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Mitogenomic phylogeny of Trochoidea (Gastropoda: Vetigastropoda): New insights from increased complete genomes

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Abstract

Increased mitochondrial (mt) genomes can provide more sets of genome-level characteristics for resolving deeper phylogeny. Limited information with respect to the Trochoidea mitochondrial genome organization is available; besides, monophyly and internal relationships of the superfamily still remain a matter of discussion. To resolve the monophyly and internal phylogenetic controversies of Trochoidea and expand our understanding for mt genomic characteristic evolution among Trochoidea, the phylogenetic trees were reconstructed using 13 newly sequenced complete mt genomes and 35 genomes from GenBank, and both the maximum likelihood and Bayesian inference analyses were highly supported. Vetigastropoda phylogenetic analyses recovered the monophyly of Trochoidea. Trochoidea phylogenetic analyses and genetic distances supported the non-monophyly of Tegulidae and *Tegula*, indicating that the taxonomic status of several genera (*Rochia*, *Tectus* and *Cittarium*) should be revised and *Tegula*, *Omphalius* and *Chlorostoma* should be placed as a same genus. The close affinity between *Tectus virgatus* and *Rochia* was also revealed. Three-nucleotide insertion in *nad1*, nine-nucleotide insertion and six-nucleotide deletion in *nad5* are detected in Tegulidae, *Tectus* and *Rochia*, respectively. Gene orders within Trochoidea are stable, with gene rearrangements exclusive to tRNA genes observed. Homoplasious convergences because of trnT rearrangement display translocation in Turbinidae and reversion in Trochidae and Calliostomatida. For *trnE* and *trnG*, we identify 11 arrangement types, suggesting that the gene rearrangement history needs to be further evaluated. Our study emphasizes the importance of mt genomes in resolving phylogenetic relationships within Trochoidea. In addition, the mt genomic characters would contribute new insights into the classification of Trochoidea.

KEYWORDS

character evolution, mitochondrial genomes, phylogenetic analysis, Trochoidea

1 | INTRODUCTION

Superfamily Trochoidea (Rafifinesque, 1815) distributes worldwide and conquers almost all latitudes and bathymetric ranges from the high intertidal to bathyal depths (Hickman & McLean, 1990; Williams et al., 2008). By far it is the largest and most megadiverse superfamily within Vetigastropoda, with in excess of 2,000 living species that are grouped

into about 500 recognized genera (Geiger et al., 2008; Hickman, 1996). Trochoidea is a splendid group for studying the phylogeny underlying biological diversification. The classification of Trochoidea has a long contentious history and is still in considerable flux. Hickman and McLean (1990) first produced the comprehensive morphological monograph of the Trochoidea and maintained the three families traditionally recognized within the superfamily, namely Trochidae, Turbinidae and Skeneidae, based on detailed morphological data including anatomical, radula and shell characteristics. According to the recent phylogenetic studies, the Trochoidea as traditionally defined was revealed to be polyphyletic which prompted important changes for Trochoidea taxonomy (Heß et al., 2008; Kano, 2008; Williams, 2012; Williams et al., 2008; Williams & Ozawa, 2006). For example, some taxa were transferred to the superfamily Seguenzioidea (Kano, 2008; Kano et al., 2009), whereas others were assigned to their own new superfamilies: Angarioidea and Phasianelloidea (Williams et al., 2008; Williams & Ozawa, 2006). Williams (2012) restricted Trochoidea up to eight families: Trochidae, Turbinidae, Solariellidae, Calliostomatidae, Liotiidae, Skeneidae, Margaritidae and Tegulidae. The current classification of Trochoidea (Bouchet et al., 2017) was classified into 13 families, including Angariidae, Areneidae, Calliostomatidae, Colloniidae, Conradiidae, Skeneidae, Liotiidae, Margaritidae, Phasianellidae, Solariellidae, Tegulidae, Trochidae and Turbinidae. The final composition of Trochoidea and taxonomic internal classification is still under debate and in flux.

Tegulidae, owing to the unusual distribution of character states of its members, has a long and debatable taxonomic history (Hickman & McLean, 1990). Initially, it was treated as the subfamily of Trochidae (Hickman & McLean, 1990), subsequent as an enigmatic group located somewhere between Trochidae and Turbinidae (Hickman, 1996) and finally placed as a subfamily of Turbinidae by both Bouchet et al. (2005) and Williams et al. (2008). More recently, it was elevated to familial rank ultimately by Kano (2008) and Williams (2012). However, some studies (Uribe et al., 2017; Williams, 2012) revealed Tegulidae was paraphyletic and the attribution 'problematic' genera including *Cittarium*, *Tectus*, *Rochia* and *Tegula* ought to be further lucubrated. At first, Hickman and McLean (1990) included *Tectus* and *Cittarium* within Trochinae and Gibbulinae, respectively. Afterwards, Bouchet et al. (2005) placed both genera in Tegulidae; Williams et al. (2008) showed a sister relationship between *Cittarium* and *Tectus* and Williams (2012) recovered *Tectus*, *Cittarium* and possibly *Rochia* as a distinct clade without precedent, although with low support. Recently, Uribe et al. (2017) proposed they might be better to be considered as a separate new family, but their familial rank pending further studies with more taxa. Otherwise, the speciose genus *Tegula* is likewise non-monophyletic; alternatively, the genera *Omphalius* and *Chlorostoma* were supposed to be grouped together with *Tegula* (Uribe et al., 2017). Thus, monophyly and internal classification of Tegulidae still remain elusive and await further phylogenetic analyses.

Highlights

- Thirteen new mt genomes representing twelve Trochoidea and one Seguenzioidea were sequenced;
- The arrangements of *trnT* result in homoplasious convergences and that of *trnE* and *trnG* are diversity with eleven types;
- Three-nucleotide insertion in *nad1*, nine-nucleotide insertion and six-nucleotide deletion in *nad5* were observed in Tegulidae, *Tectus* and *Rochia*, respectively within Trochoidea, polyphyly of Tegulidae and *Tegula* is supported;
- The phylogenetic relationship between *Tectus*, *Rochia* and *Cittarium* should be further confirmed.

Turbinidae, whose representatives can be found in almost all latitudes and bathymetric ranges of every ocean, is a diverse and ecologically important group of molluscs (Hickman & McLean, 1990). Radular features or the calcified operculum used to be considered as the dominating characters defining Turbinidae (Williams & Ozawa, 2006); however, some studies revealed some radular characters may be highly plastic (Reid & Osorio, 2000; Robertson, 1985); beyond that, the ability to calcify opercula has arisen several times independently in non-sister gastropod clades (e.g. marine Naticidae; marine, freshwater, and terrestrial Neritimorpha; marine and freshwater Littorinidae; terrestrial Pomatiasidae and Ampullariidae) (Williams & Ozawa, 2006). Interestingly, the result of shell microstructure studies revealed Turbinidae has no unique characters compared with Trochidae (Hedegaard, 1990) and both families may be polyphyletic in the later phylogenetic studies based on partial gene fragments (Williams & Ozawa, 2006). The group has been the most formidable to resolve using either morphological or molecular data, and as such, systematics of the family has not been confirmed yet (Williams, 2012); especially, the genus *Turbo* may not be monophyletic. However, within Turbinidae, only five complete mt genomes were sequenced and no one of *Turbo* was available.

Trochidae is the largest and most diverse in Trochoidea. The family is comprising up to eleven subfamilies and remains a large family including well in excess of 600 species and more than 60 genera (Hickman & McLean, 1990; Williams et al., 2008; Williams et al., 2010; Williams, 2012). High levels of homoplasy in anatomical, radular and shell

characters (Hickman & McLean, 1990) generate disputable taxonomy of this grouping; furthermore, possibly due to unrepresentative taxon sampling and less information are contained in gene fragments, the phylogenetic relationships are highly debatable all the time that it is obvious in recent molecular studies (Kano, 2008; Williams et al., 2008; Williams et al., 2010; Williams, 2012). Hickman and McLean (1990) assigned the different genera within Trochidae to 13 subfamilies, lately, thorough taxonomic revision of the family was carried out, and at present, Trochidae remains ten subfamilies according to the new classification for Trochoidea proposed by Williams (2012) and Bouchet et al. (2017). Compared to gene fragments, complete mt genomes contain more information and have been proven useful in recovering internal nodes with high statistical support.

Complete mt genomes are helpful for understanding phylogenetic relationships and have been widely used to reconstruct phylogenetic relationships in different gastropod groups, including the Neritimorpha (Uribe et al., 2016), Heterobranchia (Sevigny et al., 2015; White et al., 2011) and Caenogastropoda (Cunha et al., 2009; Osca et al., 2015); what's more, phylogenetic analyses of complete mt genomes have resulted in good resolutions among vetigastropoda superfamilies (Lee et al., 2016; Uribe et al., 2016, 2017) and were regarded as good candidates to resolve phylogenetic relationships within Trochoidea (Uribe et al., 2017). Within Trochoidea, the past century classifications were based solely on shell, radula and anatomical characters (Hickman & McLean, 1990). More recently, molecular data have helped to clarify the phylogenetic relationships of major lineages within Trochoidea (Williams, 2012; Uribe, et al., 2016; Uribe et al., 2017; Williams & Ozawa, 2006), although most of the works were either based on mitochondrial and nuclear gene fragments or later based on mitogenomic data with limited taxon sampling (Lee et al., 2016; Uribe, et al., 2016; Uribe et al., 2017; Wort et al., 2016). These studies highlighted effectivity and significance of complete mt genomes and called for the inclusion of more taxa into phylogenetic reconstruction. Addition to sequences, mt genome can provide numerous genome-level features that bear much signal for gaining insights into evolutionary relationships of Trochoidea. Some studies showed that genome-level characters, such as gene order and composition, were promoted as having potential to resolve molluscan relationships (Simison & Boore, 2008); furthermore, mitochondrial gene orders were used with increasing frequency as robust characters in deep-level metazoan phylogenetic studies over the time (Rawlings et al., 2003). However, thus far, there are only 22 Trochoidea complete or almost complete mt genomes available in GenBank, covering six of 14 families referring to Phasianellidae, Margaritidae, Angariidae, Trochidae, Turbinidae, Tegulidae and several genera (e.g. *Rochia*, *Cittarium* and *Tectus*) which has not been assigned.

