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Research Paper

Mitochondrial genomes of *Tapes dorsatus* and *Cardita variegata*: insights into Heteroconchia phylogeny*

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Abstract Heteroconchia, a widespread and abundant aquatic invertebrate, is an important clade of bivalve mollusks. The relationship between the three branches of Heteroconchia, Palaeoheterodonta, Archiheterodonta, and Euheterodonta has become a main controversy in molecular studies of the relationships between bivalves. In the present study, we assembled the complete mitochondrial genomes of *Tapes dorsatus* (Veneridae) and *Cardita variegata* (Carditidae) using high-throughput sequencing. *C. variegata* is the first mitochondrial genome belonging to the family Carditidae to be reported. We used 12 protein coding genes (excluding *atp8*) from the complete mitochondrial genomes of 146 species to recover the internal relationships of Heteroconchia. Our results support the traditional view of early branching of Palaeoheterodonta and the recovery of the monophyly of Palaeoheterodonta, Anomalodesmata, Imparidentia. Rearrangement analysis show that gene arrangement within Venerida was highly variable. Time-calibrated phylogenetic studies based on a relaxed molecular clock model suggested that Veneridae originated approximately 337.62 million years ago (Ma) and split into two major clades, whereas Carditidae originated approximately 510.09 Ma. Our results provide evidence of the internal relationships of Heteroconchia.

Keyword: *Tapes dorsatus*; *Cardita variegata*; mitochondrial genome; phylogeny

1 INTRODUCTION

Bivalves are the second most species-rich class of mollusks after gastropods. Bivalves have long been a focus of research. The clade Heteroconchia has been defined in subsequent studies (Cope, 2002; Bieler and Mikkelsen, 2006). As a major taxon in recent seas, it includes all bivalves with heterodont hinges that date back to the Early Ordovician. Members of this subclass have a wide variety of shell structure forms, and in terms of distribution, they are under strict environmental control (Cope, 2004). Currently, the Heteroconchia (consisting of Archiheterodonta, Euheterodonta and Palaeoheterodonta) is widely supported (Bieler et al., 2014; González et al., 2015). However, the evolutionary relationships between the three subclasses remain controversial. Previous phylogenetic analyses have confirmed either the traditional view of the early branching of Palaeoheterodonta (Giribet and Wheeler, 2002; Taylor et al., 2007) or the sister group relationship between Palaeoheterodonta and Archiheterodonta (Wilson et al., 2010), or the placement of Archiheterodonta in a more basal position (Bieler et al., 2014). It is worth noting that these studies use many datasets represented by

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ribosomal genes or only by genes coding for nuclear proteins. However, Heteroconchia has not been studied using mitochondrial data and the internal relationships are not yet clear. Therefore, it is necessary to conduct an in-depth study of the phylogeny of Heteroconchia.

Veneridae (Rafinesque, 1815) is a large and species-rich family in the class Bivalvia comprising over 800 species (Kappner and Bieler, 2006). They are mainly distributed throughout temperate tropical seas. Although it includes some economically important bivalve species, such as *Pismo clams*, *Mercenaria mercenaria* (Linnaeus, 1758), and *Manila clams*, it is one of the least understood mollusca taxa. Early fossil records of Veneridae can be traced back to the Cenozoic Era and suggest extensive species radiation, possibly of polyphyletic origin (Passamonti et al., 1999). In recent years, Veneridae have received extensive attention (Canapa et al., 1996, 2003; Chen et al., 2009). Thus far, approximately 33 complete mitochondrial genomes of Veneridae have been sequenced (including unverified), covering eight subfamilies.

Carditidae is a large family within the Phylum Mollusca distributed worldwide, but it is one of the least studied and neglected bivalve families (Huber, 2010). Recently, the family was divided into two subfamilies, Carditinae and Thecaliinae, which have been confirmed to contain approximately 140 species (Dall, 1903). Thecaliinae females have an incubatory chamber, which is the major distinction between the two subfamilies. The Carditidae family poses challenges for characterization due to its extensive intraspecies and interspecies morphological variation (Coan, 1977). The first phylogenetic analysis of the Carditidae family was based on morphological shell features (Pérez, 2019). Furthermore, our understanding of the mitochondrial genome of this family is currently very limited. However, as we sequence more mitogenomes, we will gain a deeper understanding of the family's phylogeny and evolution.

Mitochondrial genome data has been proved useful in solving the phylogenetic relationships of various metazoans (Osigus et al., 2013), including mollusks (Grande et al., 2008; Mikkelsen et al., 2018; Kong et al., 2020). This is because the mitochondrial genome is one of the most conserved regions, meaning that it changes relatively little over time compared to other regions of the genome (Boore, 1999). However, some mollusk species, especially bivalves, have been reported to show great variation in the size, number, and structure of their mitotic genomes (Serb and Lydeard, 2003; Wu et al., 2009). The mitochondrial DNA of mollusks is generally a closed circular molecule that contains 37 genes, including 13 protein-coding genes (PCGs), 22 transfer RNA genes (tRNA), two ribosomal RNA genes (rRNA), and a noncoding control region (Hu and Wang, 2019; Feng et al., 2021). Some bivalves, such as *Mytilus edulis* (Boore et al., 2004), *Mactra veneriformis* (Meng et al., 2013), lack the *atp8* gene. In contrast, all other mollusk species studied so far have the *atp8* gene (Gissi et al., 2008; Ren et al., 2009). Due to their rapid evolution, mitochondrial genomes are prone to amplification and have a higher mutation rate than nuclear sequences. In recent years, numerous studies have focused on using mitochondrial genomes to analyze gene rearrangements and study the phylogenetics of bivalves.

In this study, we sequenced two complete mitochondrial genomes: *Tapes dorsatus* (Veneridae) and *Cardita variegata* (Carditidae). The dataset for *C. variegata* is the first complete mitochondrial genome representing Carditidae reported on the National Center for Biotechnology Information (NCBI) database, GenBank. We then conducted a detailed investigation of the unique characteristics of these mitochondrial genomes. In addition, we performed a phylogenetic analysis of 12 PCGs (except *atp8*) from 146 complete mitogenomes of Heteroconchia retrieved from the GenBank to further understand their evolutionary relationships. Subsequently, we explored the evolution of mitochondrial gene rearrangements in Venerida and assessed the divergence times between the Veneridae and Carditidae families.

2 MATERIAL AND METHOD

2.1 Sample collection and DNA extraction

For this research, *T. dorsatus* and *C. variegata* specimens were obtained from a single sampling event conducted in Hainan Province, China. Total genomic DNA was extracted from fresh tissues using the Marine Animal DNA Kits (DP324, Tiangen Biotech Beijing, Co., Ltd., China). Subsequently, genomic DNA was observed on a 1.0% agarose gel, and the DNA was stored at -20 °C before sequencing.

2.2 High-throughput sequencing, assembly, and annotation

The complete mitochondrial genomes of *T. dorsatus* and *C. variegata* were sequenced on an Illumina HiSeq platform (Lingen Biotechnology Co., Shanghai, China). Sequencing libraries were constructed using 1 μg of genomic DNA as the starting volume. The biological information (BI) method was used for quality control, genome assembly, and functional annotation of the two complete mitochondrial genomes. The web server MITOS (http://mitos.bioinf.uni-leipzig.de/index.py) was used to predict the tRNA genes in the mitochondrial genome, applying the invertebrate genetic code. Sequence alignment and relative synonymous codon usage (RSCU) were conducted using MEGA V7.0 software (Kumar et al., 2016). The genome was displayed on a circle map using Organellar Genome DRAW (Lohse et al., 2007).

