The complete mitochondrial genome of a cold seep gastropod *Phymorhynchus buccinoides* (Neogastropoda: Conoidea: Raphitomidae)

Lvpei Du¹,², Shanya Cai¹, Jun Liu¹, Ruoyu Liu¹,², Haibin Zhang¹

¹ Institute of Deep-sea Science and Engineering, Chinese Academy of Sciences, Sanya, China, ² University of Chinese Academy of Sciences, Beijing, China

* liujun@idsse.ac.cn

Abstract

*Phymorhynchus* is a genus of deep-sea snails that are most distributed in hydrothermal vent or cold seep environments. In this study, we presented the complete mitochondrial genome of *P. buccinoides*, a cold seep snail from the South China Sea. It is the first mitochondrial genome of a cold seep member of the superfamily Conoidea. The mitochondrial genome is 15,764 bp in length, and contains 13 protein-coding genes (PCGs), 2 rRNA genes, and 22 tRNA genes. These genes are encoded on the positive strand, except for 8 tRNA genes that are encoded on the negative strand. The start codon ATG and 3 types of stop codons, TAA, TAG and the truncated termination codon T, are used in the 13 PCGs. All 13 PCGs in the 26 species of Conoidea share the same gene order, while several tRNA genes have been translocated. Phylogenetic analysis revealed that *P. buccinoides* clustered with *Typhlosyrinx* sp., *Eubela* sp., and *Phymorhynchus* sp., forming the Raphitomidae clade, with high support values. Positive selection analysis showed that a residue located in *atp6* (18S) was identified as the positively selected site with high posterior probabilities, suggesting potential adaption to the cold seep environment. Overall, our data will provide a useful resource on the evolutionary adaptation of cold seep snails for future studies.

Introduction

Conoidea are venomous marine gastropods in Neogastropoda [1, 2], which are found in all oceans, from the tropics to the poles, and from shallow waters to abyssal depths [3]. The superfamily includes 15 families (Borsoniidae, Bouchetispiridae, Clathurellidae, Clavatulidae, Cochlespiridae, Conidae, Conorbidae, Driliidae, Fusiturridae, Mangeliidae, Marshallenidae, Pseudomelatomidae, Raphitomidae, Terebridae, Turridae) [4, 5], and has more than 300 accepted genera and about 5,000 species in total [3, 6, 7]. Raphitomidae, elevated to a full family by Bouchet et al. in 2011 [4], is the largest and most diverse family of Conoidea [8]. Phylogenetic relationships and evolution of Conoidea are very challenging because of its high diversity [9]. In this context, the complete mitochondrial genome analysis can bring new information to the phylogenetic analysis of Conoidea.

For most molluscs, the mitochondrial genome is a closed circular DNA molecule ranging from 15 Kbp to 20 Kbp in length [10], which generally contains 37 genes: 13 protein-coding
genes (PCGs) (cox1-3, nad1-6, nad4L, atp6, atp8 and cob), 2 encoding ribosomal RNA genes (rrnS and rrnL), and 22 encoding transfer RNA genes (tRNAs) [10, 11]. In recent years, mitochondrial genome sequences have been widely used in phylogenetic reconstruction and species identification for many marine animal groups [11, 12]. In addition, as energetic centers of cells, all the 13 mitochondrial PCGs are involved in the oxidative phosphorylation, and mutations in these genes can directly influence metabolic performance [10, 13]. Increasing evidence has shown that mitochondrial PCGs are subject to positive selection in response to extreme environmental stress. For example, selective signatures have been detected for mitochondrial PCGs in marine animals inhabiting extreme environments: the nad5 and nad2 in Pacific salmon [14], the nad2 and nad4 in deep-sea sea cucumber [15], the atp8 and nad5 in deep-sea Starfish [13], and the cox1, cox3, cob, nad2, nad4 and nad5 in cold seeps clams [16].

Cold seeps is one of the extreme deep-sea environments, where fluid migrates upward from deep stratum to the seafloor under pressure that result from plate subduction or gravity compression [17–19]. It mostly occurs in geologically active and passive continental margins and trenches [19]. This environment is characterized by darkness, high hydrostatic pressure, variable temperatures and high levels of toxins [16, 20]. Despite the harsh conditions, dense communities of fauna have been observed in the cold seep ecosystems, which are supported by chemosynthetic symbionts [21]. Cold seep communities have a high level of endemism with common specific lineages at levels of family, genus and species [22]. Evidence of adaptations has been found in species inhabiting these chemosynthetic environments, such as clams [16], mussels [23], tubeworms [24] and shrimps [25]. Recent studies have also identified potentially adaptive residues in mitochondrial PCGs in cold-seep clams [16].

In the present study, we have reported the mitochondrial genome of Phymorhynchus buccinoides Okutani, Fujikura & Sasaki, 1993 in the family Raphitomidae, a gastropod collected from the Haima cold seeps in the South China Sea at depth of 1388 m. This species was first described by Okutani et al. in 1993, based on specimens collected from a cold seep off Hatsushima, Japan (S1 Fig) [26]. Here, we first presented the mitochondrial genome organization, codon usage and gene order information of P. buccinoides. Phylogenetic relationships between P. buccinoides and other species from the superfamily Conoidea were examined based on mitochondrial PCGs. Finally, we performed positive selection analyses in order to understand the adaptive evolution of mitochondrial genes in P. buccinoides to the cold seeps.

Materials and methods

Ethics statement

The snails collected in this study required no specific permits. The sampling locations were not privately owned or protected in any way and the collection did not involve endangered or protected species.

Sample collection and DNA extraction

The specimen (S1 Fig) was collected at depth of 1388 m by Human Occupied Vehicle “ShenHaiYongShi” during an expedition in Haima cold seeps in the South China Sea (16.73°N, 114.46°E) in 2018. The active Haima methane seeps, which have recently been discovered, are located at depths of 1370–1390 m on the northwestern slope of the South China Sea [27]. Methane-derived authigenic carbonates, abundant gas hydrates and chemosynthetic communities are observed in this seep area [27, 28].

The specimen was morphologically identified to P. buccinoides according to keys of Okutani et al. [26]. The sample (voucher no. IDSSE-EEMB-L02) was stored at -80°C in Institute of
Deep-sea Science and Engineering, CAS. Total genomic DNA was extracted from preserved foot tissues using the TIANGEN marine animal DNA kit (TIANGEN, China).

**PCR amplification and sequencing**

The complete mitochondrial genome of this sample was obtained by PCR amplification. The short fragments of *cox1*, *rrnL* and *nad5* were amplified with primers LCO1490+HC02198 [29], 16sinicioF2+16sfinR [30] and QW58ND5F4+QW58ND5R3 (designed in this study based on the sequences from other closely related species from NCBI), respectively. The new sequences were used to design specific primers, which were combined with the *cox3* (forward), *cox1* (forward and reverse), and *rrnL* (forward and reverse) primers published by Uribe et al. [2] for long PCR amplification. The remaining unknown fragments were amplified by using the new designed specific primers (S1 Table).

