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# Compositional properties and codon usage pattern of mitochondrial cytochrome c oxidase I gene among potamidids

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## Abstract

Codon usage bias (CUB) refers to an unequal occurrence of specific synonymous codons in a genome with variations within and among species. Findings suggest that CUB is significant in interpreting evolutionary trends of species and/or genes. However, information of CUB remained unclear for organisms in the family Potamididae. This study examined compositional features and codon usage patterns of *coI* gene among potamidids to identify factors shaping CUB. Quantification of CUB and the identification of causative factors were performed by assessing nucleotide composition, effective number of codons (ENC), relative synonymous codon usage (RSCU), parity rule (PR2) analysis and neutrality plot analysis as well as correspondence analysis (CoA). Results from neutrality plot suggested that natural selection is the dominant evolutionary factor for CUB in the *coI* gene among the different genera of potamidids. In contrast, CoA, PR2 and ENC analyses indicated the existence of mutational pressure with minor contribution. Nucleotide composition investigations indicated that most codons are AT rich; with T nucleotides count being highly presented. Thus, compositional restraint was also among the reasons behind the pattern of codon usage in *coI* gene of species in the potamidids. Although, the present study involved one mitochondrial gene, *coI*, the findings have demonstrated that various genera of potamidids are differentially adapted in the environment, and that *coI* gene may be differentially expressed and remain to be elucidated.

## Introduction

Evolutionary processes involve a change in heritable traits over successive generations of a biological population (Fowler et al. 2018). Therefore, evolutionary processes give rise to diversity at every biological organization level (Adams and Engel 2021; Cassidy 2020; Hopcroft 2018). The major driving mechanism of heredity in all individuals is in form of genes received from parents and passed to offspring (Alberts 2015; Fowler et al. 2018). At the molecular level, that is, molecular evolution, changes in the number of nucleotides or amino acid substitution per site per year are what influence evolutionary changes (Nei et al. 2000; Ruse and Travis 2009). However, some changes of a single DNA nucleotide within a protein-coding portion of a gene may not affect the sequence of amino acids that make up the gene's protein a phenomenon called silent sites mutation (Klasen and Wabl 2004; Czech et al. 2010). Comparing the rates of evolution among genes and patterns of silent site evolution as revealed, for example, by codon usage studies can tell more about the forces behind genome evolution.

Codon usage refers to the frequency in which the available codons of a gene are being used by a particular organism (Behura and Severson 2013; Zhou et al. 2016; Parvathy et al. 2022). Although each codon is specific for only one amino acid, some amino acids are said to be encoded by more than one codon, making the genetic code described as degenerate. The different codons that specify the same amino acids are referred to as synonymous codons (Mitra et al. 2016). Even though they encode the same amino acid, it has been shown for all organisms that there is an unequal occurrence of synonymous codons in a genome, a phenomenon called codon usage bias (CUB) (Behura and Severson 2013). The major factors influencing CUB are genetic drift, mutation pressure, and natural selection (Salim and Cavalcanti 2008; Choudhury et al. 2017; Gun et al. 2018; Parvathy et al. 2022). Therefore, when studied in detail, CUB echoes the origin of mutation patterns as well as the evolution of species or genes, and can help to categorize a gene (or species) and interpret its evolutionary trends.

Mitochondria is an important organelle that plays a central role in cellular energy provision, containing their genome with a modified genetic code (Crimi and Rigolio 2008; Osellame et al. 2012; Chinnery and Hudson 2013; Friedman and Nunnari 2014). Having simple genomic organization, lack of recombination, and a high rate of evolution than that of the nuclear genome, mitochondria serve as a vital source of genetic information (Arif et al. 2011). Since mitochondrial genes are known to be conserved evolutionarily, it is possible to weigh related species in terms of their evolutionary pattern and molecular changes (Nabholz et al. 2008; Chinnery and Hudson 2013). Consequently, mitochondrial genome studies assist in understanding species diversity as well as population genetics (Castro et al. 1998; Nabholz et al. 2008). Cytochrome c oxidase includes a complex of genes (*coI*, *coII* and *coIII*) that concludes the ultimate step of the mitochondrial respiratory chain (Ferguson-Miller 2013; Shimada et al. 2017). The gene, *coI* has been revealed to have a distinct phylogenetic signal than other mitochondrial genes as a result of its satisfactorily hasty evolution (Townsend et al. 2012; Souza et al. 2016). Analyzing the manner of codon usage of the *coI* gene with the help of various parameters could thereby be crucial in discriminating genes (or species) and investigating their intraspecific diversity.

