

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

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## Research Article

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# Abstract

Codon usage bias refers to the phenomenon where there is unequal occurrence of specific synonymous codons in a genome, the frequency of which varies within and among species. Molecular evolutionary researches suggest that CUB studies are significant in interpreting evolutionary trends of species or genes. A number of studies on codon usage bias are emerging in different organisms. However, such information remained unclear for organisms in the family Potamididae. This study systematically examined compositional features and codon usage patterns in MT-COI gene among potamidids to identify factors shaping codon usage bias. Quantification of codon usage bias and the identification of causative factors was performed by assessing various parameters such as nucleotide composition, effective number of codons (ENC), relative synonymous codon usage (RSCU), parity rule (PR2) analysis, neutrality plot analysis as well as a multivariate statistical approach of correspondence analysis (CoA). The results from neutrality plot suggested that natural selection is the dominant evolutionary factor for CUB in MT-COI gene among the different genera of potamidids. Correspondence analysis (CoA), parity plot 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 shown as among the reasons behind the pattern of codon usage in MT-COI gene of species in the family Potamididae.

# Introduction

Evolutionary processes involves 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 is what influences 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 pattern 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 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; Nath Choudhury et al. 2017; Gun et al. 2018; Parvathy et al. 2022). Therefore, when studied in details, CUB echoes the origin of mutation pattern as well as 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 own 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 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 is a complex (COI, COII and COIII) that concludes the ultimate step of mitochondrial respiratory chain (Ferguson-Miller 2013; Shimada et al. 2017). However, COI has been revealed to have 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 MT-COI gene with the help of various parameters could thereby be crucial in discriminating genes (or species) and investigate 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 waste water (Ratsimbazafy and Kochzius 2018; Wells and Keesing 2019). Some potamidids are also used as a food source for human (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 then important in understanding their diversity and genetic evolution. In this study, nucleotide composition and CUB in MT-COI gene for organisms in the four different genera of Potamididae family were investigated to explore potamidids diversification as no work has been done yet.

## Materials And Methods

### Retrieval of sequences

Retrieval of 16 MT-COI gene sequences for the different species belonging to six 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 species of potamidids with accession number

S/N	SPECIES NAME	GENUS	ACCESSION NUMBER
1	<i>Cerithidea obusta</i>	<i>Cerithidea</i>	NC_039951
2	<i>Cerithidea sinensis</i>	<i>Cerithidea</i>	AB219355
3	<i>Cerithidea rhizophorarum</i>	<i>Cerithidea</i>	AB219333
4	<i>C. reidi</i>	<i>Cerithidea</i>	HE680238
5	<i>C. alata</i>	<i>Cerithideopsilla</i>	JF694693
6	<i>C. cingulate</i>	<i>Cerithidiopsilla</i>	JF694666
7	<i>C. costata</i>	<i>Cerithidiopsis</i>	GQ273849
8	<i>C. montagnei</i>	<i>Cerithidiopsis</i>	GQ273847
9	<i>C. californica</i>	<i>Cerithideopsis</i>	GQ273835
10	<i>C. microptera</i>	<i>Cerithideopsilla</i>	HE680612
11	<i>C. conica</i>	<i>Cerithideopsilla</i>	AM932759
12	<i>C. insica</i>	<i>Cerithideopsilla</i>	MZ831994
13	<i>C. scalariformis</i>	<i>Cerithideopsis</i>	HE680627
14	<i>T. palustris</i>	<i>Terebralia</i>	LN649639
15	<i>T. semistriata</i>	<i>Terebralia</i>	HE680660
16	<i>T. sulcata</i>	<i>Terebralia</i>	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 literature (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 influence CUB among members of the family potamididae. Lower ENC value is 20 denoting highly biased gene, whilst the upper limit being 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 MT-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**

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**

### **Nucleotide composition**

The trend of average nucleotide composition for MT-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 (Fig. 1). The overall nucleobase T composition was the highest in all the four genera within Potamididae family.

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 with exception to genus *Terebralia*. Again, nucleobase C was the least prevalent except for *Cerithideopsilla* where G nucleobase had shown low prevalence (Fig. 2). Mean GC content was 38.32%, 39.68%, 39.12%, and 41.84% in *Cerithidea*, *Cerithideopsilla*, *Cerithideopsis* and *Terebralia* genera, respectively (Fig. 2). MT-COI gene was relatively AT-rich in all genera of Potamididae family.

