

<http://dx.doi.org/10.11646/zootaxa.3779.5.4>  
<http://zoobank.org/urn:lsid:zoobank.org:pub:3DC207B8-7F92-45E3-8A93-96E98240E510>

## Intrageneric phylogenetics based on mitochondrial DNA variation among fifteen harpactorine assassin bugs with four ecotypes and three morphs (Hemiptera: Reduviidae: Harpactorinae)

DUNSTON P. AMBROSE<sup>1</sup>, E. AROCKIA LENIN & D. ANGELINE KIRUBA<sup>2</sup>

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

E-mail eruxavier@gmail.com

<sup>1</sup>Corresponding author

<sup>2</sup>Present address: Department of Industrial Biotechnology, Government College of Technology, Coimbatore-641 013, Tamil Nadu, India

### Abstract

Available mitochondrial DNA sequences viz., 16S, Cyt b, Cyt c oxidase subunit – I, and Cyt c subunit-like – I gene of *Rhynocoris* (Kolenati) species were subjected to phylogenetic analysis to understand the intrageneric and intraspecific variations and the role of geographical isolation on speciation; using CLUSTAL W in MEGA version 5.1. This analysis includes fifteen species and four ecotypes of *R. kumarii* Ambrose and Livingstone and three morphs of *R. marginatus* (Fabricius) from four countries viz., Canada, China, Korea, and South Africa. The pairwise genetic distances were calculated and phylogenograms were constructed using Maximum Likelihood, Maximum Parsimony, and Neighbor-Joining methods. These preliminary analyses not only demarcated the fifteen species of *Rhynocoris*, the four ecotypes of *R. kumarii*, and the three morphs of *R. marginatus*, but also revealed phylogenetic relationships and the role of geographical isolation and polymorphism on speciation.

**Keywords:** *Rhynocoris*, assassin bugs, biocontrol agents, molecular biosystematics, phylogenetic relationship, speciation, ecotypism, polymorphism, geographical isolation.

### Introduction

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 (Usinger, 1943; Putshkov & Putshkov, 1985; Maldonado, 1990; Schuh & Slater, 1995) 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 reduviids and employ them against a particular insect pest (Ambrose, 1999, Ambrose & Ambrose, 2003, 2009). Although multidisciplinary biosystematics including molecular tools has been attempted on Oriental reduviids (Weirauch, 2008), such an analysis is wanting on non-Oriental reduviids.

This study was undertaken based on available mitochondrial sequences of fifteen species of *Rhynocoris* Kolenati (Table 1), four ecotypes of *R. kumarii* Ambrose and Livingstone, and three morphs of *R. marginatus* (Fabricius). The inclusion of both Indian and non-Indian species of *Rhynocoris* with ecotypes and morphs will further enhance the scope of the work at the intraspecific level and the understanding on the role of geographical isolation in biosystematics.

revealed, the genetic diversity among them suggests ongoing speciation among them. The present findings on the affinity of *Rhynocoris* species do not corroborate with the existing literature on identification of these *Rhynocoris* species based on morphological characters, as Garcia *et al.* (2001) observed in *Triatoma* species.

## Conclusion

The results obtained not only have enriched our knowledge on biosystematics but have also supplemented multidisciplinary data. The results further reveals the utility of 16 S, mtCyt b, Cyt C oxidase subunit I, and Cyt c oxidase subunit I-like DNA sequences in phylogenetic analysis. The findings clearly suggest the intraspecific and interspecific phylogenetic affinity and diversity not only in the Indian and non-Indian species of *Rhynocoris* but also among the ecotypes of *R. kumarii* and the morphs of *R. marginatus*. Moreover, the genetic diversity observed among ecotypes and morphs suggesting progression of speciation warrant further studies in this direction that could lead to meaningful revision, regrouping, or replacement of species with new revelations through molecular analysis.

## Acknowledgments

The authors are grateful to the authorities of St. Xavier's College (Autonomous), Palayamkottai, Tamil Nadu, India for facilities. We are grateful to the Council of Scientific and Industrial Research (CSIR), Government of India, New Delhi, for financial assistance (Ref. No. 21(0865)/11/EMR-II, 2012-2013 dated 28.12.2011). The authors thank Prof. Carl W. Schaefer, University of Connecticut, USA, who reviewed the manuscript, and Prof. Wanzhi Cai, China Agricultural University, China for his timely support.