The family Potamididae is one of the common molluscan fauna of mangrove forests in the Indo-West Pacific area (Reid et al. 2008). They play a significant role in the mangrove ecosystem, as bioindicators of health and ecological changes, but also as biofilters in wastewater (Ratsimbazafy and Kochzius 2018; Wells and Keesing 2019). Some potamidids are also used as a food source for humans (Ratsimbazafy and Kochzius 2018). Traditionally, potamidids have been confused because they have similar shells and occupy almost common habitats, thus, in the past, their identification has been problematic (Willan 2013). This raises the need to understand the evolutionary forces behind their differences, which is important in understanding their diversity and genetic evolution. In this study, nucleotide composition and CUB in the *coI* gene for organisms in the four different genera of the Potamididae family were investigated to explore potamidids diversification.

## Material and methods

### *Retrieval of sequences*

Retrieval of 16 coI gene sequences for the different species belonging to four genera of the family potamididae was performed from NCBI (http://www.ncbi.nlm.nih.gov/Genbank/).

Table 1 lists the species, their accession number, and the genus to which they belong.

**Table 1.** List of 16 *coI* gene sequences for species of potamidids with accession numbers

| S/N | SPECIES NAME | GENUS | ACCESSION NUMBER |
|---|---|---|---|
| 1 | *C. obusta* | *Cerithidea* | NC_039951 |
| 2 | *C. sinensis* | *Cerithidea* | AB219355 |
| 3 | *C. rhizophorarum* | *Cerithidea* | AB219333 |
| 4 | *C. reidi* | *Cerithidea* | HE680238 |
| 5 | *C. alata* | *Cerithideopsilla* | JF694693 |
| 6 | *C. cingulate* | *Cerithidiopsilla* | JF694666 |
| 7 | *C. costata* | *Cerithidiopsis* | GQ273849 |
| 8 | *C. montagnei* | *Cerithidiopsis* | GQ273847 |
| 9 | *C. californica* | *Cerithideopsis* | GQ273835 |
| 10 | *C. microptera* | *Cerithideopsilla* | HE680612 |
| 11 | *C. conica* | *Cerithideopsilla* | AM932759 |
| 12 | *C. insica* | *Cerithideopsilla* | MZ831994 |
| 13 | *C. scalariformis* | *Cerithideopsis* | HE680627 |
| 14 | *T. palustris* | *Terebralia* | LN649639 |
| 15 | *T. semistriata* | *Terebralia* | HE680660 |
| 16 | *T.sulcata* | *Terebralia* | MZ832021 |

### *Nucleotide composition analysis*

Base nucleotide composition constraint is among the determinants of CUB across genomes, the relationship of which has been reported in various literatures (Barbhuiya et al. 2021; Chakraborty et al. 2022). Therefore, to reveal significant factor(s) shaping CUB in potamidids nucleotide compositional characteristics including: (i) nucleotide counts (A, C, T, and G %) (ii) Third position nucleotide count (A3, C3, T3, and G3%) (iii). Total GC% as well as positional GC content (GC1, GC2, and GC3%), were calculated from (https://genomes.urv.es/CAICal) web service.

### *Effective number of codons (ENC)*

Effective number of codons is termed as the extent of species-independent synonymous codon bias in genes (Wright 1990; Sun et al. 2013). The values represent the number of equally used codons that would generate the same codon usage bias as that observed and are thereby used to quantify CUB in a gene (Wright 1990). The DnaSP v.6 software was used to calculate ENC values, to investigate whether its natural selection or mutation pressure that influences CUB among members of the family Potamididae. Lower ENC value is 20 denoting a highly biased gene, whilst the upper limit is 61 for equally used codons (Yang et al. 2014).

### *Relative Synonymous Codon Usage (RSCU)*

Relative synonymous codon usage refers to the ratio of the observed codon frequency to the frequency expected if codon usage was uniform (Gun et al. 2018). The values are used to quantify the relative usage of synonymous codons within a codon family of a gene. RSCU > 1 indicates positive codon usage i.e. more frequently used (De Mandal et al. 2020; Yu et al. 2021). On the contrary, RSCU < 1 reveals a negative codon usage (less frequently used) (De Mandal et al. 2020; Gun et al. 2018). Over-represented and under-represented codons have their RSCU values above 1.6 and below 0.6 respectively (Xu et al. 2013; Shafat et al. 2022). In this study, RSCU values of coI gene of the various species in the family Potamididae were estimated using DnaSP v.6 software.

