Nassarius* is estimated near 34 MYA. Their ancestor originated from the Atlantic and the migration might have happened when the Mediterranean was still opened to the proto-Indo-Pacific during the Eocene (Galindo et al., 2016). According to Galindo et al. (2016), the hypothetical ancestor of *Reticunassa* experienced a major dispersion from the Atlantic and colonized the Indo-Pacific during the Palaeocene. However, the clade of *N. jacksonianus* + *N. acuticostus* was not included in Galindo et al. (2016). In the present study, the diversification between *N. jacksonianus* + *N. acuticostus* and *Reticunassa* is estimated around Miocene (36 MYA), indicating that the two clades share the same ancestor immigrated from the Atlantic. Within *Nassarius*, our results imply that these species diversified rapidly during the Miocene, as suggested by Galindo et al. (2016), as well as Haasl (2000) and Lozouet and Galindo (2015) based on paleontological data.

In an ultrametric tree, the length of the branches is proportional to the time of divergence, and therefore, the branch length of different lineages can be roughly compared to provide a criterion for taxonomic level delimitation above species (Johns & Avise, 1998; Uribe, Puillandre et al., 2017). Hence, a better coverage of nassariid mitogenomic data is needed to reconstruct a well-resolved time-calibrated tree that could be used to discuss taxonomic ranks, especially at subgenus level.

4 | CONCLUSION

This study presents mt genomes of nine nassariid species. Compared with other nassariid mt genomes reported before, all newly sequenced mt genomes in the present study share a very similar pattern with respect to genome size, nucleotide composition and AT contents. When the *nad6* genes of 17 nassariids are aligned, a deletion of three nucleotides, which was considered to be a specific character of genus *Reticunassa*, is also detected in *N. jacksonianus* and *N. acuticostus*. Both genetic distance analysis and phylogenetic analysis support a distant relationship between *N. jacksonianus* or *N. acuticostus* and other members in *Nassarius*. Our results reveal that the complete mitogenomes are a very promising tool to reconstruct a statistically robust phylogeny of genus *Nassarius*. The present study also demonstrates the inadequacy of shell or radula characters traditionally used in nassariid taxonomy at subgenus level. In the future, a better coverage of nassariid mt genomic data is needed to reconstruct a well-resolved phylogeny that could be used to discuss taxonomic ranks within *Nassarius*.

ACKNOWLEDGEMENTS

This study was supported by research grants from Fundamental Research Funds for the Central Universities (201762014) and National Natural Science Foundation of China (31772843).

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How to cite this article: Yang Y, Li Q, Kong L, Yu H. Mitogenomic phylogeny of *Nassarius* (Gastropoda: Neogastropoda). *Zool Scr*. 2019;48:302–312. https://doi.org/10.1111/zsc.12343