Here, we newly sequenced *Herpetopoma lischkei* (Seguenzioidea: Chilodontidae) and 12 Trochoidea complete mt genomes which represent four main lineages including Calliostomatidae, Trochidae, Turbinidae and two unassigned genera (*Rochia* and *Tectus*). Our aims were as follows: (a) to reconstruct a phylogeny of the Vetigastropoda allowing assessment of the monophyly of the Trochoidea; (b) to reconstruct a phylogeny of the Trochoidea to address phylogenetic relationships within the superfamily (in particular, the relative positions of Tegulidae and the genera *Tectus*, *Rochia* and *Cittarium*); and (c) to gain insights into characters evolution in terms on Trochoidea mt genomes in which some has shown gene rearrangements (Lee et al., 2016; Uribe, et al., 2016; Uribe et al., 2017). Comparative analysis of the mitochondrial genomes will offer evidences for future classification among Trochoidea. The genetic distance and phylogenetic analyses inferred in this study will provide help to further understand the evolutionary relationships within Trochoidea.

2 | MATERIALS AND METHODS

2.1 | Sample collection

All the species of Trochoidea were collected along Hainan Island, China (Table 1). After collection, specimens were immediately preserved in 95% ethanol. The total genomic DNA was isolated from 5 to 10 mg of foot tissue following a modified CTAB method (Winnepenninckx et al., 1994), and the quality of the genomic DNA was visualized on 1.0% agarose gel. The extracted DNA was preserved in TE solution and frozen at −30℃ until used.

2.2 | Mitochondrial genome sequencing, assembly and annotation

Genomic DNA was submitted to Novogene Company for library construction and high-throughput sequencing. Sequencing libraries with average insert sizes of approximately 300 bp were prepared and then sequenced as 150 bp paired-end runs on the Illumina HiSeq X platform. Finally, about 8 Gb of raw data were generated for each library. The raw reads were filtered using Trimmomatics v0.39 (Bolger et al., 2014). Short-read DNA sequences were assembled using de novo assembly in SPAdes (used in *Tectus triserialis*, *Astralium petrosum* and *Rochia conus*) (Bankevich et al., 2012) and Ray (used in remaining species) (Boisvert et al., 2010) with a k-mer of 31. The new mitochondrial protein-coding genes (PCGs) were annotated by identifying their open reading frames with ORF Finder (https://www.ncbi.nlm.nih.gov/orffinder/). Gene boundaries were examined and subsequently adjusted manually by comparison

TABLE 1 Mitogenomes newly sequenced and used in this study

Species	Family	Superfamily	Length (bp)	GenBank acc. no.	Locality
Trochus histrio	Trochidae	Trochoidea	16,965	MT752948	Yonglequndao, Hainan Province
Trochus maculatus	Trochidae	Trochoidea	16,899	MT752949	Luhuitou, Hainan Province
Clanculus denticulatus	Trochidae	Trochoidea	16,861	MT752944	Qiziwan, Hainan Province
Monodonta labio	Trochidae	Trochoidea	16,388	MT752946	Danzhou, Hainan Province
Calliostoma unicum	Calliostomatidae	Trochoidea	16,421	MT752943	Nanjidao, Hainan Province
Astralium rhodostomum	Turbinidae	Trochoidea	18,098	MT752942	Jinqingdao, Hainan Province
Astralium petrosum	Turbinidae	Trochoidea	17,449	MT663150	Ganquandao, Hainan Province
Turbo chrysostomus	Turbinidae	Trochoidea	17,031	MT752953	Luhuitou, Hainan Province
Turbo argyrostomus	Turbinidae	Trochoidea	16,928	MT752951	Yagongdao, Hainan Province
Turbo bruneus	Turbinidae	Trochoidea	16,904	MT752952	Luhuitou, Hainan Province
Rochia conus	Unassigned	Trochoidea	17,863	MT752950	Luhuitou, Hainan Province
Tectus triserialis	Unassigned	Trochoidea	18,897	MT752947	Shanhudao, Hainan Province
Herpetopoma lischkei	Chilodontidae	Seguenzioidea	18,659	MT752945	Dongfang, Hainan Province

Note: The length of each mt genome is indicated in base pairs (bp), and GenBank accession number and sampling locality are provided.

with sequenced Trochoidea mt genomes. The transfer RNA (tRNA) genes were further identified with both ARWEN (Laslett & Canbäck, 2008) and MITOS web servers (http://mitos.bioinf.uni-leipzig.de/index.py) (Bernt et al., 2013), using the invertebrate mitochondrial genetic code and the default search mode. The rRNA genes were identified by sequences comparison with previously reported Trochidae mt genomes by BLAST search.

2.3 | Sequence alignment

The newly determined complete mt genomes were aligned with all orthologous Vetigastropoda mt genomes available in GenBank (www.ncbi.nlm.nih.gov/; Table 2). Three data sets were constructed and analysed. The first (hereafter referred to as the VA data set) and second (VN) data sets were aimed to test the monophyly of Trochoidea and included another five superfamilies (Seguenzioidea, Lepetodriloidea, Haliotoidea, Fissurelloidea and Pleurotomarioidea) within Vetigastropoda. One species *Chrysomallon squamiferum* from superfamily Neomphaloidea was selected as outgroup (Uribe, et al., 2016; Uribe et al., 2017). The VA data set included the deduced amino acid sequences of the 13 mt protein-coding genes, and the VN data set included the nucleotide sequences of the 13 mt protein-coding genes and two rRNA genes. The third data set (hereafter referred to as the TN data set) was aimed to test the internal phylogenetic relationships of Trochoidea. The TN data set included 13 mt protein-coding genes and two rRNA genes, both were analysed at the nucleotide level. The 13 PCGs were aligned separately using Translator X (Abascal et al., 2010), according to the Invertebrate Mitochondrial genetic code, whereas rRNA genes were aligned separately using MAFFT v7 (Katoh & Standley, 2013) with default parameters. Ambiguously aligned positions were removed using Gblocks v.0.91b (Castresana, 2000) under default setting. Finally, the different single alignments were concatenated using Sequence Matrix 1.7.8 (Vaidya et al., 2011). Sequence format was converted using DAMBE (Xia and Xie, 2001) for further analyses. Pairwise genetic distances were calculated using NT data set in MEGA 7 (Kumar et al., 2016).

2.4 | Phylogenetic analyses

The best partition schemes and best-fit models of substitution for the data sets for phylogenetic analyses were identified using PartitionFinder 2 (Lanfear et al., 2017) according to the Bayesian information criterion (BIC; Schwarz, 1978). For the PCGs analysed at both nucleotide and amino acid levels, the partitions tested were all genes combined; all genes separated (except *atp6-atp8* and *nad4-nad4L*); and genes grouped by subunits (*atp*, *cob*, *cox* and *nad*) (followed by Uribe, et al., 2016). In addition, these three partition schemes at nucleotide level were tested considering first, second and third codon positions separately. For the mt rRNA genes, the two genes were tested both combined and separated. The selected best-fit partitions and models are provided in Tables S1, S2 and S3.

Phylogenetic analyses were conducted with maximum likelihood (ML, Felsenstein, 1981) and Bayesian inference (BI, Huelsenbeck & Ronquist, 2001) using the VA, VN and TN data sets. ML analyses were carried out using RAxML v. 8.2.1 (Stamatakis, 2006) with the rapid hill-climbing

TABLE 2 Mitogenomes downloaded from NCBI and analysed in this study. The length of each mt genome is indicated in base pairs (bp), and the GenBank accession number is provided

Species	Family	Superfamily	Length (bp)	GenBank acc. no.
Angaria delphinus	Angariidae	Trochoidea	19,554	NC_031860
Angaria neglecta	Angariidae	Trochoidea	19,470	NC_028707
Astralium haematragum	Turbinidae	Trochoidea	16,310	NC_031858
Bolma rugosa	Turbinidae	Trochoidea	17,432	NC_029366
Lunella aff cinerea	Turbinidae	Trochoidea	17,670	KF700096
Lunella correensis	Turbinidae	Trochoidea	17,308	MN604179
Lunella granulata	Turbinidae	Trochoidea	17,632	NC_031857
Chlorostoma argyrostomum	Tegulidae	Trochoidea	17,780	NC_031859
Omphalius nigerrimus	Tegulidae	Trochoidea	17,755	NC_031862
Omphalius rusticus	Tegulidae	Trochoidea	17,799	MG836833
Tegula brunnea	Tegulidae	Trochoidea	17,690	NC_016954
Tegula lividomaculata	Tegulidae	Trochoidea	17,375	NC_029367
Cittarium pica	Unassigned	Trochoidea	17,949	KY212109
Rochia nilotica	Unassigned	Trochoidea	16,966	MK284240
Tectus pyramis	Unassigned	Trochoidea	18,439	NC_036068
Tectus virgatus	Unassigned	Trochoidea	Partial	KY205709
Clanculus margaritarius	Trochidae	Trochoidea	-	-
Gibbula umbilicalis	Trochidae	Trochoidea	16,277	KY661530
Stomatella planulata	Trochidae	Trochoidea	17,151	NC_031861
Umbonium thomasi	Trochidae	Trochoidea	15,998	NC_041307
Calliostoma zizyphinum	Calliostomatidae	Trochoidea	-	-
Margarites vorticiferus	Margaritidae	Trochoidea	Partial	KY205708
Phasianella australis	Phasianellidae	Trochoidea	Partial	KX298888
Phasianella solida	Phasianellidae	Trochoidea	16,698	NC_028709
Granata lyrata	Chilodontidae	Seguenzioidea	17,632	NC_028708
Lepetodrilus nux	Lepetodrilidae	Lepetodriloidea	Partial	LC107880
Lepetodrilus schrolli	Lepetodrilidae	Lepetodriloidea	Partial	KR297250
Haliotis rubra	Haliotidae	Haliotoidea	16,907	NC_005940
Haliotis tuberculata	Haliotidae	Haliotoidea	16,521	NC_013708
Diodora graeca	Fissurellidae	Fissurelloidea	17,209	KT207825
Fissurella volcano	Fissurellidae	Fissurelloidea	17,575	NC_016953
Variegemarginula punctata	Fissurellidae	Fissurelloidea	14,440	KX298889
Bayerotrochus teramachii	Pleurotomariidae	Pleurotomarioidea	13,473	MH837533
Perotrochus caledonicus	Pleurotomariidae	Pleurotomarioidea	14,082	MH837539
Chrysomallon squamiferum	Peltospiridae	Neomphaloidea	15,388	AP013032

algorithm and 10,000 bootstrap pseudoreplicates (BP). The BP values <50, between 50 and 70, and >70 are considered to indicate non-significant, moderate and high statistical support, respectively. BI analyses were performed with MrBayes v.3.2 (Ronquist et al., 2012) by default (the Temp parameter is 0.2), running four simultaneous Monte Carlo Markov chains (MCMC) for 10 million generations (sampling every 1,000 generations), and discarding the first 25% generations as burn-in. Parameter convergence was achieved within ten million generations, and the standard deviation of split frequencies was less than 0.01. Two independent Bayesian inference runs were performed. All parameters were checked with Tracer v. 1.7 (Rambaut et al., 2018), and the effective sample size (ESS) was more than 200. Node support was assessed based on Bayesian posterior probabilities (BPP). We consider BPP values higher than 0.95 as high statistical support. The resulting phylogenetic trees were visualized in FigTree v1.4.2 (Rambaut, 2014).

