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Molecular phylogeny of geographically isolated assassin bugs based on mitochondrial COI gene

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Abstract
Available mitochondrial DNA sequences of Cytochrome oxidase subunit – I gene of assassin bugs were subjected to phylogenetic analysis to understand the intergeneric and interspecific variations and the role of geographical isolation on speciation; using CLUSTAL W in MEGA version 5.1. This analysis includes twenty five species belonging to fourteen genera from five countries viz., India, USA, China, Brunei and South Korea. The pairwise genetic distances were calculated and phylograms were constructed using Maximum Likelihood, Maximum Parsimony, and Neighbor-Joining methods. This analysis revealed phylogenetic relationships and the role of geographical isolation on speciation.

Keywords: Assassin bugs, phylogeny, speciation, geographical isolation

1. Introduction
The family Reduviidae is a family of predaceous cimicomorpha bugs called the assassin bugs with cosmopolitan distribution (Maldonado, 1990) [6]. They are abundant, occur worldwide, voracious and polyphagous predators. Hence, they are referred to as assassin bugs (Ambrose, 1999) [1-4]. Classifications of Reduviidae based on morphological characters (Usinger, 1943) [15]. Putshkov, Putshkov, 1985; Maldonado, 1990 [6]. Schuh, Slater, 1995 [10]. May at times become insufficient, and there is an urgent need for a cohesive meaningful classification of Reduviidae based on ecological, morphological, behavioural, cytological, and biochemical data. Moreover, a multidisciplinary biosystematics understanding is imperative to accurately identify reduviidae and employ them against a particular insect pest (Ambrose, 1999, Ambrose & Ambrose, 2003, 2009) [1-4]. Although multidisciplinary biosystematics including molecular tools has been attempted on Oriental reduviidae (Weirauch, 2008) [16]. Such an analysis is wanting on non-Oriental reduviidae. The inclusion of both Indian and non-Indian species of assassin bugs will further enhance the scope of the work at the intraspecific level and the understanding on the role of geographical isolation in biosystematics (Ambrose, 2014) [1-4]. This study was undertaken based on available mitochondrial sequences of twenty five assassin bugs (Table 1).

2. Material and methods
Taxon sampling
To understand the phylogeny of twenty five assassin bugs based on Cytochrome oxidase subunit I gene, DNA sequences of these species (Table 1) were subjected to phylogenetic analysis. The sequences of Indian (excluding Karnataka) species from our work are deposited in the National Centre for Biotechnology (NCBI). The sequences of non-Indian species were retrieved from NCBI (Table 2) and all these sequences were taken into consideration.

Phylogenetic analysis
The DNA sequences were subjected to pairwise distance analysis and the following phylogenetic trees were constructed: Maximum Parsimony, Maximum Likelihood and Neighbor-Joining using MEGA 5 software (Tamura, 2011) [13].

Pairwise alignment
Pairwise distances were carried out with gap opening penalty 15 and gap extension penalty 6.66 (Clustal W) (Thompson, 1994) [14].

Maximum Parsimony
The Maximum Parsimony analyses were analysed with MEGA5 (Tamura, 2011) [13]. Bootstrap method was used with 100 replications and gap/missing data treatment by complete selection and the search method was Subtree-Pruning-Regrafting (SPR) and substitution based on nucleotide sequences. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (100 replicates) was used (Felsenstein, 1985) [5]. The Maximum parsimony tree was obtained using the Subtree-Pruning-Regrafting (SPR) algorithm (Nei, Kumar, 2000) [9, 11, 7]. using search level 1. The substitution type based nucleotide sequences and the codon positions included were1st+2nd+3rd+Noncoding and all the positions containing gaps and missing data were eliminated.

Maximum Likelihood
Maximum Likelihood analyses were run in MEGA 5 (Tamura, 2011) [13]. The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model (Tamura, Nei, 1993) [13, 9, 11]. Initial tree for the heuristic search was obtained automatically by applying Neighbor-Joining and Bio NJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach and then selecting the topology with superior log likelihood value. The substitution type based nucleotide sequences and the codon positions included were 1st+2nd+3rd+Noncoding and all the positions containing gaps and missing data were eliminated.