The PCR amplifications were carried out using TaKaRa LA Taq® and the thermal cycling was: a denaturing step at 94˚C for 5min; 45 cycles of denaturation at 98˚C for 10s, annealing temperatures of 40–50˚C for 30s and extension at 68˚C for 60s per kb; and a final extension step at 68˚C for 12min. A total reaction volume of 50 μl included 33.6 μl ddH₂O, 5 μl 10× LA PCR buffer (Mg²⁺ plus, TaKaRa), 6 μl dNTP mix (2.5 mM each), 2 μl each primer (10 μM), 0.4 μl LA Taq DNA polymerase (5 U/μl, Takara), and 1 μl DNA template (100 ng/μl). For the annealing temperatures see S1 Table. PCR products were confirmed visually on a 1.0% agarose gel (1× TAE) and purified with gel extraction kit (Omega Bio-tek). The purified product was then sequenced on the ABI 3730x1 DNA analyzer (Applied Biosystems Inc.).

**Sequence analysis and gene annotation**

Raw sequences were assembled with the program Seqman within the Lasergene software [31]. Then, the mitochondrial genome were preliminarily annotated by the MITOS webserver (http://mitos.bioinf.uni-leipzig.de/index.py) [32]. NCBI BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and ORF finder (www.ncbi.nlm.nih.gov/projects/gorf/orfig.cgi) were used to identify PCGs. The locations of rRNA genes were determined by alignment with the homologous genes of other species of Neogastropoda. The tRNA genes and their secondary structures were identified by the program tRNAscan-SE 1.21 (http://lowelab.ucsc.edu/tRNAscan-SE/) [33] and ARWEN 1.2.3.c (http://130.235.244.92/ARWEN/) [34]. The mitochondrial genome map was drawn with GenomeVx [35]. The codon usage analysis was estimated with MEGA7.0 [36]. The skew values of AT and GC were used to describe the base composition difference between different families of Conoidea, with the following formulae: AT skew = (A – T) / (A + T) and GC skew = (G – C) / (G + C) [37].

**Phylogenetic analyses**

Phylogenetic relationships of families within the superfamily Conoidea were estimated with sequences of mitochondrial genomes. To balance the number of species in each family, 1–4 species (average 3) of one family were used. Finally, a total of 26 conoid species, belonging to 12 families, were analyzed (Table 2). Phylogenetic relationships were constructed by using Bayesian inference (BI) [38] and Maximum Likelihood (ML) [39] methods. *Nassarius festivus* (Nassariidae) (NC_037607) [40] and *Neptunea arthritica* (Buccinidae) (KU246047) [41] were used as outgroups according to previous phylogenetic studies [2, 42]. All mitochondrial genome sequences used in the analyses are shown in Table 2. Multiple alignments of the 13 PCGs were conducted using MEGA v7.0 [36]. Poorly aligned regions and gaps were removed by using Gblocks v0.91b [43] with the default options. Jmodeltest v2.1.7 [44] was used to
calculate the best-fit substitution models for each PCGs partition. The best-fit models are shown in S2 Table.

The BI analyses were performed with MrBayes v3.1.2 [45]. Two parallel runs each with four simultaneous MCMC chains were conducted for 5,000,000 generations, sampling every 1000 generations, and the first 25% of generations were removed as burn-in. Convergence was checked in Tracer v1.6 [46] with effective sample size for all the parameters > 200. For ML, we used the software RaxmlGUI v1.3 [47] with the settings "ML + rapid bootstrap", 1,000 bootstrap replicates and the GTR+I+G model. Visualization of BI tree and ML tree were realized in FigTree v1.4.3 [48].

Positive selection analysis
Comparing the synonymous/nonsynonymous substitution ratios (ω = dN/dS) of genes in different evolutionary lineages provides an important mean for understanding mechanisms and driving forces of gene evolution [49]. ω > 1 indicates positively selected where some favorable mutation is being fixed; ω = 1 indicates neutrality; ω < 1 indicates purifying selection where most of the non-synonymous mutations were eliminated [50]. We used the “branch models” and “branch-site models” of ‘CodeML’ program in the pamlX package [50, 51] to estimate potential adaptive evolution in the mitochondrial genes of P. buccinoides. The ML tree was constructed by MEGA v7.0 [36] as the working topology for all CodeML analyses.

The 13 individual and concatenate PCGs dataset were involved in the positive selection analysis, and all the models have corrected the average nucleotide frequency at three codon positions (CodonFreq = 2, icode = 4). In order to compare the selection pressure acting on the mitochondrial genomes of cold seep P. buccinoides and other 22 species (S3 Table) of Conoidea inhabiting normal seafloor environments, we used the “one-ratio” (M0), “free-ratio” (M1) and “two ratios” models in the “branch models” to estimate the ω (dN/dS) ratios [50]. Since positive selection usually acts on a few sites within a short period of evolutionary time [52], the “branch site models” (model A and null model A) were used to detect positive selection affecting individual site of cold seep P. buccinoides. Bayes Empirical Bayes (BEB) [53] analysis was adopted to calculate the posterior probabilities of the positively selected sites.

Results and discussion
Mitochondrial genome content and gene organization
The mitochondrial genome of P. buccinoides is a 15,764 bp circular molecule (Fig 1). The genome comprises 37 genes, including 13 PCGs, 2 rRNA genes, and 22 tRNA genes (trnL\text{CUN}, trnL\text{UUR}, trnS\text{AGN} and trnS\text{UCN} is denoted as trnL1, trnL2, trnS1 and trnS2, respectively). Among them, 29 genes are encoded on the heavy (H) strand, whereas the other 8 tRNA genes are encoded on the light (L) strand (Fig 1 and Table 1). A total of 24 noncoding regions are found (Table 1), and the largest region (519 bp) is between trnF and cox3 (Fig 1 and Table 2) and is identified as the putative control region due to the AT richness (77.64%) (Table 2) and its location [3, 54]. The complete mitochondrion has been deposited in GenBank (GenBank accession ID: MN583349).