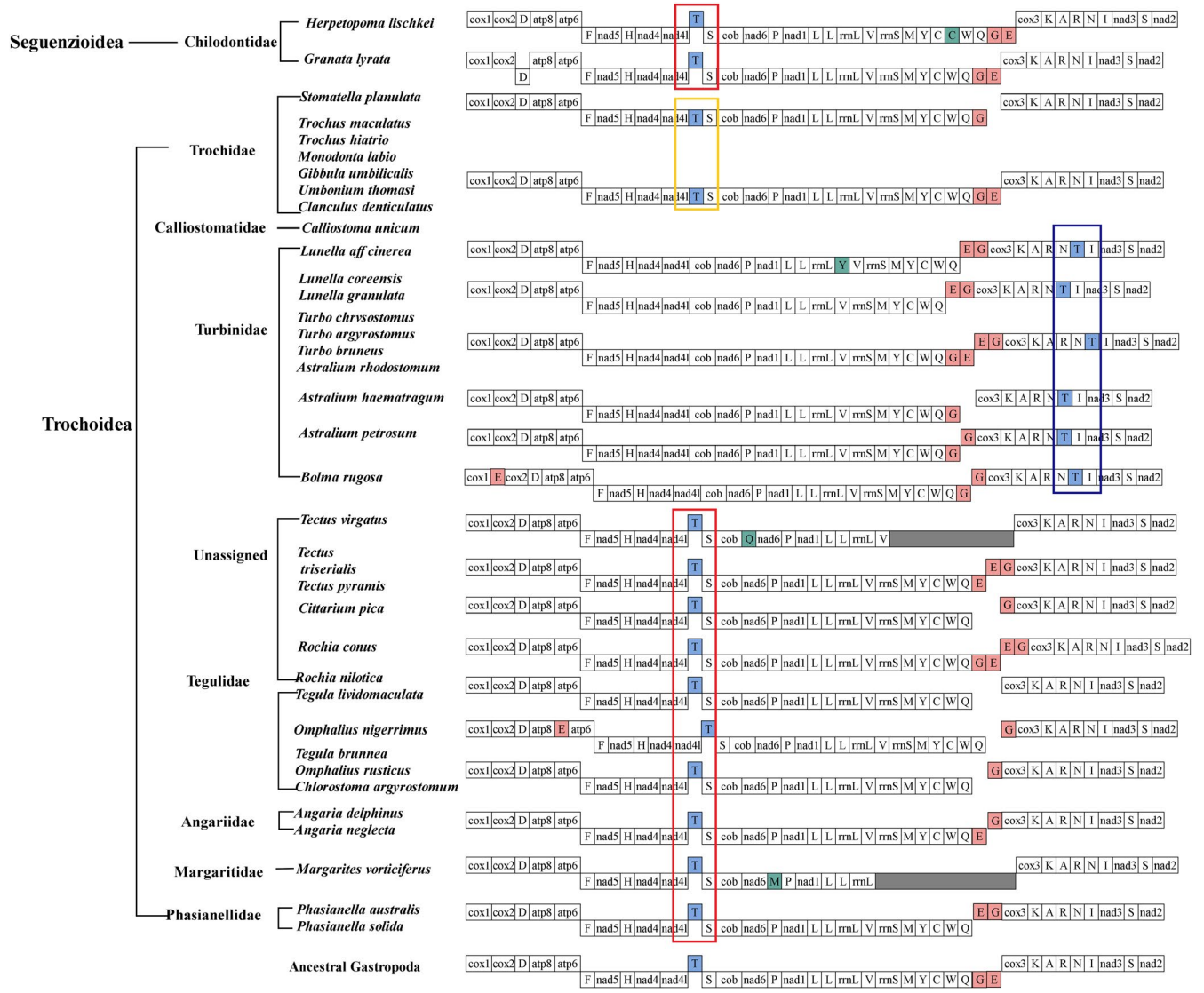

FIGURE 1 Mitochondrial gene orders of the main lineages of Trochoidea. Two Seguenzioidea are also shown for comparison. Genes encoded in major and minor strands are shown in the top and the bottom lines, respectively. Rearrangement genes are colored. Varied types arrangements of *trnT* are highlighted with different color boxes: type 1 is indicated in red and type 2 is yellow, the blue color applies to type 3

3 | RESULTS AND DISCUSSION

3.1 | Genome structure and organization

The 12 newly sequenced Trochoidea mt genomes are found to be from 16,904 bp (*Turbo bruneus*) to 18,897 bp (*T. triserialis*) possessing the typical Trochoidea mt gene content of 13 PCGs, 20 ~ 24 tRNA genes and 2 rRNA genes. The sequence of *H. lischkei* (Seguenzioidea) is 18,659 bp in size possessing 13 PCGs, 23 tRNA genes and 2 rRNA genes. These mt genomes share the same gene order with regard to the relative position of protein-coding genes but with rearrangements of some tRNAs (Figure 1). The major strand encodes 7 PCGs (*cox1*, *cox2*, *atp6*, *atp8*, *cox3*, *nad3* and *nad2*) and 7 tRNAs (*trnD*, *trnK*, *trnA*, *trnR*, *trnN*, *trnI* and *trnS2*). The remaining protein-coding genes (*nad5*, *nad4*, *nad4L*, *cytb*, *nad6* and *nad1*) and 12 tRNAs (*trnF*, *trnH*, *trnS1*, *trnP*, *trnL1*, *trnL2*, *trnV*, *trnM*, *trnY*, *trnC*, *trnW* and *trnQ*) are disposed in the minus strand. The *trnE*, *trnG* and *trnT* are disposed either in the minus strand or in the major strand. Both rRNA genes are transcribed from the minus strand that 16S rRNA is flanked by *trnL* and *trnV*, while 12S rRNA is located between *trnV* and *trnM*. For Seguenzioidea species, the genome structure and gene composition are same with Trochoidea except for certain tRNA arrangement (Figure 1).

3.2 | Gene rearrangement

The complete mt genomes of all species possess 20–24 tRNA genes. When compared to the hypothetical ancestral gastropod gene order (Osca et al., 2014a; Stöger & Schrödl, 2013),

Granata lyrata and *H. lischkei* mt genomes presented slight differences that only a *trnD* inversion and a *trnC* duplication, respectively (Figure 1). The species are the only two representatives whose mt genome is available of Seguenzioidea; it is hard to determine whether these distinctive gene orders are an autapomorphy or a more general feature of Seguenzioidea.

Within Trochoidea, in relation to the most common mt gene orders, rearrangements mainly resulted from translocation, inversion, duplication or loss of three tRNA genes (*T*, *E*, *G*) (Figure 1).

A total of three major genome arrangement types are detected only based on the *trnT* gene rearrangements. Type 1, identified in Tegulidae, Angariidae, Margaritidae, Phasianellidae and three unassigned genera (*Tectus*, *Rochia* and *Cittarium*), is identical to the ancestral gastropoda condition which *trnT* is located between *nad4l* and *trnS* in the major strand. The gene order of Trochidae and Calliostomatidae (type 2) closely resembles that of the ancestral gastropoda mitochondrial genome, only with *trnT* inverted to the minor strand. Turbinidae (type 3) possesses a novel rearrangement of *trnT* which is shifted to a new relative position between *trnN* and *trnI* in the major strand. Gene order rearrangements in mt genomes have been widely found, and if shared by two taxa can be considered molecular synapomorphies that may provide useful data for phylogenetic reconstruction (Grande et al., 2008). In these analyses, Trochidae and Calliostomatidae share *trnT* reversion and all Turbinidae genera (*Turbo*, *Astralium* and *Lunella*) possess *trnT* translocation, suggesting the *trnT* rearrangement may be molecular synapomorphies in family level. Beyond that, the results reveal that though *trnT* gene has three coding positions, and their codons are concordant; therefore, there is no correlation between tRNA gene location and codon use. These new findings may provide implications for understanding the phylogenetic relationships between Trochoidea families.