2.3 Phylogenetic analysis and divergence time estimation

Phylogenetic trees were constructed using 12 PCGs (except *atp8*) from 144 bivalve mitogenomes from the GenBank database and two newly sequenced species, with *Solemya velesiana* as the outgroup. Nucleotide sequences were aligned using MAFFT (Katoh and Standley, 2013) in PhyloSuite V1.2.2 (Zhang et al., 2020). Computer program Gblocks_V0.91b (Talavera and Castresana, 2007) was used to remove divergent and ambiguously aligned blocks, after which 9 802 of the original 15 543 nucleotides were retained for subsequent analyses. Phylogenetic analyses were performed using Bayesian methods implemented in MrBayes V3.2.6 (Ronquist et al., 2012) and maximum likelihood (ML) methods using IQ-TREE V2.2.0 (Minh et al., 2020). ModelFinder (Kalyaanamoorthy et al., 2017) was used to select the most suitable model. The ML analysis was executed using the ultrafast bootleg method, which was used for 1 000 repetitions. The BI analysis used two independent runs with four Markov chains, which were run for 1 000 000 generations until the average SD of the split frequencies was below 0.01. In addition, samples were taken every 1 000 generations and the first 25% were discarded as burn-ins. The phylogenetic tree was visualized and annotated using Interactive Tree of Life software (iTOL V6) (Letunic and Bork, 2021).

The divergence time of the infraclass Heteroconchia was estimated based on nucleotide levels and combined with relaxed molecular clock models in BEAST V1.10.4 (Suchard et al., 2018) with the Yule process as the tree prior. Two independent MCMC runs, each running for 500 million generations, and sampling once every 50 000 generations. LogCombiner V1.10.4 (Suchard et al., 2018) was used to combine the trees of these six runs and to remove 25% of the generations as burn-in. In addition, we selected the following calibration points: (1) a lambda of 20 for the minimum age of 152 million years ago (Ma) calibration date for Unionidae, resulting in a 95% credibility interval of 153–226 Ma (Graf et al., 2015); (2) it is recorded that the family Margaritiferidae first appeared during the Upper Triassic period in modern southern (Fang et al., 2009; Van Damme et al., 2015). Therefore, we set a minimum age of 230 Ma as the stem age of this family (exponential prior, min=230, lambda=30) (Huang et al., 2018); (3) the root age of Bivalvia was constrained between 520 and 541 Ma, the superfamilies Solenoidea, Cardioidea, and Tellinoidea were restricted to 228–247, 444–446, and 323–330 Ma, respectively (Rahuman et al., 2020); (4) the minimum age for the most recent common ancestor (MRCA) of *Margaritifera margaritifera–Margaritifera dahurica* was set to 34 Ma (exponential prior, min=34, lambda=9.3); the minimum age of the *Lamprotula* was set to 34 Ma (exponential prior, min=34, lambda=9.3); the minimum age of the Margaritiferidae was set to 129.4 Ma (exponential prior, min=129.4, lambda=35.1) (Bolotov et al., 2017); (5) priors for fossil ages were drawn from normal distributions, and the Anomalodesmata was constrained between 478.6 and 488.3 Ma (Bieler et al., 2014).

3 RESULT AND DISCUSSION

3.1 Genome feature and composition

The complete mitochondrial genomes of *T. dorsatus* and *C. variegata* were 19 380 bp and 17 070 bp in length, and were submitted to GenBank with accession numbers of OP066992 and OP021896, respectively (Table 1). The complete mitogenomes of *T. dorsatus* contained 13 PCGs, 22 tRNAs, and two rRNAs. The complete mitogenomes of *C. variegata* encodes a total of 38 genes, including two *trnM* genes (Fig.1), as is also the case in the mitogenomes of many other bivalves (Xu et al., 2012), which may arise from gene duplication events early in the radiation of bivalves. Furthermore, as in many previously reported species, all genes

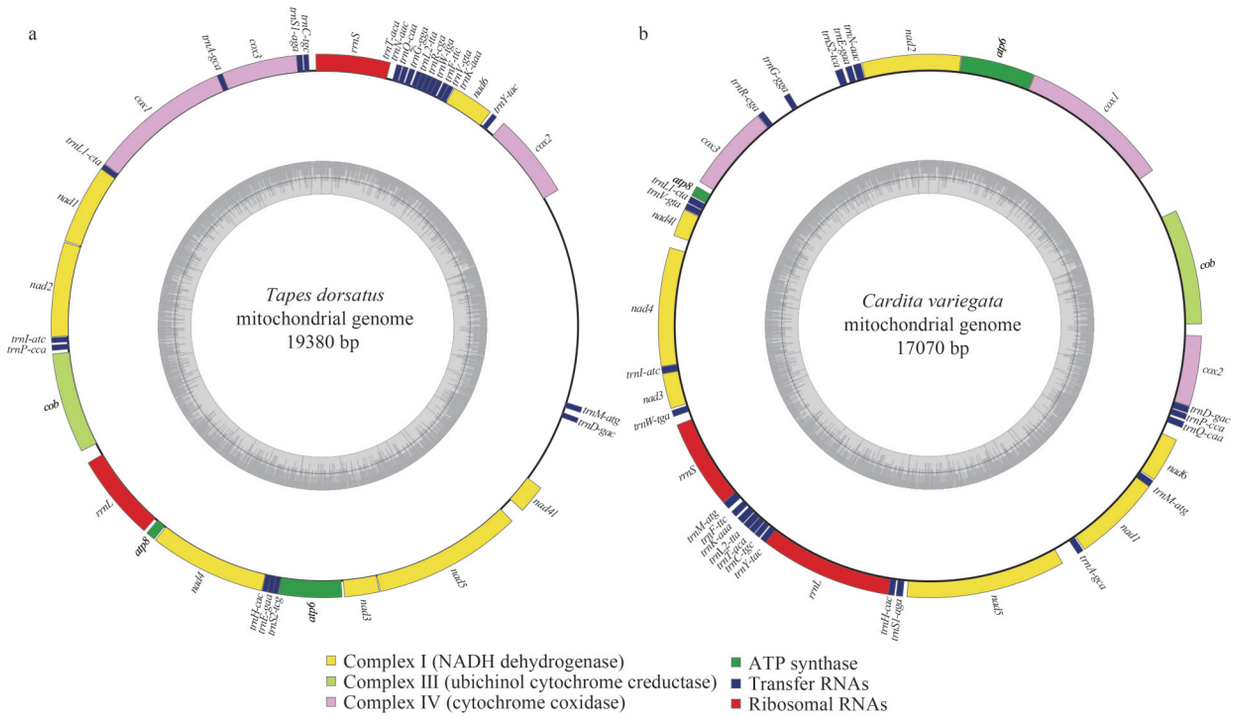


Fig.1 Gene map of the complete mitochondrial genome of *T. dorsatus* (a) and *C. variegata* (b)

The outer side of the loop indicates that the gene is encoded by the heavy chain of mitochondrial gene, and the inner side of the loop indicates that the gene is encoded by the light chain of mitochondrial gene. The genome length is shown in the middle of each map.

were transcribed in the same direction on the forward strand (Xu et al., 2010; Williams et al., 2017; Yang et al., 2019; Sun et al., 2020). Overlapping of neighboring genes is also frequently observed in the mitochondria of bivalve mollusks; our newly assembled species is no exception, with the longest overlap occurring between *trnY* and *rrnL* in *C. variegata* (Tables 2 & 3).

