**Comparative Genomic and Mitochondrial Phylogenetic Relationships of Ovulidae (Mollusca: Gastropoda) Along the Chinese Coast**

Qiong Wu1,2, Peng Xiang1, ShiHao Fan3, GuangCheng Chen1, BingPeng Xing1\*

1、Third Institute of Oceanography Ministry of Natural Resources, Xiamen, Fujian, China

2、College of Life Sciences, Beijing Normal University, Beijing, China

3、Independent Researcher, BeiJing, China

**\* Corresponding author:** Bingpeng Xing; Email: xingbpeng@gmail.com, Address: No.178, Daxue Road, Siming District. 361005 Xiamen, Fujian Province, China

**Abstract**

The Ovulidae family, closely related to Cypraeidae (cowries), comprises approximately 260–280 species predominantly inhabiting tropical and subtropical shallow marine environments. Traditional morphological classification within Ovulidae has been challenging due to significant variability influenced by their host organisms. In this study, we collected 15 Ovulidae species from China's offshore waters, including the first regional record of *Habuprionovolva aenigma* (M. Azuma & C. N. Cate, 1971). We sequenced the mitochondrial genomes of 14 species and found that, except for *H. aenigma*, they exhibit typical metazoan mitochondrial structures. Phylogenetic analyses based on mitochondrial genome data were conducted to elucidate relationships among Ovulidae genera. Notably, we discovered that the subfamily Prionovolvinae exhibits a unique mitochondrial tRNA gene order. Our results suggest that mitochondrial gene rearrangements occurred after the divergence of the Prionovolvinae and Ovulinae subfamilies. Additionally, we found that Ovulidae species display significantly higher Ka/Ks ratios compared to Cypraeidae, indicating different selective pressures possibly linked to their unique feeding habits. Based on these findings, we propose reclassifying certain genera from the Simniinae subfamily to the Prionovolvinae subfamily. This research enhances the understanding of Ovulidae phylogeny and provides genomic resources for future studies.

**Key words:** molecular phylogeny, mitochondrial genome, gastropod, Ovulidae,

**Introduction**

The family Ovulidae (Superfamily: Cypraeoidea), also known as ovulids, cowry allies, or false cowries, is one of the families most closely related to Cypraeidae (Rosenberg, 1992; Schiaparelli, Barucca, Olmo, Boyer, & Canapa, 2005). The Ovulidae originated in the early Eocene (Schiaparelli et al., 2005), and currently includes approximately 260 to 280 extant species (Galli, 2024; WoRMS, 2011; Zvonareva et al., 2020). Most species within this family inhabit depths of less than 50 meters (Felix Lorenz & Fehse, 2009), with distributions largely coinciding with those of Cypraeidae, primarily in tropical and subtropical marine regions (Reijnen & van der Meij, 2019; Rosenberg, 1992).

Most Ovulidae species feed on octocorals as ectoparasites, while a few consume sponges, crinoids, and antipatharians (Felix Lorenz & Fehse, 2009; Reijnen, Hoeksema, & Gittenberger, 2010). The size and coloration of ovulids often vary with their host, exhibiting significant variability that complicates species identification (Felix Lorenz & Fehse, 2009; Reijnen & van der Meij, 2017; Rosenberg, 1992).

The nomenclature of Ovulids can be traced back to the time of Linnaeus (C. N. Cate, 1973). Since then, species of Ovulidae have been classified into various taxonomic units, including Bulla Linnaeus, 1758; “Amphiperas” Gronovius, 1781 (non-binomial); Ovula Bruguière, 1789; Volva and Cyphoma Röding, 1798; and Radius Schumacher, 1817. The family Ovulidae was established by the British taxonomist Fleming (1822). In his publication, History of British Animals, Fleming described two genera within this family—*Volva* and *Calpurnus*—characterizing *Volva* by its "canal at each extremity, produced" and *Calpurnus* by its "canals abbreviated, external lip simple" (Fleming, 1828).

Early attempts to classify the Ovulidae based on morphological features include works by F. A. Schilder (1932), M. Schilder and Schilder (1971), C. N. Cate (1973), and C. Cate (1974). Corresponding to Fleming's two genera, taxonomists generally classify Ovulidae into two subfamilies: Ovulinae and Volvinae (Schiaparelli et al., 2005; F. A. Schilder, 1932).

With the advent of molecular biology, researchers have sought to clarify the phylogenetic relationships within Ovulidae using various genetic markers, including 16S rRNA (Schiaparelli et al., 2005), cox1 (Reijnen et al., 2010), 28S (Reijnen & van der Meij, 2019; Zvonareva et al., 2020), histone H3 gene (Reijnen & van der Meij, 2019), and ITS1-5.8S-ITS2 (Wu, Xing, Lin, Chen, & Wang, 2022). Molecular phylogenetics appears to yield different conclusions from morphological systematics; for example, the study by Schiaparelli et al. (2005) suggests that apart from the subfamily Ovulinae (excluding *Ovula ovum* (Linnaeus, 1758)), the remaining Ovulidae species can be allocated to four subfamilies of uncertain taxonomic status (Reijnen & van der Meij, 2019; Schiaparelli et al., 2005). Furthermore, Fehse (2007) expanded on this by dividing Ovulidae into four subfamilies. The most comprehensive and systematic phylogenetic study of Ovulidae to date is by Nocella et al. (2024), which utilized a nuclear gene (28S rDNA) and two mitochondrial genes (cox1 and 16S rRNA) covering 36 genera.