Protein-coding genes
In this study, all the PCGs of P. buccinoides are located on the positive strand, and this feature is observed in all Conoidea mitochondrial genomes published so far. In the typical metazoan mitochondrial genomes, most PCGs initiate with the standard start codon ATN and terminate with the stop codon TAG or TAA [59]. In P. buccinoides, all the PCGs are initiated with the
ATG codon. For the stop codons, they are ended by a complete TAA (coxl, cox2, cox3, nad1, nda2, nad4, nad5, nad6) or TAG (nad4L, atp8, cob, nad3), except for nad6 which is ended with a truncated stop codon T (S4 Table). Similarly, the genes nad4 and nad6 in Eubela sp. and Typhlosyrinx sp. (family Raphitomidae) are also ended with the truncated termination codon T (S4 Table). Previous studies have shown that truncated stop codon is a common phenomenon in the mitochondrial genomes of metazoans [60], and it doesn’t affect the transcription and translation of mitochondrial genes, since the complete stop codon might be obtained by posttranscriptional polyadenylation [16]. Previous studies have provided evidence that metazoan mitochondrial genomes usually have different codon usage bias [16, 61]. The amino acid usage and relative synonymous codon usage (RSCU) values in the PCGs of P. buccinoides are shown in Fig 2. There is a total of 3,741 amino acids (excluding stop codons) in the 13 PCGs of P. buccinoides, and the amino acid composition is consistent with the other 14 species of Conoidea (Fig 2A). Among PCGs, Leu is the most frequently used amino acid and Cys is the least frequently used, accounting for approximately 15.53% and 1.09% of the total amino acids, respectively. The RSCU indicates
the seven most commonly used codons: TTA (Leu), TCT (Ser), GCT (Ala), GTA (Val), CCT (Pro), TCA (Ser), and ATT (Ile) (Fig 2B). Besides, the codons with A and T in the third position are the most frequently used when compared with other synonymous codons. This feature has been observed in many marine invertebrates, such as crab [62], sea cucumber [15], bivalves [16, 63, 64] and gastropods [65, 66].

Table 1. Characteristics of the mitochondrial genome of *P. buccinoides*.

| Gene | Location | Size | Codon | Intergenic nucleotide(bp)* | Strand |
|------|----------|------|-------|--------------------------|--------|
|      | Start | Stop | Nucleotides(bp) | Amino acids | Start | Stop |
| cox3 | 1   | 780  | 780   | 260         | ATG   | TAA  | 519   | +   |
| trnK | 792  | 858  | 67    | 11          |       |      |       | +   |
| trnA | 863  | 929  | 67    | 4           |       |      |       | +   |
| trnR | 932  | 999  | 68    | 2           |       |      |       | +   |
| trnN | 1001 | 1066 | 66    | 1           |       |      |       | +   |
| trnI | 1072 | 1138 | 67    | 5           |       |      |       | +   |
| nad3 | 1141 | 1494 | 354   | 118         | ATG   | TAG  | 2     | +   |
| trnS1| 1496 | 1563 | 68    | 1           |       |      |       | +   |
| nad2 | 1564 | 2622 | 1059  | 333         | ATG   | TAA  | 0     | +   |
| cox1 | 2627 | 4165 | 1539  | 513         | ATG   | TAA  | 4     | +   |
| cox2 | 4240 | 4926 | 687   | 229         | ATG   | TAA  | 74    | +   |
| trnD | 4951 | 5015 | 67    | 23          |       |      |       | +   |
| atp8 | 5084 | 5245 | 162   | 54          | ATG   | TAG  | 67    | +   |
| atp6 | 5262 | 5954 | 693   | 231         | ATG   | TAA  | 16    | +   |
| trnM | 5991 | 6056 | 66    | 36          |       |      |       | -   |
| trnY | 6062 | 6126 | 65    | 5           |       |      |       | -   |
| trnC | 6131 | 6193 | 63    | 4           |       |      |       | -   |
| trnW | 6194 | 6257 | 64    | 0           |       |      |       | -   |
| trnQ | 6261 | 6313 | 64    | 0           |       |      |       | -   |
| trnG | 6325 | 6389 | 65    | 3           |       |      |       | -   |
| trnE | 6390 | 6455 | 66    | 0           |       |      |       | -   |
| rns  | 6456 | 7387 | 932   | 0           |       |      |       | +   |
| trnV | 7388 | 7451 | 65    | 0           |       |      |       | +   |
| rmlL | 7456 | 8776 | 1321  | 0           |       |      |       | +   |
| trnl1| 8777 | 8845 | 69    | 0           |       |      |       | +   |
| trnl2| 8856 | 8923 | 68    | 10          |       |      |       | +   |
| nad1 | 8925 | 9866 | 942   | 314         | ATG   | TAA  | 1     | +   |
| trnP | 9867 | 9930 | 64    | 0           |       |      |       | +   |
| nad6 | 9932 | 10436| 505   | 169         | ATG   | T—   | 1     | +   |
| cob  | 10437| 11576| 1140  | 380         | ATG   | TAG  | 0     | +   |
| trns2| 11587| 11650| 64    | 10          |       |      |       | +   |
| trnT | 11651| 11714| 64    | 0           |       |      |       | -   |
| nad4L| 11722| 12018| 297   | 99          | ATG   | TAG  | 7     | +   |
| nad4 | 12012| 13391| 1380  | 460         | ATG   | TAA  | -7    | +   |
| trnH | 13387| 13448| 62    | -5          |       |      |       | +   |
| nadS | 13449| 15170| 1722  | 574         | ATG   | TAA  | 0     | +   |
| trnF | 15179| 15242| 64    | 8           |       |      |       | +   |

*Intergenic nucleotide refer to non-coding bases between two genes, and the negative number indicating gene overlap.

https://doi.org/10.1371/journal.pone.0242541.t001
### Table 2. Genomic characteristics of Conoidea mtDNAs.