### *Correspondence analysis*

To determine the major factors affecting the pattern of codon usage in each genus, Correspondence analysis, a multivariate statistical technique was executed using R program. In this case, the large-scale trend of codon usage bias in the sequence of nucleic acids could be estimated.

### *Parity rule 2 (PR2) analysis*

A parity plot was created to evaluate Chargaff`s second parity rule (A=T and G=C) by plotting AT bias [A/ (A+T)] on the y-axis versus GC bias [G/ (G+C)] on the x-axis. Any deviation from PR2 indicates biasness arbitrated by either natural selection, mutation pressure, or both forces (Nasrullah et al. 2015). This was created using OriginPro software.

### *Neutrality plot analysis*

This is the most widely used method to accurately reveal the prime driving force of CUB as either mutation pressure or natural selection. The plots were constructed utilizing average GC12 values on the y-axis against GC3 values on the x-axis in R program. When the codon bias is absolutely under the action of neutrality or directional mutational force the quantitative relation between GC3 and GC12 becomes almost equal and the slope is nearer to 1(He et al. 2016; Shen et al. 2020).

## Results and Discussion

Codon usage bias is a common feature in most organism genomes including prokaryotes and eukaryotes, and may contribute to genome evolution in a profound manner (Behura and Severson 2013; Zhou et al. 2016). The two widely referred theories that explained the foundation of codon usage bias in organisms are the neutral theory and the selection-mutation drift model (Duret 2002; Deb et al. 2020). According to neutral theory, silent mutations in the codons` third position raise the phenomenon of codon preference (Subramanian 2008). Whereas, the selection-mutation drift model suggests that, CUB occurs as an equilibrium event to balance natural selection, mutation pressure, and genetic drift (Bulmer 1991; Duret 2002). Individual genes show variation in codon usage patterns; however, even within a gene, the pattern of codon usage may vary depending on the position of the codon (Gerdol et al. 2015). The coding sequences of a gene and/or genome provide information on gene function and hence the evolution of the organism (Behura and Severson 2013; Zhou et al. 2016). Thus, studies on the patterns of codon usage including their causes, consequences, as well as selective forces shaping their evolution are significant in understanding genome biology and population diversification. In this study, compositional aspects and codon usage properties for *coI* gene in *Cerithidea*, *Cerithideopsilla*, *Cerithideopsis*, and *Terebralia* genera of the family Potamididae were analyzed.