Mitochondrial genomes have long been employed to infer the phylogenetic relationships of bilaterian animals due to their relatively rapid evolutionary rates (Boore, 1999; Wang, Yang, Kong, Sasaki, & Li, 2023), providing substantial genetic information (Irisarri, Uribe, Eernisse, & Zardoya, 2020). Their single-copy nature reduces the difficulty of assessing homology, while gene rearrangements and duplications can provide additional genetic data for phylogenetic analysis (Boore & Brown, 1998). However, as of September 2024, only one mitochondrial genome sequence of Ovulidae species available in the GenBank database (*Volva habei*, OR492307).

In this study, we collected 15 Ovulidae species from the offshore waters of China, providing the first record of *Habuprionovolva aenigma* (M. Azuma & C. N. Cate, 1971) in Chinese waters. We completed the mitochondrial genome sequencing for 14 Ovulidae species and constructed phylogenetic trees based on mitochondrial genome data to further elucidate the phylogenetic relationships among Ovulidae genera.

**Materials and Methods**

**Sample Collection and Identification**

Sample collection locations, dates, and methods are detailed in Table 1. Species identification primarily relied on “The Living Ovulidae: A Manual of the Families of Allied Cowries” (Felix Lorenz & Fehse, 2009), “Cowries and Their Relatives of China” (S.-P. Zhang, 2011), and “Hardy's Internet Guide to Marine Gastropods” (Hardy, 2023). Specimen photographs were captured using a Leica S9D stereo microscope.

**DNA Extraction and First-Generation Sequencing**

DNA extraction was performed following the animal tissue extraction protocol of the DNeasy Blood & Tissue Kit (QIAGEN). After extraction, the nucleic acid concentration of the DNA extracts was measured using a BioDrop spectrophotometer. Due to the difficulty of separating polysaccharide components from tissues using the kit, the DNA was diluted to 0.2 µg/ml to mitigate the inhibitory effects of polysaccharides on PCR reactions (Wu et al., 2022). Each 25 µl PCR reaction consisted of 12.5 µl PCR mixture (Taq Plus Master Mix II (Dye Plus)), 1 µl of each primer (10 µM), 2.5 µl diluted DNA extract, and 8 µl ultra-pure water. The cox1 amplification primers were dgLCO: 5′-GGT CAA CAA ATC ATA AAG AYA TYG G and dgHCO2198: 5′-TAA ACT TCA GGG TGA CCA AAR AAY CA (Meyer, 2003). The annealing temperature was set at 45°C, increasing by 0.5°C per cycle for 15 cycles, followed by 49°C for 20 cycles, targeting a product length of 680 bp. The 16S rRNA amplification primers were 16Sar: 5′-CGC CTG TTT ATC AAA AAC AT and 16Sbr: 5′-CCG GTC TGA ACT CAG ATC ACG T (Hillis, Moritz, & Mable, 1996), with an annealing temperature of 52°C, targeting a product length of 550 bp.

**Library Preparation and Second-Generation Sequencing**

Total genomic DNA was sent to Majorbio Bio-pharm Technology Co., Ltd. (Shanghai, China) and Novogene Co., Ltd. for library preparation and high-throughput sequencing. Libraries were prepared with an average fragment size of approximately 300 bp. The DNA libraries were sequenced on the Illumina Novaseq platform using paired-end reads of 150 bp. The quality of the raw sequencing data was assessed using Fastp (S. Chen, Zhou, Chen, & Gu, 2018).

**Sequence Assembly and Annotation**

Sequence assembly was performed using NOVOPlasty 4.3.5 (Dierckxsens, Mardulyn, & Smits, 2017) with the cox1 or 16S rRNA sequences obtained from first-generation sequencing as seeds, and MitoZ (Meng, Li, Yang, & Liu, 2019). If circularization was unsuccessful, SeqMan Pro (DNAStar) (Burland, 1999) was used to concatenate results from different software. Annotation was conducted using MitoZ (Meng et al., 2019) and the MITOS2 web server (Bernt et al. (2013); Galaxy server: usegalaxy.eu). Protein-coding gene (PCG) boundaries were manually adjusted using SnapGene (GLS Biotech). Mitochondrial circle map was generated using Proksee (Grant et al. (2023), <https://proksee.ca/>). The linearized mitochondrial gene arrangement patterns were generated using PhyloSuite (D. Zhang et al., 2020) and visualized with iTOL (Letunic and Bork (2021), https://itol.embl.de/).