## References

- Almeida, C.E., Pacheco, R.S., Haag, K., Dupas, S., Dotson, E.M. & Costa, J. (2008) Inferring from the Cyt B gene the *Triatoma brasiliensis* Neiva, 1911 (Hemiptera: Reduviidae: Triatominae) genetic structure and domiciliary infestation in the state of Paraiba, Brazil. *The American Journal of Tropical Medicine and Hygiene*, 78, 791–802.
- Ambrose, D.P. (1999) *Assassin bugs*. Science Publishers, New Hampshire, USA and Oxford and IBH Publishing Company Private Limited, New Delhi, India, 337 pp.
- Ambrose, A.D. & Ambrose, D.P. (2003) Linear regression coefficient ( $r$ ) of postembryonic developmental morphometry as a tool in the biosystematics of Reduviidae (Insecta: Hemiptera). *Shaspa*, 10, 57–66.
- Ambrose, A.D. & Ambrose, D.P. (2009) Predation, copulation, oviposition and functional morphology of tibia, rostrum and eggs as tools in the biosystematics of Reduviidae (Hemiptera). *Indian Journal of Entomology*, 71, 1–17.
- Baskar, A. (2010) *Molecular genetic variation and phylogeny of Rhynocoris spp. .based on mitochondrial gene analysis (Heteroptera: Reduviidae)*, Ph.D. Thesis, University of Madras.
- Baskar, A., Ambrose, D.P., Tirumurugan, K.G. & Fleming, A.T. (2012a) Ecotypic diversity in the assassin bug, *Rhynocoris marginatus* Fabricius (Heteroptera: Reduviidae). *Hexapoda*, 19, 38–46.
- Baskar, A., Ambrose, D.P. & Tirumurugan, K.G. (2012b) Ecotypic diversity in the assassin bug, *Rhynocoris kumari* Ambrose and Livingstone (Heteroptera: Reduviidae). *Journal of Entomological Research*, 36, 369–375.
- Baskar, A., Ambrose, D.P., Tirumurugan, K.G. & Fleming, A.T. (2012c) Ecotypic diversity in the assassin bug, *Rhynocoris fuscipes* Fabricius (Heteroptera: Reduviidae). *Journal of Advanced Zoology*, 33, 133–121.
- Cui, A.M. & Huang, Y. (2012) Phylogenetic relationships among Orthoptera insect groups based on complete sequences of 16S ribosomal RNA. *Yi Chuan*, 34, 597–608.
- Felsenstein, J. (1985) Confidence limites on phylogenies : An approach using the bootstrap. *Evolution*, 39, 783–791.
- Garcia, B.A., Moriyama, E.N. & Powell, J.R. (2001) Mitochondrial DNA sequences of Triatomines (Hemiptera: Reduviidae): Phylogenetic Relationships. *Journal of Medical Entomology*, 38, 675–683.  
<http://dx.doi.org/10.1603/0022-2585-38.5.675>
- Giordano, R., Cortez, J.C.P., Paulk, S. & Stevens, L. (2005) Genetic diversity of *Triatoma infestans* (Hemiptera: Reduviidae) in Chuquisaca, Bolivia based on the mitochondrial cytochrome b gene. *Memórias do Instituto Oswaldo Cruz*, 100, 753–760.  
<http://dx.doi.org/10.1590/s0074-02762005000700014>
- Mahendran, B., Ghosh, S.K. & Kundu, S.C. (2006) Molecular phylogeny of silk-producing insects based on 16S ribosomal RNA and cytochrome oxidase subunit I genes. *Journal of Genetics*, 85, 31–38.  
<http://dx.doi.org/10.1007/bf02728967>

- Maldonado, J.C. (1990) Systematic Catalogue of the Reduviidae of the World (Insecta: Heteroptera). *Special Edition of Caribbean Journal of Science*, 1–694.
- Naranjo, B.M., Zuriaga, M.A., Azofeifa, G., Zeledon, R. & Bargues, M.D. (2010) Molecular evidence of intraspecific variability in different habitat-related populations of *Triatoma dimidiata* (Hemiptera: Reduviidae) from Costa Rica. *Parasitological Research*, 106, 895–905.  
<http://dx.doi.org/10.1007/s00436-010-1762-9>
- Nei, M. & Kumar, S. (2000) *Molecular Evolution and Phylogenetics*. Oxford University Press, New York, 333 pp.
- Putshkov, P.V. & Putshkov, V.G. (1985) A catalogue of the assassin bug genera of the world (Heteroptera: Reduviidae). *Viniti, Lyubertsy*, 1–138.
- Saitou, N. & Nei, M. (1987) The neighbor-joining method. A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*, 4, 406–425.
- Schuh, R.T. & Slater, J.A. (1995) True Bugs of the World (Hemiptera: Heteroptera). Classification and Natural History, Cornell University Press, Ithaca, 336 pp.
- Tajima, F. & Nei, M. (1984) Estimation of evolutionary distance between nucleotide sequences. *Molecular Biology and Evolution*, 1, 269–285.
- Tamura, K. & Nei, M. (1993) Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution*, 10, 512–526.
- Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011) MEGA5 : Molecular Evolutionary Genetic Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods, *Molecular Biology and Evolution*, 28, 2731–2739.  
<http://dx.doi.org/10.1093/molbev/msr121>
- Thompson, J.D., Higgins, D.G. & Gibson, T.J. (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice. *Nucleic Acids Research*, 22, 4673–4680.  
<http://dx.doi.org/10.1093/nar/22.22.4673>
- Usinger, R.L. (1943) A revised classification of the Reduviidae with a new subfamily from S. America. *Annals of the Entomological Society of America*, 36, 602–618.
- Weirauch, C. (2008) Cladistic analysis of Reduviidae (Heteroptera: Cimicomorpha) based on morphological characters. *Systematic Entomology*, 33, 229–274.  
<http://dx.doi.org/10.1111/j.1365-3113.2007.00417.x>
- Zhao, Q. & Zhu, Q. (2011) Taxonomic and genetic status of lancelet in Weihai coastal waters based on mitochondrial DNA sequence. *Chinese Journal of Oceanology and Limnology*, 29, 623–32.  
<http://dx.doi.org/10.1007/s00343-011-0131-6>