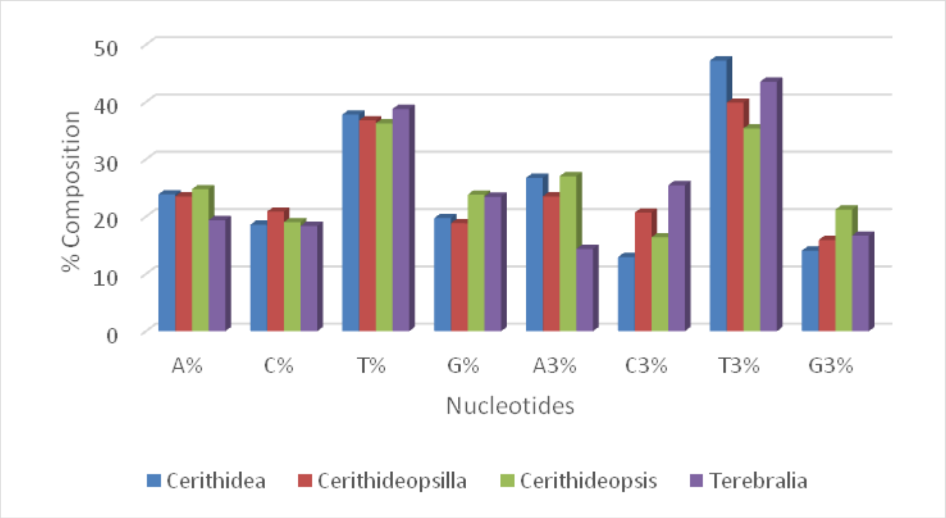


**Figure 1.** Overall nucleotide composition for *coI* gene in different genus of potamidids

*Nucleotide composition*

The trend of average nucleotide composition for *coI* gene was T >A >G >C in *Cerithidea* and *Cerithideopsis*, whereas, in *Terebralia* and *Cerithideopsilla* the trend was T >G >A >C and T >A >C >G respectively (Figure 1). The overall nucleobase T composition was the highest in all the four genera within the Potamididae family. Compositional feature parameters that explain the possible codon usage pattern of the *coI* gene among different species of the family Potamididae were found to be interesting. For example, the third position of codons was mostly occupied by nucleobase T (Figure 1) suggesting that codons ending with T were more preferred as compared to other nucleotides. It is known that the third codon position is subjected to silent mutation pressure (Chamary and Hurst 2009; Alberts 2015) therefore, when studied in detail such investigations play a vital role in the interpretation of codon trend of the various genes. In the present study, silent mutations at third codon positions involving T nucleobase was observed (Figure 1) to be common as a result codon ending with T nucleobase were highly favored.

The fashion of nucleotide composition at the third position (A3, C3, T3, and G3) was as

T3>A3>C3>G3, thus nucleobase T ranked the highest followed by A except for genus *Terebralia*. Again, nucleobase C was the least prevalent except for *Cerithideopsilla* where G nucleobase had shown low prevalence (Figure 2). Mean GC content was 38.32%, 39.68%, 39.12%, and 41.84% in *Cerithidea*, *Cerithideopsilla*, *Cerithideopsis*, and *Terebralia* genera, respectively (Figure 2). The *coI* gene was relatively AT-rich in all genera of the Potamididae family. A slight difference was observed in positional GC contents (GC1, GC2, and GC3) among the four genera of Potadimidae (Figure 2).

These findings agree with the postulate that compositional properties might have effects on the codon usage pattern of a gene (Deka and Chakraborty 2014; Choudhury et al. 2018; Barbhuiya et al. 2020).

### *Effective number of codons (ENC)*

The effective number of codons was calculated to analyze codon usage patterns in the different species belonging to the family Potamididae.

The ENC values found in the present study for the six genera of Potamididae are displayed in Table 2. Although for all genera CUB, as determined by ENC values, was moderate, the lowest CUB was observed in genus *Cerithideopsis* as compared to other genera (Table 2). Moreover, codon usage bias of the *coI* gene was found to be weak because the ENC values in the present study were greater than 35, but less than 50. These findings are not surprising because, other studies in mitochondrial ATP, ND, and CO genes of amphibians, arthropods, and reptiles, respectively revealed similar low CUB (Barbhuiya et al. 2019; Barbhuiya et al. 2021; Chakraborty et al. 2022).

**Table 2.** ENC values of *coI* gene in different genera of the family Potamididae

| S/N | Genus | ENC Value |
|---|---|---|
| 1 | *Cerithidea* | 45.65% |
| 2 | *Cerithideopsilla* | 46.43% |
| 3 | *Cerithideopsis* | 49.86% |
| 4 | *Terebralia* | 48.86% |