According to recent studies, many Trochoidea mt genomes showed rearrangements affecting *trnG* and *trnE* genes, and in some instances, one or both genes were missing (Lee et al., 2016; Uribe, et al., 2016; Uribe et al., 2017). To determine whether other species of Trochoidea share the same rearrangements, we analysed the all Trochoidea complete mitochondrial genomes referred in the study (Figure 1). All genomes were observed arrangement diversity regarding the *trnE* and *trnG* with the exception of Trochidae (without *Stomatella planulata*) and Calliostomatidae whose gene orders are identical with the hypothetical ancestral gastropoda. For remaining species, ten types were inferred as follows: *S. planulata* and *Astralium haematragum* genomes (type A) possess congruent condition with genomes of *Cittarium pica*, *Tegula brunnea*, *Chlorostoma argyrostomum* and *Omphalius rusticus* (type B) which only lack *trnE* gene compared with ancestral gastropoda (Osca et al., 2014a; Stöger & Schrödl, 2013); however, the *trnG* gene is encoded on different strands in the both types. The *trnG* arrangement of *Omphalius nigerrimus* (type C) and *Bolma rugosa* (type D) is identical to that of the previously type except for the location of *trnE* (Figure 1). The identical gene order exhibited by the genomes of type E (*Angaria*) and type *F* (*Lunella* and *Phasianella solida*) that is *trnQ-trnE-trnG-cox3*, but with different coding strand of *trnE* (Figure 1). There is considerable variation in *Rochia nilotica* and *Tegula lividomaculata* (type G) that both genes are not observed; every remaining type possesses unique arrangement, but they share duplication of *trnE* and/or *trnG*. In *A. petrosum* (type H), only *trnG* is duplicated and both *trnG* are arranged on the opposite strand. Within *Tectus* species, both taxa (*Tectus pyramis* and *T. triserialis*) (type I) share the condition that *trnG* is located at the major strand and *trnE* is duplicated and located on the two strands. In *Turbo, Astralium rhodostomum*, *R. conus* (type J), both genes are duplicated and appear in inverse order on the opposite strand (*trnE–trnG* in the major strand, *trnG–trnE* in the minor strand). It is impossible to infer the evolution of these rearrangements, given that this part of the mt genome could not be sequenced in *T. virgatus* and *Margarites vorticiferus*, and is not available for *C. margaritarius* and *C. zizyphinum* (Uribe et al., 2017). Adding *trnG–trnE* to the end of the ancestral cluster *trnM–trnY–trnC–trnW–trnQ* might be synapomorphic for gastropod and the location is known for having relatively high rates of gene rearrangement, before the hypothesized control region of gastropod mt genomes (Duarte et al., 2008). The translocation and loss of *trnE* and *trnG* have been described frequently (Lee et al., 2016; Uribe, et al., 2016; Uribe et al., 2017), and only the duplication of *trnE* first found in *T. pyramis* (Zhao et al., 2018), but in our study, one or both genes are duplicated in eight species referring to type H, I and J (Figure 1). The duplicated genes have a completely similarity, some studies have shown the same mitochondrial genes in the group were generated by replication, and the higher the similarity rate, the later the replication event occurred (Xu et al., 2012). Similarly, in *H. lischekei*, the similarity between *trnC* genes was found up to 94%. The duplication of extremely high similarity reveals these multiple copies of the tRNA genes are most likely due to recent replication events. Beyond that, the unexpected loss of tRNAs appears to be enigmatic, while compensatory mechanisms seem to be an acceptable hypothesis to a functional tRNA during the translation and the interaction with the ribosome (Amaral et al., 2016; Domes et al., 2008; Masta & Boore, 2008; Schneider, 2001). As discussed by Clayton (1992), the tRNA losses may happen because protein encoded in the nucleus could interact with the mitochondria during the translation step. Indeed, tRNAs, which are encoded simultaneously in the nucleus, could suffer a selective pressure for deletion providing the reduction of mt genome size (Amaral et al., 2016; Domes et al., 2008). The comparative analyses for diversity arrangements of *trnE* and *trnG* reveal that both

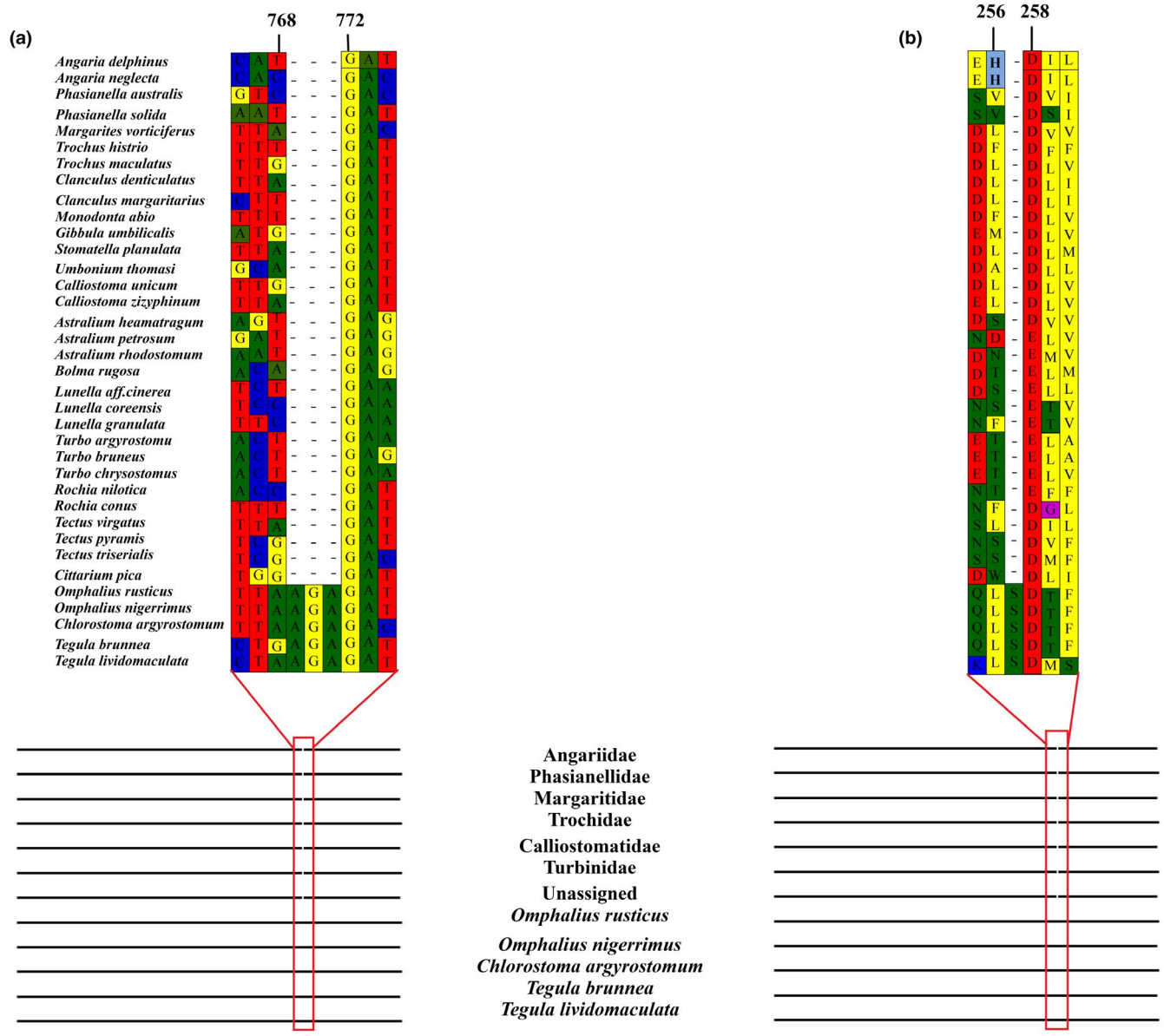

FIGURE 2 *Nad1* sequence differences in 36 Trochoidea. Nucleotide (a) and amino acid (b) alignments of a portion of *nad1* gene indicate that the three-nucleotide insertion present in Tegulidae

of replication and loss are coded separately, the combination of replication and loss results in *trnE* and/or *trnG* encoded by major strand that displayed in five types (B, C, D, E and F) instead of only inversion event seems to be an acceptable hypothesis. Comparative analyses of *trnE* and *trnG* gene evolution characteristics may provide useful information for future revisions on Trochoidea taxonomy and evolutionary process research.

3.3 | Protein-coding genes

All PCGs of 12 newly sequenced Trochoidea mt genomes start with the typical codon ATG except *nad4* in *Clanculus denticulatus*, *A. rhodostomum*, *Calliostoma unicum* and *nad6* in *T. triserialis*, which employ alternative initiation codon GTG. Within Seguenzioidea, initiation codon GTG is also used in *nad4* gene of *H. lischkei*, and has been reported as a start codon in *G. lyrata* (Uribe, et al., 2016).

All of PCGs analysed in the present study used conventional initiation codons, and both ATG and GTG have reported in many mollusc groups (Marquez et al., 2016; Osca et al., 2015; White et al., 2011; Xu et al., 2012). The 13 PCGs end in complete termination codons TAG and TAA which are the most common termination codons, and no incomplete stop codons are found in our study.

When the PCGs of 36 Trochoidea species (TN data set) are aligned, Tegulidae does not contain continuous

three-nucleotide deletion present in the other species in *nad1* genes (Figure 2), as in this case, in *nad5* genes (Figure 3), continuous nine-nucleotide insertion is found only in *Tectus* sequences (*T. pyramis* and *T. triserialis*). Interestingly, *Rochia* species (*R. nilotica* and *R. conus*) and *T. virgatus* contain continuous six-nucleotide deletion in another separate loci. A translated amino acid alignment confirms the locations. As a result, the insertion or deletion only leads to the insertion or deletion of corresponding amino acid and does not bring other changes in the deduced amino acid sequences. Nucleotide insertions and deletions (indels) are one of the major sources of evolutionary change at the molecular level (Tao et al., 2008). The dramatic observation of the variation seen in the *nad1* gene of Tegulidae, *nad5* gene of *Rochia* species and *Tectus* species is elucidated in this study. The mitochondrial gene deletions among related species in *cox1* gene of *Melibe* (Gastropoda) taxa and in *nad6* gene of Reticunassa have been reported and the deletions were explained as a derived trait, which may reflect unusual constraints on the protein in those taxa (Sevigny et al., 2015; Yang et al., 2018). Hence, the *nad5* deletions may be a conservative trait. Besides, the insertions may be a derived trait as well, but few nucleotide insertion events were reported which probably because that insertions occur less frequently. In these analyses, limited taxa in same genus or family and ambiguous classification status make us unable to expand our understanding of these traits. Nevertheless, our study potentially contributes to study patterns of indels and further deciphers the evolutionary process of Trochoidea genomes.