Neighbor-Joining
Neighbor-Joining analyses were determined with MEGA5 (Tamura, 2011) [13]. The evolutionary history was inferred

using the Neighbor-Joining method (Saitou & Nei, 1987) [9, 11]. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (100 replicates) was used (Felsenstein, 1985) [5]. The evolutionary distances were computed using the Tajima-Nei method (Tajima & Nei, 1984) [13, 9, 11]. Codon positions included were 1st+2nd+3rd+Noncoding and all positions containing gaps and missing data were eliminated.

Table 1: Twenty five assassin bugs were subjected to phylogenetic analyses.

Species	Locality
Acanthaspis quinquespinosa	Tamil Nadu, India
Acanthaspis pedestris	Tamil Nadu, India
Acanthaspis siva	Tamil Nadu, India
Empyrocoris annulata	Tamil Nadu, India
Edocla slateri	Tamil Nadu, India
Velitra sinensis	Tamil Nadu, India
Catamiarus brevipennis	Tamil Nadu, India
Ectomocoris tibialis	Tamil Nadu, India
Ectomocoris cardiger	Tamil Nadu, India
Ectomocoris quadriguttattus	Tamil Nadu, India
*Ambastus villosus	Columbia, USA
*Cimbus tenax	Brunei
*Coranus dilatatus	Beijing, China
*Coranus emodicus	Beijing, China
*Coranus fuscipennis	Beijing, China
*Coranus hammarstroemi	Beijing, China
*Coranus lativentris	Beijing, China
*Coranus marginatus	Beijing, China
*Coranus sichuensis	Beijing, China
*Coranus spiniscutis	Beijing, China
*Emesaya brevipennis	New York, USA
Endochus albomaculatus	Karnataka, India
*Isyndus obscures	Seoul, South Korea
*Peirates turbis	Seoul, South Korea
Rihirbus trochantericus	Karnataka, India

Species denoted by * are non-Indian species and the rest of the species are Indian species.

Table 2: Mitochondrial DNA sequences of twenty five assassin bugs subjected to phylogenetic analysis.

Cytochrome oxidase subunit I- gene, partial sequence; mitochondrial	
Species	**GenBank accession number**
Acanthaspis quinquespinosa	KF443082
Acanthaspis pedestris	KF443083
Acanthaspis siva	KC130938.1
Empyrocoris annulata	KC130940.1
Edocla slateri	KC130939.1
Velitra sinensis	KF443084
Catamiarus brevipennis	KF056931.1
Ectomocoris tibialis	KF056932.1
Ectomocoris cardiger	KF056933.1
Ectomocoris quadriguttattus	KF056934.1
Ambastus villosus	GQ869659.1
*Cimbus tenax	GQ869658.1
*Coranus dilatatus	EU128701.1
*Coranus emodicus	EU128703.1
*Coranus fuscipennis	EU128704.1
*Coranus hammarstroemi	EU128700.1
*Coranus lativentris	EU128699.1
*Coranus marginatus	EU128702.1
*Coranus sichuensis	EU128705.1
*Coranus spiniscutis	EU128706.1
*Emesaya brevipennis	EU683231.1
Endochus albomaculatus	KC834737.1
*Isyndus obscures	GQ292195.1
*Peirates turbis	JQ888642.1
Rihirbus trochantericus	KC834736.1

Species denoted by * are non-Indian species and the rest of the species are Indian species.

3. Results and Discussion

Based on Cyt oxidase subunit I gene sequences, three phylograms were constructed. The results of Maximum Parsimony, Maximum Likelihood, and Neighbor-Joining trees were analyzed based on the arrangement of each species on the tree.

Observations were made from ML tree and compared with MP and NJ as follows: Intrageneric affinity is observed from the single cluster constituting the eight non-Indian species belonging to the genus, *Coranus*. This also suggests the geographical role of speciation at the generic level since all the eight species analyzed are from Beijing, China. Out of these eight species, sub-clusters are comprised of *Coranus fuscipennis* and *Coranus marginatus*; *Coranus spiniscutes* and *Coranus sichuensis* and *Coranus emodicus* and *Coranus hammasstroemi* revealing interspecific affinity.