Table 1 Complete mitochondrial genomes and their GenBank accession number used for phylogenetic analysis

Order	Family	Species	Accession No.	Size (bp)	AT content (%)
Unionida	Mutelidae	*Mutela dubia*	NC_034844	16 168	63.7
	Hyriidae	*Echyridella menziesii*	NC_034845	16 031	60.0
	Margaritiferidae	*Cumberlandia monodonta*	NC_034846	16 099	59.5
		Pseudunio marocanus	NC_034911	16 001	60.4
		Margaritifera dahurica	NC_023942	16 112	61.5
		Margaritifera falcata	NC_015476	16 121	61.7
		Margaritifera margaritifera	NC_043836	16 122	60.7
	Unionidae	*Quadrula quadrula*	NC_013658	16 033	62.6
		Pleurobema oviforme	NC_050057	15 852	61.4
		Amblema plicata	NC_050056	15 946	60.9
		Toxolasma parvum	NC_015483	15 949	61.8
		Potamilus leptodon	NC_028522	16 133	62.4
		Potamilus streckersoni	NC_057093	16 293	61.3
		Potamilus alatus	KU559011	16 067	60.5
		Lampsilis ornata	NC_005335	16 060	62.4
		Venustaconcha ellipsiformis	FJ809753	15 975	62.5
		Lampsilis siliquoidea	NC_037721	16 094	63.0
		Lampsilis powellii	NC_037720	16 043	63.3

To be continued

Table 1 Continued

Order	Family	Species	Accession No.	Size (bp)	AT content (%)
Unionida	Unionidae	*Chamberlainia hainesiana*	NC_044110	16 746	58.2
		Hyriopsis schlegelii	NC_015110	15 939	60.3
		Hyriopsis cumingii	NC_011763	15 954	60.2
		Rectidens sumatrensis	NC_059765	15 983	64.5
		Hyriopsis bialata	NC_059764	15 919	63.6
		Physunio superbus	NC_059763	16 057	61.2
		Contradens contradens	NC_059762	15 956	61.0
		Solenaia oleivora	NC_022701	16 392	59.9
		Solenaia carinata	NC_023250	16 716	60.9
		Ptychorhynchus_pfisteri	KY067440	16 040	60.8
		Parvasolenaia rivularis	NC_039839	16 613	57.7
		Potomida littoralis	NC_030073	15 789	58.2
		Lamprotula leaii	NC_023346	16 530	60.2
		Lamprotula scripta	NC_030258	16 250	59.0
		Lamprotula cornuumlunae	NC_058232	16 635	58.8
		Lamprotula caveata	NC_030336	16 641	58.9
		Sinanodonta woodiana	NC_024943	16 256	65.9
		Sinanodonta lucida	NC_026673	16 285	64.0
		Anemina arcaeformis	NC_026674	15 672	64.6
		Anemina euscaphys	NC_026792	15 741	64.8
		Anodonta anatina	NC_022803	15 653	66.0
		Anodonta exulcerata	NC_057110	15 619	63.6
		Anodonta cygnea	NC_036488	15 613	63.9
		Anodonta nuttalliana	NC_057111	16 015	65.4
		Utterbackia imbecillis	NC_015479	16 103	65.9
		Pyganodon grandis	NC_013661	15 848	64.3
		Alasmidonta heterodon	NC_037431	15 909	65.9
		Lasmigona compressa	NC_015481	15 903	66.9
		Alasmidonta varicosa	NC_038155	15 693	66.8
		Lanceolaria gladiola	KY067441	15 732	63.7
		Lanceolaria lanceolata	NC_023955	15 782	62.8
		Lanceolaria grayii	NC_026686	15 736	62.9
		Aculamprotula coreana	NC_026035	15 697	63.1
		Aculamprotula scripta	NC_045529	15 688	62.9
		Unio tumidus	KY021078	15 769	66.4
		Unio elongatulus	NC_057113	15 763	65.2
		Unio crassus	NC_033976	15 781	66.8
		Cuneopsis heudei	NC_042471	15 892	62.5
		Cuneopsis celtiformis	NC_060431	15 922	64.5
		Schistodesmus lampreyanus	NC_042470	15 855	64.6
		Nodularia douglasiae	NC_060593	15 746	65.7
		Nodularia breviconcha	MT955592	15 741	65.7
Anomalodesmata	Poromyidae	*Tropidomya abbreviata*	NC_034304	16 829	68.9
	Cuspidariidae	*Cuspidaria undata*	ON360998	16 266	68.2
	Myochamidae	*Myadora brevis*	NC_034303	19 292	67.6
	Laternulidae	*Laternula truncata*	NC_034305	16 363	60.7
		Laternula elliptica	NC_022846	14 622	55.1
	Lyonsiidae	*Lyonsia norwegica*	NC_034302	14 673	50.7

To be continued

Table 1 Continued

Order	Family	Species	Accession No.	Size (bp)	AT content (%)
Anomalodesmata	Clavagellidae	*Bryopa lata*	NC_034300	31 969	59.3
Carditida	Carditidae	*Cardita variegata*	OP021896	17 070	65.8
Lucinida	Lucinidae	*Lucinella divaricata*	NC_013275	18 940	63.7
		Loripes lacteus	NC_013271	17 321	62.1
Adapedonta	Pharidae	*Sinonovacula constricta*	NC_011075	17 225	67.0
	Solenidae	*Solen strictus*	NC_017616	16 535	62.6
		Solen grandis	NC_016665	16 784	64.8
	Hiatellidae	*Panopea globosa*	NC_025636	15 469	63.7
		Panopea generosa	NC_025635	15 585	63.7
		Panopea abrupta	NC_033538	15 381	64.4
Cardiida	Cardiidae	*Cerastoderma edule*	NC_035728	14 947	58.2
		Acanthocardia tuberculata	NC_008452	16 104	60.0
		Hippopus hippopus	MT755622	21 565	60.3
		Tridacna gigas	NC_050683	19 558	57.7
		Tridacna derasa	NC_039945	20 760	65.3
		Tridacna squamosa	NC_026558	20 930	62.4
		Tridacna crocea	NC_057530	18 266	62.4
	Psammobiidae	*Nuttallia olivacea*	NC_018373	18 182	65.3
		Gari elongata	NC_042422	16 766	62.2
		Hiatula acuta	NC_042421	16 352	63.3
		Sanguinolaria ovalis	NC_042423	16 460	61.0
		Hiatula chinensis	NC_042420	16 333	61.3
	Donacidae	*Donax variegatus*	NC_035986	17 195	60.4
		Donax trunculus	NC_035985	17 365	58.9
		Donax vittatus	NC_035987	17 070	63.5
		Donax semiestriatus	NC_035984	17 044	61.9
	Tellinidae	*Limecola balthica*	NC_046519	17 492	63.6
		Iridona iridescens	NC_018371	16 799	65.8
	Solecurtidae	*Solecurtus divaricatus*	NC_018376	16 749	60.1
Myida	Dreissenidae	*Mytilopsis leucophaeata*	MW727517	15 981	69.4
	Xylophagaidae	*Xylonora corona*	NC_063753	15 083	57.1
		Xylophaga dorsalis	NC_063754	16 787	67.2
		Xylophaga washingtona	NC_063756	18 624	59.9
		Xylophaga oregona	NC_063755	18 220	59.8
	Teredinidae	*Kuphus polythalamius*	NC_063762	18 094	57.6
		Teredothyra matocotana	NC_063767	17 364	62.1
		Lithoredo abatanica	NC_063763	16 084	57.7
		Bactronophorus thoracites	NC_063758	16 562	56.9
		Neoteredo reynei	NC_063764	18 035	58.5
		Bankia setacea	NC_063760	16 986	60.2
		Teredo bartschi	NC_063766	16 962	60.1
		Bankia gouldi	NC_063759	16 795	60.9
Venerida	Mactridae	*Lutraria rhynchaena*	NC_023384	16 927	62.3
		Lutraria maxima	NC_036766	17 082	63.9
		Mactra antiquata	NC_021375	17 199	64.2
		Mactra quadrangularis	NC_058202	16 848	67.7
		Mactra chinensis	NC_025510	17 285	63.7