**Phylogenetic Analysis**

Given the close relationship between Cypraeidae and Ovulidae, we included 11 Cypraeidae species in the analysis, whose distribution areas largely overlap with those of the Ovulids collected in this study (Q. Ma et al., 2023). Two species from the family Tonnidae were selected as outgroups (Li et al., 2024; Pu et al., 2019). Using PhyloSuite (D. Zhang et al., 2020), different genes were extracted. Multiple sequences were aligned with MAFFT (Katoh & Standley, 2013) using the 'auto' strategy and codon alignment for PCGs or normal alignment for rRNA. The PCG sequences were refined using MACSE (Ranwez, Douzery, Cambon, Chantret, & Delsuc, 2018), and ambiguously aligned fragments were removed with Gblocks (Talavera & Castresana, 2007). Different gene sequences were concatenated using PhyloSuite (D. Zhang et al., 2020). Two datasets (PCGs, PCGs + rRNA) were used to construct phylogenetic trees. The best-fit partition model was selected for IQ-TREE (Nguyen, Schmidt, Von Haeseler, & Minh, 2015) using the AIC criterion with ModelFinder (Kalyaanamoorthy, Minh, Wong, Von Haeseler, & Jermiin, 2017) and for MrBayes (Ronquist et al., 2012) using the BIC criterion, with the codon model chosen for PCGs.

Bayesian inference(BI) phylogenies were constructed using MrBayes 3.2.6 (Ronquist et al., 2012) under a partition model (two parallel runs, 2,000,000 generations), discarding the initial 25% of sampled data as burn-in. Maximum likelihood (ML) phylogenies were inferred using IQ-TREE (Nguyen et al., 2015) with an edge-linked partition model for 2,000 ultrafast bootstraps (Minh, Nguyen, & Von Haeseler, 2013) and the Shimodaira–Hasegawa-like approximate likelihood-ratio test (Guindon et al., 2010).

Only species with complete sequences for all 13 PCGs were selected for selection pressure analysis, along with Cypraeids sequences obtained from GenBank. Ka/Ks ratios (nonsynonymous to synonymous substitution rates) were calculated using MEGA with the Jukes-Cantor model (Nei & Gojobori, 1986; Tamura, Stecher, & Kumar, 2021). For Ovulidae species, the ka/ks ratio for each PCG was calculated in relation to other species within the family, while for Cypraeidae species, no individual statistics were shown. The K2P distance matrix for the cox1 gene was produced by MEGA.

**Results**

A total of 15 Ovulidae species were collected (Fig. 1). Among them, *Procalpurnus lacteus* (Lamarck, 1810) did not yield contigs longer than 1000 bp and was therefore omitted from subsequent analyses. *Cuspivolva queenslandica* (C. N. Cate, 1974) and *Prosimnia semperi* (Weinkauff, 1881) only produced incomplete mitochondrial genome fragments. Additionally, approximately 500 bp of the l-rRNA sequence were missing for *Prionovolva brevis* (G. B. Sowerby I, 1828). As a result, 14 Ovulidae species from 13 genera were included in the analysis, along with the mitochondrial genome sequence of *Volva habei* Oyama, 1961, retrieved from GenBank. The mitochondrial genome lengths ranged from 15,894 bp to 18,642 bp. With the exception of *Habuprionovolva aenigma*, the mitochondrial genomes of the other Ovulidae species displayed the typical structure of metazoan mitochondrial genomes (Fig. 2), comprising two rRNAs, 13 protein-coding genes, and 22 tRNAs (Boore, 1999).

The detail of best partition models are provided in Supplementary Table 1. The phylogenetic trees for the Cypraeidae portion, constructed using two datasets and two methods, were completely consistent. In the Ovulidae portion, apart from the ML tree for the 13 PCGs, the remaining three phylogenetic trees showed similar topologies (Fig. 3; Supplementary Fig. 1), albeit with minor differences: the BI and ML phylogenetic trees for the 13 PCGs + 2 rRNAs were nearly identical, differing only in the positioning of the crown group comprising *Sandalia triticea* (Lamarck, 1810) and *Contrasimnia xanthochila* (Kuroda, 1928). The ML tree for the 13 PCGs + 2 rRNAs and the BI tree for the 13 PCGs differed only in the interchange of *Cuspivolva queenslandica* and *Prionovolva brevis*. All three phylogenetic trees strongly supported the sister group relationship between *Sandalia triticea* and *Contrasimnia xanthochila*, while the topology of the ML tree for the 13 PCGs differed but had low support at the nodes.

The mitochondrial gene arrangement results (Fig. 3) revealed that all analyzed Cypraeidae species share the same gene count and arrangement. Among the Ovulidae subfamily, *Ovula ovum*, *Volva habei*, and *Phenacovolva rosea* exhibited the same gene order as Cypraeidae. In contrast, the remaining species (traditionally classified under the Prionovolvinae and Simniinae subfamilies) had trnF located between cox1 and cox2. *Habuprionovolva aenigma* exhibited unique characteristics, with a trnD between cox1 and trnF, and a trnM between rrnS and trnV (Fig. 2 & Fig. 3). Overall, the mitochondrial genomes of Ovulidae and Cypraeidae displayed similar PCGs arrangements and distribution patterns: with the exception of eight tRNAs, the remaining genes were primarily located on the major strand.

The Ka/Ks analysis indicated that, while all analyzed species had Ka/Ks values less than 1, there were significant differences between the Ka/Ks values of cowries and Ovulids, as detailed in Fig. 4. Among the analyzed Ovulids, the minimum K2P distance was 10.94% (between *Sandalia triticea* and *Prionovolva brevis*), and the maximum was 23.75% (between *Ovula ovum* and *Diminovula alabaster* (Reeve, 1865)), with an average K2P genetic distance of 17.11% across the 14 Ovulidae species, as summarized in Table 2.