| Species                  | Family              | Accession number | Whole mitochondrial genome Length (bp) | Protein coding genes A+T% | Protein coding genes GC skewness | rRNAs A+T% | rRNAs Length (bp) | tRNAs A+T% | tRNAs Length (bp) | Non-coding regions A+T% | Non-coding regions Length (bp) | Reference |
|--------------------------|---------------------|------------------|----------------------------------------|--------------------------|----------------------------------|------------|-------------------|------------|-------------------|----------------------------|--------------------------------|-----------|
| Phymorhynchus buccinoides | Raphitomidae        | MN583439         | 15754 71.14 0.11 0.03 11236 69.87 84.40 2357 74.57 1466 70.50 519 77.46 this study |
| Eubela sp.               | Raphitomidae        | MH308406         | 1513 69.82 0.11 0.04 11234 74.87 80.97 2355 69.44 1432 66.51 - - [2] |
| Typhlosyrinx sp.         | Raphitomidae        | MH308407         | 15894 70.43 0.11 0.04 11235 69.62 81.50 2357 74.19 1440 71.25 - - [34] |
| Phymorhynchus sp.        | Raphitomidae        | MT111940         | 15631 69.17 0.12 0.04 11236 69.34 79.72 2356 73.65 1442 69.84 379 74.93 [55] |
| Clavatula tripartita     | Clavatulidae        | MH308391         | 15743 68.48 0.11 0.03 11236 70.87 79.03 2328 70.65 1435 68.67 - - [2] |
| Clionella kraussii       | Clavatulidae        | MH308390         | 15760 68.78 0.11 0.01 11188 71.87 78.90 2341 71.29 1415 68.67 964 72.51 [2] |
| Turricula spurius        | Clavatulidae        | MK25198          | 16453 69.21 0.10 0.03 11223 68.11 81.72 2235 71.70 1480 69.39 1144 69.14 [56] |
| Conus quercinus          | Conidae              | MH400188         | 16380 66.58 0.15 0.12 11265 72.87 76.08 2328 67.44 1482 65.41 415 62.41 [57] |
| Conus capitaneus         | Conidae              | NC030354         | 15829 62.20 0.18 0.14 11262 73.87 67.65 2206 68.44 1487 65.51 - - [54] |
| Fusiturris similis       | Fusiturbidae        | NC_013234        | 15595 66.37 0.12 0.04 11244 75.87 73.63 2309 70.44 1458 67.51 - - [1] |
| Pinguigemmula sp.        | Turridae             | MH308408         | 15238 67.71 0.11 0.04 11226 66.53 77.80 2310 70.78 1480 68.24 - - [2] |
| Lophiotoma cerithiformis | Turridae             | DQ284754         | 15380 67.88 0.12 0.03 11217 66.68 77.11 2318 71.77 1494 69.41 394 58.88 [58] |
| Gemmuloborsonia moosai   | Turridae             | MH308392         | 15352 68.16 0.12 0.02 11229 67.13 79.27 2218 73.20 1623 68.58 313 68.37 [2] |
| Inquisitor sp.           | Pseudomelatomidae   | MH308403         | 15533 70.10 -0.12 0.12 11229 69.57 84.56 2327 73.49 1493 69.59 920 67.07 [2] |
| Otitoma sp.              | Pseudomelatomidae   | MH308405         | 15380 67.88 -0.12 0.03 11217 66.68 77.11 2318 71.77 1494 69.41 394 58.88 [58] |
| Oxymeris dimidiata       | Terebridae           | NC_013234        | 15595 66.37 -0.12 0.03 11226 66.53 77.80 2310 70.78 1480 68.24 - - [2] |
| Splendrillia sp.1        | Drilliidae           | MH308395         | 15358 65.65 -0.22 0.27 11231 65.02 76.67 2352 65.86 1651 66.43 689 73.62 [1] |
| Splendrillia sp.2        | Drilliidae           | MH308396         | 15321 71.57 -0.11 0.05 11217 70.74 84.90 2344 73.91 1479 74.79 1414 69.04 655 73.42 [2] |
| Bathytoma punicea        | Borsoniidae          | MH308389         | 16037 65.98 -0.11 0.04 11228 68.45 66.36 2398 70.28 1470 67.96 752 72.81 [54] |
| Tomopleura sp.           | Borsoniidae          | MH308390         | 15078 69.82 0.19 0.19 11229 69.57 84.56 2327 73.49 1493 69.59 920 67.07 [2] |
| Anguloclavus sp.1        | Horaiclidae          | MH308397         | 15103 66.98 -0.13 0.13 11201 65.89 76.04 2309 70.81 1403 68.07 - - [2] |
| Anguloclavus sp.2        | Horaiclidae          | MH308399         | 15076 71.67 0.11 0.02 11221 69.87 79.64 2310 71.76 1399 71.76 - - [2] |
| Benthomangelia sp.       | Mangeliidae          | MH308400         | 15071 71.67 -0.12 0.12 11201 65.89 79.64 2310 71.76 1399 71.76 - - [2] |
| Toxicochleispira sp.     | Mangeliidae          | MH308401         | 15076 71.67 -0.12 0.12 11201 65.89 79.64 2310 71.76 1399 71.76 - - [2] |
| Cochlespira sp.          | Cochlespiridae       | MH308394         | 15381 63.87 -0.02 0.17 11220 61.86 66.73 2304 69.65 1416 67.80 - - [2] |

*Represents the species sequenced by this study
● is the mitochondrial genome without complete genes.
https://doi.org/10.1371/journal.pone.0242541.t002
Ribosomal RNA and transfer RNA genes

The boundaries of rRNA genes are determined by sequence alignment with that of Typhlosyrinx sp. and Eubela sp.. As in most Conoidea mitochondrial genomes, the *rrnS* and *rrnL* genes in *P. buccinoides* are located between *trnE* and *trnV* and between *trnV* and *trnL*1, respectively (Fig 1).

Based on potential secondary structures, 22 tRNA genes are identified for *P. buccinoides*. Generally, a typical cloverleaf of secondary structure includes an aminoacyl acceptor stem, a TψC stem and loop (T-arm), an anticodon stem and loop, and a DHU stem and loop (D-arm) [11]. Here, all the 22 tRNA genes of *P. buccinoides* can be folded into the typical cloverleaf secondary structures (S2 Fig). However, D-stem absence of tRNA genes is common in most Caenogastropoda species [58, 67, 68] and most other metazoans [69–71].

Gene arrangement

Mitochondrial gene arrangements of metazoans are relatively conserved within major lineages but may be variable between them, and comparisons of these gene arrangements have potential for resolving some deep lineage divergences [10, 72]. In the present study, we compared the mitochondrial genome sequence of *P. buccinoides* with that of other species in the

---

**Fig 2. Amino acid contents and codon usage of 13 mitochondrial genes.** (A) Relative amino acid contents within the mitochondrial genome of the Conoidea. The X-axis shows the percentage of each amino acid, and the Y-axis shows the name of each species. (B) The relative synonymous codon usage (RSCU) of *P. buccinoides* mitochondrial genome. The total number of the RSCU value are provided on the Y-axis, and codon families are on the X-axis.