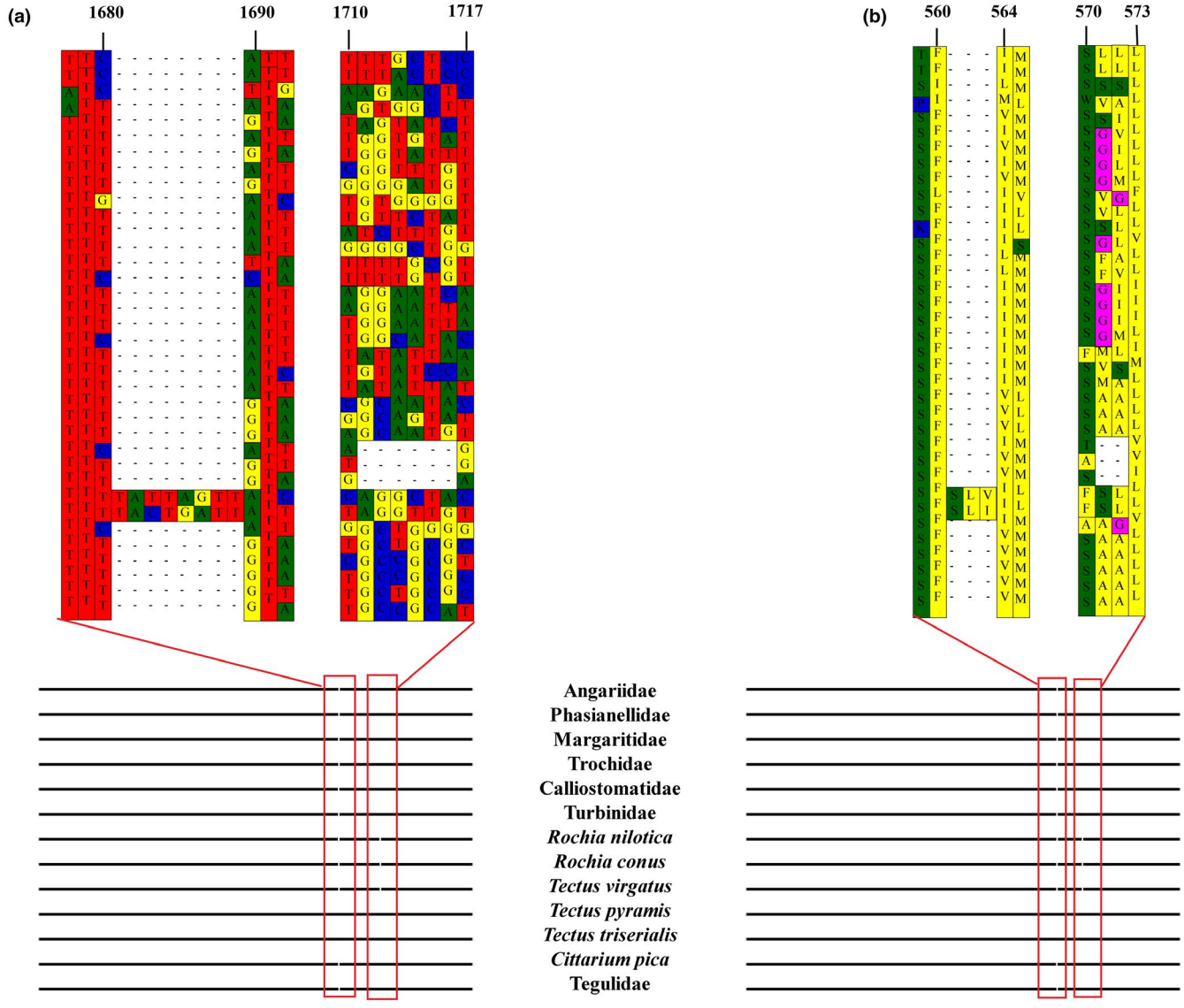

FIGURE 3 *Nad5* sequence considerable differences in 36 Trochoidea (their order is same with Figure 2). Nucleotide (a) and amino acid (b) alignments of a portion of *nad5* gene indicate that all species lack the nine-nucleotide insertion present in *Tectus pyramis* and *T. triserialis* and on the basis, *Rochia nilotica*, *R. conus* and *T. virgatus* which have another six-nucleotide deletion

3.4 | Interspecific genetic distance and phylogenetic relationship

According to the Bayesian information criterion (BIC), the best partition scheme for PCGs at nucleotide level was the one combining genes by subunits but analysing the three codons separately. At the amino acid level, the best partition scheme for PCGs was also the one combining genes by subunits. For the rRNA genes, the best partition scheme was combining 12S and 16S rRNA genes together. The best substitution model for each partition is shown in Tables S1, S2 and S3. Phylogenetic analyses included Vetigastropoda tree (hereafter designed the Tree V) and Trochoidea tree (hereafter designed the Tree T). Vetigastropoda phylogenetic analyses were conducted to test monophyly of Trochoidea with maximum likelihood (ML, Felsenstein, 1981) and Bayesian inference (BI, Huelsenbeck & Ronquist, 2001) using the VA data set (contain 2,371 sites) and VN data sets (contain 7,927 sites). Phylogenetic analyses with ML and BI rendered rather unresolved trees based on VN data sets (see Figures S1 and S2) due to the extremely long branches of *Perotrochus caledonicus* that caused significant instability of the trees. The ML and BI phylogenetic analyses based on VA data set arrived at the almost identical topologies, only differing in the relationships within Trochoidea (Figure 4). Most nodes were strongly supported but some nodes received moderate and low support. We incorporated two representatives of the superfamily Pleurotomarioidea including *Bayerotrochus teramachii* (MH837533) and *Perotrochus caledonicus* (MH837539), whose clade was placed as sister group to all other Vetigastropoda lineages with high statistical support (BP 99%, PP 1), keeping with other phylogenies (Kano, 2008; Williams et al., 2008; Zapata et al., 2014). Our analyses found strong support for the placement of Fissurelloidea as the next clade to diverge from other vetigastropoda lineages; however, the members exhibited relatively long branches. Fissurelloidea also have usually been recovered as sister group of the remaining Vetigastropoda lineages in previous mt genome phylogenies (Lee et al., 2016; Uribe, et al., 2016; Wort et al., 2016) where no representatives of Pleurotomarioidea were included. However, the result clearly differed from a phylogeny recently reconstructed by Uribe et al. (2017) that the position of Lepetodriloidea as sister group of the remaining Vetigastropoda lineages was supported with moderate statistical support. The possibility of a long-branch attraction effect by the outgroup cannot be dismissed. The lineage Seguenzoidea was the sister group of the clade formed by Trochoidea and Haliotoidea + Lepetodriloidea and the relationship received relatively high support in ML tree (BP: 74.2%) whereas moderate support in BI tree (PP: 0.65), similar to a phylogeny recently reconstructed by Zapata et al. (2014), based on nuclear transcriptomic data, in which phylogenetic relationships within Vetigastropoda were fully resolved (i.e. all nodes received maximal statistical support). In that study, Seguenzoidea were recovered as the sister group of a clade in which Lepetodriloidea was sister to Lepetelloidea and Haliotoidea was sister to

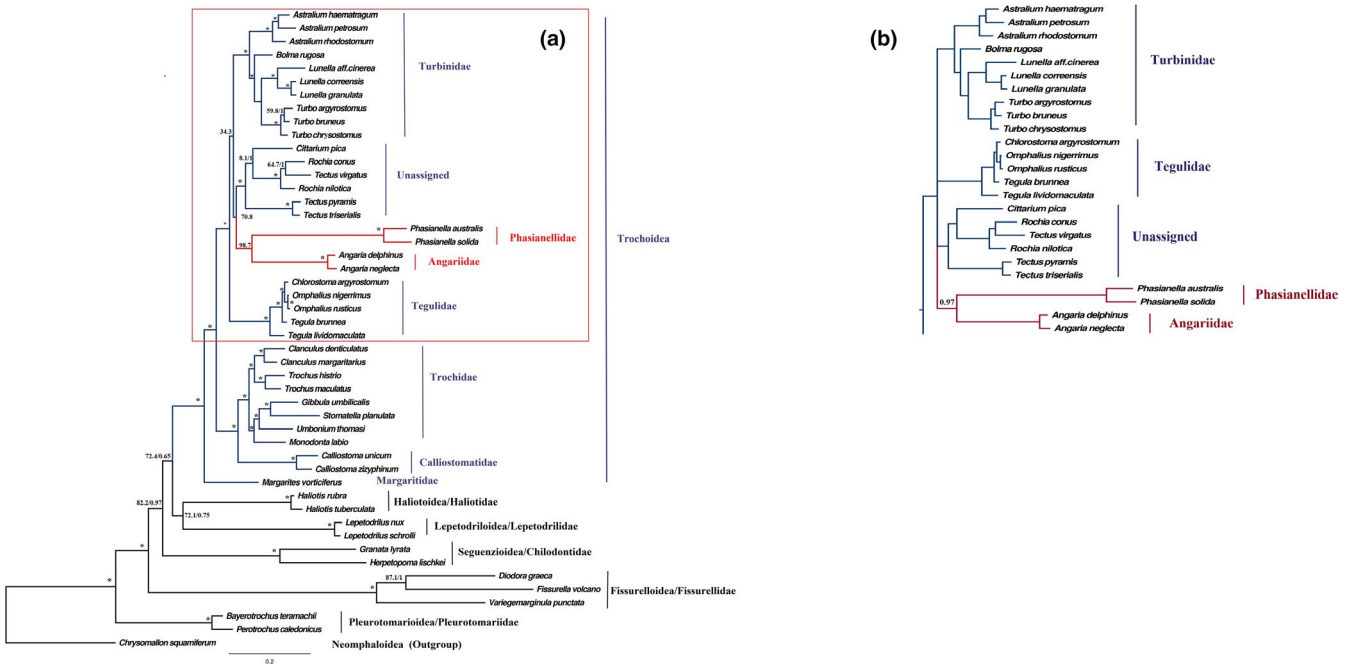

FIGURE 4 Phylogenetic relationships of Vetigastropoda based on 12 protein-coding genes at the amino acid level. The ML phylograms (a) are shown. Topology differences in BI are shown in the inset (b). The superfamily Trochoidea is indicated in blue (Phasianellidae and Angariidae are red); the black colour in the tree applies to the other superfamilies. Numbers at nodes are statistical support values for ML (bootstrap proportions in percentage)/BI (posterior probabilities). An asterisk represents nodes with posterior probabilities ≥0.95 and bootstrap proportions ≥90

