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Intrageneric and Intergeneric Phylogenetics Based on Available Mitochondrial Genes and Nuclear Gene Variation among Ten Peiratine Species: Nine Species of Ectomocoris Mayr and One Species of Catamiarus (Serville) (Hemiptera: Reduviidae: Peiratinae)

Muthumari Manimuthu, Arumugam Ganesh Kumar and Dunston Philamon Ambrose*

Entomology Research Unit, St. Xavier's College (Autonomous), Palayamkottai-627 002, Tamil Nadu, India

Abstract

The phylogenetic analysis of a nuclear gene, 28S ribosomal RNA of two determined and three undetermined species of *Ectomocoris* Mayr and the mitochondrial gene, cytochrome C of three determined and one undetermined species of *Ectomocoris* and one species of *Catamiarus* (Serville) from Australia and Asia was made. It reveals the interspecific and intrageneric phylogenetic affinity among *Ectomocoris* and intergeneric affinity between *Ectomocoris* and *Catamiarus*. Moreover, the Cty c sequence analysis warrant further studies on the undetermined species of *Ectomocoris* and the validation of *Catamiarus* as genus or subgenus of *Ectomocoris*. The study also proves the usefulness of 28S rRNA and Cyt c oxidase subunit I genes as useful molecular markers in the phylogenetic analysis of the peiratine genera *Ectomocoris* and *Catamiarus*.

Keywords: Ectomocoris; 28S rRNA; COI; *Peiratinae; B*iocontrol agents; *I*ntrageneric and intergeneric molecular biosystematics

Introduction

Some assassin bugs have different morphs, biotypes, and ecotypes with various colours and shapes which often mislead a museum entomologist in recognizing the morphs and ecotypes of a particular species.

Hence, classifications of Reduviidae based on morphological characters [1-3] 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 [4-6]. Moreover, a multidisciplinary biosystematics is imperative to accurately identify reduviids and employ them against a particular insect pest [4-7]. Literature available on multidisciplinary biosystematics of Reduviidae including molecular tools is very meagre [6,8-10].

Though assassin bugs of subfamily Peiratinae are abundant and have worldwide distribution with about 32 genera and 300 species [3,11-17] only a few taxa have been studied in detail [18]. Cai and Lu [19] studied the Chinese species of *Ectomocoris*. But no such work is available on the *Ectomocoris* and *Catamiarus* species of other geographical regions. Since classification of Peiratinae is being subjected to constant changes an attempt is made here to understand the intrageneric phylogenetic relationship of species of *Ectomocoris* from Australia and Asia and their relationship with *Catamiarus*, represented by only one species *Catamiarus* brevipennis (Serville) endemic to India.

This study was undertaken based on the available a mitochondrial gene, COI and a nuclear gene, 28S rRNA of ten peiratine species: nine species of *Ectomocoris* Mayr and one species of *Catamiarus* (Serville) [Table 1]. The inclusion of *Ectomocoris* species from Asian countries and Australia, two continents further enhances the scope of the work at the intraspecific level and the understanding on the role of geographical isolation in biosystematics.

Material and Methods

Taxon sampling

To understand the intrageneric and intergeneric biosystematics and phylogenetics, the sequences of one mitochondrial gene, Cyt c oxidase subunit I gene of four *Ectomocoris* species and of Catamiarus brevipennis (Serville) and one nuclear gene, 28S rRNA of five species of *Ectomocoris* [Tables 1 and 2] were subjected to phylogenetic analysis. The genes of species of *Ectomocoris viz., E. cordiger Stal, E. quadriguttatus (Fabricius)* and *E. tibialis* Distant and *C. brevipennis* were sequenced by the authors and the remaining sequences were obtained from the GenBank.