**Discussion**

Compared to Cypraeidae, the Ovulidae are a relatively young group, with the oldest fossils dating back only to 56.0 - 47.8 Ma (Paleobiology Database: <https://paleobiodb.org>). In contrast to the fossil record of Cypraeidae, which is more extensive, the fossils of Ovulidae are fewer and mainly concentrated in the genus *Simnia*. This may be related to the later emergence of Ovulidae and the more fragile nature of their shells, which makes fossil preservation more challenging. Genus with fossil records are rarely distributed in China; therefore, we were unable to locate suitable specimens for dating.

In this study, 15 Ovulidae species were collected, traditionally classified into three subfamilies: Ovulinae, Prionovolvinae, and Simniinae. Among these samples, two species only yielded partial mitochondrial gene fragments (*Cuspivolva queenslandica* and *Prosimnia semperi*). Notably, this study represents the first collection of *Habuprionovolva aenigma* in China. Additionally, *Cuspivolva bellica* (C. N. Cate, 1973) was first collected in China in 2019 (Z. Chen, Guo, Liu, Wei, & Zhang, 2022), and genus *Naviculavolva* was first reported in China in 2022 (Wu et al., 2023).

The phylogenetic trees constructed based on the 13 PCGs using two methods exhibited structural inconsistencies, particularly concerning the relationships among *Cuspivolva queenslandica*, *Contrasimnia xanthochila*, *Sandalia triticea*, and *Cuspivolva bellica*. However, both trees did not support the classification of *Cuspivolva queenslandica* and *Cuspivolva bellica* within the same genus. Although Nocella et al. (2024) did not include *Cuspivolva bellica* in their phylogenetic tree, their analysis still indicated that *Cuspivolva queenslandica* was not grouped with other species of the *Cuspivolva* genus. Some shell collectors, such as Kijineko (2024), propose that *C. queenslandica* may be more closely related to the genus *Primovula*. Therefore, we suggest that *Cuspivolva queenslandica* may not belong to the *Cuspivolva* genus, and a thorough systematic review of the genus's validity may be warranted. Due to the instability in topology, determining which phylogenetic tree is more reliable remains challenging. Similarly, in Nocella et al. (2024), the bootstrap values for the clade containing these four species (defined as the Prionovolvinae subfamily, excluding *Calpurnus verrucosus*) were also low. This instability may stem from insufficient divergence among their most common recent ancestors (MCRA), suggesting minimal base differences, likely resulting from rapid diversification that may have occurred approximately 13 Myr ago, according to Nocella et al. (2024).

Given the structural discrepancies in the phylogenetic trees derived from different datasets, the following discussion focuses on clades that are consistent and exhibit high confidence across the various phylogenetic trees. The phylogenetic tree reconstructed in this study aligns with that of Nocella et al. (2024) on the following points: 1) It confirms the phylogenetic positions of *Ovula ovum*, *Volva habei*, *Phenacovolva rosea*, and *Calpurnus verrucosus*. 2) It corroborates the close relationship between *Habuprionovolva aenigm*a and *Diminovula alabaster*. 3) It validates the close relationship between *Naviculavolva* and *Crenavolva*, suggesting that the Simniinae subfamily, as defined by Felix Lorenz and Fehse (2009), is not monophyletic. The position of *Contrasimnia xanthochila* also refutes the monophyly of the Simniinae subfamily. Since the present study did not cover more basally-diverging lineages like genus *Simnia*, we cannot test the broader applicability of the definition of the “Simniinae” subfamily. However, based on the evidence gathered in this study, both *Naviculavolva* and *Contrasimnia* should be reclassified into the subfamily Prionovolvinae, forming an independent clade with a distinct tRNA arrangement.

The primary differences between this study and Nocella et al. (2024) are as follows: 1) The phylogenetic tree from Nocella et al. (2024) suggests that genera *Volva* and *Ovula* are sister groups, with *Phenacovolva* as the outgroup, while in the present study, *Phenacovolva rosea* and *Ovula ovum* are sister groups. 2) In this study, genus *Primovula* (represented by *Primovula formosa*) forms a clade with genera *Naviculavolva* and *Crenavolva*, excluding *Prosimnia semperi*. 3) The branch containing *Prosimnia semperi* diverged earlier than those of *Sandalia*, *Diminovula*, and *Habuprionovolva*. Regarding the phylogenetic relationships among *Ovula ovum*, *Volva habei*, and *Phenacovolva rosea*, all four phylogenetic trees in this study exhibit strong support, while Nocella et al. (2024) provide weak support for the MCRA nodes of these three species, leading us to conclude that our trees are more reliable. As for the relationship of genus *Primovula* with *Naviculavolva* and *Crenavolva*, all phylogenetic trees in this study show high support for the sister group relationship between *Crenavolva traillii* and *Naviculavolva deflexa*, although support for the position of *Primovula formosa* in the PCGs-based tree is low. Nocella et al. (2024) also provide low support for the branches of these species. Regarding *Prosimnia semperi*, all four phylogenetic trees in this study strongly support that it is the earliest diverging branch compared to *Sandalia triticea*, *Primovula formosa*, and *Habuprionovolva aenigma*.