A kind of phylogenetic hierarchy is observed among this particular genus as *Coranus dilatatus* followed by *Coranus lativentris.* This is followed by the clusters comprising of *Coranus fuscipennis* and *Coranus marginatus; Coranus emodicus* and *Coranus hammasstroemi* and *Coranus spiniscutes* and *Coranus sichuensis.* Ambrose (2006) [1-4] has reported that various species belonging to *Coranus* genus are abundantly found in native localities. But genetic data on this particular native genus is not available which has to be explored to depict the impact of biogeographical isolation on this genus.

Another line of phylogeny shows intergeneric affinity between *Catamiarus brevipennis* and *Velitra sinensis* since these two genera constitute a single cluster. Moreover, they are evolved at a uniform evolutionary rate, indicating their similar rate of evolution. Interestingly, these two different genera might possess certain unique characteristics that make them to constitute a single cluster rather than being a part of the other cluster constituted by the rest of the genera.

Generic specificity is observed for the genera, *Emesaya brevipennis, Cimbus tenax, Rihirbus trachantericus, Isyndus obscures, Ambastus villosus* and *Endochus albamaculatus* since they stood independently and do not show affinity towards any other genera belonging to the same family itself.

In addition to this, phylogenetic hierarchy is observed constituting both Indian and non-Indian species: *Endochus albomaculatus* stands independently followed by *Ambastus villosus, isyndus obscures, Rihirbus* trochantericus. This is followed by a cluster comprising of *Empyrocoris annulata, Acanthaspis siva, Edocla slateri.* This is followed by *Cimbus tenax, Emesaya brevipennis* and a cluster comprising of two sub-clusters, of which one consists of *Ectomocoris tibialis, Ectomocoris quadriguttatus, peiratus turbis* and another sub-cluster is constituted by *Acanthaspis pedestris, Acanthaspis quinquespinosa* and *Ectomocoris cardiger.* The sub-clusters reveal intergeneric affinity between the genera, *Acanthaspis* and *Ectomocoris*; *Acanthaspis* and *Empyrocoris*, *Ectomocoris* and *Peiratus* since they share a common clade. A kind of multiple combination of affinity factor between different genera is observed suggesting that they all universally share certain features of the family Reduviidae. These observations were the same in case of ML and NJ phylograms whereas there are minor variations noted in MP tree in the level of phylogeny. Moreover in MP phylogram, the intergeneric clustering has not been repeated exactly as in ML and NJ trees but they are closer to each other by the property of forming a uniform pattern of sub-clusters. Except for few varied observations, all the three phylograms show the similar trend of clustering between any two genera revealing uniform phylogenetic relationship.