Phylogenetic analysis

The gene sequences were subjected into pairwise distance analysis

Species	Distribution	Reference
Catamiarus brevipennis (Seville)	India	3
Ectomocoris atrox (Stål)	Bhamao Island, Burma, Cambodia, China, India, Indonesia (Borneo, Celebes, Java and Sumatra), Malaysia, Philippines, Sri Lanka	3, 24
Ectomocoris ornatus (Stål)	Australia	3, 25
Ectomocoris cordiger Stål	India, Iraq (Hinaidi, near Baghdad), Iran and Sri Lanka	3
Ectomocoris quadriguttatus (Fabricius)	India	3
Ectomocoris tibialis Distant	India	3
Ectomocoris sp.	Australia	26
Ectomocoris sp.$_1$	Australia	
Ectomocoris sp.$_2$	Australia	
Ectomocoris sp.$_3$	Australia	

Table 1: Ten species of Peiratinae: nine species of *Ectomocoris* and *Catamiarus brevipennis* subjected to phylogenetic analyses with their distribution.

***Corresponding author:** Dunston Philamon Ambrose, Entomology Research Unit, St. Xavier's College (Autonomous), Palayamkottai-627 002, Tamil Nadu, India, Tel: 0462 2560744; E-mail: eruxavier@gmail.com

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and the phylogenetic trees were constructed based on maximum likelihood and neighbor-joining, maximum evolution, UPGMA and maximum parsimony methods with MEGA 5 software [20]. The five different methods were used to understand the utility of each method in the biosystematics.

Pairwise alignment

Pairwise distances were carried out with gap opening penalty 15 and gap extension penalty 6.66 (Clustal W) [21].

Maximum parsimony

The maximum parsimony analyses were analysed with MEGA5

Genes	Species	Genbank Accession Number
28S ribosomal RNA	*Ectomocoris atrox* (Stål)	KP236926.1
	Ectomocoris ornatus (Stål)	FJ230595.1
	Ectomocoris sp.$_1$	JQ942214.1
	Ectomocoris sp.$_2$	FJ230761.1
	Ectomocoris sp.$_3$	FJ230682.1
Cytochrome c oxidase subunit I	*Ectomocoris cordiger* Stål	KF056933.1
	Ectomocoris quadriguttatus (Fabricius)	KF056934.1
	Ectomocoris tibialis Distant	KF056932.1
	Ectomocoris sp.	GU198517.1
	Catamiarus brevepennis (Serville)	KF056931.1

Table 2: Gene sequences of ten species of Peiratinae: nine species *Ectomocoris* species and *Catamiarus brevipennis* with their GenBank accession number.

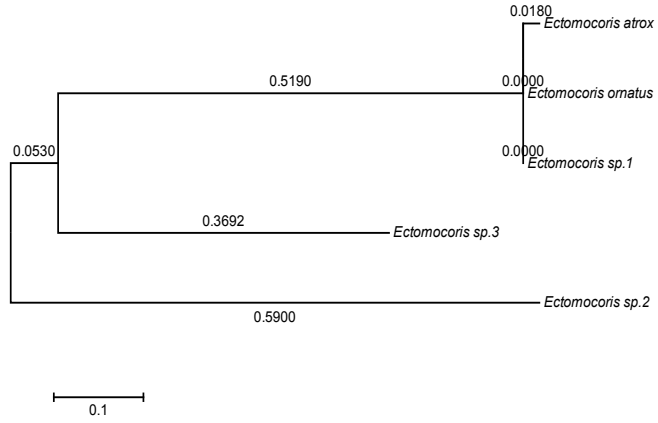

Figure 1: ML tree based on 28S rRNA gene variations showing the relationships of five *Ectomocoris* species.

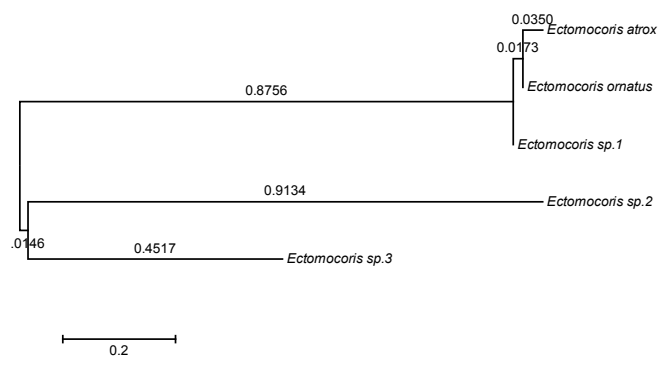

Figure 2: NJ tree based on 28S rRNA gene variations showing the relationships of five *Ectomocoris* species.