To be continued

Table 1 Continued

Order	Family	Species	Accession No.	Size (bp)	AT content (%)
Venerida	Arcticidae	*Arctica islandica*	NC_022709	18 289	69.3
	Cyrenidae	*Villorita cyprinoides*	NC_050989	15 880	68.0
		Corbicula sandai	NC_061685	16 758	69.4
		Corbicula leana	MW646295	17 041	69.4
		Corbicula japonica	NC_060659	17 432	70.3
		Corbicula fluminea	NC_046410	17 423	70.5
	Vesicomyidae	*Ectenagena elongata*	NC_051454	16 827	67.7
		Calyptogena magnifica	NC_028724	19 738	68.4
		Calyptogena extenta	MF981085	16 106	65.5
		Calyptogena marissinica	NC_044766	17 374	65.4
	Veneridae	*Meretrix lyrata*	NC_022924	21 625	70.5
		Meretrix meretrix	NC_013188	19 826	68.4
		Meretrix lamarckii	NC_016174	21 209	65.7
		Cyclina sinensis	KU097333	21 799	73.0
		Saxidomus purpurata	NC_026728	19 637	66.2
		Callista chinensis	NC_056193	19 703	67.6
		Dosinia altior	NC_037916	17 536	69.6
		Dosinia troscheli	NC_037917	17 229	69.7
		Dosinia japonica	NC_038063	17 693	70.0
		Mercenaria mercenaria	NC_048487	18 365	69.0
		Antigona lamellaris	MT254059	17 532	68.0
		Venus verrucosa	MW662610	18 077	68.8
		Chamelea striatula	MW662611	25 658	66.1
		Tapes dorsatus	OP066992	19 380	65.7
		Venerupis aspera	NC_060434	18 519	63.4
		Macridiscus melanaegis	NC_045870	20 738	67.1
		Macridiscus semicancellata	NC_060435	19 984	67.7
		Macridiscus multifarius	NC_045888	20 171	67.6
		Ruditapes decussatus	NC_035757	18 995	63.0
		Paratapes undulatus	NC_016891	18 154	64.9
		Paratapes textilis	NC_016890	18 561	64.3
		Paphia euglypta	NC_014579	18 643	66.9
		Paphia amabilis	NC_016889	19 629	63.3

The nucleotide composition of the two mitogenomes was as follows: A: 25.72%, C: 10.04%, G: 24.21%, and T: 40.03% (*T. dorsatus*); A: 23.42%, C: 10.99%, G: 23.15%, and T: 42.44% (*C. variegata*). The high A and T contents indicated that the use of codons was biased toward A and T, and the AT skew was negative for *T. dorsatus* (-0.191 8) and *C. variegata* (-0.288 8). The AT contents of the two mitochondrial genomes were 65.75% (*T. dorsatus*) and 65.86% (*C. variegata*), which was within the AT content range for Heteroconchia (50.7% to 73%) (Table 1). The base composition of *T. dorsatus* was compared with that of the other 22 species of Veneridae; the AT range of the 21 mitochondrial genomes was 60.15%–68.35%, and the AT skew was negative, ranging from -0.111 3 (*P. amabilis*) to -0.281 9 (*C. sinensis*). The coding ranges of the two mitochondrial genomes were 14 655 and 14 946, accounting for 77.12% (*T. dorsatus*) and 85.85% (*C. variegata*) of the corresponding genomes, respectively.

3.2 PCGs, tRNA gene, rRNA gene, and codon usage

The AT and GC content of the PCGs, tRNAs, and rRNAs in both species had the same base bias as the whole genome (Fig.2). The total nucleotide lengths of the 13 PCGs in the two mitochondrial genomes

Table 2 Summary of the gene features of *T. dorsatus*

Gene	Position (bp)	Strand	Length (bp)	Intergenic nucleotides	Start/stop codon
cox2	1 613–2 599	+	987	101	TTG/TAG
trnY	2 701–2 764	+	64	27	–
nad6	2 792–3 289	+	498	6	ATG/TAA
trnK	3 296–3 360	+	65	1	–
trnV	3 362–3 423	+	62	14	–
trnF	3 438–3 501	+	64	0	–
trnW	3 502–3 564	+	63	0	–
trnR	3 565–3 628	+	64	0	–
trnL2	3 629–3 692	+	64	0	–
trnG	3 693–3 754	+	62	19	–
trnQ	3 774–3 840	+	67	14	–
trnN	3 855–3 919	+	65	7	–
trnT	3 927–3 995	+	69	70	–
rrnS	4 066–4 927	+	862	78	–
trnC	5 006–5 068	+	63	9	–
trnS1	5 078–5 144	+	67	0	–
cox3	5 145–6 018	+	874	0	TTG/T**
trnA	6 019–6 082	+	64	-6	–
cox1	6 077–7 720	+	1 644	3	ATG/TAA
trnL1	7 724–7 786	+	63	-6	–
nad1	7 787–8 710	+	924	10	ATA/TAA
nad2	8 721–9 815	+	1 095	5	ATT/TAG
trnI	9 821–9 884	+	64	21	–
trnP	9 906–9 971	+	66	35	–
cytb	10 007–11 167	+	1 161	148	ATT/TAA
rrnL	11 316–12 323	+	1 008	35	–
atp8	12 359–12 472	+	114	14	ATG/TAG
nad4	12 487–13 836	+	1 350	2	ATA/TAA
trnH	13 839–13 902	+	64	0	–
rrnE	13 903–13 967	+	65	-4	–
trnS2	13 964–14 026	+	63	1	–
atp6	14 028–14 756	+	729	23	ATG/TAG
nad3	14 780–15 184	+	405	8	GTG/TAA
nad5	15 193–16 914	+	1 722	162	ATT/TAA
nad4L	17 077–17 412	+	336	841	ATG/TAA
trnD	18 254–18 315	+	62	62	–
trnM	18 378–18 444	+	67	–	–

+ indicates that the gene is on the forward strand; – indicates that the gene is on the reverse strand; ** indicates an incomplete termination codon, the gene ends with a single codon T.

Table 3 Summary of the gene features of *C. variegata*

Gene	Position (bp)	Strand	Length (bp)	Intergenic nucleotides	Start/stop codon
cytb	19–1 191	+	1 173	463	ATT/TAG
cox1	1 655–3 193	+	1 539	3	ATT/TAA
atp6	3 197–3 949	+	753	4	ATG/TAG
nad2	3 954–4 961	+	1 008	6	ATG/TAA
trnN	4 968–5 048	+	81	14	–
trnE	5 063–5 129	+	67	30	–
trnS2	5 160–5 237	+	78	507	–
trnG	5 745–5 809	+	65	253	–
trnR	6 063–6 129	+	67	3	–
cox3	6 133–7 014	+	882	53	ATG/TAA
atp8	7 068–7 175	+	108	9	ATG/TAG
trnL1	7 185–7 255	+	71	6	–
trnV	7 262–7 329	+	68	4	–
nad4L	7 334–7 615	+	282	122	ATG/TAA
nad4	7 738–8 943	+	1 206	2	ATT/TAA
trnI	8 946–9 014	+	69	1	–
nad3	9 016–9 375	+	360	18	ATG/TAG
trnW	9 394–9 461	+	68	85	–
rrnS	9 547–10 455	+	909	1	–
trnM	10 457–10 537	+	81	40	–
trnF	10 578–10 645	+	68	22	–
trnK	10 668–10 742	+	75	7	–
trnL2	10 750–10 821	+	72	3	–
trnT	10 825–10 891	+	67	5	–
trnC	10 897–10 959	+	63	13	–
trnY	10 973–11 040	+	68	-50	–
rrnL	10 991–12 404	+	1 414	-25	–
trnH	12 380–12 445	+	66	15	–
trnS1	12 461–12 530	+	70	39	–
nad5	12 570–14 189	+	1 620	175	GTG/TAG
trnA	14 365–14 433	+	69	11	–
nad1	14 445–15 353	+	909	0	ATG/TAA
trnM	15 354–15 423	+	70	11	–
nad6	15 435–15 896	+	462	128	ATG/TAG
trnQ	16 025–16 093	+	69	20	–
trnP	16 114–16 176	+	63	8	–
trnD	16 185–16 253	+	69	2	–
cox2	16 256–16 972	+	717	–	ATG/TAA

+ indicates that the gene is on the forward strand; – indicates that the gene is on the reverse strand.

were 11 019 and 11 839, accounting for 64.55% and 61.09% of the total mitochondrial genome, respectively. We show the start and stop codons of 13 PCGs in the two mitochondrial genomes, most of which start with the typical codon ATN. However, others start with the codons GTG (*T. dorsatus*: *nad3*, *C. variegata*: *nad5*) or TTG (*T. dorsatus*: *cox2*, *cox3*) (Tables 2 & 3). The starting of genes with GTG or TTG is also found in other bivalve species such as *Paphia textilis* (*nad1* and n*ad4L*: GTG), *Donax semistriatus* (*nad4* and *nad4L*: TTG). Such codon usage is considered a flexible or random situation (Wu et al., 2009; Xu et al., 2012). Most PCG in two mitochondrial genomes are terminated