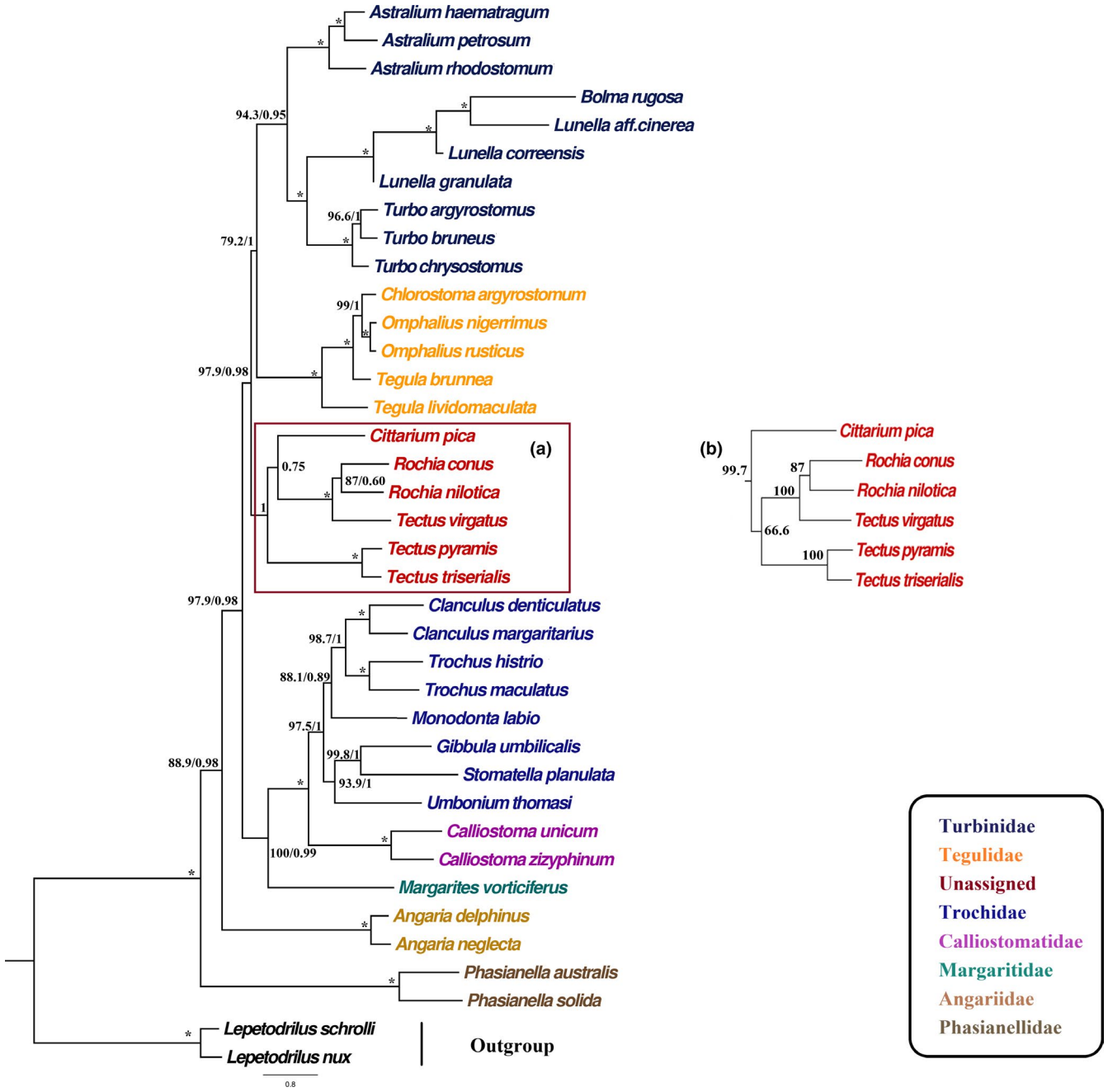

FIGURE 5 Phylogenetic relationships of Trochoidea based on 12 protein-coding genes and 2 rRNA at the nucleotide level. The BI phylograms (a) are shown. Topology differences in ML are shown in the inset (b). Numbers at nodes are statistical support values for ML (bootstrap proportions in percentage)/BI (posterior probabilities). An asterisk indicates maximal support in ML (BP: 100%) and BI (PP 1)

Trochoidea (including Phasianelloidea); however, no representatives of Fissurelloidea were included (Zapata et al., 2014). The differences may be due to the missing of Lepetelloidea in our study, whereas in recent phylogenies (Lee et al., 2016; Uribe, et al., 2016; Uribe et al., 2017; Wort et al., 2016), superfamilies Seguenzoidea and Haliotoidea formed a well-supported clade. In this study, the branches of these superfamilies are relatively long, but almost all nodes receive high statistical support; besides, superfamilies Scissurelloidea are also missing (Uribe, et al., 2016; Uribe et al., 2017). Several molecular phylogenies based on partial gene sequences have recovered a close relationship between Scissurelloidea and Lepetodriloidea (Kano, 2008; Williams & Ozawa, 2006; Yoon & Kim, 2005), while the relative phylogenetic position of Lepetelloidea remains controversial (Aktipis & Giribet, 2012).

The main focus of the present phylogenetic analysis was Trochoidea which the monophyly was recovered and received maximal support in Uribe et al. (2017), and our result (Tree V) also supported the monophyly of the group with maximal support (Figure 4). The initial recognition of Phasianellidae

and Angariidae as valid superfamilies (Phasianelloidea and Angarioidea) distinct from Trochoidea (Hickman & McLean, 1990) was based on phylogenetic analyses of partial mt and nuclear genes, which placed these two lineages in early-diverging positions in the Vetigastropoda tree (Williams et al., 2008; Aktipis & Giribet, 2012; see also position of Phasianelloidea in Kano, 2008). The phylogenies, based on mt (Lee et al., 2016; Uribe, et al., 2016; Uribe et al., 2017; Wort et al., 2016) and nuclear (Zapata et al., 2014) genomic datasets, have likewise recovered a clade grouping Trochoidea together with Phasianellidea and Angariidea (the latter were missing in Zapata et al., 2014). Until recently, Phasianellidae and Angariidae were accepted as members of Trochoidea (http://www.marinespecies.org/) (Bouchet et al., 2017) instead of distinct superfamilies. Our results displayed that the phylogenetic position of Phasianellidae and Angariidae was rather unstable. In Tree V, Phasianellidae and Angariidae were recovered as a clade grouping with the several unassigned genera, diverse with Uribe et al. (2017). However, Phasianellidae and Angariidae failed to form a clade; instead, Phasianellidae was as the sister group of Angariidae + remaining families in Tree T. Tree T, a molecular phylogeny of Trochoidea, was reconstructed using above methods based on nucleotide sequences of 13 PCGs and two rRNAs of Trochoidea main lineages to resolve the internal phylogenetic relationships, *Lepetodrilus schrolli* and *Lepetodrilus nux* were used as outgroup. Most nodes were strongly supported but some nodes received moderate support. Except for Angariidae and Phasianellidae, two major lineages could be distinguished. The reconstructed phylogenetic trees recovered the Trochidae as sister group of the Calliostomatidae with maximal statistical support and the resulting clade was sister group of the *M. vorticiferus,* which formed a monophyletic group with high support (BP: 100%, PP: 0.99). The next lineage was Turbinidae + (paraphyletic) Tegulidae, in which some certain Turbinidae members (*Lunella* and *B. rugosa*) exhibited relatively long branches that may have produced a long-branch attraction effect and the pulling of these two branches to more basal positions. Tegulidae has been regarded as the most formidable to resolve using either morphological or molecular data, and as such, systematics of the family has not been confirmed yet (Williams, 2012); especially, the genus *Turbo* may not be monophyletic. Within the group, so far, only five complete mt genomes were sequenced and no representatives of *Turbo* were available, a situation that has been addressed in the present analyses, we increased two *Astralium* and three *Turbo* mt genomes that formed tight groups with Maximum support, respectively. In terms of higher-level relationships, the monophyly of Turbinidae received maximal support as well. Within the lineage, the internal relationships of Tegulidae are equivocal, and no evidence supported monophyly of Tegulidae which indicated (a) the *Tectus*, *Cittarium* and *Rochia* should be assigned to a new family; and (b) the taxonomic status of *Tegula*, *Omphalius* and *Chlorostoma* needs to be further analysed. Williams (2012) first recovered *Tectus*, *Cittarium* and *Rochia* as a distinct clade, although with low support. The clade was not formally described, but she suggested familial rank pending further studies and the suggestion was supported by Uribe et al. (2017). Nevertheless, our result revealed that the topologies of BI and ML tree were different among the clade of *Tectus*, *Rochia* and *Cittarium* (Figure 5) due to the unstable placement of *C. pica*. Otherwise, the speciose genus *Tegula* are likewise non-monophyletic; alternatively, the genera *Omphalius* and *Chlorostoma* were supposed to be grouped together with *Tegula* (Uribe et al., 2017). Our results supported *Tegula* is non-monophyletic, whereas the taxonomic status needs further analysing combined with genetic distances (will be analysed below). In addition, according to the phylogenetic results that *T. virgatus* shared a closer affinity with *Rochia* and comparative mt genome evidence (the deletions of *nad5* genes observed in *T. virgatus* and *Rochia* as mentioned above), we supported the validity of the classification of *T. virgatus* as a species of *Rochia*.

In order to gain insights into the phylogenetic relationships, the genetic distances of 13 PCGs and 12 rRNA genes between Trochoidea species were conducted and part of the heatmaps are provided in Figure 6. The close affinity of *Tegula* to *Omphalius*, *Tegula* to *Chlorostoma* and *Omphalius* to *Chlorostoma* was indicated with genetic distances of 0.13–0.20, 0.13–0.20 and 0.11, respectively, lower than the distances between congeneric species of *Trochus* (0.21); thus, we supported the genera *Tegula*, *Omphalius* and *Chlorostoma* should be grouped together with *Tegula*. The genetic distance between *T. virgatus* and *Rochia* was 0.22–0.23, yet with Tectus was 0.3 that accorded with the phylogenetic analysis and supported the validity of the classification of *T. virgatus* as a species of *Rochia*. Genetic distance values detected between Tegulidae and several unassigned genera (Rochia, Tectus, Cittarium) (0.28–0.31) were similar to values between Tegulidae and Trochidae (0.30–0.34). This is another evidence that support these genera should be considered as a distinct clade instead of being assigned to Tegulidae (Uribe et al., 2017; Williams, 2012). However, it is worth noting that the intergeneric genetic distances (0.28–0.30) between the several unassigned genera were quite similar to distances between them and Tegulidae (0.28–0.31), respectively. Hence, whether they should be placed into a same family is equivocal because of indeterminate topology (Figure 5) and relatively distant genetic distance. But, pairwise genetic distances among Tegulidae and these unassigned genera analyses may provide implications for future revisions on Trochoidea internal taxonomy. Although the complete mitochondrial sequences provided support for a robust phylogeny in our study, they are to a certain extent 'single genes' from a vantage of incomplete lineage sorting and evolution, and hence, it is essential to add nuclear genes for further study.