(20). Bootstrap method was used with 100 replications and gap/missing data treatment by complete selection and substitution based on nucleotide sequences [22]. The maximum parsimony tree was obtained using the Subtree-Pruning-Regrafting (SPR) algorithm [23] with search level 1 [Table 1].

Maximum likelihood

The evolutionary history was inferred based on the Tamura-Nei model [27]. Initial tree for the heuristic search was obtained automatically by applying neighbor-joining and BioNJ 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 [Table 2].

Neighbor-joining

The evolutionary history was inferred using the neighbor-joining method [28]. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (100 replicates) was used [22]. The evolutionary distances were computed using the Tajima-Nei method [29].

Minimum evolution

The evolutionary history was inferred using the minimum evolution method (30). The optimal tree with the sum of branch length =8.45674115 is shown. The confidence probability (multiplied by 100) was estimated using the bootstrap test [30,31].

UPGMA

The evolutionary history was inferred using the UPGMA method [32]. The optimal tree with the sum of branch length =8.42786450 is shown.

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 in all the five methods. Five phylograms were thus constructed based on maximum likelihood (ML), neighbor-joining (N-J), maximum evolution (ME), UPGMA and maximum parsimony (MP) methods for one mitochondrial gene, Cyt c oxidase subunit I and one nuclear gene, 28S rRNA. The trees were analyzed based on the arrangement of each species in the tree.

Results and Discussion

28S rRNA

The ML tree based on 28S rRNA sequence shows a major cluster and two separate minor branches of the first cluster shows the affinity between *E. atrox, E. ornatus* and Ectomocoris sp.$_1$ whereas the Ectomocoris sp.$_3$ stands distinctly as a separate branch but closer to the major cluster. The *Ectomocoris* sp.$_2$ also stands as a separate branch but distinctly away from other species (Figure 1).

The NJ (Figure 2) and ME trees based on 28S rRNA sequences almost replicate the affinity observed in ML tree. But *Ectomocoris* sp.2 took the position of *Ectomocoris* sp.3 and vice versa. However, the intergeneric affinity among *E. atrox, E. ornatus* and *Ectomocoris* sp.1 is maintained (Figure 3).

Though the UPGMA tree based on 28S rRNA exhibits a similar affinity between *E. atrox*, *E. ornatus* and *Ectomocoris* sp.$_1$ the uniqueness of *E. atox* and the closer affinity between *E. ornatus* and *Ectomocoris* sp.$_1$ by forming a cluster with *E. ornatus* and *Ectomocoris* sp.$_1$ and a separate branch of *E. atrox* are visible. Moreover, the affinity between these three species with *Ectomocoris* sp.$_2$ and sp.$_3$ is similar to that exhibited by ML tree (Figure 4).

The MP tree based on 28S rRNA also shows the intrageneric affinity of *E. atrox*, *E. ornatus* and *Ectomocoris* sp.$_1$ (Figure 5) as observed in the ML, NJ, ME and UPGMA trees but the closer affinity exhibited between *E. ornatus* and *Ectomocoris* sp.$_1$ is revealed.