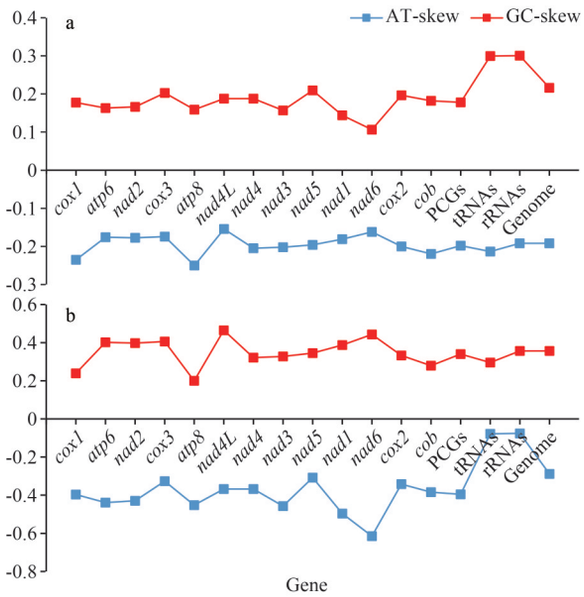


Fig.2 The graphs showing the AT- and GC- skew in protein-coding genes (PCG), rRNA, tRNA and genome of *T. dorsatus* (a) and *C. variegata* (b)

with complete stop codon: TAA or TAG, while *cox3* gene in *T. dorsatus* is terminated with incomplete stop codon T. The RSCU values of the 13 PCGs showed that UUA (Leu1) and CCU (Pro) were the two most common codons in *T. dorsatus* species, whereas the most common codons in *C. variegata* species were UUG (Leu1) and CCU (Pro) (Fig.3).

In the mitogenomes of metazoans, almost all amino acids (excluding leucine and serine), are decoded by only one tRNA each (Podsiadlowski et al., 2008). There were 22 tRNAs that were distributed in both mitochondrial genomes, ranging from 62 to 81 nucleotides, and an additional *trnM* gene was detected in *C. variegata* (Table 1), which has also been reported in other bivalves (Milbury and Gaffney, 2005; Dreyer and Steiner, 2006; Wu et al., 2009; Xu et al., 2010). In this study, most tRNAs were folded into typical secondary structures. Most of the putative secondary structures contained 7-bp receptor arms, and DHU stems were present only in the *C. variegata trnS2* gene. In addition, we found that almost of tRNAs (except for *trnK* in *T. dorsatus*) contained at least one G-U base pair (Feng et al., 2021) (Fig.4). The *rrnL* lengths of the two species were 1 008 and 1 414 bp, whereas the *rrnS* lengths were 862 and 909 bp, respectively. In addition, the A and T contents of the rRNA genes in the two species were higher than their G and C contents.

3.3 Gene arrangement

Molluscan bivalves exhibit greater gene order variation in their mitogenomes and have higher mutation rates than other metazoans (Serb and Lydeard, 2003; Ren et al., 2010). In this study, we selected *T. dorsatus* and 37 other species from GenBank to study mitochondrial gene rearrangements in Venerida (Fig.5). As in other bivalves (Lee et al., 2019), the order of genes and number of tRNAs

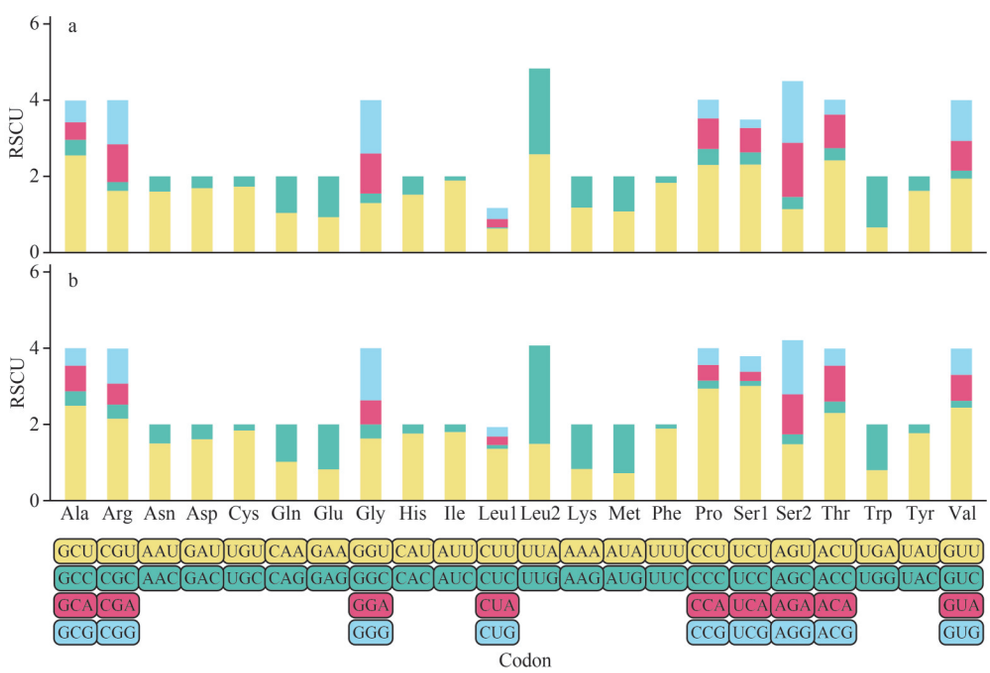


Fig.3 The relative synonymous codon usage (RSCU) in the mitogenomes of *T. dorsatus* (a) and *C. variegata* (b)

vary considerably in the order Venerida. We found that species belonging to the family Cyrenidae and two subfamilies, Meretricinae and Dosininae, had the same order of protein-coding genes. However, in other families, the order of protein-coding genes is highly variable, such as the translocation of the *nad1* gene which occurs in the family Mactridae. In addition to tRNA repeats, we found a conserved gene order at the genus level in the order Venerida (purging *atp8*), such as *Paphia* and *Macridiscus*. The gene order of all species of the same genus is represented by a synteny block. The order of gene arrangement for all the species in the order Venerida demonstrated that the subfamily Tapetinae possessed the greatest variability. The gene arrangement order of *T. dorsatus* was more like that of the neighboring subfamily Venerinae. Excluding the tRNA genes, *T. dorsatus* is identical in gene order to *Antigona lamellaris* of the subfamily Venerinae. Compared to other species of this subfamily, they all contain the *cox1-nad1-nad2-cytb-rrnL* gene fragment.