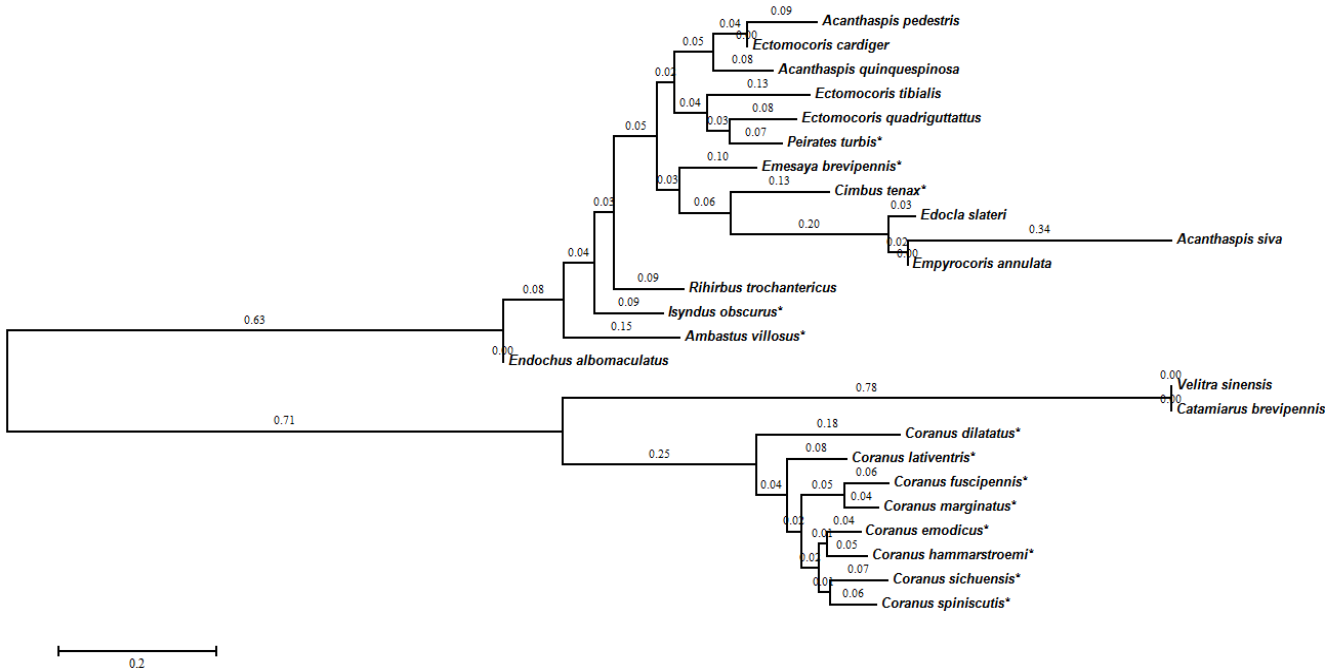

Fig 1: Maximum Likelihood tree based on COI gene variations showing the relationships twenty five assassin bugs

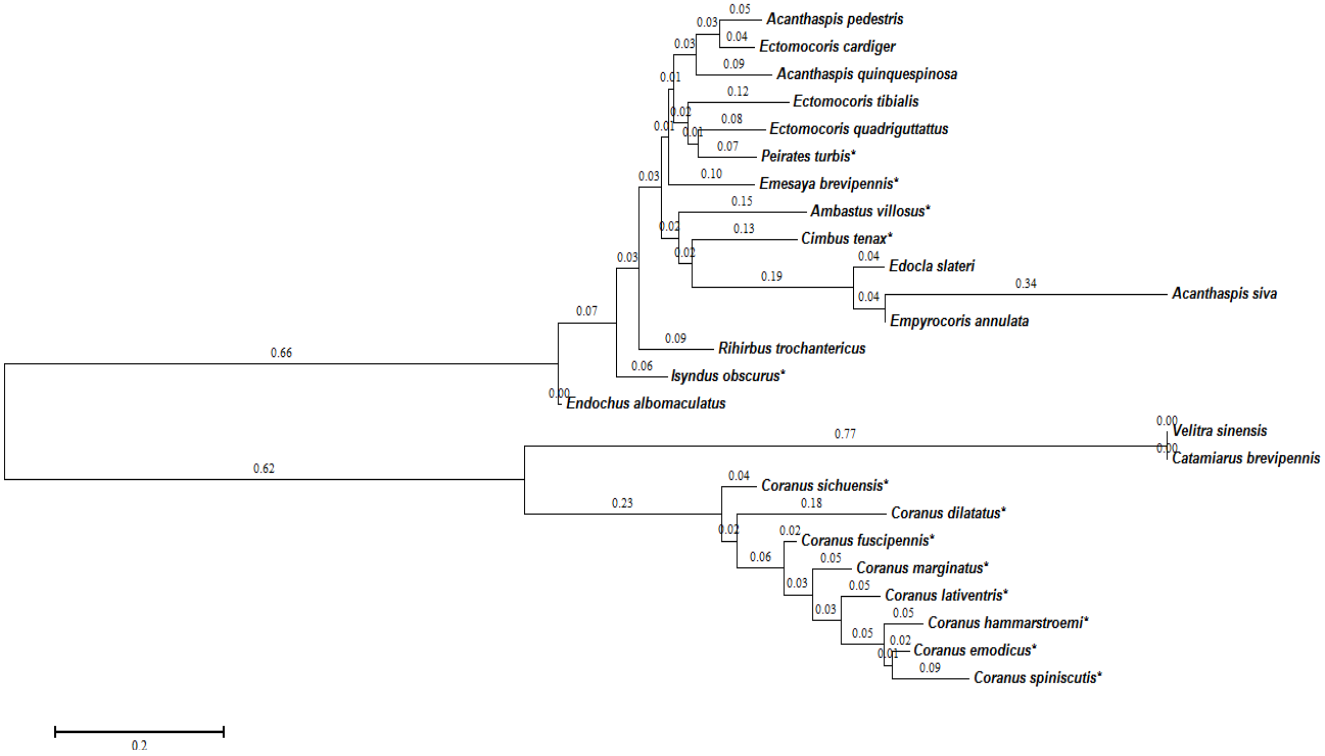

Fig 2: Neighbour -Joining tree based on COI gene variations showing the relationships twenty five assassin bugs