### Effective Number of Codons (ENC).

The effective number of codons was calculated to analyze codon usage pattern in the different species belonging to 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).

**Table 2** ENC values of MT-COI gene in different genera of the family Potamididae.

S/N	Genus	MT-COI
1	<i>Cerithidea</i>	45.65%
2	<i>Cerithideopsilla</i>	46.43%
3	<i>Cerithideopsis</i>	49.86%
4	<i>Terebralia</i>	48.86%

### Pattern of relative synonymous codon usage (RSCU)

This was calculated to quantify the relative usage of synonymous codons within a codon family of MT-COI gene. The RSCU values were as presented in Table 3 below.

**Table 3** RSCU values of codons for MT-COI gene in the six genera of Potamididae

CODON	RSCU VALUES			
	<i>Cerithidea</i>	<i>Cerithideopsilla</i>	<i>Cerithideopsis</i>	<i>Terebralia</i>
UUU	1.55	1.40	1.57	1.60
UUC	<i>0.36</i>	0.60	<i>0.43</i>	<i>0.40</i>
UUA	0.84	0.69	1.21	<i>0.00</i>
UUG	0.82	1.28	1.00	<b>2.00</b>
CUU	<b>2.20</b>	<b>3.39</b>	<b>2.54</b>	<b>3.20</b>
CUC	1.56	<i>0.31</i>	1.02	<i>0.58</i>
CUA	<i>0.50</i>	<i>0.23</i>	<i>0.23</i>	<i>0.25</i>
CUG	<i>0.22</i>	<i>0.11</i>	<i>0.23</i>	<i>0.00</i>
AUU	1.49	1.44	1.53	1.24
AUC	<i>0.53</i>	0.93	0.70	0.84
AUA	0.79	0.63	0.77	<i>0.59</i>
AUG	0.96	1.00	1.00	1.00
GUU	1.57	<b>2.40</b>	<b>2.75</b>	<b>2.65</b>
GUC	0.61	1.20	<i>0.50</i>	0.71
GUA	0.87	<i>0.00</i>	<i>0.46</i>	<i>0.44</i>
GUG	0.92	0.76	<i>0.29</i>	<i>0.21</i>
UCU	<b>1.69</b>	<b>1.71</b>	1.57	<b>2.04</b>
UCC	1.36	0.81	0.77	1.12
UCA	1.22	<i>0.49</i>	0.74	0.46
UCG	<i>0.46</i>	0.83	<i>0.21</i>	0.79
CCU	<b>2.28</b>	1.59	<i>0.52</i>	1.36
CCC	<i>0.29</i>	0.95	1.49	0.84
CCA	1.36	0.63	1.25	1.42
CCG	<i>0.40</i>	0.70	<i>0.00</i>	0.71
ACU	1.50	1.43	<b>1.91</b>	<b>2.10</b>
ACC	<i>0.25</i>	0.93	<i>0.47</i>	<i>0.38</i>
ACA	1.28	<i>0.50</i>	0.82	0.80

ACG	<i>0.50</i>	0.85	0.81	1.00
GCU	1.40	1.35	1.38	1.18
GCC	0.73	1.17	<i>0.34</i>	1.19
GCA	1.37	<i>0.37</i>	<b>1.81</b>	<i>0.55</i>
GCG	0.90	<i>0.47</i>	<i>0.47</i>	0.70
UAU	1.25	1.39	1.33	1.47
UAC	<i>0.25</i>	0.61	0.84	0.36
UAA	<i>0.56</i>	1.04	1.26	1.15
UAG	<i>0.16</i>	<i>0.58</i>	1.52	<i>0.52</i>
CAU	1.39	1.19	1.17	<i>0.33</i>
CAC	<i>0.49</i>	0.62	0.77	<b>1.67</b>
CAA	0.75	1.20	1.06	<b>1.89</b>
CAG	1.25	<i>0.40</i>	<i>0.50</i>	<i>0.11</i>
AAU	0.89	0.76	0.84	1.58
AAC	0.61	1.10	0.66	1.24
AAA	<i>0.58</i>	0.90	1.11	0.71
AAG	1.21	0.98	0.72	1.14
GAU	0.82	0.83	1.07	0.98
GAC	<b>1.83</b>	1.20	0.77	0.60
GAA	<i>0.42</i>	0.65	1.29	0.78
GAG	<i>0.52</i>	<i>0.50</i>	0.84	0.86
UGU	<i>0.52</i>	1.30	0.74	1.27
UGC	<b>2.24</b>	1.01	1.41	0.87
UGA	1.13	1.12	0.91	<i>0.00</i>
UGG	0.75	1.17	0.81	0.95
CGU	<i>0.00</i>	1.06	0.63	1.00
CGC	1.34	0.72	<i>0.49</i>	0.95
CGA	<i>0.52</i>	0.68	0.74	<i>0.38</i>
CGG	<i>0.24</i>	0.63	1.13	0.73

