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Research Article

Variations in Nucleotide Frequency Distribution in Spider Families

Sehrish Ashraaf[1], Hafiz Muhammad Tahir[1], Muhammad Akram Qazi[2], Shaukat Ali[1*]

[1]*Department of Zoology, Government College University, Lahore, Pakistan.*
[2]*Punjab Agriculture Research Board, Imam Hussain Road, Gulberg III, Lahore, Pakistan.*

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SA and HMT designed and conducted the study. SA, HMT, MAQ and SA wrote the manuscript.

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Abstract | The study was designed to test the hypothesis, variations in nucleotide frequency distribution in *cytochrome c oxidase subunit I* (*COI*) in spiders used as a marker to delimit species. Based on morphological identification and sequencing of *CO1* gene, a total of 22 species of spiders belonging to five families were investigated and compared within each species and among families for nucleotide frequency distribution of all species collected. We had found non-significant difference in frequency of nucleotides in *COI* within the members of species and within families (thymine 41.30-44.30%, adenine 24.96-27.19%, guanine 17.18-19.48% and cytosine 12.28-13.40% while GC 29.53-32.84%). Therefore, it was concluded that variation in nucleotide frequency distribution could not to bee used as a criterion for species delimitation in spiders.

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Introduction

Modern molecular techniques are efficient, affordable (Padial and De La Riva, 2007) and have been greatly accepted for identification and delimitation of species (Hebert *et al.*, 2004; Smith *et al.*, 2007; Kerr *et al.*, 2009). DNA barcoding is being widely used to identify and discover new species in a wide range of taxa (Wang *et al.*, 2018). Cytochrome c oxidase subunit I (*COI*) gene of mitochondrial DNA (mtDNA) was used to separate the insect species (Gotoh and Arabuli, 2019). Species delimitation is a necessary part of evolution, conservation and molecular ecology. Although there are several molecular markers (COI, CYTB, 16S, 18S, 28S, ITS1, and ITS2) available for species delimitation but no universal marker has been accepted yet (White *et al.*, 2014). A molecular marker becomes ideal if it has a few properties like presence of single copy in haploid genome, easy to align, all sites equally free to vary, equal base composition, and high substitution rate (Cruickshank *et al.*, 2001). Recently, a handful number of universal markers are reported but finding all properties in a single marker is difficult (Cao *et al.*, 2016). However, there are different molecular markers to delimit distinct species i.e., ITS1, ITS2 (second internal transcribed spacer regions) of the nuclear ribosomal gene cluster (18S rDNA, 5.8S rDNA and 28S rDNA), mitochondrial genes, 16S rDNA, 12S rDNA, CO1, COIII, nuclear ribosomal genes, 18S rDNA, nuclear protein-coding genes, 28S rDNA and others (Yli-Mattila et al., 2000).

Corresponding author: Shaukat Ali
shuakatali134@yahoo.com

Table 1: Frequency distribution of nucleotide of *cytochrome c oxidase subunit I* among different families of spiders

Spider Families	Frequency distribution (%)				
	G	C	A	T	GC
Araneidae	19.48±0.64	13.4±0.05	24.96±0.05	41.30±0.14	32.84±0.1
Lycosidae	17.31±0.06	12.60±0.02	25.88±0.06	44.25±0.0.2	32.84±0.1
Salticidae	18.68±0.04	12.28±0.02	25.74±0.04	43.3±0.03	30.95±0.05
Clubionidae	18.01±0.06	12.3±0.06	26.32±0.06	42.73±0.045	30.95±0.08
Oxyopidae	17.18±0.09	12.34±0.055	27.19±0.13	43.28±0.04	29.53±0.12
P- Value	*> 0.05*	*> 0.05*	*> 0.05*	*> 0.05*	*> 0.05*

Each value in the table represents the mean of 5 replicates and SEM (Standard Error of Mean).

Table 2: Frequency distribution of nucleotide of *cytochrome c oxidase subunit I* among different species of spider