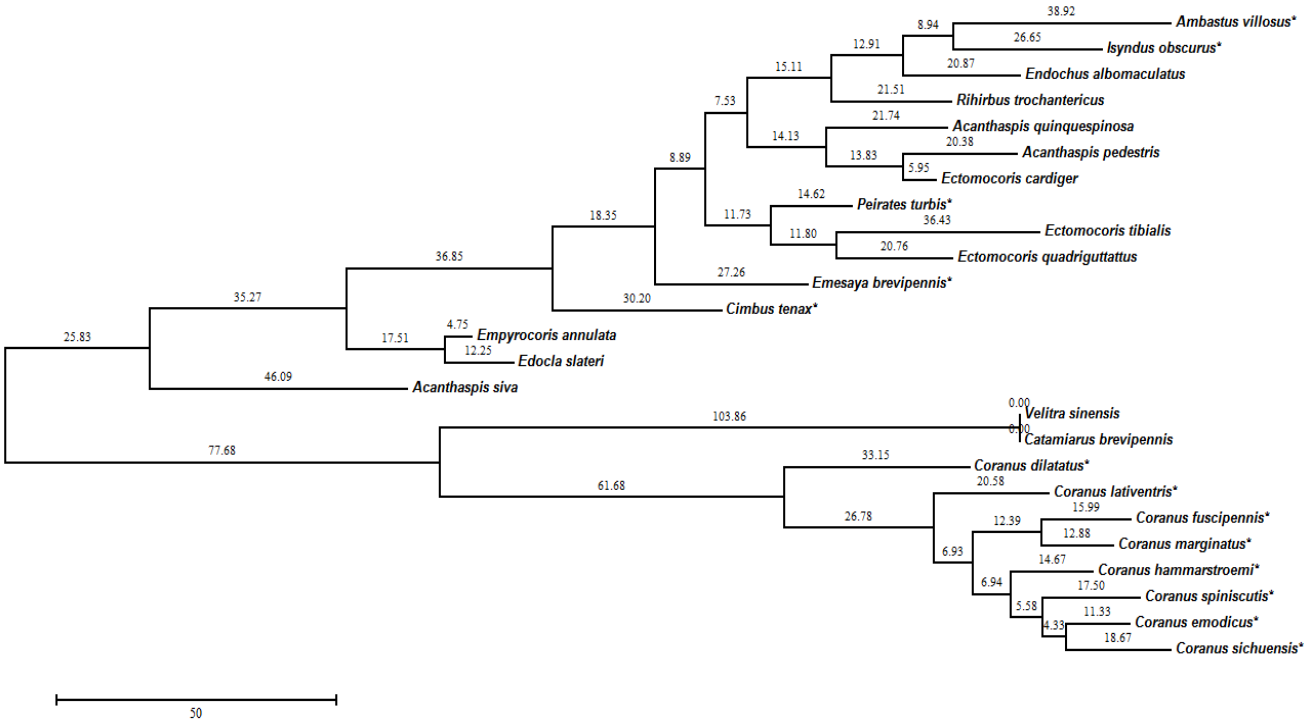

Fig 3: Maximum Parsimony tree based on COI gene variations showing the relationships twenty five assassin bugs

4. Conclusion

The results obtained not only have enriched our knowledge on biosystematics but have also supplemented multidisciplinary data. The results further reveal the utility of Cytochrome oxidase subunit I DNA sequences in phylogenetic analysis. The findings clearly suggest the intergeneric and intrageneric phylogenetic affinity and diversity of Indian and non-Indian genera. Moreover, the genetic diversity observed among various genera of the family Reduviidae warrant further studies in this direction that could lead to meaningful revision, regrouping, or replacement of species with new revelations through molecular analysis.

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