AGU	<i>0.48</i>	<i>0.52</i>	1.57	<i>0.41</i>
AGC	1.09	1.42	1.40	1.14
AGA	<b>1.78</b>	1.20	1.08	1.49
AGG	1.44	<b>2.68</b>	<b>1.77</b>	<b>1.69</b>
GGU	1.32	<i>0.19</i>	0.89	1.09
GGC	1.56	1.04	<b>1.65</b>	<b>1.81</b>
GGA	0.75	<i>0.19</i>	<i>0.43</i>	<i>0.20</i>
GGG	<i>0.00</i>	1.18	1.34	1.42

RSCU value above 1.6 is indicated by bold, RSCU value below 0.6 is indicated by italics whereas RSCU value between 0.6 and 1.6 is indicated by roman.

### Correspondence analysis (CoA)

Correspondence analysis showing the distribution pattern of codons in the four genera is as shown in Figure 5. The distribution depicted variation in codon usage across the different species in the family Potamididae for MT-COI gene. Most codon positions were found to concentrate close to the axes and at the center of the plot.

### Parity plot analysis

To estimate the effects of mutation pressure and natural selection on the codon usage, 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) i.e. A and T were not proportionate with C and G (Fig. 4).

### Neutrality plot

Neutrality plots executed using RStudio, based on GC3% and GC12% were as represented on Figure 5 below. A negative slope in *Cerithideopsis Cerithideopsilla* and *Terebralia* indicated negative association between GC3% and GC12%. However, a slight positive slope indicating slight positive correlation between GC3 and GC12 was found in *Cerithidea*.

## Discussion

Codon usage bias is a common feature in most organism genome 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 neutral theory and selection-mutation drift model (Duret 2002; Deb et al. 2020). According to neutral theory, silent mutations in the codons' third position rise the phenomenon of codon preference (Subramanian 2008). Whereas, selection-mutation drift model suggest 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 pattern; 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 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 MT-COI gene in *Cerithidea*, *Cerithideopsilla*, *Cerithideopsis* and *Terebralia* genera of Potamididae were analyzed. Findings from the present study have demonstrated that both natural selection and mutation pressure were contributing factors on CUB and evolutionary variations among Potamidids. From PR2 plot (Fig. 4) it was found that, gene spots deviated from base composition rule (Chargaff's second parity rule) that is, A and T were not proportionate with C and G (Fig. 4). This suggested that a combinatorial action of mutation pressure and natural selection influenced CUB in the species studied. Although 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. From neutrality plots for all genera (Fig. 5), negative correlation between GC3% and GC12% was found, corroborating that natural selection was significant factor to evolutionary changes and CUB for MT-COI gene. However, the exception was in genus *Cerithidea*, where 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 MT-COI gene. Narrow GC content, which was less than 50%, further suggests that natural selection was the most significant factor for codon usage pattern of MT-COI gene in Potamidids. In support of these findings, other studies in reptiles (Chakraborty et al. 2022) and amphibians (P. A. Barbhuiya et al. 2021) concluded that natural selection and mutation pressure were the major and minor evolutionary forces respectively, responsible for shaping CUB in mitochondrial genes.

Furthermore, other compositional features parameters that explain possible codon usage pattern of MT-COI gene among different species of the family Potamididae were found to be interesting. For example, third position of codons was mostly occupied by nucleobase T suggesting that codons ending with T were more preferred as compared to other nucleotides. It is known that third codon position is subjected to silent mutations pressure (Chamary and Hurst 2009; Alberts 2015) therefore, when studied in details such investigations play a vital role in interpretation of codon trend of the various genes. In the present study, silent mutations at third codon positions involving T nucleobase was observed to be common as a result codon ending with T nucleobase were highly favored. Additionally, slight difference was observed in positional GC contents (GC1, GC2 and GC3) among the four genera of Potamididae (Fig 02). 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). Moreover, codon usage bias of MT-COI gene was found to be weak because the ENC values in the present study was greater 35, but was less 50. These findings are supported by earlier investigations which revealed

low CUB in mitochondrial ATP, ND and CO genes of amphibians, arthropods and reptiles respectively (R. I. Barbhuiya et al. 2019; P. A. Barbhuiya et al. 2021; Chakraborty et al. 2022).