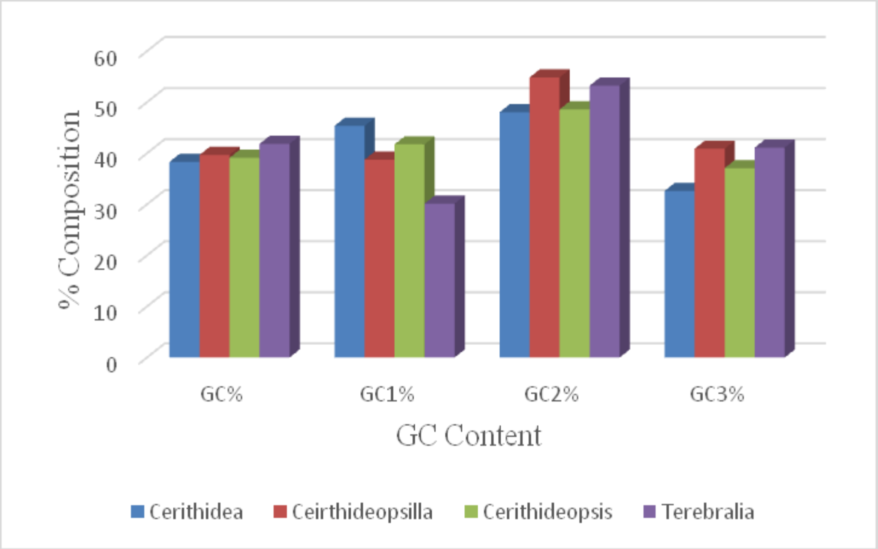


**Figure 2.** Overall GC content and positional GC content of *coI* gene among the different genera of Potamidids

*Pattern of relative synonymous codon usage (RSCU)*

The RSCU values were established to quantify the relative usage of synonymous codons within a codon family of the *coI* gene. The RSCU values were as presented in Supplementary table 1. There was variability in RSCU values among and between four genera of Potamididae in the present study. Interestingly, some codons were overrepresented across different genera while others were underrepresented (Supplementary table 1). Specifically, five codons (CUU, GUU, UCU, ACU, and AGG) were found to be commonly overrepresented across all four genera of the family Potamididae, and six codons (GGA, CGA, GAG, UAC, CUG, CUA) were commonly underrepresented across the four different genera in the family Potamididae (Supplementary table 1). It has been reported that similar codon usage patterns including over and underrepresented codons in genomes ensure translational efficiency (Tuller et al. 2010), thus, the observed similar pattern in codon preference might be due to translational selection. Further, a multivariate statistical approach of correspondence analysis (CoA), utilizing RSCU values, was performed to define the varying trends in codon usage among potamidids.

*Correspondence analysis (CoA)*

Correspondence analysis showing the distribution pattern of codons in the four genera is shown in Figure 3. The distribution depicted variation in codon usage across the different species in the family Potamididae for the *coI* gene.

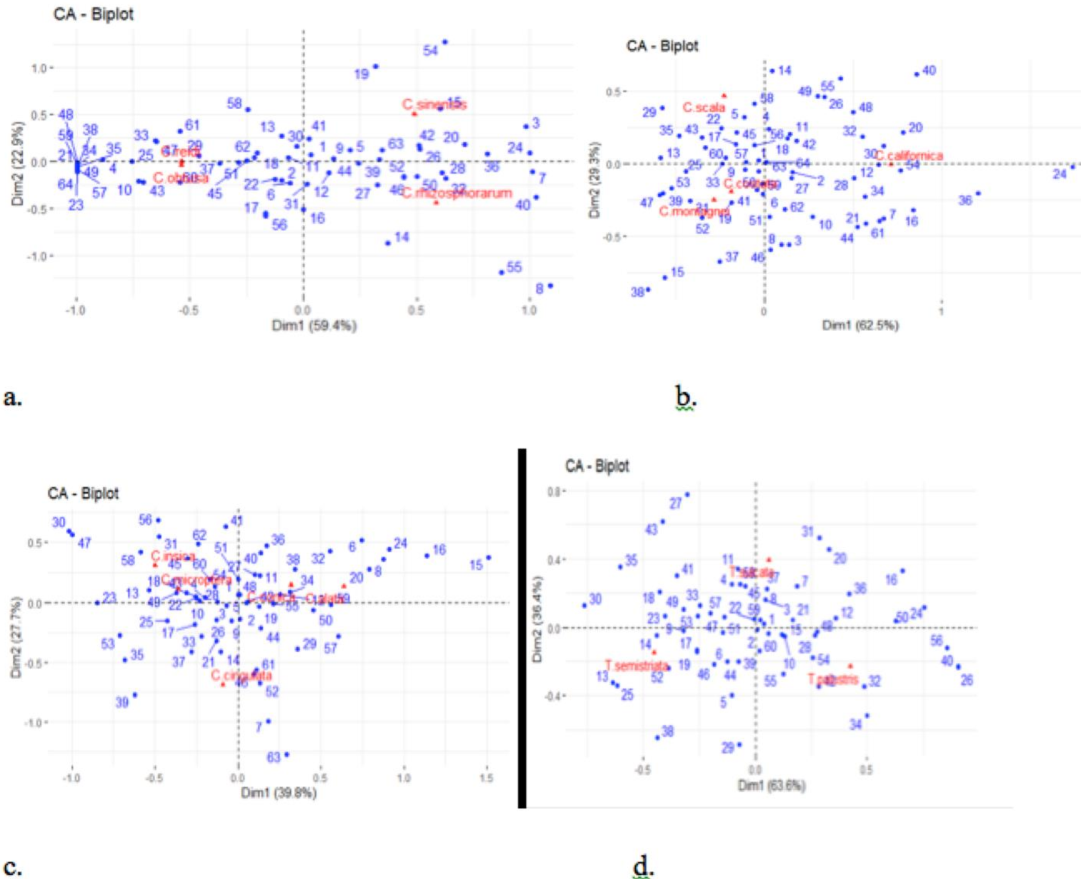


**Figure 3.** Correspondence analysis (CoA) of codon usage bias in *coI* gene for *Cerithidea* (a); *Cerithideopsis* (b); *Cerithideopsilla* (c) and *Terebralia* (d)