https://doi.org/10.1371/journal.pone.0242541.g002
superfamily Conoidea (Fig 3). All 13 PCGs in Conoidea share the same gene order, while several tRNAs are translocated. The gene order of families Raphitomidae, Conidae, Mangeliidae, Pseudomelatomidae, and Drilliidae (red box in Fig 3) is completely identical. Some species in families Turridae, Clavatulidae and Borsoniidae have the same gene order as Raphitomidae (red box in Fig 3), but tRNA genes in some species of the former three families have been translocated. Comparing the gene order of these eight families (red box in Fig 3) with the species *G. moosai* of Turridae shows a translocation of the *trnF* gene from a position between *nad5* and *cox3* to a position between *trnS2* and *trnT*. When these eight families (red box in Fig 3) compared with the species *Tomopleura* sp. of Borsoniidae, the *trnT* gene translocated from a position between *trnS2* and *nad4L* to a position between *cox1* and *cox2*. Comparing the gene order of these eight families (red box in Fig 3) with the species *Tomopleura* sp. of Borsoniidae, the *trnT* gene translocated from a position between *trnS2* and *nad4L* to a position between *cox1* and *cox2*. Comparing the gene order of these eight families (red box in Fig 3) with Terebridae shows a translocation of the *trnS2* gene from a position between *cob* and *trnT* to a position between *nad6* and *cob* in Clavatulidae, Horaiclavidae and Fusiturridae (green box in Fig 3). There are two tRNA genes translocated, when comparing the gene order of Clavatulidae, Horaiclavidae and Fusiturridae (green box in Fig 3) with Terebridae. One shows a translocation of the *trnV* gene from a position between *rrnS* and *rrnL* to a position between *trnS2* and *trnT*. *TrnK-trnR, trnN, trnL* and *trnS2* are translocated, when comparing the gene order of these eight families (red box in Fig 3) with Cochlespiridae. The gene order of these eight families (red box in Fig 3) also shows a translocation of the *trnS2* gene from a position between *cob* and *trnT* to a position between *nad6* and *cob* in Clavatulidae, Horaiclavidae and Fusiturridae (green box in Fig 3). There are three and five tRNA genes translocated, when comparing Cochlespiridae with the gene order of Clavatulidae, Horaiclavidae and Fusiturridae (green box in Fig 3) and Terebridae, respectively. These results together with findings from previous studies [1, 73, 74] indicate that the tRNA gene rearrangement of Caenogastropoda occurs occasionally.

**Phylogenetic relationships**

Phylogenetic analysis was performed based on nucleotide sequences of 13 mitochondrial PCGs. The BI and ML analyses generated similar tree topologies with most clades strongly supported (BI posterior probabilities \( \geq 0.98 \); ML bootstrap values \( \geq 85\% \) ) (Fig 4). The best supported phylogenetic relationship of Conoidea is as follows: (((Raphitomidae + Mangeliidae) + (Conidae + Borsoniidae)) + Cochlespiridae) + (((Clavatulidae + (Fusiturridae + Horaiclavidae)) + Turridae) + Terebridae) + ((Pseudomelatomidae + Clavatulidae) + Drilliidae)). This relationship between Raphitomidae and other Conoidea families is also supported by previous studies [2, 75]. Raphitominae has been recognized as a subfamily of Conidae [76], but a recent study upgrades Raphitominae to a full family [4]. In this study, the result shows that Raphitomidae is separated from Conidae, supporting its distinct role as a family.

**Positive selection analysis**

Since the cold seep environments may affect the function of mitochondrial genomes [16], we used positive selection analysis to detect potential selection in cold seep *P. buccinoides*. "Branch models" analysis showed no significant (\( p > 0.05 \)) difference between *P. buccinoides* and other 22 Conoidea species (\( \omega_0 = 0.02215, \omega_1 = 0.02444 \)) (Table 3). However, when we used the "branch site models" to analyze individual genes, a residue, 18 S in *atp6*, was identified as the positively selected site with high posterior probabilities (BEB values > 95%) (Table 3), suggesting potential positive selection in these amino acid sites.

The harsh chemosynthetic environment of cold seeps can influence the mitochondrial aerobic respiration [16], and thus survival of cold seep animals may require adaptation of
mitochondrial PCGs which play important roles in the oxidative phosphorylation [10, 13]. In the present study, one site of atp6 is identified to be positively selected. Recent studies have also found positive selection in ATP genes for many deep-sea animals, such as sea anemone [77] and shrimp [25], indicating potential adaptation to marine extreme environments. ATP dehydrogenase not only is the last enzyme complex in the respiratory chain, but also is a part of the regulatory system of complex V [78]. The atp6 subunit plays an important role in the assembly of F0 proton in ATP synthase, which suggest that mutation in the atp6 gene may affect the production of ATP [79]. Therefore, we predict that the atp6 gene may play an important role in P. buccinoides’s adaptation to cold seep environments.

The deep-sea cold seeps are chemosynthetic ecosystems, which are mainly characterized by high concentrations of methane, sulfide and heavy metals, and low levels of oxygen [80, 81]. These harsh conditions can affect various biological processes, including respiration, reproduction and development [82]. As the energetic centers of eukaryotic cells, mitochondria have proved to be subject to these environmental stress conditions. For example, a previous study based on transcriptomes of shrimps (Alvinocaris longirostris) from reducing environments (cold seeps and hydrothermal vents) identified differentially expressed genes including genes associated with mitochondria, which may contribute to adaptation to the harsh conditions [83]. A recent study focusing on vesicomyid clams inhabiting cold seeps and hydrothermal vents found ten potentially adaptive residues in several mitochondrial genes [16]. In this study, one residue in atp6, was identified as the positively selected site, suggesting potential adaption
of the mitogenome for the cold-seep gastropod *P. buccinoides*. Different positively selected genes are detected between this and previous studies [e.g., 16], which may be caused by the fact that different animal groups might have different adaptation mechanisms, or that the results of positive selection are probably inaccurate due to limited species. Nevertheless, more species from cold seeps and other reducing environments such as hydrothermal vents are required to understand the mitochondrial adaptation for this important gastropod group.

**Conclusion**

In this study, the complete mitochondrial genome of a cold seep snail, *P. buccinoides*, is presented. It is a 15,764 bp circular molecule and encodes 37 typical genes including 13 PCGs, 2 rRNA genes, and 22 tRNA genes. We analyzed the mitochondrial genome content and gene organization, codon usage, gene arrangement, phylogenetic relationships, and positive selection of *P. buccinoides*. The mitogenomic features and codon usage of *P. buccinoides* are similar to other Conoidea species. We found a completely identical arrangement of PCGs in the mitochondrial genomes of the superfamily Conoidea, when the tRNA genes were not considered. The residue located in *atp6* was inferred to be positively selected. This study is the first determination of the mitochondrial genome of a cold seep member of the Conoidea and may provide evidence for the adaptive evolution of *P. buccinoides* in the cold seep environments.

**Supporting information**

S1 Fig. The morphological image of *P. buccinoides*. (EPS)

S2 Fig. Secondary structures of tRNAs in the *P. buccinoides* mitochondrial genome. The tRNAs are labeled with the abbreviations of their corresponding amino acids. (EPS)

S1 Table. Primers used for amplifying of *P. buccinoides* mitochondrial genome. (DOCX)

S2 Table. The information of the best fitting substitution model applied to each gene. (DOCX)

S3 Table. List of taxa used and the environment of species in the Positive selection analysis. (XLSX)

The residue located in *atp6* was inferred to be positively selected. This study is the first determination of the mitochondrial genome of a cold seep member of the Conoidea and may provide evidence for the adaptive evolution of *P. buccinoides* in the cold seep environments.
S4 Table. The length, start codon and stop codon of the PCGs of Conoidea. (XLSX)

Acknowledgments
The authors want to express their gratitude to the captains and crews of the R/V Tansuo 01 and the pilots of HOV "shenhaiyongshi" for their technical support.