The PCGs sequences of the mitochondrial genomes in Cypraeidae and Ovulidae exemplify the typical mitochondrial genome order found in Caenogastropoda (Li et al., 2024). The uniform tRNA arrangement across various Cypraeidae suggests a lack of gene rearrangement events, possibly due to the conservative nature of their mitochondrial sequences or the limited range of genera studied. *Tonna galea* and *Tonna dolium*, serving as outgroups, exhibit the same gene order as Cypraeidae, suggesting that this gene arrangement may represent a synapomorphic trait of this clade. Three Ovulidae species—*Ovula ovum*, *Volva habei*, and *Phenacovolva rosea*—share the same gene order as those found in Cypraeidae. Based on their phylogenetic placements, we infer that mitochondrial gene rearrangements in Ovulidae likely occurred after the divergence of the Prionovolvinae and Ovulinae subfamilies, as defined by Nocella et al. (2024), which is estimated to have taken place between 42.7 and 30.25 million years ago. Dietary preferences may contribute to these rearrangements; discussions with aquarists during sample collection suggested that these three species could adapt their diets through domestication, whereas other species demonstrated a strong dietary specialization towards octocorals.

The Ka/Ks analysis revealed that the 13 PCGs of the studied 13 genera exhibited significantly higher Ka/Ks values than those of Cypraeidae. Factors potentially influencing selection pressure on mitochondrial PCGs include diet, climate (Mishmar et al., 2003), generation time, locomotion capabilities, and effective population size (Jakovlić et al., 2023). Given that *Ovula ovum* and *Volva habei* are of medium size and *Calpurnus verrucosus* is similar in size to smaller Cypraeidae, the differences in Ka/Ks values cannot be solely attributed to size. Additionally, since the Cypraeidae and Ovulidae species are geographically proximate, temperature differences are also unlikely to explain the variations. We suggest that the unique feeding habits of Ovulidae may contribute to some of the Ka/Ks discrepancies, potentially reducing the purifying selection pressure on their mitochondria(Jakovlić et al., 2023).

Notably, there appears to be a pattern in the distribution of the Ka/Ks ratios for mitochondrial genes, particularly for the nad2 gene, which may correlate with species size or population scale (Jakovlić et al., 2023). The species with the five highest median Ka/Ks values for nad2 are *Crenavolva traillii*, *Calpurnus verrucosus*, *Phenacovolva rosea*, *Volva habei*, and *Ovula ovum*, all significantly higher than the others. Except for *Crenavolva traillii*, these four are also the largest among the samples and have close phylogenetic relationships. Regarding population size, as noted by Bouchet (2009), quantitative data for most mollusks are lacking. However, driven by shell specimen trade, common Ovulidae species tend to have stable market prices, which, when controlling for sampling and logistics costs, could serve as a proxy for collection difficulty and population size. According to Kijineko's Cowries Collection (www.cypraea.jp), the two most expensive species are *Primovula formosa* and *Cuspivolva bellica*, with distribution areas and depths comparable to those of *Crenavolva traillii* and *Sandalia triticea* (white individuals), suggesting potentially smaller populations. Correspondingly, these two species have lower median nad2 Ka/Ks values. Given the multifactorial influences on Ka/Ks ratios, and the close phylogenetic relationships among several large Ovulids, a larger-scale study encompassing various environmental factors is necessary to ascertain the determinants of Ka/Ks ratios.

The K2P genetic distance results indicate that *Phenacovolva rosea* and *Volva habei* are more closely related among the three Ovulinae species, both exhibiting elongated anterior and posterior terminals. Following them, *Ovula ovum* and *Volva habei* are distinguished by their large sizes, which rank among the largest within the Ovulidae family. An interesting observation is the relatively small genetic distance between *Sandalia triticea* and both *Primovula formosa* and *Prionovolva brevis*. *S. triticea* may be the most cold-tolerant Ovulidae species found in China, with its northern limit extending to the waters near Qingdao (approximately 36° N). Although our collected specimens and those in the trade are typically found in the intertidal zone, there are records of this species at depths of 150–200 m according to F Lorenz (2009) (*S. bridgesi* is a synonym of *S. triticea* (Wu et al., 2022)). The relationship between *S. triticea* and *Primovula formosa* is expected, as our samples of *Primovula formosa* were collected from the intertidal zone, utilizing the same host as *S. triticea*. However, the close relationship between *S. triticea* and *Prionovolva brevis* was unexpected. *Prionovolva brevis* samples in this study were obtained from fishing trawls at depths of around 150–200 m. Although both species share thin, inflated dorsal shells and broad apertures, they differ significantly in size. Furthermore, there is a difference in their host associations: the host of *Prionovolva brevis* is *Dendronephthya* sp. (Vadher, Kardani, & Beleem, 2022), whereas the hosts of *S. triticea* are *Hicksonella* sp. and *Melitodes flabellifera* (Kükenthal, 1908)(X. Ma, 1997).