The coding sequences of MT-COI gene were evaluated for their relative synonymous codon usage (RSCU). 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 (Table 3). Specifically, five codons (CUU, GUU, UCU, ACU, and AGG) were found to be commonly overrepresented across all four genera of family Potamididae, and six codons (GGA, CGA, GAG, UAC, CUG, CUA) were commonly underrepresented across the four different genera in the family Potamididae (Table 3). It has been reported that similar codon usage patterns including over and underrepresented codons in genomes ensures 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 clearly define the varying trends in codon usage among potamidids. A chunk of codons were situated close to the axes and some concentrated in the center of the plot proposing that nucleotide compositional constraints under mutation pressure might associated with the CUB of MT-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).

This study was performed to deduce factors that might favor compositional properties of MT-COI gene in family potamidids. Furthermore, the evolutionary pattern of MT-COI gene in the family Potamididae was evaluated. Low level of CUB was observed in MT-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 MT-COI gene in different genera within the family Potamididae.

## Declarations

There is no conflict of interest.

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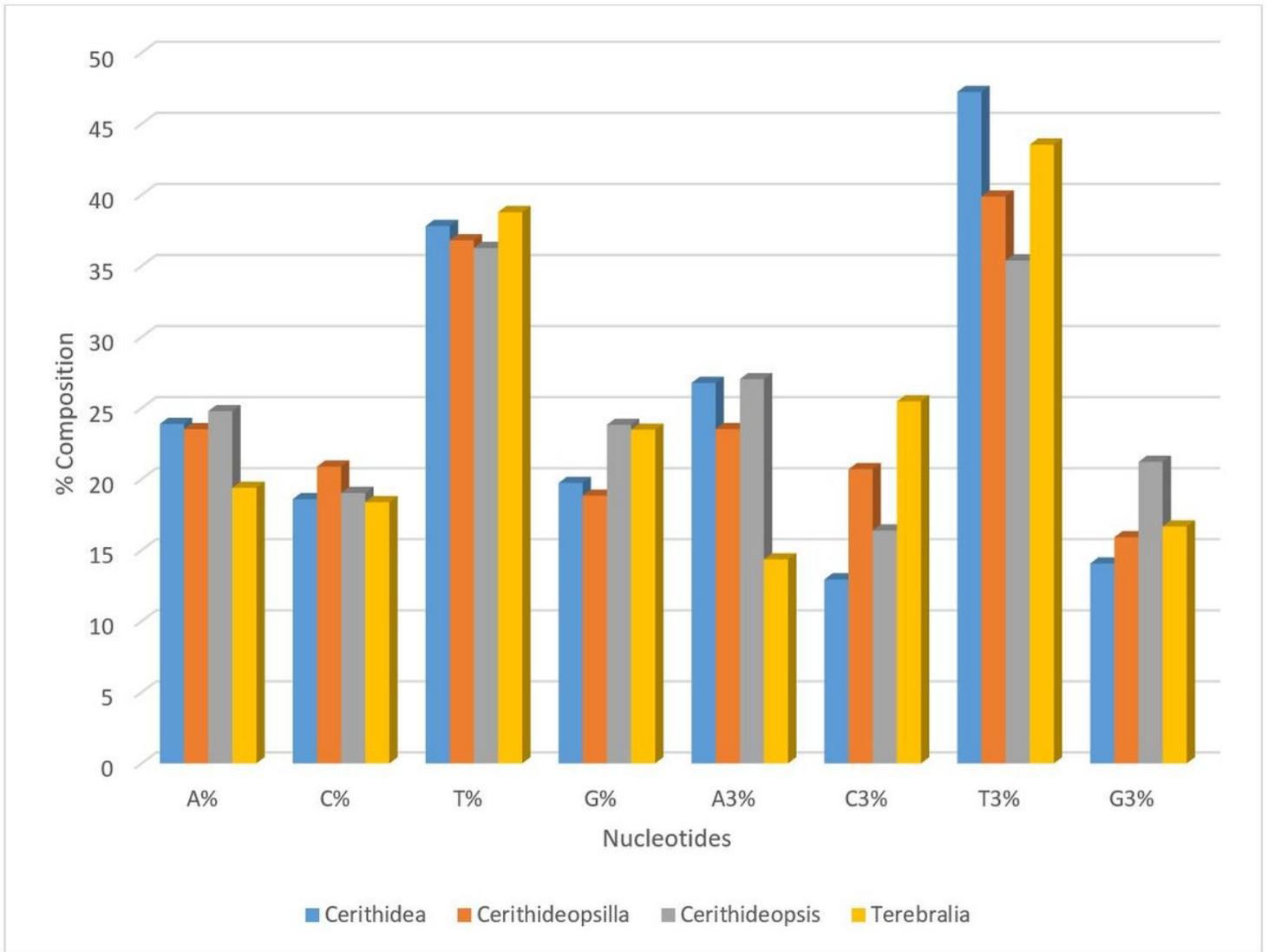
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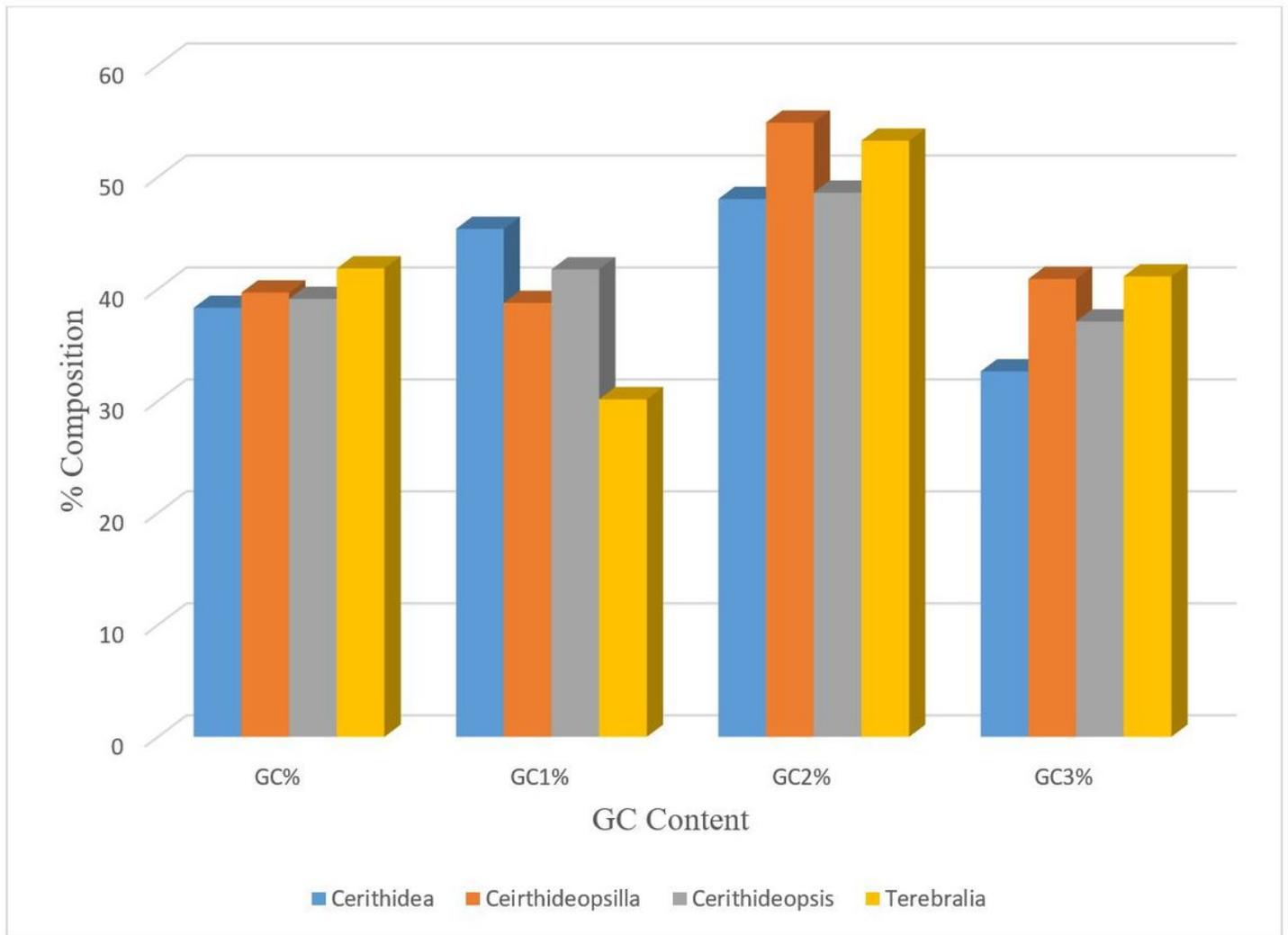
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## Figures