Two species of *Ectomocoris* viz., *E. ornatus* and *Ectomocoris* sp.$_1$ from Australia form the first major cluster with *E. atrox* from Asia where as the remaining two species from Australia diversified into two separate lines. The results reveal monophyly though they belong to two continents as observed by Liu et al., [33] in *Coranus* (Reduviidae: Harpactorinae), Cui and Huang [34] in Orthoptera and Ambrose et al., [6] in *Rhynocoris* Kolenati species of Reduviidae from India. They exhibit affinity despite their geographical isolation as observed by Mahendran et al., [35] in silk-producing insects and Ambrose et al., [6] in *R. fuscipes* of India with *R. segmentarius* (Germar) of South Africa. However, we admit that it is premature to suggest the rold of geographical isolation without knowing the molecular characteristics such as number of segregating sites, nucleotide diversity and haplotype diversity and the geographical genetic structure. Although Liu et al., [33] reported that 28S rRNA is highly conserved gene and may not be an optimum molecular marker for phylogenetic studies on a Harpactorine genus, *Coranus* Curtis, the present analysis contradicts their view and suggests its usefulness in the phylogenetic analysis of *Ectomocoris*.

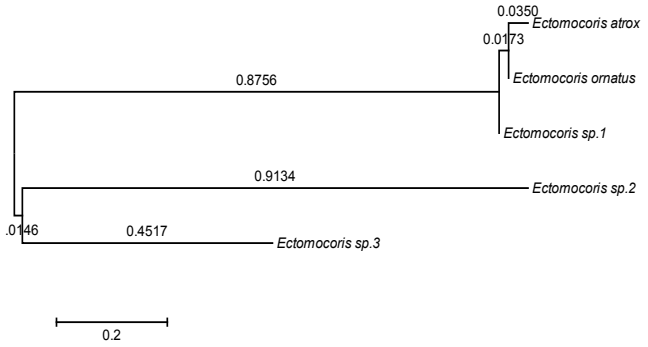

Figure 3: ME tree based on 28S rRNA gene variations showing the relationships of five *Ectomocoris* species.

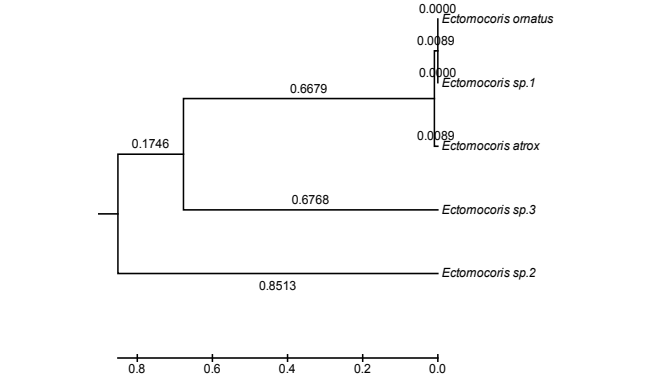

Figure 4: UPGMA method tree based on 28S rRNA gene variations showing the relationships of five *Ectomocoris* species.

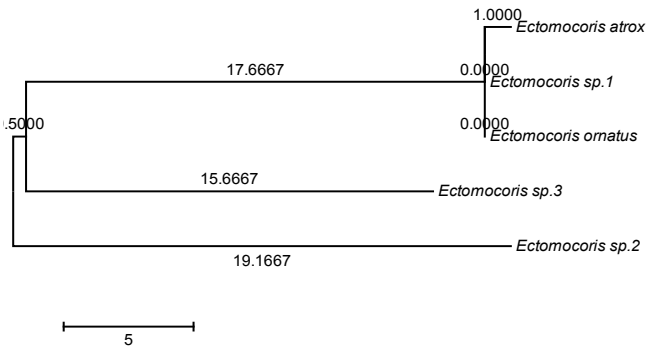

Figure 5: MP method tree based on 28s rRNA gene variations showing the relationships of five *Ectomocoris* species.

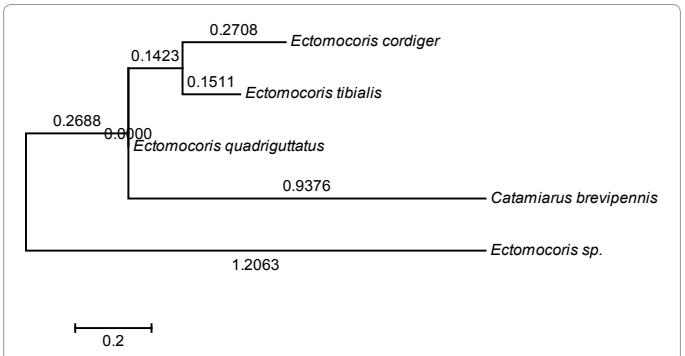

Figure 6: ML tree based on Cyt c subunit like 1 gene variations showing the relationships of five *Ectomocoris* species.