4 | CONCLUSION

Phylogenetic signal born in complete mt genome sequences is useful in addressing phylogenetic relationships within major lineages of Trochoidea. Here, we newly sequenced 13 complete

	Trm	*Trh*	*Cld*	*Clm*	*Mol*	*Umt*	*Giu*	*Stp*	*Omn*	*Omr*	*Cha*	*Teb*	*Tel*	*Cip*	*Roc*	*Ron*	*Tev*	*Tep*	*Tet*
Trm																			
Trh	0.21																		
Cld	0.25	0.24																	
Clm	0.24	0.23	0.21																
Mol	0.27	0.27	0.27	0.25															
Umt	0.27	0.28	0.28	0.27	0.27														
Giu	0.27	0.27	0.28	0.28	0.28	0.26													
Stp	0.28	0.29	0.28	0.28	0.30	0.27	0.25												
Omn	0.32	0.32	0.32	0.32	0.33	0.32	0.30	0.31											
Omr	0.32	0.31	0.32	0.32	0.33	0.31	0.30	0.32	0.07										
Cha	0.33	0.32	0.32	0.33	0.34	0.31	0.30	0.32	0.11	0.11									
Teb	0.32	0.32	0.32	0.32	0.34	0.32	0.30	0.32	0.13	0.13	0.13								
Tel	0.33	0.32	0.32	0.33	0.33	0.31	0.30	0.32	0.20	0.19	0.20	0.19							
Cip	0.33	0.33	0.32	0.33	0.34	0.33	0.32	0.33	0.29	0.29	0.29	0.30	0.28						
Roc	0.33	0.33	0.33	0.33	0.36	0.32	0.32	0.32	0.30	0.30	0.29	0.29	0.29	0.29					
Ron	0.34	0.34	0.32	0.33	0.36	0.33	0.32	0.32	0.30	0.30	0.30	0.30	0.29	0.28	0.20				
Tev	0.34	0.34	0.33	0.33	0.36	0.35	0.33	0.34	0.31	0.31	0.31	0.30	0.30	0.30	0.22	0.23			
Tep	0.32	0.33	0.33	0.33	0.36	0.33	0.32	0.32	0.29	0.28	0.28	0.29	0.29	0.29	0.29	0.28	0.30		
Tet	0.32	0.32	0.33	0.33	0.35	0.33	0.33	0.32	0.29	0.29	0.29	0.29	0.29	0.29	0.28	0.28	0.30	0.13	

FIGURE 6 Pairwise genetic distances of 12 PCGs and 2 rRNA of *Trochus maculatus* (*Trm*), *Trochus histrio* (*Trh*), *Clanculus denticulatus* (*Cld*), *Clanculus margaritarius* (*Clm*), *Monodonta labio* (*Mol*), *Gibbula umbilicalis* (*Giu*), *Stomatella planulata* (*Stp*), *Umbonium thomasi* (*Umt*), *Omphalius nigerrimus* (*Omn*), *Omphalius rusticus* (*Omr*), *Chlorostoma argyrostomum (Cha), Tegula brunnea* (*Teb*), *Tegula lividomaculata* (*Tel*), *Cittarium pica* (*Cip*), *Rochia conus* (*Roc*), *Rochia nilotica* (*Ron*), *Tectus virgatus* (*Tev*), *Tectus pyramis* (*Tep*) and *Tectus triserialis* (*Tet*)

mt genomes referring to one Seguenzioidea and 12 Trochoidea species. By comparative analyses of all sequences, three major characters were detected: homoplasious convergences of *trnT* gene rearrangement in Trochidae + Calliostomatidae and Turbinidae; considerable variation in location and number of *trnE* and *trnG* within Trochoidea; and three-nucleotide insertions in Tegulidae *nad1* genes, for *nad5* genes, nine-nucleotide insertions and six-nucleotide deletions in *Tectus* and *Rochia,* respectively. These characters could provide new ideas for further solving phylogenetic relationships in future. Comprehensive analyses for phylogenetic trees and genetic distances support non-monophyly of Tegulidae and *Tegula* which is identical with preceding studies (Lee et al., 2016; Uribe et al., 2017), indicating taxonomic status of *Rochia*, *Tectus* and *Cittarium* should be further studies and *Tegula*, *Omphalius* and *Chlorostoma* should be assigned to a same genus. The close affinity between *T. virgatus* and *Rochia* is also revealed. This phylogeny provides a robust backbone to further understand the evolutionary processes and diversification of Trochoidea. Future mt genome phylogenetic studies await incorporating a denser taxon sampling at the family level which will contribute to expand our understanding of the evolutionary processes.

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CONFLICT OF INTEREST

The authors declare no competing financial interests.

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REFERENCES

Abascal, F., Zardoya, R., & Telford, M. J. (2010). TranslatorX: Multiple alignment of nucleotide sequences guided by amino acid translations. *Nucleic Acids Research*, *38*, W7–W13. https://doi.org/10.1093/nar/gkq291

Aktipis, S. W., & Giribet, G. (2012). Testing relationships among the vetigastropod taxa: A molecular approach. *Journal of Molluscan Studies*, *78*, 12–27. https://doi.org/10.1093/mollus/eyr023

Amaral, D. T., Mitani, Y., Ohmiya, Y., & Viviani, V. R. (2016). Organization and comparative analysis of the mitochondrial genomes of bioluminescent Elateroidea (Coleoptera: Polyphaga). *Gene*, *586*, 254–262. https://doi.org/10.1016/j.gene.2016.04.009

Bankevich, A., Nurk, S., Antipov, D., Gurevich, A. A., Dvorkin, M., Kulikov, A. S., Lesin, V. M., Nikolenko, S. I., Pham, S., Prjibelski, A. D., Pyshkin, A. V., Sirotkin, A. V., Vyahhi, N., Tesler, G., Alekseyev, M. A., & Pevzner, P. A. (2012). SPAdes: A new genome assembly algorithm and its applications to single-cell sequencing. *Journal of Computational Biology*, *19*, 455–477. https://doi.org/10.1089/cmb.2012.0021

Bernt, M., Donath, A., Jühling, F., Externbrink, F., Florentz, C., Fritzsch, G., Pütz, J., Middendorf, M., & Stadler, P. F. (2013). MITOS: Improved de novo metazoan mitochondrial genome annotation. *Molecular Phylogenetics and Evolution*, *69*, 313–319. https://doi.org/10.1016/j.ympev.2012.08.023

Boisvert, S., Laviolette, F., & Corbeil, J. (2010). Ray: Simultaneous assembly of reads from a mix of high-throughput sequencing

technologies. *Journal of Computational Biology*, *17*, 1401–1415. https://doi.org/10.1089/cmb.2009.0238

Bolger, A. M., Lohse, M., & Usadel, B. (2014). Trimmomatic: A flexible trimmer for Illumina sequence data. *Bioinformatics*, *30*, 2114–2120. https://doi.org/10.1093/bioinformatics/btu170

Bouchet, P., Rocroi, J. P., Frýda, J., Hausdorf, B., Ponder, W. F., Valdés, Á., & Wáren, A. (2005). Classification and nomenclator of gastropod families. *Malacologia*, *47*, 1–397.

Bouchet, P., Rocroi, J.-P., Hausdorf, B., Kaim, A., Kano, Y., Nützel, A., Parkhaev, P., Schrödl, M., & Strong, E. E. (2017). Revised classification, nomenclator and typification of gastropod and monoplacophoran families. *Malacologia*, *61*, 1–526. https://doi.org/10.4002/040.061.0201

Castresana, J. (2000). Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Molecular Biology and Evolution*, *17*, 540–552. https://doi.org/10.1093/oxfordjournals.molbev.a026334

Clayton, D. A. (1992). Transcription and replication of animal mitochondrial DNAs. *International Review of Cytology*, *141*, 217–232. https://doi.org/10.1016/S0074-7696(08)62067-7

Cunha, R. L., Grande, C., & Zardoya, R. (2009). Neogastropod phylogenetic relationships based on entire mitochondrial genomes. *BMC Evolutionary Biology*, *9*, https://doi.org/10.1186/1471-2148-9-210

Domes, K., Maraun, M., Scheu, S., & Cameron, S. L. (2008). The complete mitochondrial genome of the sexual oribatid mite *Steganacarus magnus*: Genome rearrangements and loss of tRNAs. *BMC Genomics*, *9*, 13. https://doi.org/10.1186/1471-2164-9-532

Duarte, G. T., De Azeredo-Espin, A. M. L., & Junqueira, A. C. M. (2008). The mitochondrial control region of *Blowflies* (Diptera: Calliphoridae): A hot spot for mitochondrial genome rearrangements. *Journal of Medical Entomology*, *45*, 667–676. https://doi.org/10.1093/jmedent/45.4.667

Felsenstein, J. (1981). Evolutionary trees from DNA sequences: A maximum likelihood approach. *Journal of Molecular Evolution*, *17*, 368–376. https://doi.org/10.1007/BF01734359

Geiger, D. L., Nützel, A., & Sasaki, T. (2008). Vetigastropoda. In W. F. Ponder, & D. R. Lindberg (Eds.), *Phylogeny and evolution of the Mollusca* (pp. 297–330). Berkeley, CA: University of California Press.

Grande, C., Templado, J., & Zardoya, R. (2008). Evolution of gastropod mitochondrial genome arrangements. *BMC Evolutionary Biology*, *8*, https://doi.org/10.1186/1471-2148-8-61

Hedegaard, C. (1990). *Shell structure of the Recent Archaeogastropoda*. Ph.D: University of Aarhus, Department of Ecology and Genetics, Denmark.

Heß, M., Beck, F., Gensler, H., Kano, Y., Kiel, S., & Haszprunar, G. (2008). Microanatomy, shell structure and molecular phylogeny of *Leptogyra*, *Xyleptogyra* and *Leptogyropsis* (Gastropoda: Neomphalida: Melanodrymiidae) from sunken wood. *Journal of Molluscan Studies*, *74*, 383–401. https://doi.org/10.1093/mollus/eyn030

Hickman, C. S. (1996). Phylogeny and patterns of evolutionary radiation in trochoidean gastropods. In J. D. Taylor (Ed.), *Origin and evolutionary radiation of the Mollusca* (pp. 177–198). Oxford: Oxford University Press.