3.4 Phylogenetic analysis and divergence times

We performed a phylogenetic analysis of the

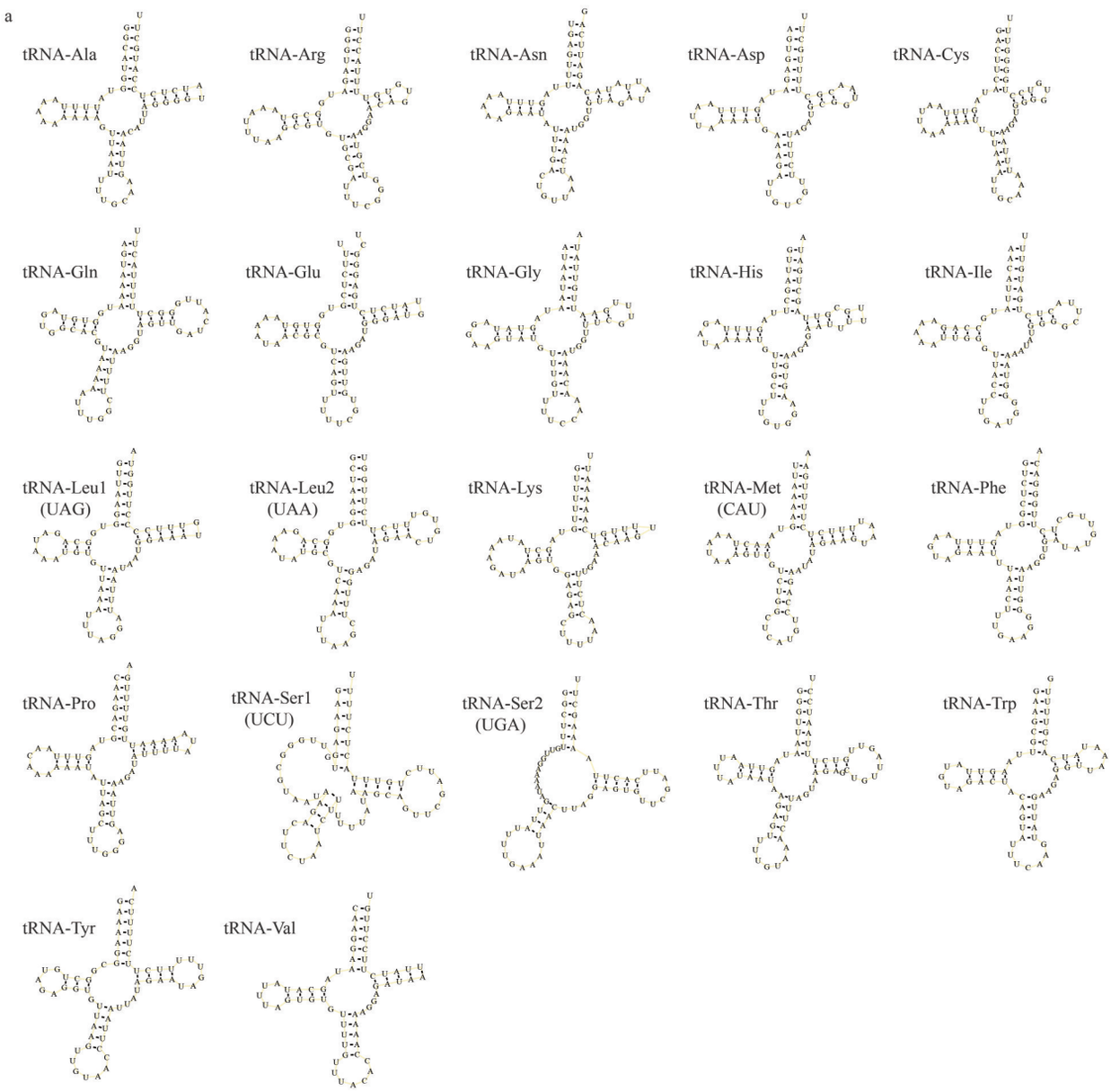
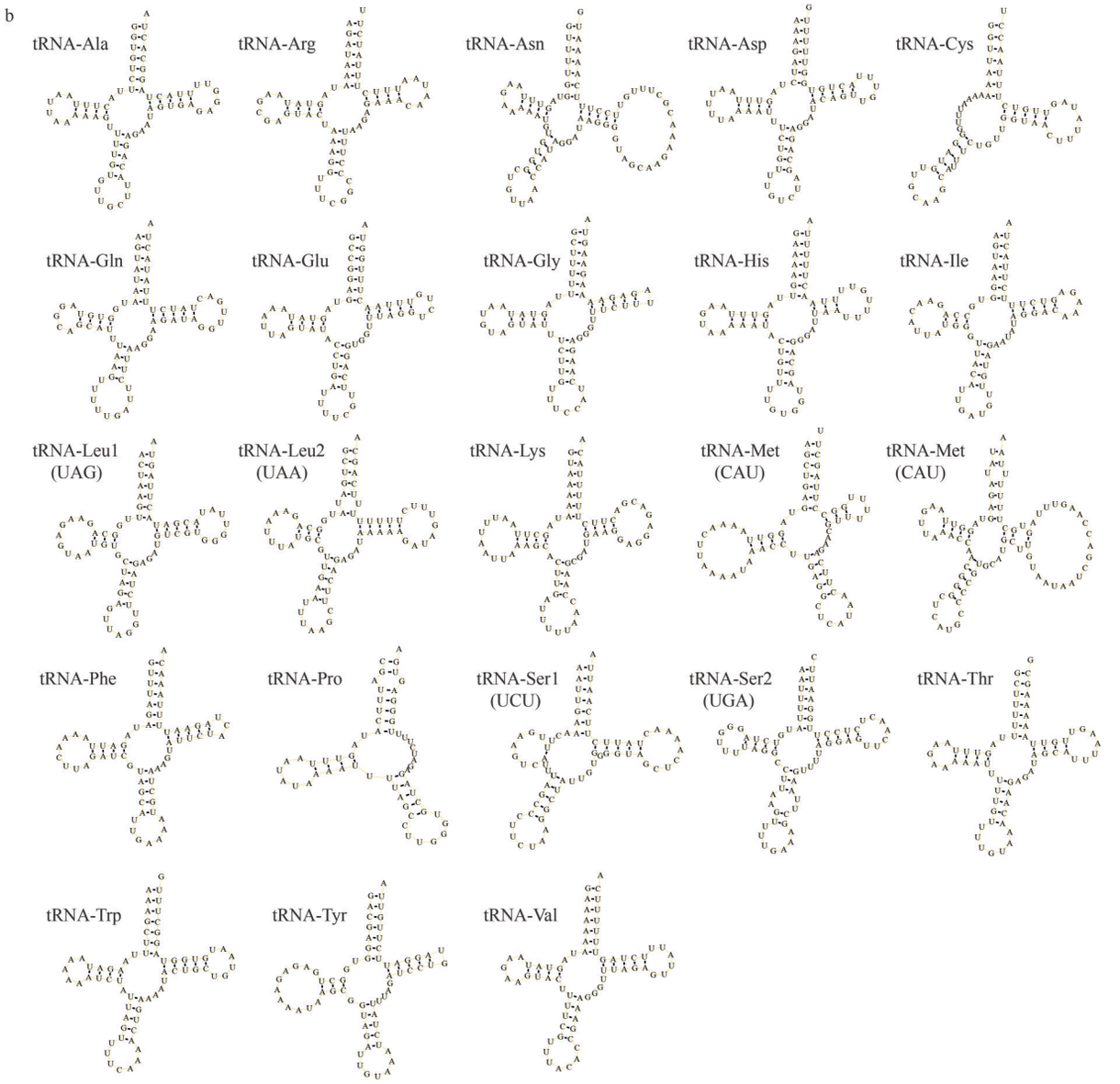


Fig.4 Secondary structure of the tRNA genes in the mitogenome of *T. dorsatus* (a) and *C. variegata* (b)

To be continued

Fig.4 Continued



Heteroconchia using 12 PCGs (except *atp8*), including seven orders and one superorder, for a total of 146 species, with *Solemya velesiana* of the order Solemyida as the outgroup (Fig.6). Maximum likelihood (ML) and Bayesian inference (BI) analyses based on nucleotide sequences produced almost the same topologies with strong bootstrapping and posterior probability values.