Author Contributions
Conceptualization: Lvpei Du, Shanya Cai, Jun Liu, Haibin Zhang.
Data curation: Lvpei Du, Shanya Cai, Jun Liu, Ruoyu Liu.
Formal analysis: Lvpei Du.
Funding acquisition: Haibin Zhang.
Investigation: Lvpei Du, Jun Liu.
Methodology: Lvpei Du, Shanya Cai, Ruoyu Liu.
Project administration: Jun Liu, Haibin Zhang.
Resources: Haibin Zhang.
Software: Lvpei Du, Shanya Cai, Ruoyu Liu.
Supervision: Lvpei Du, Shanya Cai, Jun Liu, Ruoyu Liu, Haibin Zhang.
Validation: Lvpei Du, Shanya Cai, Jun Liu.
Writing – original draft: Lvpei Du.
Writing – review & editing: Lvpei Du, Jun Liu, Haibin Zhang.

References
1. Cunha RL, Grande C, Zardoya R. Neogastropod phylogenetic relationships based on entire mitochondrial genomes. BMC Evolutionary Biology. 2009; 9(1):210. https://doi.org/10.1186/1471-2148-9-210 PMID: 19698157
2. Uribe J, Zardoya R, Puillandre N. Phylogenetic relationships of the conoidean snails (Gastropoda: Caenogastropoda) based on mitochondrial genomes. Molecular Phylogenetics and Evolution. 2018; 127:896–906. https://doi.org/10.1016/j.ympev.2018.06.037 WOS:000446021900079. PMID: 29959984
3. Puillandre N, Fedosov AE, Kantor YI. Systematics and Evolution of the Conoidea. Evolution of Venomous Animals and Their Toxins. 2015:1–32. https://doi.org/10.1007/978-94-007-6727-0_19–1
4. Bouchet P, Kantor YI, Sysoev A, Puillandre N. A new operational classification of the Conoidea (Gastropoda). Journal of Molluscan Studies. 2011; 77:273–308. https://doi.org/10.1093/mollus/eyr017 WOS:000293305300009.
5. World Register of Marine Species (WoRMS) [Internet]. WoRMS Editorial Board. 2019 [cited 2019-12-28]. Available from: http://www.marinespecies.org.
6. Puillandre N, Samadi S, Boisselier MC, Sysoev AV, Kantor YI, Cracaud C, et al. Starting to unravel the toxoglossan knot: Molecular phylogeny of the "turrids" (Neogastropoda: Conoidea). Molecular Phylogenetics and Evolution. 2008; 47(3):1122–34. https://doi.org/10.1016/j.ympev.2007.11.007 WOS:000257128900020. PMID: 18180170
7. Tucker JK. Catalog of Recent and fossil turrids (Mollusca: Gastropoda). Zootaxa. 2004;(682):1–1295. WOS:000225568700001.
8. Manousis T, Kontadakis C, Mbazios G, Polyzoulis G. The family Raphitomiidae (Mollusca: Gastropoda: Conoidea) in the Greek Seas with the description of two new species. Journal of Biological Research-Thessaloniki. 2018; 25. https://doi.org/10.1186/s40709-018-0085-3 WOS:000437990500001. PMID: 30003064
9. Grandcolas P, Maurel M-C. The Conoidea and Their Toxins: Evolution of a Hyper-Diversified Group. Grandcolas P, Maurel MC, editors 2018. 227–49 p.

10. Boore JL. Animal mitochondrial genomes. Nucleic Acids Research. 1999; 27(8):1767–80. https://doi.org/10.1093/nar/27.8.1767 WOS:000079966900001. PMID: 10101183

11. Boore JL, Macey JR, Medina M. Sequencing and comparing whole mitochondrial genomes of animals. In: Zimmer EA, Roalson EH, editors. Molecular Evolution: Producing the Biochemical Data, Part B. Methods in Enzymology. 395 2005. p. 311–48.

12. Cui L, Dong Y, Cao R, Gao J, Cen J, Zheng Z, et al. Mitochondrial genome of the garfish Hyporhamphus quoyi (Beloniformes: Hemiramphidae) and phylogenetic relationships within Beloniformes based on whole mitogenomes. Plos One. 2018; 13(11). https://doi.org/10.1371/journal.pone.0240525 WOS:000463055400006. PMID: 30439749

13. Mu W, Liu J, Zhang H. The first complete mitochondrial genome of the Mariana Trench Freyastera benthophila (Asteroidea: Brisingida: Brisingidae) allows insights into the deep-sea adaptive evolution of Brisingida. Ecology and Evolution. 2018; 8(22):10673–86. https://doi.org/10.1002/ece3.4427 WOS:000451611000004. PMID: 30519397

14. Garvin MR, Bielawski JP, Gharrett AJ. Positive Darwinian Selection in the Piston That Powers Proton Pumps in Complex I of the Mitochondria of Pacific Salmon. Plos One. 2011; 6(9). https://doi.org/10.1371/journal.pone.0242127 WOS:000295936900005. PMID: 21969854

15. Mu W, Liu J, Zhang H. Complete mitochondrial genome of Benthodytes marianensis (Holothuroidea: Elasipodida: Psychropotidae): Insight into deep sea adaptation in the sea cucumber. Plos One. 2018; 13(11). https://doi.org/10.1371/journal.pone.0208051 WOS:000451883700020. PMID: 30500836

16. Yang M, Gong L, Sui J, Li X. The complete mitochondrial genome of Calypogena marissinica (Heteroidea: Veneroida: Vesicomyidae): Insight into the deep-sea adaptive evolution of vesicomyids. PloS one. 2019; 14(9):e0217952–e. https://doi.org/10.1371/journal.pone.0217952 MEDLINE:31536521. PMID: 31536521

17. Wei J, Li J, Wu T, Zhang W, Li J, Wang J, et al. Geologically controlled intermittent gas eruption and its impact on bottom water temperature and chemosynthetic communities-A case study in the "HaiMa" cold seeps, South China Sea. Geological Journal. 2020. https://doi.org/10.1002/gj.3780 WOS:000513856900001.

18. Gibson R AR, Gordon J. Ecology of cold seep sediments: interactions of fauna with flow, chemistry and microbes. Oceanography and Marine Biology: an Annual Review. 2005; 43:1–46. https://doi.org/10.1201/9781420037449.ch1 WOS:000232951200001.