Hickman, C. S., & Mclean, J. H. (1990). Systematic revision and suprageneric classification of trochacean gastropods. *Natural History Museum of Los Angeles County, Science Series*, *35*, 1–169. https://doi.org/10.1016/0006-8993(78)90452-3

Huelsenbeck, J., & Ronquist, F. (2001). MrBayes: Bayesian inference of phylogenetic trees. *Bioinformatics*, *17*, 754–755. https://doi.org/10.1093/bioinformatics/17.8.754

Kano, Y. (2008). Vetigastropod phylogeny and a new concept of Seguenzioidea: Independent evolution of copulatory organs in the deep-sea habitats. *Zoologica Scripta*, *37*, 1–21. https://doi.org/10.1111/j.1463-6409.2007.00316.x

Kano, Y., Chikyu, E., & Warén, A. (2009). Morphological, ecological and molecular characterization of the enigmatic planispiral snail genus *Adeuomphalus* (Vetigastropoda: Seguenzioidea). *Journal of Molluscan Studies*, *75*, 397–418. https://doi.org/10.1093/mollus/eyp037

Katoh, K., & Standley, D. M. (2013). MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Molecular Biology and Evolution*, *30*, 772–780. https://doi.org/10.1093/molbev/mst010

Kumar, S., Stecher, G., & Tamura, K. (2016). MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution*, *33*, 1870–1874. https://doi.org/10.1093/molbev/msw054

Lanfear, R., Frandsen, P. B., Wright, A. M., Senfeld, T., & Calcott, B. (2017). PartitionFinder 2: New methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. *Molecular Biology and Evolution*, *34*, 772–773. https://doi.org/10.1093/molbev/msw260

Laslett, D., & Canbäck, B. (2008). ARWEN: A program to detect tRNA genes in metazoan mitochondrial nucleotide sequences. *Bioinformatics*, *24*, 172–175. https://doi.org/10.1093/bioinformatics/btm573

Lee, H., Samadi, S., Puillandre, N., Tsai, M. H., Dai, C. F., & Chen, W. J. (2016). Eight new mitogenomes for exploring the phylogeny and classification of Vetigastropoda. *Journal of Molluscan Studies*, *82*, 534–541. https://doi.org/10.1093/mollus/eyw027

Marquez, E. J., Castro, E. R., & Alzate, J. F. (2016). Mitochondrial genome of the endangered marine gastropod *Strombus gigas* Linnaeus, 1758 (Mollusca: Gastropoda). *Mitochondrial DNA Part A*, *27*, 1516–1517. https://doi.org/10.3109/19401736.2014.953118

Masta, S. E., & Boore, J. L. (2008). Parallel evolution of truncated transfer RNA genes in arachnid mitochondrial genomes. *Molecular Biology and Evolution*, *25*, 949–959. https://doi.org/10.1093/molbev/msn051

Osca, D., Irisarri, I., Todt, C., Grande, C., & Zardoya, R. (2014a). The complete mitochondrial genome of *Scutopus ventrolineatus* (Mollusca: Chaetodermomorpha) supports the Aculifera hypothesis. *BMC Evolutionary Biology*, *14*, 1–10. https://doi.org/10.1186/s12862-014-0197-9

Osca, D., Templado, J., & Zardoya, R. (2015). Caenogastropod mitogenomics. *Molecular Phylogenetics and Evolution*, *93*, 118–128. https://doi.org/10.1016/j.ympev.2015.07.011

Rambaut, A. (2014). *Fig Tree. Version 1.4.2*. http://tree.bio.ed.ac.uk/software/figtree/

Rambaut, A., Drummond, A. J., Xie, D., Baele, G., & Suchard, M. A. (2018). Posterior summarization in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology*, *67*, 901–904. https://doi.org/10.1093/sysbio/syy032

Rawlings, T. A., Collinst, T. M., & Bieler, R. (2003). Changing identities: tRNA duplication and remolding within animal mitochondrial genomes. *Proceedings of the National Academy of Sciences of the United States of America*, *100*, 15700–15705. https://doi.org/10.1073/pnas.2535036100

Reid, D. G., & Osorio, C. (2000). The shallow-water marine Mollusca of the Estero Elefantes and Laguna San Rafael, southern Chile. *Bulletin of the Natural History Museum London (Zoology)*, *66*, 109–146.

Robertson, R. (1985). Archaeogastropoda biology and the systematics of the genus *Tricolia* (Trochacea: Tricoliidae) in the Indo-West Pacific. *Monographs of Marine Molluscs*, *3*, 1–103.

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D. L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M. A., & Huelsenbeck, J. P. (2012). MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology*, *61*, 539–542. https://doi.org/10.1093/sysbio/sys029

Schneider, A. (2001). Does the evolutionary history of aminoacyl-tRNA synthetases explain the loss of mitochondrial tRNA genes? *Trends in Genetics*, *17*, 557–559. https://doi.org/10.1016/S0168-9525(01)02439-8

Schwarz, G. (1978). Estimating the dimension of a model. *Annals of Statistics*, *6*, 461–464. https://doi.org/10.1214/aos/1176344136

Sevigny, J. L., Kirouac, L. E., Thomas, W. K., Ramsdell, J. S., Lawlor, K. E., Sharifi, O., Grewal, S., Baysdorfer, C., Curr, K., Naimie, A. A., Okamoto, K., Murray, J. A., & Newcomb, J. M. (2015). The mitochondrial genomes of the nudibranch mollusks, *Melibe leonina* and *Tritonia diomedea*, and their impact on gastropod phylogeny. *PLoS One*, *10*, https://doi.org/10.1371/journal.pone.0127519

Simison, W. B., & Boore, J. L. (2008). Molluscan evolutionary genomics. In W. F. Ponder, & D. R. Lindberg (Eds.), *Phylogeny and Evolution of the Mollusca* (pp. 447–461). London: University of California Press. https://doi.org/10.2172/982432

Stamatakis, A. (2006). RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics*, *22*, 2688–2690. https://doi.org/10.1093/bioinformatics/btl446

Stöger, I., & Schrödl, M. (2013). Mitogenomics does not resolve deep molluscan relationships (yet?). *Molecular Phylogenetics and Evolution*, *69*, 376–392. https://doi.org/10.1016/j.ympev.2012.11.017

Tao, S. H., Fan, Y. H., Wang, W. J., Ma, G. J., Liang, L. J., & Shi, Q. (2008). Patterns of insertion and deletion in mammalian genomes. *Current Genomics*, *8*, 370–378. https://doi.org/10.2174/138920207783406479

Uribe, J. E., Colgan, D., Castro, L. R., Kano, Y., & Zardoya, R. (2016). Phylogenetic relationships among superfamilies of Neritimorpha (Mollusca: Gastropoda). *Molecular Phylogenetic Evolution*, *104*, 21–31. https://doi.org/10.1016/j.ympev.2016.07.021

Uribe, J. E., Kano, Y., Templado, J., & Zardoya, R. (2016). Mitogenomics of Vetigastropoda: Insights into the evolution of pallial symmetry. *Zoologica Scripta*, *45*, 145–159. https://doi.org/10.1111/zsc.12146

Uribe, J. E., Williams, S. T., Templado, J., Abalde, S., & Zardoya, R. (2017). Denser mitogenomic sampling improves resolution of the phylogeny of the superfamily Trochoidea (Gastropoda: Vetigastropoda). *Journal of Molluscan Studies*, *83*, 111–118. https://doi.org/10.1093/mollus/eyw049

Vaidya, G., Lohman, D. J., & Meier, R. (2011). SequenceMatrix: Concatenation software for the fast assembly of multi-gene datasets with character set and codon information. *Cladistics*, *27*, 171–180. https://doi.org/10.1111/j.1096-0031.2010.00329.x

White, T. R., Conrad, M. M., Tseng, R., Balayan, S., Golding, R., De Frias Martins, A., & Dayrat, B. A. (2011). Ten new complete mitochondrial genomes of pulmonates (Mollusca: Gastropoda) and their impact on phylogenetic relationships. *BMC Evolutionary Biology*, *11*, 295. https://doi.org/10.1186/1471-2148-11-295

Williams, S. T. (2012). Advances in molecular systematics of the vetigastropod superfamily Trochoidea. *Zoologica Scripta*, *41*, 571–595. https://doi.org/10.1111/j.1463-6409.2012.00552.x

Williams, S. T., Donald, K. M., Spencer, H. G., & Nakano, T. (2010). Molecular systematics of the marine gastropod families Trochidae and Calliostomatidae (Mollusca: Superfamily Trochoidea). *Mol. Phylogenet. Evol.*, *54*, 783–809. https://doi.org/10.1016/j.ympev.2009.11.008

Williams, S. T., Karube, S., & Ozawa, T. (2008). Molecular systematics of Vetigastropoda: Trochidae, Turbinidae and Trochoidea redefined. *Zoologica Scripta*, *37*, 483–506. https://doi.org/10.1111/j.1463-6409.2008.00341.x

Williams, S. T., & Ozawa, T. (2006). Molecular phylogeny suggests polyphyly of both the turban shells (family Turbinidae) and the superfamily Trochoidea (Mollusca: Vetigastropoda). *Molecular Phylogenetics and Evolution*, *39*, 33–51. https://doi.org/10.1016/j.ympev.2005.12.017

Winnepenninckx, B., Backeljau, T., & Dewachter, R. (1994). Extraction of high molecular weight dna from molecular weight DNA in molluscs. *Trends in Genetics*, *9*(12), 407. https://doi.org/10.1016/0168-9525(93)90102-n

Wort, E. J. G., Fenberg, P. B., & Williams, S. T. (2016). Testing the contribution of individual genes in mitochondrial genomes for assessing phylogenetic relationships in Vetigastropoda. *Journal of Molluscan Studies*, *83*, 123–128. https://doi.org/10.1093/mollus/eyw044

Xia, X., & Xie, Z. (2001). DAMBE: Software package for data analysis in molecular biology and evolution. *Journal of Heredity*, *92*, 371–373. https://doi.org/10.1093/jhered/92.4.371

Xu, X. D., Wu, X. Y., & Yu, Z. N. (2012). Comparative studies of the complete mitochondrial genomes of four *Paphia* clams and reconsideration of subgenus *Neotapes* (Bivalvia: Veneridae). *Gene*, *494*, 17–23. https://doi.org/10.1016/j.gene.2011.12.002

Yang, Y., Li, Q., Kong, L. F., & Yu, H. (2018). Comparative mitogenomic analysis reveals cryptic species in *Reticunassa festiva* (Neogastropoda: Nassariidae). *Gene*, *662*, 88–96. https://doi.org/10.1016/j.gene.2018.04.001

Yoon, S. H., & Kim, W. (2005). Phylogenetic relationships among six vetigastropod subgroups (mollusca, gastropoda) based on 18s rDNA sequences. *Molecules and Cells*, *19*(2), 283–288.

Zapata, F., Wilson, N. G., Howison, M., Andrade, S. C. S., Jörger, K. M., Schrödl, M., Goetz, F. E., Giribet, G., & Dunn, C. W. (2014). Phylogenomic analyses of deep gastropod relationships reject Orthogastropoda. *Proceedings of the Royal Society B: Biological Sciences*, *281*, https://doi.org/10.1098/rspb.2014.1739

Zhao, Z. Y., Tu, Z. G., Cui, J., & Bai, L. R. (2018). The complete mitochondrial genome of an endangered reef gastropods *Trochus pyramis*. *Conservation Genet Resour*, *10*, 347–349. https://doi.org/10.1007/s12686-017-0821-2

SUPPORTING INFORMATION

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

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