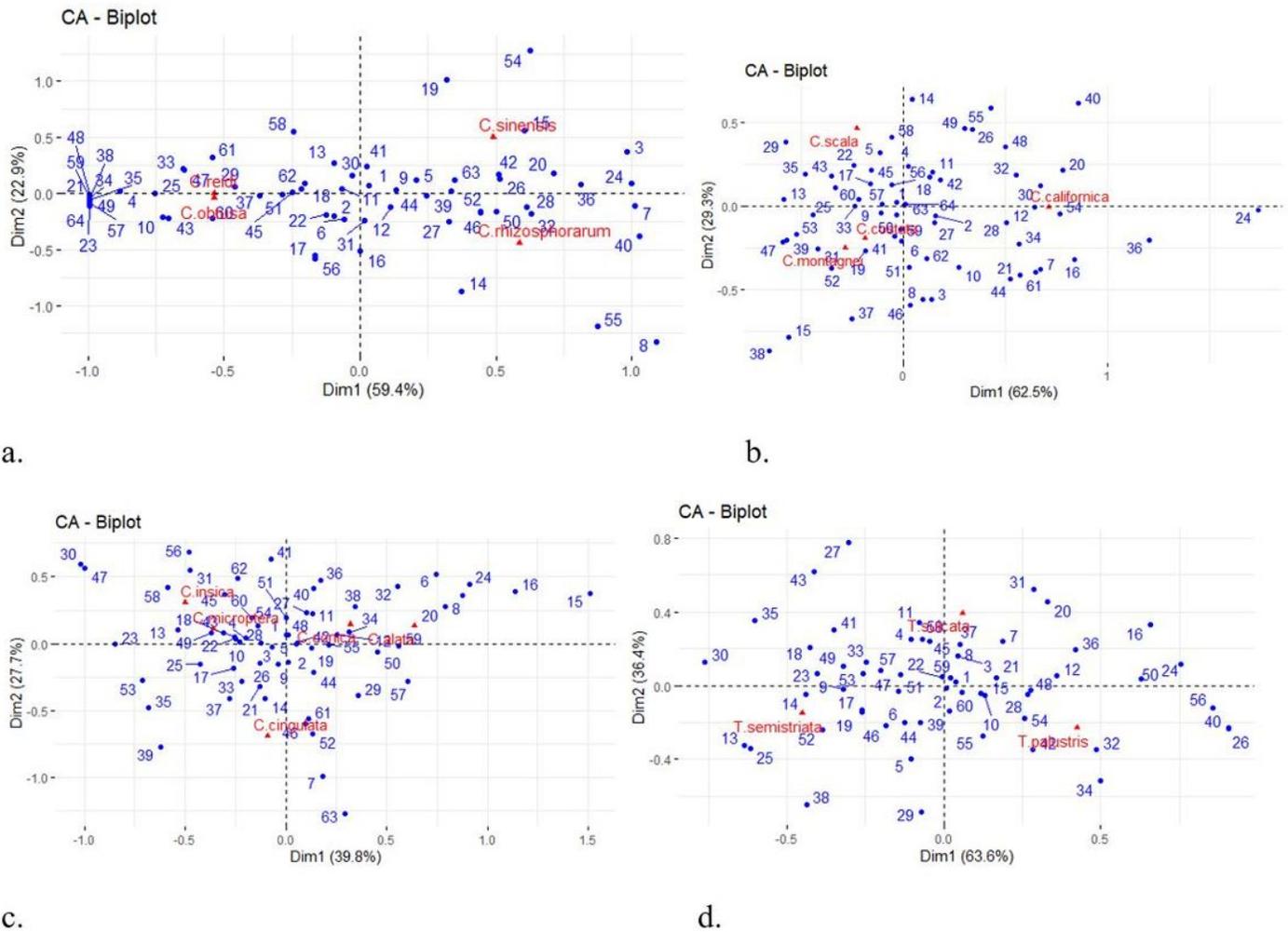
**Figure 1**

Overall nucleotide composition for MT-COI gene in different genus of potamidids