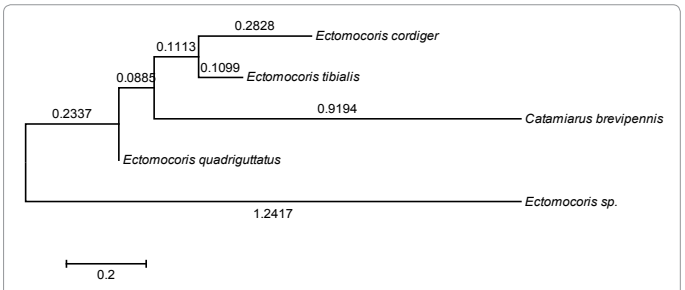

Figure 7: NJ tree based on Cyt c subunit like 1 gene variations showing the relationships of five *Ectomocoris* species.

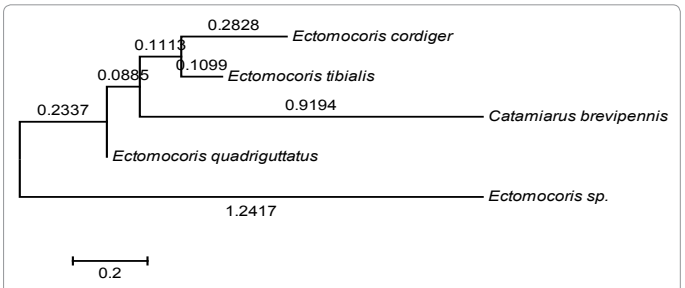

Figure 8: Minimum Evolution Method Tree Based On Cyt C Subunit Like 1 Gene Variations Showing The Relationships Of Five *Ectomocoris* Species.

Cyt c

The five phylograms (Figure 6-10) of Cyt c gene of three determined species of *Ectomocoris* viz., *E. cordiger*, *E. quadriguttatus* and *E. tibialis* from Asia, one undetermined species of *Ectomocoris* from Australia and *C. brevipennis* an endemic species from India revealed close affinity among them. The Asiatic *E. quadriguttatus* is closely allied with the other two Asiatic species viz., *E. cordiger* and *E. tibialis* but interestingly distantly related to them than to *C. brevipennis*. This is evidenced by the existence of a separate diversified lineage of *E. quadriguttatus* in ML, NJ and ME trees based on Cyt c gene sequences. In the UPGMA tree, in addition to the closer affinity exhibited between *E. quadriguttatus* and

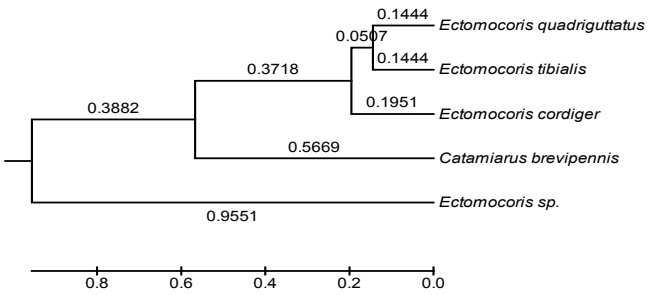

Figure 9: Upgma Method Tree Based On Cyt C Subunit Like 1 Gene Variations Showing The Relationships Of Five *Ectomocoris* Species.