Our analysis shows the phylogenetic relationship of Heteroconchia as follows: ((((Palaeoheterodonta)+Anomalodesmata)+Archiheterodonta)+Imparidentia) and is more supportive of the view of an early split of Palaeoheterodonta. The relationship between all species representing the Palaeoheterodonta from the same order Unionida was recovered as ((((((Unionidae+Anodontinae)+Gonideinae)+Ambleminae)+Margaritiferidae)+Hyriidae)+Iridinidae), which is basically consistent with the phylogenetic tree previously constructed based on mitogenomes data (Huang et al., 2018). Within the family Carditidae, *C. variegata* forms a monophyletic clade. Additional phylogenetic analyses based on more molecular data will be necessary to resolve the relationships among the other members of the family. In a previous molecular analysis, *A. tuberculata* has been evidenced to be a member of the Cardioidea superfamily (Dreyer and Steiner, 2006) and as a sister group to Tellinoidea, which

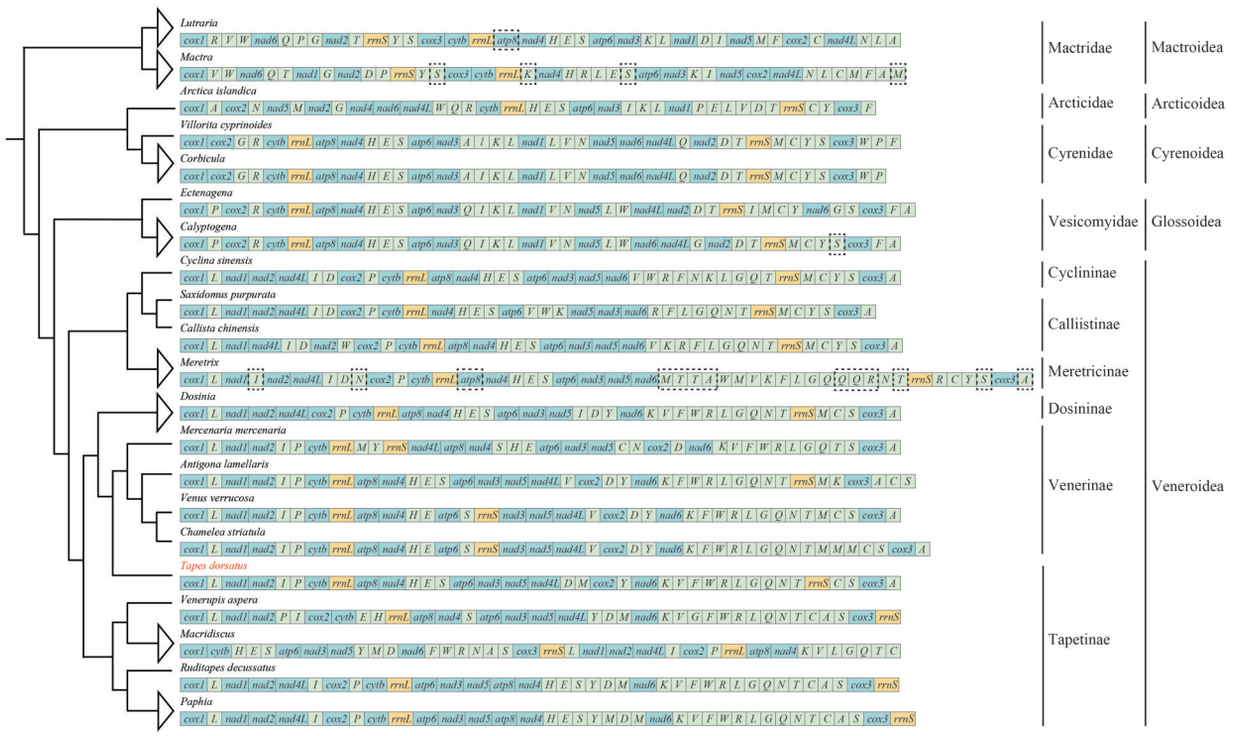


Fig.5 Mitochondrial gene arrangements in the venerida

The gene arrangement for species of the same genus is represented by a set of blocks, and dashed boxes indicate that some species of the same genus do not encode this gene.

was supported by the BI and ML trees obtained based on nucleotide data. The monophyly of Anomalodesmata and Myida is strongly supported; however, their internal relationships remain unclear. The sister taxa Poromyoidea and Pandoroidea of Anomalodesmata clustered together with posterior probability=1.0. *Myadora brevis*, which belongs to the Myochamidae family, forms a distinct evolutionary branch within the Pandoroidea superfamily. ((Clavagellidae+Lyonsiidae)+ Laternulidae), as sister taxa of Myochamidae, formed another evolutionary branch, which is consistent with previously determined phylogenetic relationships based on the amino acid sequences of the 11 mitochondrial genes (Williams et al., 2017). Thirteen species from three families comprise the order Myida, which is divided into two evolutionary branches. Our results support the monophyly of the Pholadoidea superfamily, which consists of the Xylophagidae and Teredinidae. These mitochondrial genome sequence data confirm the morphological results (Taylor et al., 2007), but contrasts with the topology obtained from the rRNA data (Monari, 2009). Neoheterodontei, defined in previous studies, have been reported to include Myida and Venerida (Taylor et al., 2007). Inconsistent with recent results obtained based on the mitochondrial genome is that our results support Cardiida as the sister group of Neoheterodontei, despite having lower support values (Wang et al., 2023). But it supports some earlier studies (Taylor et al., 2007; Lemer et al., 2019). *T. diorsatus* clusters well with other species of the family Veneridae, which first combines with Vesicomyidae, then unites with Cyrenoidea, a superfamily consisting of sister groups Cyrenidae and Arcticidae (Rahuman et al., 2020) and finally combines with Mactridae. Veneridae is the most diverse and economically important taxon of Venerida. It is divided into two distinct branches: Meretricinae, Cyclininae, and Calliistinae, which are grouped in one branch; and Dosininae, Venerinae, and Tapetinae which belong to the other branch (Canapa et al., 2003; Rahuman et al., 2020). These results support our explanation of the phylogenetic relationships among Heteroconchia.

We used ten fossil calibration points to estimate the divergence dates within Heteroconchia based on nucleotide sequences. The tree topology recovered with BEAST is essentially the same as the topology derived from the ML and BI analyses (Fig.7). The subfamily Ambleminae was separated from the Unionidae at about 118.33 Ma (95% HPD=91.18–

