BIOSYSTEMATICS OF CHOSEN PEIRATINE ASSASSIN BUGS (INSECTA: HEMIPTERA: REDUVIIDAE)

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By

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DECLARATION

I hereby declare that the thesis entitled "**BIOSYSTEMATICS OF CHOSEN PEIRATINE ASSASSIN BUGS (INSECTA: HEMIPTERA: REDUVIIDAE**" submitted by me for the Degree of Doctor of Philosophy in Zoology is the result of my original and independent research work carried out under the guidance of Dr. Dunston P. Ambrose, D.Sc., Director, Entomology Research Unit, St. Xavier's College (Autonomous), Palayamkottai – 627 002 and it has not been submitted for the award of any degree, diploma, associateship, fellowship of any University or Institution.

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CONTENTS

The following four species of Peiratinae were redescribed with camera lucida diagrams, colour microphotographs and micrometry.

Redescribed species:

1. *Catamiarus brevipennis* (Serville, 1831)
2. *Ectomocoris cordiger* Stål, 1866
3. *Ectomocoris quadriguttatus* (Fabricius, 1781)
4. *Ectomocoris tibialis* Distant, 1904

Materials and Methods

This study is based on the specimens collected by Dr. Dunston P. Ambrose, the author and his colleagues in the Entomology Research Unit, St. Xavier's College (Autonomous), Palayamkottai, Tamil Nadu, India from Muppanthal Scrub Jungle (latitude 8° 26"N and longitude 77°54"E), Kanyakumari District, South India. The specimens are deposited in the Entomology Research Unit. Camera lucida illustrations of either pinned or 70% alcohol preserved specimens and genitalia were made. Colour microphotographs were made using Olympus Zooming Binocular Research Microscope and Camera. The morphometric analyses were made using ocular and stage micrometers. Body length was measured from the apex of head to the tip of hemelytra, if the hemelytra reached or extended beyond the tip of the abdomen in the resting position or from the apex of the head to the tip of the abdomen, if the hemelytra did not reach the tip of the abdomen. Maximum width of pronotum was measured across humeral angles (including spines). All measurements are given in millimeter.

Male and female genitalia of the peiratine species were prepared as follows. The tips of abdomen were soaked in hot 10% potassium hydroxide solution for approximately 5 minutes to

CHAPTER II
PROTEIN PROFILING

Introduction

Proteins are abundant in all organisms and play key roles in most biological events as catalysts, transporters, and messengers. Thus, it is crucial to note that all research related to proteins increase our understanding of their levels, interactions, functions, modifications, regulations and localization in cells (Twyman, 2004; Graves and Haystead, 2002). Proteins do not have a predictable structure as nucleic acids and thus their rates of migration are not similar to each other. They may not migrate when applying an electromotive force (when they are in their isoelectric point). In these cases, the proteins are denatured by adding a detergent such as sodium dodecyl sulfate (SDS) to separate them exclusively according to molecular weight. This technique was firstly introduced by Shapiro *et al.* (1967). Moreover, comparison of protein patterns is widely used for taxonomic purposes (Stephen, 1958; Halliday, 1975; Nunamaker *et al.*, 1984; Soares *et al.,* 1998; Suranto, 2002). The value of protein profiling is increasing daily because it provides a much better understanding of an organism (Graves and Haystead, 2002). Proteomics is a rapidly expanding discipline that aims to gain a comprehensive understanding of proteins. The term proteomics, which is a combination of protein and genomics, is used to define the large-scale analysis of a complete set of proteins - the chief components of cells that are responsible for the most significant metabolic pathways in cells or tissues (Graves and Haystead, 2002; Pandey and Mann, 2000; James, 1997). The goal underlying proteomics is not only to identify all proteins in a cell, but also to identify the correlation between the genetic sequence and three-dimensional (3D) protein structure (Graves and Haystead, 2002).

Introduction

The taxonomical studies have been undertaken with molecular evidences. Molecular evidences provide a more objective way to determine relationships than morphological taxonomy. The variations in protein or DNA sequences can be used to calculate the relationship between several organisms. They also help to estimate the ancestral history and lineages. Though, this approach generally parallels other available information it often provides new insights into the evolutionary relationships. Molecular tools have been widely used for species separation and identification since the past two decades (Baker and Palumbi, 1994; Sperling *et al.*, 1994).

The development of DNA amplification using the PCR technique has opened up possibility of examining genetic changes in population (DeSalle and Birstein, 1996). In PCR reaction, a DNA sequence can be amplified many thousand folds to provide sufficient products for restriction analysis or direct sequencing. Once appropriate primers are available, large number of individuals can be assayed quickly. Thus, it facilitates large population screening for variability. For instance, molecular identification was carried out using mt COI primer in onion trips and melon trips (Ashokan *et al.*, 2007).

Molecular systematic studies are improved with progress of molecular biological tools such as DNA hybridization, PCR-RFLP, RAPD, allozyme electrophoresis, microsatellite DNA, etc. (Liu and Cordes, 2004).

CHAPTER IV

RESTRICTION FRAGMENT LENGTH POLYMORPHISM

Introduction

Restriction fragment length polymorphisms (Botstein *et al.*, 1980) are highly polymorphic, co-dominantly- inherited markers based on the use of restriction enzymes which can be applied as single and multilocus probes with the banding patterns. The past limitations associated with the pedigree data, morphological, physiological and cytological markers for assessing genetic diversity in many species have largely been circumvented by the development of DNA markers such as restriction fragment length polymorphisms (RFLPs) (Botstein *et al.*, 1980). Polymorphic genetic markers have wide potential applications in animal and plant improvement programmes as a means for varietal and parentage identification, evaluation of polymorphic genetic loci affecting quantitative economic traits, and genetic mapping (Nagaraju *et al.*, 2001).

The restriction fragment length polymorphism, or RFLP (commonly pronounced as "rif-lip"), is a molecular technique that exploits variations in homologous DNA sequences. It refers to the difference between samples of homologous DNA molecules that come from differing locations of restriction enzyme sites, and to a related laboratory technique by which these segments can be illustrated. Several molecular techniques are available for identification such as polymerase chain reaction (Rodriguez *et al.*, 1991), randomly amplified polymorphic DNA (RAPD), DNA hybridization (Ebbehoj and Thomson,1991), gene sequencing (Chikuni *et al.*, 1994) and DNA fingerprinting (Ganai *et al.*, 2000) which have been tried as identification methods but they have their own limitations. The Polymerase Chain Reaction-Restriction

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