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Short Communication

INTRASPECIFIC SYSTEMATICS OF GENUS NEZARA (HEMIPTERA: PENTATOMIDAE) INFERRED FROM ANALYSIS OF THE MITOCHONDRIAL CYT B GENE SEQUENCES

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ABSTRACT

In the present study, the genetic divergence among genus Nezara of family Pentatomidae collected from different localities of Northern India was estimated using partial Cyt b gene sequences. Intraspecific divergence ranged from 0.2% to 7.2% with an average of 4.16±3.2% while the interspecific divergence between *N. viridula* and *N. antennata* was ranged from 6.1% to 9.8% with an average of 7.54 ± 1.2%. Mitochondrial Cyt b gene sequences clearly showed the overlapping of intra and interspecific divergence in the genus Nezara as the intraspecific divergence in *N. viridula* of the present study was quite high.

Keywords: Mitochondrial, *Nezara, Cytb,* Genetic divergence

INTRODUCTION

The genus Nezara is a widely diverse and economically important group of subfamily Pentatominae. The high reproductive rates and an exceptional ability to transmit diseases make them the worst agricultural pests (Song and Liang, 2009). Mitochondrial DNA is widely used as a valuable molecular marker for phylogenetic studies in animals, because of its simple genomic structure (Avise, 1994). It has relatively fast mutation rate, lacks introns and shows no recombination (Brown et al., 1979; Hebert et al., 2003). The mitochondrial Cytochrome b gene is considered as a powerful marker for identifying species with DNA analytical techniques (Zehner et al., 1998). Moreover, Cyt b has been proved to have the same level of sequence variation as the COI region for phylogenetic analysis of many insect orders (Simmons and Weller, 2001; Subbanna et al., 2016). The aim of the present study was to study the intra and interspecific divergence of the distant populations of Nezara species collected from the Indian subcontinent based upon Cyt b gene characterization.

MATERIALS AND METHODS

The first step subsumed the collection of bugs from different regions of North India (Himachal Pradesh, Punjab and Uttarakhand). DNA was extracted from thorax region using modified PCI method (Kambhampati and Rai, 1991). Integrity of extracted DNA was checked on 0.8% agarose gel by horizontal gel electrophoresis. Quantification of DNA was done by nano-drop spectrophotometer. The Cyt b gene was amplified using forward primer 5'-TAGGATATGTTTTACCTTGAGGACA-3' and reverse primer 5'- TCCTCCTAATTTATTAGGAATTG-3' (Muraji et al., 2000). PCR was carried out at an annealing temperature of 55°C for 40 sec and an extension at 72°C for 40 sec. The amplified products of Cyt b gene fragments were got sequenced directly from Agri Genome Pvt. Ltd, Cochin (India). The sequence data of 440bp was retrieved in the form of chromatograms. The sequences were checked manually for the exclusion of ambiguous nucleotides. Related sequences were retrieved from GenBank using Basic Local Alignment Search Tool (BLAST) algorithm (Altschul et al., 1997). All the sequences were aligned in Clustal W and divergence at population and species levels was analysed by K2P model of base substitution. Percentage divergence matrix was drawn using Kimura-2-parameter model (Kimura, 1980) in MEGA 6.06 (Tamura et al., 2013).

RESULTS AND DISCUSSION

Six sequences of *Nezara viridula* were compared which included three sequences from the present study and 3 sequences retrieved from Genbank submitted from China, Solovenia and Japan (Tables 1 & 2). Intraspecific divergence ranged from a minimum of 0.2 (between *N. viridula* of present study) to maximum of 7.2 (between *N. viridula* of the present study and the sequence of *N. viridula* submitted from China). The average intraspecific variation was in the range of 4.16 ± 3.2% (Table 3). Six sequences of *Nezara viridula* (3 sequences of the present study and 3 sequences retrieved from Genbank) were compared with two sequences of *N. antennata* (1 sequence of the present study and 1 sequence from China). Interspecific divergence ranged from a minimum of 6.1% (between *N. viridula* of the present study and *N. antennata* from China) to a maximum of 9.8% (between *N. viridula* from China and *N. antennata* from the present study) with an average of 7.54 ± 1.2% (Table 4).