19. Van Dover CL, German CR, Speer KG, Parson LM, Vijenhoek RC. Marine biology—Evolution and biogeography of deep-sea vent and seep invertebrates. Science. 2002; 295(5558):1253–7. https://doi.org/10.1126/science.1067361 WOS:000173926000031. PMID: 11847311

20. Sun J, Zhang Y, Xu T, Zhang Y, Mu H, Zhang Y, et al. Adaptation to deep-sea chemosynthetic environments as revealed by mussel genomes. Nature Ecology & Evolution. 2017; 1(5). https://doi.org/10.1038/s41559-017-0121 WOS:000417171010011. PMID: 28912709

21. Tunncliffe V, Juniper SK, Sibuet M. Reducing environments of the deep-sea floor. Ecosystems of the Deep Ocean. 2003; 28:81–110. CCC:000183545300004.

22. Le Bris N, Arnaud-Haond S, Beaulieu S, Cordes E, Hilario A, Rogers A, et al. Hydrothermal vents and cold seeps. UN Ed) First Global Integrated Marine Assessment. 2016; 18.

23. Wong YH, Sun J, He LS, Chen LG, Qiu J-W, Qian P-Y. High-throughput transcriptome sequencing of the cold seep mussel Bathymodiolus platifrons. Scientific Reports. 2015; 5. https://doi.org/10.1038/srep16597 WOS:000365058100001. PMID: 26593439

24. Li Y, Tassia MG, Waits DS, Bogantes VE, David KT, Halanych KM. Genomic adaptations to chemosymbiosis in the deep-sea seep-dwelling tube worm Lamellibrachia luyi mesi. Bmc Biology. 2019; 17(1). https://doi.org/10.1186/s12915-019-0713-x WOS:000499184800002. PMID: 31379792

25. Se Sun, Hui M, Wang M, Sha Z. The complete mitochondrial genome of the alvinocaridid shrimp Shin-kacaris leurokolos (Decapoda, Caridea): Insight into the mitochondrial genetic basis of deep-sea hydrothermal vent adaptation in the shrimp. Comparative Biochemistry and Physiology D-Genomics & Proteomics. 2018; 25:42–52. https://doi.org/10.1016/j.cbd.2017.11.002 WOS:000426535700006. PMID: 29145028

26. Takashi Okutani, Katsunori Fujikura, Takenori S. New taxon and new distribution records of deepsea gastropods collected from or near the chemosynthetic communities in Japanese water. Bull Nat Sci Mus Tokyo. 1993; Tokyo (Ser A) 19:122–43.

27. Liang Q, Hu Y, Feng D, Peckmann J, Chen L, Yang S, et al. Authigenic carbonates from newly discovered active cold seeps on the northwestern slope of the South China Sea: Constraints on fluid sources, formation environments, and seepage dynamics. Deep-Sea Research Part I-Oceanographic Research Papers. 2017; 124:31–41. https://doi.org/10.1016/j.dsr.2017.04.015 WOS:000404700200003.
28. Feng J, Li N, Luo M, Liang J, Yang S, Wang H, et al. A Quantitative Assessment of Methane-Derived Carbon Cycling at the Cold Seeps in the Northwestern South China Sea. Minerals. 2020; 10(3):256.

29. Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R. DNA primers for amplification of mitochondrial cytochrome oxidase subunit I from metazoan invertebrates. Molecular marine biology and biotechnology. 1994; 3(5):294–9. PMID: 7881515

30. Uribe JE, Puillandré N, Zardoya R. Beyond Conus: Phylogenetic relationships of Conidae based on complete mitochondrial genomes. Molecular Phylogenetics and Evolution. 2017; 107:142–51. https://doi.org/10.1016/j.ympev.2016.10.008 WOS:000394200500014. PMID: 27749464

31. Burland TG. DNASTAR's lasergene sequence analysis software. Methods Mol Biol. 2000; 132:71–91. MEDLINE:10547832. https://doi.org/10.1385/1-59259-192-2:71 PMID: 10547832

32. Bernt M, Donath A, Juehling F, Externbrink F, Florentz C, Fritzsch G, et al. MITOS: Improved de novo metazoan mitochondrial genome annotation. Molecular Phylogenetics and Evolution. 2013; 69(2):313–9. https://doi.org/10.1016/j.ympev.2012.08.023 WOS:000324508900002. PMID: 22982435

33. Chan PP, Lowe TM. tRNAscan-SE: Searching for tRNA Genes in Genomic Sequences. Methods in molecular biology (Clifton, NJ). 2019; 162:1–14. https://doi.org/10.1007/978-1-4939-9173-0_1_1. MEDLINE:31020551. PMID: 31020551

34. Laslet D, Canback B. ARWEN: a program to detect tRNA genes in metazoan mitochondrial nucleotide sequences. Bioinformatics. 2008; 24(2):172–5. https://doi.org/10.1093/bioinformatics/btm573 WOS:000254298500004. PMID: 18033792

35. Conant GC, Wolfe KH. GenomeVx: simple web-based creation of editable circular chromosome maps. Bioinformatics. 2008; 24(6):861–2. https://doi.org/10.1093/bioinformatics/btm598 WOS:000254010400017. PMID: 18227121

36. Kumar S, Stecher G, Tamura K. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. Molecular Biology and Evolution. 2016; 33(7):1870–4. https://doi.org/10.1093/molbev/msw054 WOS:000378767100018. PMID: 27004904

37. Perna NT, Kocher TD. Patterns of Nucleotide Composition at Fourfold Degenerate Sites of Animal Mitochondrial Genomes. Journal of Molecular Evolution. 1995; 41(3):353–8. https://doi.org/10.1007/BF00186547 WOS:A1995RQ42500011. PMID: 7563121

38. Huelsenbeck JP, Ronquist F. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics. 2001; 17(8):754–5. https://doi.org/10.1093/bioinformatics/17.8.754 WOS:000171021000016. PMID: 11524383

39. Felsenstein J. Evolutionary trees from DNA sequences: a maximum likelihood approach. Journal of Molecular Evolution. 1981; 17(6):368–76. https://doi.org/10.1007/BF01734359 WOS: A1981MG91100007. PMID: 7288891

40. Yang Y, Li Q, Kong L, Yu H. Comparative mitogenomic analysis reveals cryptic species in Festivina (Neogastropoda: Nassariidae). Gene. 2018; 662:88–96. https://doi.org/10.1016/j.gene.2018.04.001 WOS:000434238700011. PMID: 29627529

41. Hao ZL, Yang LM, Zhan YY, Tian Y, Mao JX, Wang L, et al. The complete mitochondrial genome of Neptunea arthritica cumingii (Gastropoda: Buccinidae). Mitochondrial DNA Part B-Resources. 2015; 93:118–28. https://doi.org/10.1016/j.mib.2015.07.011 WOS:000362613200012. PMID: 26220836