Most codon positions were found to concentrate close to the axes and at the center of the plot. Chunks of codons were situated close to the axes and some concentrated in the center of the plot (Figure 3) proposing that nucleotide compositional constraints under mutation pressure might be associated with CUB of the *coI* gene. Earlier investigations on codon usage bias in *Harpago chiragra* and *Lambis lambis* (Gastropoda: Stromboidea) also revealed that the mitochondria genes were subjected to different mutational pressures, which signified compositional constraints (Jiang et al. 2019). Similar findings were observed in reptiles (Chakraborty et al. 2022), cotton species (Wang et al. 2018), and bivalves (Gerdol et al. 2015).

### *Parity plot analysis*

To estimate the effects of mutation pressure and natural selection on codon usage, a PR2 plot was executed. It was found that the gene spots were scattered to show deviation from base composition rule (Chargaff`s second parity rule) that A and T were not proportionate with C and G (Figure 4).

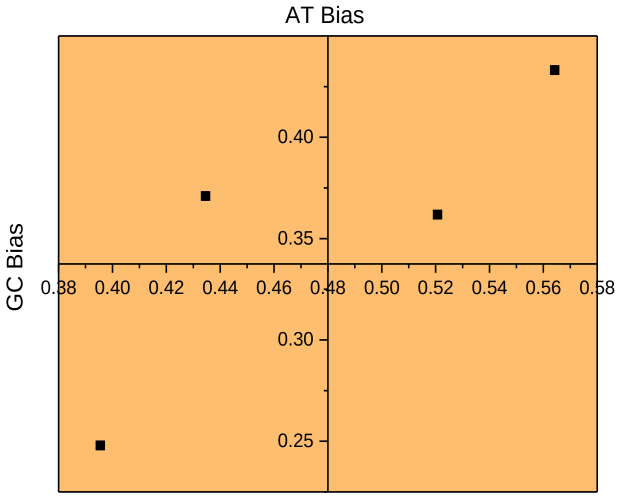


**Figure 4.** Parity plot analysis of *coI* gene in the six genera of the family Potamididae

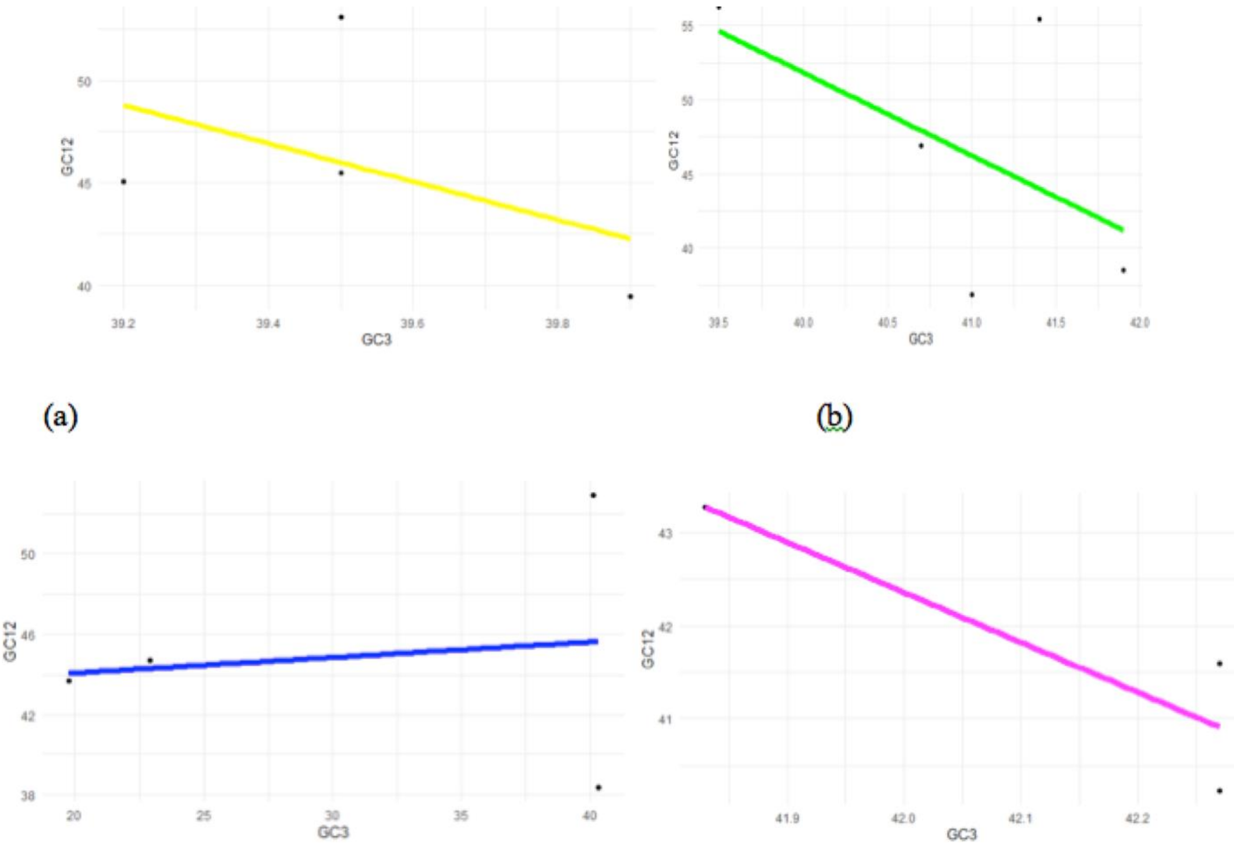
Findings from the present study have demonstrated that both natural selection and mutation pressure were contributing factors to CUB and evolutionary variations among Potamidids. This suggested that a combinatorial action of mutation pressure and natural selection influenced CUB in the species studied. Although the parity plot reflected the main factors influencing codon usage bias in different genera of Potamidids, there was no precise estimation of whether mutation pressure or natural selection was the most significant factor.

### *Neutrality plot*

Neutrality plots executed using R Studio, based on GC3% and GC12% were as represented in Figure 5. A negative slope in *Cerithideopsis*, *Cerithideopsilla*, and *Terebralia* indicated a negative association between GC3% and GC12%. However, a slight positive slope indicating a slight positive correlation between GC3 and GC12 was found in *Cerithidea*. From neutrality plots for all genera (Figure 5), a negative correlation between GC3% and