Generally, the species exhibited discriminative values of intra and interspecific divergence and the percentage divergence values revealed a clear pattern of increased nucleotide diversity from conspecific to congeneric level revealed a clear pattern of increased nucleotide diversity. But in the present study, Cyt b gene sequences showed the overlapping of intra and

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Table 1: Details of species analyzed for *Cyt b* in the present study

S. No.	Taxa	Specimen Code	Collection Place	Collection Month/Year	Accession Number
1	*Nezara antennata* Scott, 1874	P18	Punjab	June, 2013	MG994914
2	*Nezara viridula*(Linnaeus, 1758)	P9 P10 HP19	Punjab Punjab Himachal Pradesh	March, 2013 April, 2014 June, 2014	MG821868 MG821867 MG821866

Table 2: List of taxa whose *Cyt b* sequences were downloaded from Genbank for alignment

S. No.	Taxa	Family	Accession no.	Country
1	*Nezara viridula*	Pentatomidae	AB020514.1 AY839170.1 FJ418864.1	Japan Slovenia China
2	*Nezara antennata*	Pentatomidae	FJ418867.1	China

Table 3: Pairwise K2P intraspecific divergence

S.No.	Sequence I	AccessionNo.	Sequence II	Accession No.	Divergence (%)
1	Nezara antennata*	MG994914	*Nezara antennata*	FJ418867.1	2.8
2	*Nezara viridula**	MG821866	*Nezara viridula**	MG821868	0.5
3	*Nezara viridula**	MG821866	*Nezara viridula**	MG821867	0.2
4	*Nezara viridula**	MG821866	*Nezara viridula*	AB020514.1	6.4
5	*Nezara viridula**	MG821866	*Nezara viridula*	AY839170.1	6.7
6	*Nezara viridula**	MG821866	*Nezara viridula*	FJ418864.1	7.2
7	*Nezara viridula**	MG821867	*Nezara viridula*	AB020514.1	6.1
8	*Nezara viridula**	MG821867	*Nezara viridula*	AY839170.1	6.4
9	*Nezara viridula**	MG821867	*Nezara viridula*	FJ418864.1	7.0
10	*Nezara viridula**	MG821868	*Nezara viridula**	MG821867	0.2
11	*Nezara viridula**	MG821868	*Nezara viridula*	AB020514.1	6.4
12	*Nezara viridula**	MG821868	*Nezara viridula*	AY839170.1	6.7
13	*Nezara viridula**	MG821868	*Nezara viridula*	FJ418864.1	7.2
14	*Nezara viridula*	AB020514.1	*Nezara viridula*	AY839170.1	0.2
15	*Nezara viridula*	AB020514.1	*Nezara viridula*	FJ418864.1	0.7
16	*Nezara viridula*	AY839170.1	*Nezara viridula*	FJ418864.1	0.5

*indicates specimens of the present study

Table 4: Pairwise K2P interspecific divergence

S.No.	Species I	Accession No.	Species II	Accession No.	Divergence (%)
1	*Nezara viridula**	MG821866	*Nezara antennata**	MG994914	7.2
2	*Nezara viridula**	MG821866	*Nezara antennata*	FJ418867.1	6.7
3	*Nezara viridula**	MG821867	*Nezara antennata**	MG994914	7.5
4	*Nezara viridula**	MG821868	*Nezara antennata**	MG994914	7.2
5	*Nezara viridula**	MG821867	*Nezara antennata*	FJ418867.1	6.4
6	*Nezara viridula**	MG821868	*Nezara antennata*	FJ418867.1	6.1
7	*Nezara viridula*	AB020514.1	*Nezara antennata**	MG994914	9.2
8	*Nezara viridula*	AB020514.1	*Nezara antennata*	FJ418867.1	6.7
9	*Nezara viridula*	AY839170.1	*Nezara antennata**	MG994914	9.5
10	*Nezara viridula*	AY839170.1	*Nezara antennata*	FJ418867.1	7.0
11	*Nezara viridula*	FJ418864.1	*Nezara antennata**	MG994914	9.8
12	*Nezara viridula*	FJ418864.1	*Nezara antennata*	FJ418867.1	7.2

*indicates specimens of the present study

interspecific divergence in the genus Nezara. So, a clear cut distinction could not be obtained. The interspecific divergence between *N. viridula* and *N. antennata* was ranged from 6.1% to 9.8% with an average of 7.54 ± 1.2%. However, the intraspecific divergence in *N. viridula* of the present study was quite high when compared with the sequences of *N. viridula* submitted from China. Surprisingly, the overall range of intraspecific distance was observed to be 0.2% to 7.2% which overlaps with interspecific distance of 6.1% to 9.8%.

CONCLUSION

It can be concluded that the Indian sub-continent has a rich biodiversity owing to the pervasiveness of varying ecological zones. The occurrence of both tropical and temperate conditions within this region boosts the phenomenon of genetic divergence amongst organisms. The morphological and/or genetic variations can be a manifestation of the fluctuating environmental conditions. However these divergences, at times, cannot be elucidated on the basis of morphological data. The results reported here point towards the potential of Cyt b gene sequence data in assessment of genetic variability among the natural populations of Nezara and pave way for a better understanding of the population structure of this enormously diverse genus.

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