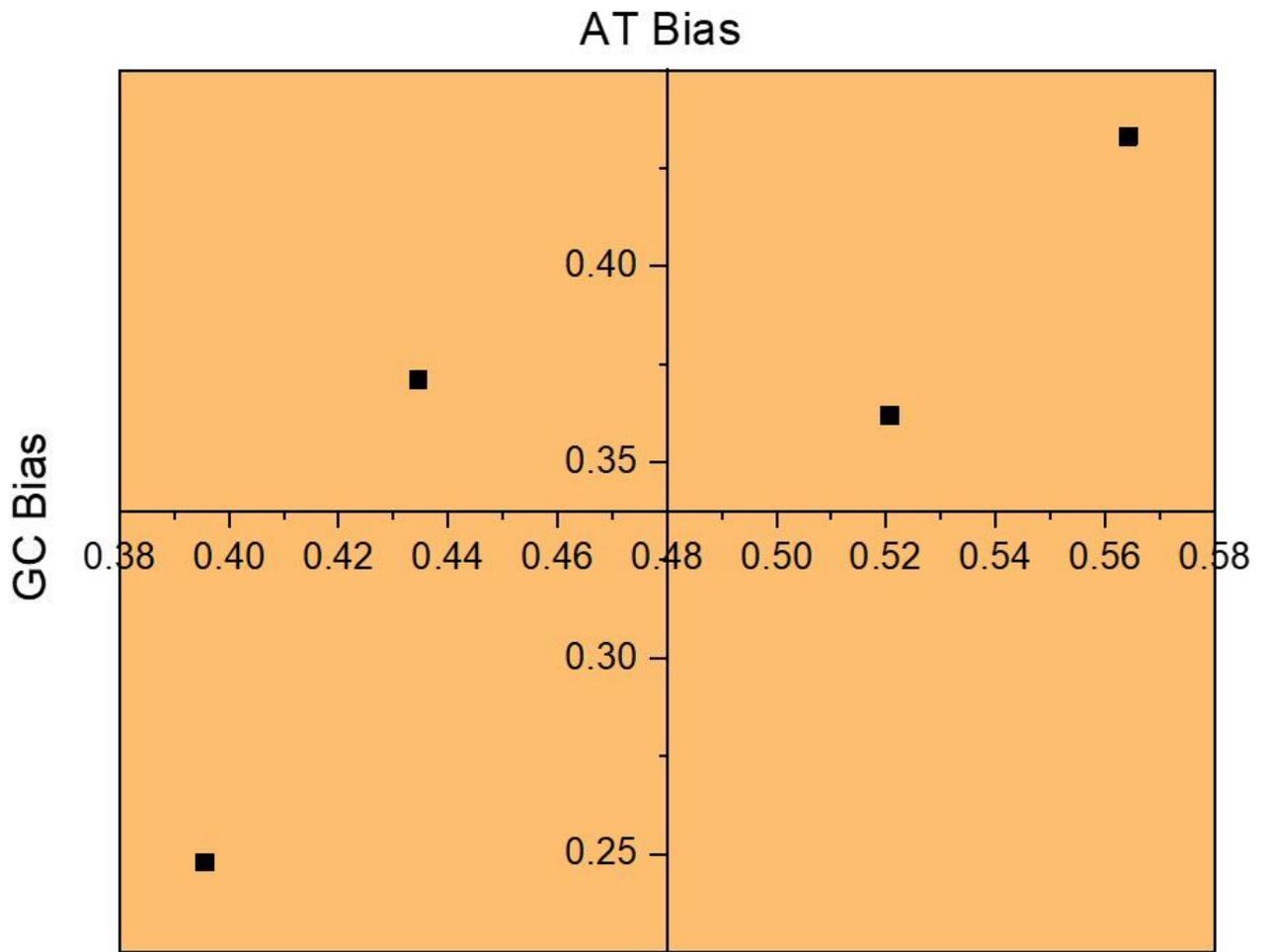
**Figure 2**

Overall GC content and positional GC content of MT-COI gene among the different genera of Potamidids.