We acknowledge the limitations of using mitochondrial genomes to reconstruct phylogenetic trees. Factors such as incomplete lineage sorting (Kimball, Guido, Hosner, & Braun, 2021; McGuire et al., 2007), introgression, maternal inheritance, and recombination can complicate mtDNA interpretations (Rubinoff, Cameron, & Will, 2006), making it less suitable as the sole data source for phylogenetic analysis. On the other hand, compared to nuclear genomes, mitochondrial genomes evolve more rapidly due to their haploid nature (DeSalle, Schierwater, & Hadrys, 2017) and exhibit greater variability at lower taxonomic levels (Kelava et al., 2024). Mitochondrial phylogenetic trees can reflect true species relationships more accurately than individual nuclear markers (Kimball et al., 2021).

In this study, despite utilizing complete mitochondrial genomes, some nodes in the phylogenetic trees remain unresolved. The rarity of fossil evidence, the instability of morphological traits, and the variability in host-parasite relationships (Schiaparelli et al., 2005) suggest that molecular techniques may be the best approach to resolving the phylogenetic relationships within this group. Future research should focus on acquiring additional genetic markers, such as nuclear genes, and exploring how to integrate nuclear and mitochondrial data to construct more reliable phylogenetic trees (Fisher-Reid & Wiens, 2011).

**Conclusion**

In this study, we obtained complete mitochondrial genomes for 12 species of the Ovulidae family and partial mitochondrial genome fragments for 2 additional species. Using two datasets, we reconstructed phylogenetic trees using Bayesian Inference and Maximum Likelihood methods. We found that Ovulinae subfamily species closely related to Cypraeidae—*Volva habei*, *Phenacovolva rosea*, and *Ovula ovum*—share the same mitochondrial gene order as Cypraeidae species, while the remaining Ovulidae species, except for *Habuprionovolva aenigma*, exhibit a similar mitochondrial gene order. We suggest reclassifying the genera *Naviculavolva* and *Contrasimnia* from the Simniinae subfamily to the Prionovolvinae subfamily. Additionally, we compared the selective pressures on mitochondrial protein-coding genes between different species of Ovulidae and Cypraeidae, finding that Cypraeidae species experience significantly higher selective pressures. Finally, we analyzed genetic distances among various Ovulidae species.

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**Data Availability Statement**

All sequencing data generated in this study are available in Table 1.

**Reference**

Bernt, M., Donath, A., Jühling, F., Externbrink, F., Florentz, C., Fritzsch, G., . . . Stadler, P. F. (2013). MITOS: improved de novo metazoan mitochondrial genome annotation. *Molecular Phylogenetics and Evolution, 69*(2), 313-319.

Boore, J. L. (1999). Animal mitochondrial genomes. *Nucleic Acids Research, 27*(8), 1767-1780.

Boore, J. L., & Brown, W. M. (1998). Big trees from little genomes: mitochondrial gene order as a phylogenetic tool. *Current opinion in genetics & development, 8*(6), 668-674.

Bouchet, P. (2009). From specimens to data, and from seashells to molluscs: the Panglao Marine Biodiversity Project. *Vita Malacologica, 8*, 1-8.

Burland, T. G. (1999). DNASTAR’s Lasergene sequence analysis software. *Bioinformatics Methods and Protocols*, 71-91.

Cate, C. (1974). The Ovulidae: A key to the genera, and other pertinent notes (Mollusca: Gastropoda). *The Veliger, 16*, 307-313.

Cate, C. N. (1973). A systematic revision of the Recent cypraeid family Ovulidae (Mollusca: Gastropoda). *The Veliger, 15*, 1-116.

Chen, S., Zhou, Y., Chen, Y., & Gu, J. (2018). fastp: an ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics, 34*(17), i884-i890.

Chen, Z., Guo, X., Liu, Y., Wei, P., & Zhang, S. (2022). A new record of genus *Cuspivolva*(Mollusca, Gastropoda, Ovulidae) in coastal waters off Xiamen (in chinese). *Journal of Tropical Oceanography, 41*(2), 189-192.

DeSalle, R., Schierwater, B., & Hadrys, H. (2017). MtDNA: The small workhorse of evolutionary studies. *Frontiers in Bioscience-Landmark, 22*, 873-887.

Dierckxsens, N., Mardulyn, P., & Smits, G. (2017). NOVOPlasty: de novo assembly of organelle genomes from whole genome data. *Nucleic Acids Research, 45*(4), e18-e18.

Fehse, D. (2007). Contributions to the knowledge of the Ovulidae. XVI. The higher systematics. *Spixiana, 30*, 121-125.

Fisher-Reid, M. C., & Wiens, J. J. (2011). What are the consequences of combining nuclear and mitochondrial data for phylogenetic analysis? Lessons from Plethodon salamanders and 13 other vertebrate clades. *BMC Evolutionary Biology, 11*, 1-20.

Fleming, J. (1822). *The philosophy of zoology, or, A general view of the structure, functions, and classification of animals* (Vol. 2): Edinburgh, Hurst, Robinson & Co.

Fleming, J. (1828). *A history of British animals*: Edinburgh, Printed for Bell & Bradfute.

Galli, C. (2024). Worldwide Mollusc Species DataBase. Worldwide Mollusc Species DataBase Retrieved 2024 <https://www.conchology.be/?t=2218&letter=A&family=OVULIDAE&p=1>

Grant, J. R., Enns, E., Marinier, E., Mandal, A., Herman, E. K., Chen, C.-y., . . . Stothard, P. (2023). Proksee: in-depth characterization and visualization of bacterial genomes. *Nucleic Acids Research, 51*(W1), W484-W492.