42. Crosse, (Gastropoda: Buccinidae). Mitochondrial DNA Part B-Resources. 2015; 93:118–28. https://doi.org/10.1016/j.mib.2015.07.011 WOS:000362613200012. PMID: 26220836

43. Castresana J. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. Molecular Biology and Evolution. 2000; 17(4):540–52. https://doi.org/10.1093/molbev/msa054 WOS:000086337700008. PMID: 10742046

44. Posada D. jModelTest: Phylogenetic model averaging. Molecular Biology and Evolution. 2008; 25 (7):1253–6. https://doi.org/10.1093/molbev/msn083 WOS:000256979100001. PMID: 18397919

45. Mau B, Newton MA, Larget B. Bayesian phylogenetic inference via Markov chain Monte Carlo methods. Biometrics. 1999; 55(1):1–12. https://doi.org/10.1111/j.0006-341x.1999.00001.x PMID: 11318142

46. Drummond AJ, Suchard MA, Xie D, Rambaut A. Bayesian Phylogenetics with BEAUti and the BEAST 1.7. Molecular Biology and Evolution. 2012; 29(8):1969–73. https://doi.org/10.1093/molbev/mss075 WOS:000307173000008. PMID: 22367748

47. Silvestro D, Michalak I. raxmlGUI: a graphical front-end for RAxML. Organisms Diversity & Evolution. 2012; 12(4):335–7. https://doi.org/10.1007/s13127-011-0056-0 WOS:000310541800002.

48. Rambaut A. FigTree 1.4. 2 software. Institute of Evolutionary Biology, Univ Edinburgh. 2014.

49. Yang Z. Likelihood ratio tests for detecting positive selection and application to primate lysozyme evolution. Molecular biology and evolution. 1998; 15(5):686–73. https://doi.org/10.1093/oxfordjournals.molbev.a025957. PMID: 9580986
69. Grande C, Templado J, Cervera JL, Zardoya R. The complete mitochondrial genome of the nudibranch *Roboastra europaea* (Mollusca: Gastropoda) supports the monophyly of opisthobranchs. Molecular Biology and Evolution. 2002; 19(10):1672–85. https://doi.org/10.1093/oxfordjournals.molbev.a003990 WOS:000178497200003. PMID: 12270894

70. Wang H, Zhang S, Xiao G, Liu B. Complete mtDNA of the *Meretrix lamarkii* (Bivalvia: Veneridae) and molecular identification of suspected *M. lamarkii* based on the whole mitochondrial genome. Marine Genomics. 2011; 4(4):263–71. https://doi.org/10.1016/j.margen.2011.06.006 WOS:000298448200004. PMID: 22118638

71. Geng X, Cheng R, Xiang T, Deng B, Wang Y, Deng D, et al. The complete mitochondrial genome of the Chinese Daphnia pulex (Cladocera, Daphniidae). Zookeys. 2016;(615):47–60. https://doi.org/10.3897/zookeys.615.8581 WOS:000383375800003. PMID: 27687940

72. Boore JL, Brown WM. Big trees from little genomes: mitochondrial gene order as a phylogenetic tool. Current Opinion in Genetics & Development. 1998; 8(6):668–74. https://doi.org/10.1016/s0959-437x(98)80035-x WOS:000077614100009. PMID: 9914213

73. Bandyopadhyay PK, Stevenson BJ, Ownby J-P, Cady MT, Watkins M, Olivera BM. The mitochondrial genome of *Conus textile*, coxI-cox II intergenic sequences and Conoidea evolution. Molecular Phylogenetics and Evolution. 2008; 46(1):215–23. https://doi.org/10.1016/j.mpev.2007.08.002 WOS:000253358800018. PMID: 17936021

74. Brauer A, Kurz A, Stockwell T, Baden-Tillson H, Heidler J, Wittig I, et al. The Mitochondrial Genome of the Venomous Cone Snail *Conus consors*. Plos One. 2012; 7(12). https://doi.org/10.1371/journal.pone.0051528 WOS:000312064100128. PMID: 23236512

75. Puillandre N, Kantor YI, Sysoev A, Couloux A, Meyer C, Rawlings T, et al. The dragon tamed? A molecular phylogeny of the Conoidea (Gastropoda). Journal of Molluscan Studies. 2011; 77: 259–72. https://doi.org/10.1093/mollus/eyr015 WOS:000293305300008.

76. Taylor J. Foregut anatomy, feeding mechanisms, relationships and classification of the Conoidea (= Toxoglossa) (Gastropoda). Bull nat Hist Mus, London (Zoology). 1993; 59(2):125–70.

77. Zhang B, Zhang Y-H, Wang X, Zhang H-X, Lin Q. The mitochondrial genome of a sea anemone *Bolocera* sp. exhibits novel genetic structures potentially involved in adaptation to the deep-sea environment. Ecology and Evolution. 2017; 7(13):4951–62. https://doi.org/10.1002/ece3.3067 WOS:000404645400009. PMID: 28690821

78. Sun Se, Sha Z, Wang Y. The complete mitochondrial genomes of two vent squat lobsters, Munidopsis launensis and *M. verrilli*: Novel gene arrangements and phylogenetic implications. Ecology and Evolution. 2019; 9(22):4230–407. https://doi.org/10.1002/ece3.5542 WOS:000488593000001. PMID: 31788185

79. da Fonseca RR, Johnson WE, O’Brien SJ, Ramos MJ, Antunes A. The adaptive evolution of the mammalian mitochondrial genome. Bmc Genomics. 2008; 9. https://doi.org/10.1186/1471-2164-9-119 WOS:000255705100001. PMID: 18318906

80. Little CT, Vrijenhoek RC. Are hydrothermal vent animals living fossils? Trends in Ecology & Evolution. 2003; 18(11):582–8. https://doi.org/10.1016/j.tree.2003.08.009

81. Sibuet M, Olu K. Biogeography, biodiversity and fluid dependence of deep-sea cold-seep communities at active and passive margins. Deep-Sea Research (Part II, Topical Studies in Oceanography). 1998; 45(1):517–67. https://doi.org/10.1016/S0967-0645(97)00074-X

82. Turnipseed M, Knick KE, Lipcius RN, Dreyer J, Dover CLV. Diversity in mussel beds at deep-sea hydrothermal vents and cold seeps. Ecology Letters. 2010; 6(6):518–23. https://doi.org/10.1111/j.1461-0248.2003.00465.x

83. Hui M, Cheng J, Sha Z. Adaptation to the deep-sea hydrothermal vents and cold seeps: insights from the transcriptomes of Alvinocaris longirostris in both environments. Deep Sea Research Part I: Oceanographic Research Papers. 2018; 135:23–33. https://doi.org/10.1016/j.dsr.2018.03.014