GC12% corroborating that natural selection was a significant factor in evolutionary changes and CUB for the *coI* gene. However, the exception was in the genus *Cerithidea,* where a positive correlation between GC3% and GC12% was observed suggesting that other evolutionary forces like mutation pressure, genetic drift, and non-random mating were contributing factors to evolutionary changes and CUB for the *coI* gene. Narrow GC content (Figure 2), which was less than 50%, further suggests that natural selection was the most significant factor for the codon usage pattern of the *coI* gene in Potamidids. In support of these findings, other studies in reptiles (Chakraborty et al. 2022) and amphibians (Barbhuiya et al. 2021) concluded that natural selection and mutation was the major and minor evolutionary forces respectively, responsible for shaping CUB in mitochondrial genes.

## Conclusion

This study was performed to deduce factors that might favor compositional properties of the *coI* gene in family potamidids. Furthermore, the evolutionary pattern of the *coI* gene in the family Potamididae was evaluated. A low level of CUB was observed in the *coI* gene of potamidids. The codon usage pattern was different across the different genera. The two evolutionary forces; mutation pressure, and natural selection were found to be significant in determining the codon usage bias of the gene under study. However, natural selection had a dominant role while mutation pressure had a submissive role in influencing codon usage bias of *coI* gene in different genera within the family Potamididae. Although, the present study involved one mitochondrial gene, *coI*, the findings have demonstrated that various genera of potamidis are differentially adapted in the environment for their survival, and indicate that *coI* gene may be differentially expressed in potamidis genera, which remain to be elucidated.



(a) (b)

(c) (d)

**Figure 5.** Neutrality plot between GC12% and GC3% in *coI* gene for a) *Cerithideopsis* b) *Cerithideopsilla* c) *Cerithidea* d) *Terebralia*

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## Conflict of interest

Authors declare no conflict of interest.

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