Guindon, S., Dufayard, J.-F., Lefort, V., Anisimova, M., Hordijk, W., & Gascuel, O. (2010). New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Systematic Biology, 59*(3), 307-321.

Hardy, E. (2023). Hardy's Internet Guide to Marine Gastropods. <https://www.conchology.be/>

Hillis, D. M., Moritz, C., & Mable, B. K. (1996). *Molecular Systematics* (Vol. 23): Sinauer.

Irisarri, I., Uribe, J. E., Eernisse, D. J., & Zardoya, R. (2020). A mitogenomic phylogeny of chitons (Mollusca: Polyplacophora). *BMC Evolutionary Biology, 20*(1), 1-15.

Jakovlić, I., Zou, H., Ye, T., Zhang, H., Liu, X., Xiang, C.-Y., . . . Zhang, D. (2023). Mitogenomic evolutionary rates in bilateria are influenced by parasitic lifestyle and locomotory capacity. *Nature Communications, 14*(1), 6307.

Kalyaanamoorthy, S., Minh, B. Q., Wong, T. K., Von Haeseler, A., & Jermiin, L. S. (2017). ModelFinder: fast model selection for accurate phylogenetic estimates. *Nature Methods, 14*(6), 587-589.

Katoh, K., & Standley, D. M. (2013). MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution, 30*(4), 772-780.

Kelava, S., Apanaskevich, D. A., Shao, R., Gofton, A. W., Mans, B. J., Teo, E. J., . . . Barker, S. C. (2024). Insights from entire mitochondrial genome sequences into the phylogeny of ticks of the genera Haemaphysalis and Archaeocroton with the elevation of the subgenus Alloceraea Schulze, 1919 back to the status of a genus. *Medical and Veterinary Entomology, 38*(2), 189-204.

Kijineko. (2024, 2024). Kijineko's Cowries Collection. Retrieved from <https://cypraea.jp/>

Kimball, R. T., Guido, M., Hosner, P. A., & Braun, E. L. (2021). When good mitochondria go bad: Cyto-nuclear discordance in landfowl (Aves: Galliformes). *Gene, 801*, 145841.

Letunic, I., & Bork, P. (2021). Interactive Tree Of Life (iTOL) v5: an online tool for phylogenetic tree display and annotation. *Nucleic Acids Research, 49*(W1), W293-W296.

Li, F., Li, W., Zhang, Y., Wang, A., Liu, C., Gu, Z., & Yang, Y. (2024). The molecular phylogeny of Caenogastropoda (Mollusca, Gastropoda) based on mitochondrial genomes and nuclear genes. *Gene, 928*, 148790.

Lorenz, F. (2009). Two new species of Ovulidae from the Western Pacific (Gastropoda: Ovulidae). *Conchylia, 40*, 38-44.

Lorenz, F., & Fehse, D. (2009). *The living Ovulidae: a manual of the families of allied cowries: Ovulidae, Pediculariidae and Eocypraeidae*: ConchBooks.

Ma, Q., Li, F., Zheng, J., Liu, C., Wang, A., Yang, Y., & Gu, Z. (2023). Mitogenomic phylogeny of Cypraeidae (Gastropoda: Mesogastropoda). *Frontiers in Ecology and Evolution, 11*, 1138297.

Ma, X. (1997). *Fauna Sinica: Phylum Mollusca: Gastropoda: Mesogastropoda: Cypraeacea (in chinese)*: China Science Publishing & Media Ltd.

McGuire, J. A., Linkem, C. W., Koo, M. S., Hutchison, D. W., Lappin, A. K., Orange, D. I., . . . Jaeger, J. R. (2007). Mitochondrial introgression and incomplete lineage sorting through space and time: phylogenetics of crotaphytid lizards. *Evolution, 61*(12), 2879-2897.

Meng, G., Li, Y., Yang, C., & Liu, S. (2019). MitoZ: a toolkit for animal mitochondrial genome assembly, annotation and visualization. *Nucleic Acids Research, 47*(11), e63-e63.

Meyer, C. P. (2003). Molecular systematics of cowries (Gastropoda: Cypraeidae) and diversification patterns in the tropics. *Biological Journal of the Linnean Society, 79*(3), 401-459.

Minh, B. Q., Nguyen, M. A. T., & Von Haeseler, A. (2013). Ultrafast approximation for phylogenetic bootstrap. *Molecular Biology and Evolution, 30*(5), 1188-1195.

Mishmar, D., Ruiz-Pesini, E., Golik, P., Macaulay, V., Clark, A. G., Hosseini, S., . . . Brown, M. D. (2003). Natural selection shaped regional mtDNA variation in humans. *Proceedings of the National Academy of Sciences, 100*(1), 171-176.

Nei, M., & Gojobori, T. (1986). Simple methods for estimating the numbers of synonymous and nonsynonymous nucleotide substitutions. *Molecular Biology and Evolution, 3*(5), 418-426.

Nguyen, L.-T., Schmidt, H. A., Von Haeseler, A., & Minh, B. Q. (2015). IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Molecular Biology and Evolution, 32*(1), 268-274.