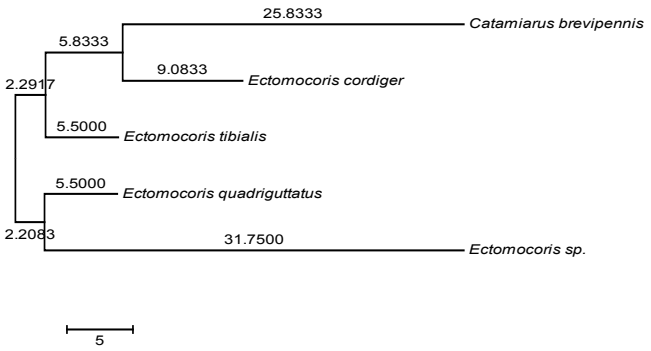

Figure 10: MP method tree based on Cyt c subunit like 1 gene variations showing the relationships of five *Ectomocoris* species.

E. tibialis and these two species are distantly aligned with *E. cordiger*. Here again, *C. brevipennis* forms a diversified branch but closer to all the three Asian *Ectomocoris* species and clearly demarcated from the *Ectomocoris* sp. of Australia. Interestingly, *C. brevipennis* and *E. cordiger* diversified as a separate cluster and *E. tibialis* form a separate branch. Moreover, *E. quadriguttatus* from Asia clustered with *Ectomocoris* sp. of Australia suggesting intrageneric affinity despite geographical isolation. However, we admit that it is premature to suggest the role of geographical isolation without knowing the molecular characteristics such as the number of segregating sites, nucleotide diversity and haplo type diversity and the geographical genetic structure. Moreover, the quantity of sampling is too small. Except in MP tree, the undetermined Australian *Ectomocoris* sp. stands as a separate line away from the three Asian *Ectomocoris* species and the lone Indian endemic *Catamiarus brevipennis* suggesting the requirement of further studies to validate the taxonomic position of Australian *Ectomocoris*. Similarly, the genetic status of *Catamiarus* has to be validated either as a separate genus as it now exists or to place it as a subgenus of *Ectomocoris*.

Thus, intrageneric phylogenetic affinity among the four *Ectomocoris* species despite geographical isolation as well as intergeneric affinity between *Ectomocoris* and *Catamiarus* are revelaed. Baskar [36], Baskar et al., [37-39] and Ambrose et al., [6] reported genetic diversity among four harpactorine reduviid species of *Rhynocoris* viz., *R. kumarii* Ambrose and Livingstone, *R. marginatus* (Fabricius), *R. longifrons* (Stal) and *R. fuscipes* (Fabricius) based on mitochondrial genes and the present results corroborate with their findings. Hence, the existence of genetic diversity, with low level of gene flow might be operating among *Ectomocoris* and *Catamiarus* species as Zhao and Zhu [40] observed in *Branchiostoma japanicum* Lonnberg. Moreover, these observations again support the monophyly of family Reduviidae as observed by Liu et al., (33) and Ambrose et al., [6]. However, these observations are contrary to those of Giordano et al., [41] in a haematophagous reduviid, *Triatoma infestans* (Klug). This contradiction might be the result of the non-dispersal haematophagous feeding behaviour of *Triatoma* in contrast to the dispersal predatory behavior of *Ectomocoris* and *Catamiarus*. The findings further suggest that the Cyt c oxidase subunit I gene is a useful marker to understand the intrageneric and intergeneric affinity [42].

Conclusion

The results obtained not only have enriched the knowledge on biosystematics of *Ectomocoris* and *Catamiarus* but also supplemented multidisciplinary data of the two genera. The results further reveal the utility of mitochondrial gene Cyt c oxidase subunit I and nuclear gene 28S rRNA sequence in phylogenetic analysis in *Ectomocoris* and *Catamiarus*, i.e., as useful markers to understand the intrageneric affinity of *Ectomocoris* and its intergeneric affinity with *Catamiarus*. The findings further suggest intraspecific and interspecific phylogenetic affinity of *Ectomocoris* species from two continents. However, our study based on the available genetic sequences of about 10 per cent of the total number of *Ectomocoris* species emphasizes the need of further studies on the genetic sequencing and analysis of the genus *Ectomocoris*. Further analyses with more number of species will reveal their better phylogenetics.

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