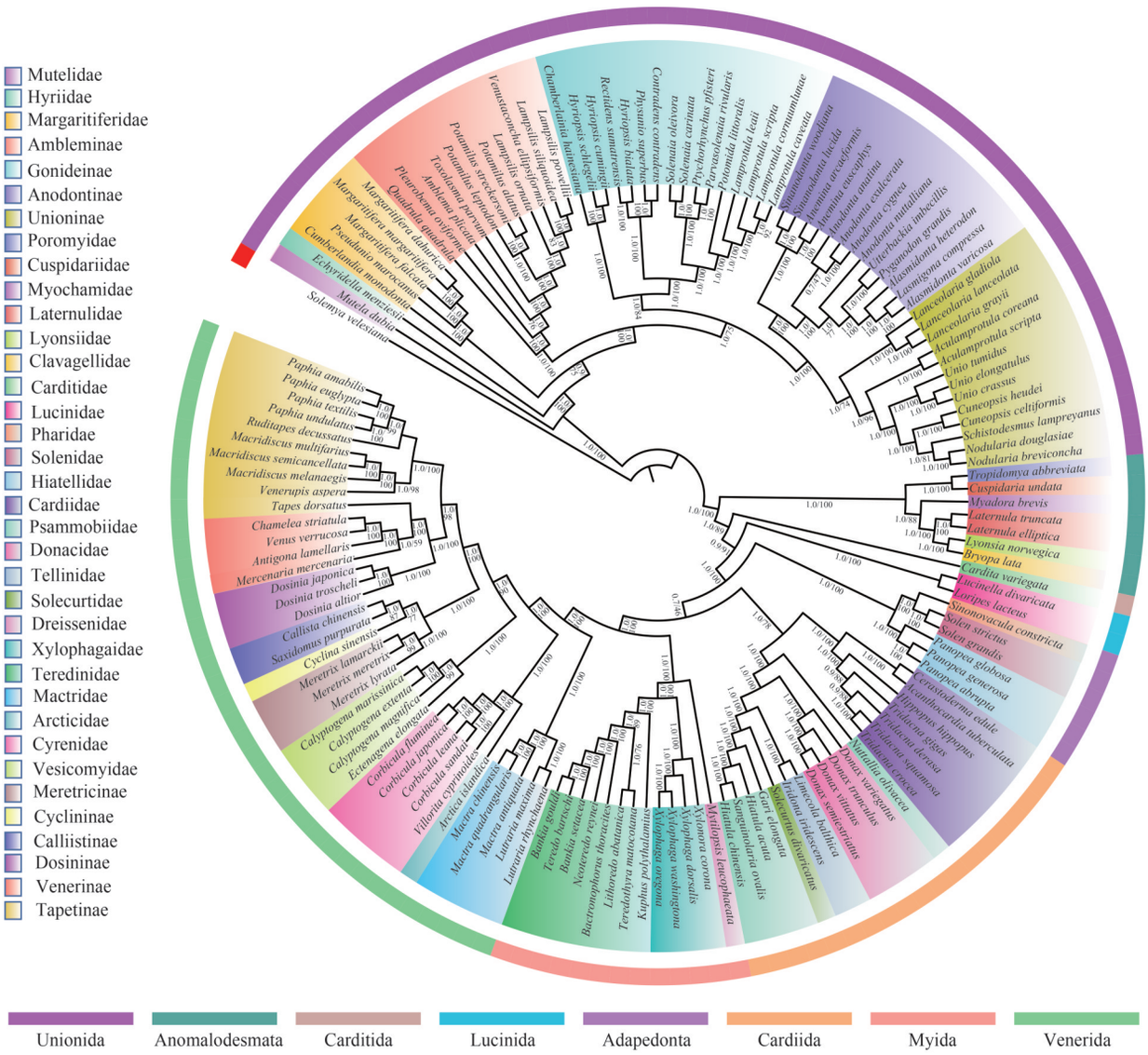


Fig.6 Phylogenetic tree of Heteroconchia based on sequences of 12 PCGs (except *atp8*)

The nucleotide sequences of 12 protein coding genes in mitochondrial genome were used to infer phylogenetic tree. The support values of each node are displayed through maximum likelihood bootstrap and Bayesian posterior probabilities. *Solemya velesiana* (GenBank: NC_034906) were included as the outgroup taxon.

163.73) as a sister group to the remaining taxa of the family, and all subfamilies appear diverse in the Cretaceous. Our finding that most crown groups of the subfamily Gonideinae originated from the Paleocene and Eocene corroborates previous studies (Zieritz et al., 2021). The divergence time of Carditidae is estimated to be approximately 510.09 Ma (95% HPD=494.88–524.3), which is younger than the fossil record that began in the Devonian (Chavan, 1969). Larger taxonomic samples may be needed in subsequent studies to estimate the divergence time of the family. Our results indicate that Veneridae originated at 337.62 Ma (95% HPD=335.95–339.29), in agreement with previous molecular studies, suggesting that Veneridae appeared in the Carboniferous period (Plazzi and Passamonti, 2010; Wang et al., 2021). The Veneridae split into two clades at about 286.61 Ma (95% HPD=248.94–317.11), with diversification occurring from Jurassic to Cretaceous. The family Vesicomyidae is estimated to separate at about 55.49 Ma (95% HPD=33.79–85.3), near the Cretaceous-Paleogene boundary, which supports the results obtained by overlaying genetic data with morphological data (Johnson et al., 2017). Divergence time estimates indicate that

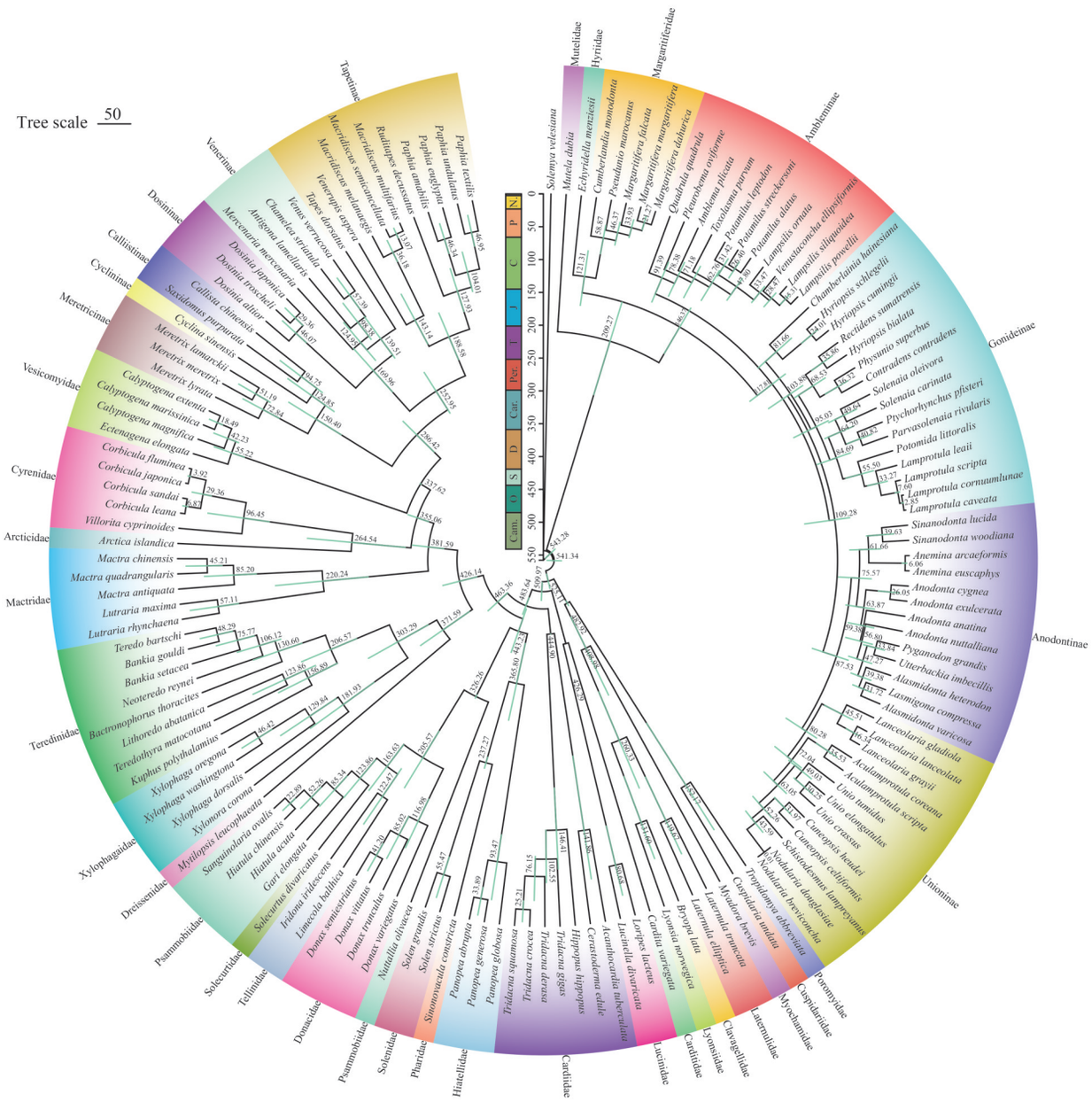


Fig.7 Divergence time estimation for Heteroconchia inferred with BEAST based on 12 PCGs (except *atp8*)

The 95% Highest Posterior Distribution (HPD) is reported using green bars. Bayesian posterior probabilities are shown at each node.

the genus *Corbicula* from the family Cyrenidae, including *C. japonica* and *C. fluminea* are relatively close split at about 3.95 Ma (95% HPD=1.66–7.48). *C. sandai* and *C. leana* diverged more distantly at about 6.87 Ma (95% HPD=3–12.83).

4 CONCLUSION

In this study, we obtained the complete mitochondrial genomes of *T. dorsatus* and *C. variegata*. Our findings revealed that, similar to most bivalve species, all genes in both species were encoded on the same strand. Notably, *C. variegata* encoded an additional *trnM* compared to *T. dorsatus*. This is the first report of a complete mitogenome in the family Carditidae, which provides valuable insights into the family's mitochondrial characteristics. Our data provide important insights into the evolution and phylogenetic relationships of the mitogenome of Heteroconchia, identifying the phylogenetic position of both species and their families within the group.

5 DATA AVAILABILITY STATEMENT

The genome sequence data that support the findings of this study are openly available in GenBank of NCBI at (https://www.ncbi.nlm.nih.gov/nuccore/OP066992, https://www.ncbi.nlm.nih.gov/nuccore/OP021896) under the accession number: OP066992, OP021896.

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