Nocella, E., Zvonareva, S. S., Fassio, G., Pica, D., Buge, B., Villa, R., . . . Oliverio, M. (2024). Spicy food for the egg-cowries: the evolution of corallivory in the Ovulidae (Gastropoda: Cypraeoidea). *Frontiers in Marine Science, 10*, 1323156.

Pu, L., Liu, H., Yang, M., Li, B., Xia, G., Shen, M., & Wang, G. (2019). Complete mitochondrial genome of tiger cowrie *Cypraea tigris* (Linnaeus, 1758). *Mitochondrial DNA Part B, 4*(2), 2755-2756.

Ranwez, V., Douzery, E. J., Cambon, C., Chantret, N., & Delsuc, F. (2018). MACSE v2: toolkit for the alignment of coding sequences accounting for frameshifts and stop codons. *Molecular Biology and Evolution, 35*(10), 2582-2584.

Reijnen, B. T., Hoeksema, B. W., & Gittenberger, E. (2010). Host specificity and phylogenetic relationships among Atlantic Ovulidae (Mollusca: Gastropoda). *Contributions to Zoology, 79*(2), 69-78.

Reijnen, B. T., & van der Meij, S. E. (2017). Coat of many colours—DNA reveals polymorphism of mantle patterns and colouration in Caribbean Cyphoma Röding, 1798 (Gastropoda, Ovulidae). *PeerJ, 5*, e3018.

Reijnen, B. T., & van der Meij, S. E. (2019). Systematics of the subfamily Aclyvolvinae (Caenogastropoda: Ovulidae) based on molecular and morphometric analyses. *Journal of Molluscan Studies, 85*(3), 336-347.

Ronquist, F., Teslenko, M., Van Der Mark, P., Ayres, D. L., Darling, A., Höhna, S., . . . Huelsenbeck, J. P. (2012). MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology, 61*(3), 539-542.

Rosenberg, G. (1992). An introduction to the Ovulidae (Gastropoda: Cypraeacea). *American Conchologist, 20*(1), 4-7.

Rubinoff, D., Cameron, S., & Will, K. (2006). A genomic perspective on the shortcomings of mitochondrial DNA for “barcoding” identification. *Journal of heredity, 97*(6), 581-594.

Schiaparelli, S., Barucca, M., Olmo, E., Boyer, M., & Canapa, A. (2005). Phylogenetic relationships within Ovulidae (Gastropoda: Cypraeoidea) based on molecular data from the 16S rRNA gene. *Marine Biology, 147*, 411-420.

Schilder, F. A. (1932). The living species of Amphiperatinae. *Proceedings of the Malacological Society London, 20*(1), 46-64.

Schilder, M., & Schilder, F. A. (1971). *A Catalogue of Living and Fossil Cowries: Taxonomy and Bibliography of Triviacea and Cypraeacea, (Gastropeda Prosebranehia)* (Vol. 85): Institut royal des sciences naturelles de Belgique.

Talavera, G., & Castresana, J. (2007). Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. *Systematic Biology, 56*(4), 564-577.

Tamura, K., Stecher, G., & Kumar, S. (2021). MEGA11: molecular evolutionary genetics analysis version 11. *Molecular Biology and Evolution, 38*(7), 3022-3027.

Vadher, P., Kardani, H., & Beleem, I. (2022). First record of marine gastropod Prionovolva brevis (GB Sowerby I, 1828) from Gujarat coast, India. *Indian Journal of Geo-Marine Sciences (IJMS), 50*(09), 709-713.

Wang, Y., Yang, Y., Kong, L., Sasaki, T., & Li, Q. (2023). Phylogenomic resolution of Imparidentia (Mollusca: Bivalvia) diversification through mitochondrial genomes. *Marine Life Science & Technology*, 1-11.

WoRMS. (2011). World Register of Marine Species. Retrieved 2024 <https://www.marinespecies.org/aphia.php?p=taxdetails&id=1747>

Wu, Q., Xing, B., Fan, S., Chen, X., Sun, R., Chen, G., & Wang, C. (2023). A New Recorded Genus of the Family Ovulidae from China (in chinese). *Journal of Applied Oceanography, 42*(01), 1-6.

Wu, Q., Xing, B., Lin, M., Chen, G., & Wang, C. (2022). ﻿Molecular phylogeny suggests synonymy of *Sandalia bridgesi* Lorenz, 2009 with *S. triticea* (Lamarck, 1810)(Gastropoda, Ovulidae). *ZooKeys, 1096*, 189.

Zhang, D., Gao, F., Jakovlić, I., Zou, H., Zhang, J., Li, W. X., & Wang, G. T. (2020). PhyloSuite: An integrated and scalable desktop platform for streamlined molecular sequence data management and evolutionary phylogenetics studies. *Molecular Ecology Resources, 20*(1), 348-355.

Zhang, S.-P. (2011). *Cowries and their relatives of China (in chinese, 中国宝贝总科图鉴)*: Ocean Press.

Zvonareva, S. S., Mekhova, E. S., Fedosov, A., Hoang, D., Nguyen, T., & Vo, H. (2020). Diversity and relationships of shallow-water Ovulidae (Mollusca: Gastropoda) of Vietnam. *Archiv fur Molluskenkunde, 149*(2), 113-146.