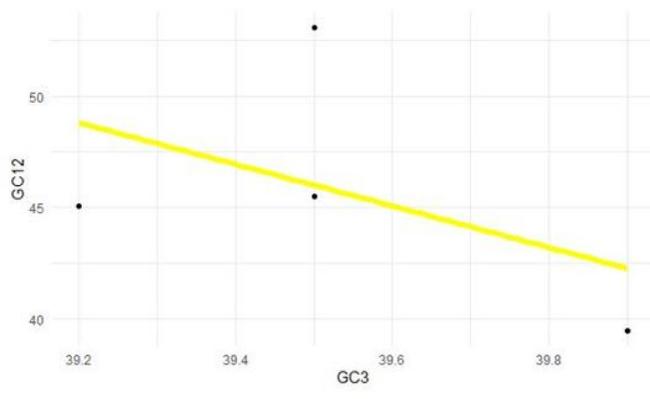
**Figure 3**

Correspondence analysis (CoA) of codon usage bias in MT-COI gene for *Cerithidea* (a); *Cerithideopsis* (b); *Cerithideopsisilla* (c) and *Terebralia* (d).

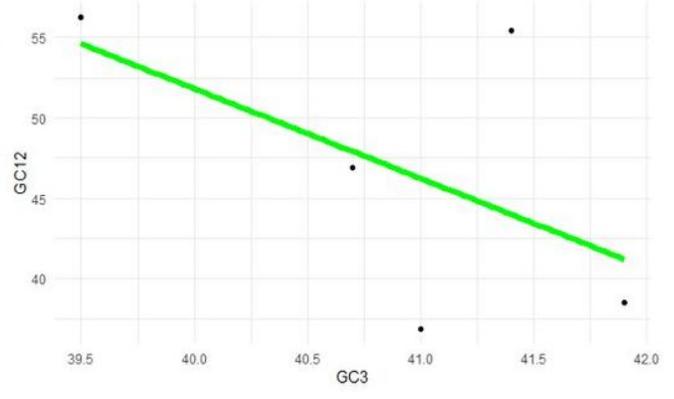


**Figure 4**

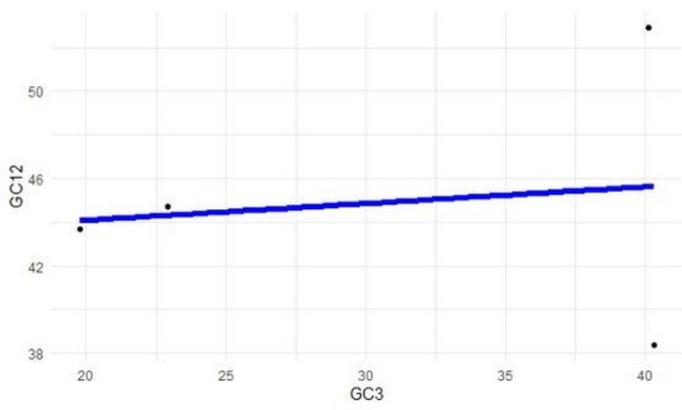
Parity plot analysis of MT-COI gene in the six genera of the family Potamididae.



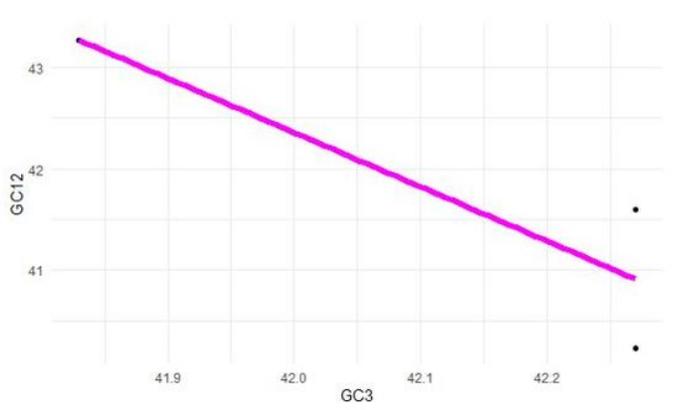
(a)



(b)



(c)



(d)

**Figure 5**

Neutrality plot between GC12% and GC3% in MT-COI gene for a) *Cerithideopsis* b) *Cerithideopsisilla* c) *Cerithidea* d) *Terebralia*.