	Frequency distribution (%)				
Species of family Araneidae	**G**	**C**	**A**	**T**	**GC**
Araneus mitficans	19.58 ±0.05	13.16 ±0.05	24.98 ±0.07	42.28 ±0.04	32.74 ±0.09
Argiope pulchella	19.07± 0.04	13.41 ±0.1	26.29 ± 0.06	41.22± 0.16	32.48 ± 0.1
Argiope trifasciata	18.06±0.1	12.84±0.11	26.29±0.23	42.92±0.09	30.8± 0.2
Cheiracanthium inornatum	17.2±0.15	12.44±0.1	27±0.07	43.36±0.14	29.64±0.2
Cyclosa chichawatnensis	21.95±0.15	15.21±0.1	24.7± 0.07	38.14±0.14	37.16±0.2
Cyrtophora citricola	18.33±0.04	14.44±0.07	27.29±0.05	39.67±0.06	33.04±0.1
Eriovixia exceisa	23.59±0.09	14.42±0.03	21.32±0.03	40.67±0.1	38.01±0.09
Neoscona thesi	18.3±0.04	12.19±0.02	28.18±0.05	41.32±0.04	30.5±0.03
Neoscona vigilans	18.75±0.04	12.52±0.03	26.57±0.04	42.16±0.04	31.27±0.07
P- Value	*0.04*	*>0.05*	*>0.05*	*>0.05*	*>0.05*
Species of family Lycosidae					
Wadicosa fedalis	17.79±0.05	12.65±0.03	25.52±0.05	44.04±0.04	30.44±0.06
Trocosa sp.	17.33±0.09	12.77±0	25.84±0.09	43.92±0	29.94±0.09
Trocosa aquatic	16.7 ±0.03	12.58±0.02	25.85±0.03	44.86±0.03	29.29±0.02
Draposa oakleyi	17.34±0.07	12.82±0.04	26.15±0.07	43.7±0.05	30.15±0.07
Lycosa Terrestris	17.26±0.04	12.77±0.01	25.45±0.04	44.52±0.02	30.03±0.04
Hippasa pisaurina	17.48±0.08	12.06±0.03	25.99±0.08	44.47±0.03	29.54±0.1
P- Value	*> 0.05*	*> 0.05*	*> 0.05*	*> 0.05*	*> 0.05*
Species of family Salticidae					
Trite sp.	19.36±0.05	12.29±0.02	24.13±0.04	44.23±0.03	31.64±0.06
Thynes imparialis	19.12±0.03	12.68±0.03	25.93±0.04	42.28±0.03	31.8±0.05
Talemonia dimidiate	17.56±0.05	11.87±0.03	27.17±0.04	43.4±0.04	29.43±0.04
P- Value	*> 0.05*	*> 0.05*	*> 0.05*	*> 0.05*	*> 0.05*
Species of family Clubionidae					
Clubiona drassodes	17.77±0.05	13.21±0.09	26.55±0.04	42.48±0.12	30.98±0.12
Clubiona filicata	18.25±0.07	12.66±0.03	26.09±0.05	42.99±0.04	30.92±0.08
P- Value	*> 0.05*	*> 0.05*	*> 0.05*	*> 0.05*	*> 0.05*
Species of family Oxyopidae					
Oxyopes hindastanicus	17.38±0.08	12.32±0.05	26.93±0.1	43.37±0.04	29.7±0.1
Oxypes ozyrae	16.99±0.1	12.37±0.06	27.45±0.16	43.19±0.04	29.36±0.14
P- Value	*> 0.05*	*> 0.05*	*> 0.05*	*> 0.05*	*> 0.05*

Each value in the table represents the mean of 5 replicates and SEM (Standard Error of Mean).

CO1 is the most popular marker used in DNA barcoding for delimiting species in the whole animal kingdom (Avise, 2012; White *et al.*, 2014). This genetic region is important and widely used due to some distinguishing characteris-

tics like presence across the animal kingdom, rare mutations (insertions and deletions) and enough divergence in sequence adequate to differentiate closely related species (Hebert *et al.,* 2003a; Hebert *et al.,* 2003b).

Despite of presence of many markers for species identification and delimitation, still there is unmet need to find more validated markers. For this purpose, the current study was designed to test whether variation in frequency distribution of nucleotide in *CO1* in spiders could be used as a marker to delimit species or not.

Methodology

For the study, 658 base pair sequences of *COI* from 25 species of spiders belonging to five families were used. The study was conducted at Government College University Lahore in 2017. Spiders were collected by using pitfall traps and handpicking method. All the specimens were preserved in 70% alcohol for morphological study. The specimens used for molecular study were preserved in 95% alcohol and kept at -20°C in the refrigerator of Molecular Laboratory of Zoology Department, Government College University Lahore. Morphological identification of spiders was completed following the keys provided by Barrion and Litsinger (1995). Each species was represented by at least five individuals. Tissues for DNA extraction was taken from first leg of left side from each individual and after completing the necessary formalities tissue samples were sent to Centre for Biodiversity Genomics, University of Guelph, Canada for sequencing. A total of 110 samples belonging to 22 species and five families were processed for sequencing of *CO1* gene. The nucleotide frequency distribution was computed using online sequence analysis tool available on Barcode of Life Data System (www.boldsystems.org). Nucleotide frequency distribution of *CO1* gene among all members of same species and between different families was compared using one-way Analysis of variance (SPSS 16). GC composition within members of same species and between different families was also compared. The differences were considered significant if $P < 0.05$.

Results and Discussion

Results showed that the frequency of nucleotides among members of same species, within different species of same family and even among different families differed non-significantly ($P > 0.05$). The frequency of thymine nucleotide among members of all families was highest (about 41%), followed by adenine, guanine and cytosine (Table 1). Sequence composition of GC base pairs among individuals of five studied families also varied non-significantly. No clear variation within different species of same family or among families was found.

When different species of family Araneidae were compared for nucleotide frequency distribution significant difference was observed in G% distribution only ($P = 0.04$). However, the variations in other nucleotides among different species were statistically non-significant ($P > 0.05$; Table 2). Although, there was variation in nucleotide frequency distribution among species of family Lycosidae, Salticidae, Clubionidae and Oxyopidae but statistically non-significant differences were found (Table 2).

Cytochrome c oxidase subunit I (*CO1*) gene of mitochondrial DNA (mtDNA) was used to separate the insect species (Gotoh and Arabuli, 2019) but in this study no significant variation was found in nucleotide frequency distribution within members of same species or even within different species of same family. Previously this aspect has not been discussed in literature by researchers. The samples were compared collected from agricultural fields of central Punjab, Pakistan. Although spiders were collected from different habitats but the difference in the climatic conditions and microhabitats were almost the same. This might be the possible difference of non-significant difference in nucleotide frequency distribution in members of same species or different species of same family. There is need to collect samples from different geographical ranges to establish the role of nucleotide frequency distribution of nucleotide in species delimitation.

It is concluded that the variation in nucleotide frequency distribution in *CO1* gene may not be useful for identification of spiders in the study area.

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