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Comparative phylogenetics of Pentatomidae and Dinidoridae using morphological traits and DNA sequences

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Abstract
The insect families Pentatomidae and Dinidoridae, both part of the order Hemiptera (true bugs), are closely related and share numerous similarities, often leading to misidentifications. Phylogenetic analysis based on DNA sequencing offers a powerful method for reconstructing evolutionary histories, tracing genetic lineages, and gaining deeper insights into species diversity and their relationships. Morphological characteristics of the specimens were meticulously examined using a Leica M205 C stereo zoom microscope, with identification guided by established taxonomic keys (Salini, S. & Viraktamath, C. A., 2015). Genomic DNA was isolated and sequenced, and phylogenetic and molecular evolutionary analyses were performed using MEGA Version 11. While most previous taxonomic studies in this group have predominantly relied on morphological data, this study integrates both morphological and molecular tools to address and resolve the taxonomic uncertainties surrounding Pentatomidae and Dinidoridae.

Keywords: Pentatomidae, Dinidoridae, Phylogeny, Hemiptera, Monophylum

Introduction

The infraorder Pentatomomorpha, part of the Heteroptera order, is home to a large group of insects, including both phytophagous and seed-eating species commonly known as "plant bugs." The name Pentatomomorpha is derived from Pentatoma, a term introduced by Pendergrast, Leston, and Southwood in 1954. This diverse group includes five superfamilies: Aradoidea, Coreoidea, Lygaeoidea, Pentatomoidea, and Pyrrhocoroidea (Schaefer, C. W., 1993) [10]. The Pentatomoidea superfamily is particularly noteworthy, comprising approximately 7,000 species divided into 21 families—16 extant and 5 extinct. Among these, families like Pentatomidae (stink bugs), Dinidoridae, and others are of significant economic importance.

Pentatomidae, commonly known as stink bugs, are present in every major zoogeographic region, making them the most diverse family of pentatomomorphan bugs (S. Salini, 2019) [9]. These insects show a wide range of variation in terms of size, shape, and color. Species like Sepontia Stål and and Spermatodes Bergroth are only a few millimeters long, while others, such as Catacanthus Spinola and Placosternum Amyot & Serville, grow to lengths of 22 to 28 mm or more (S. Salini, 2019) [9]. Most members of this family feed on plants. They use their piercing and sucking mouthparts to inject saliva into soft plant tissues like buds or seeds, after which they suck up the liquefied food using their maxillary stylets. While Pentatomidae are generally minor pests, their feeding can be quite damaging, especially when they target economically important plant parts.

Dinidoridae, a family of the pentatomoid group, contains about a hundred species classified into sixteen genera (Durai 1987 [1]; J.A. Lis 1990; Rolston et al. 1996; Kocorek and J.A. Lis 2000, 2008). The members are generally large and robust, with some species reaching lengths of over 27 mm. Their bodies range from ovoid to oblong in shape, and the head and pronotum possess lateral keels. The scutellum is moderately short, blunt, and typically accounts for about 50% of the abdominal length (Schuh & Slater 1995).

These bugs are economically important, making their rapid identification essential. Since the current classification system is based largely on morphological similarities, there is little consensus on their relationships. However, DNA barcoding is a powerful tool for identifying serious pest species and conducting their phylogenetic analysis.

This study focuses on the DNA barcoding and phylogenetic analysis of two economically important bugs: SA8- *Nazara viridula* (Pentatomidae) and SA5- *Coridius chinensis* (Dinidoridae), both of which belong to the same superfamily, Pentatomoidea.

Materials and Methods

The adult bugs were collected with an insect net and transferred to clean bottles, then brought to the lab where they were stored at -20°C for both DNA extraction and future reference as voucher specimens. The morphological features of the specimens were studied under a Leica (M205 C) stereo zoom microscope (Sruthi et al., 2024) [11] and their identification was carried out using standard keys (Salini, S., & Viraktamath, C. A. 2015) [8]. Genomic DNA was extracted from the specimens and sequenced. The sequence was compared for similarity using BLASTn from NCBI, and the closest matches were selected. These sequences were then aligned with the query sequence using ClustalW. The phylogenetic and molecular evolutionary analysis was performed with MEGA Version 11 (Tamura et al., 2021).

Result and Discussion

Stál in 1867 and 1870 established the Dinidoridae as a subfamily of Pentatomidae (Stal, 1867, 1870) Kirkaldy in 1909 catalogued the world fauna and maintained their subfamily status (Kirkaldy, 1909) [3] Leston in 1955 [4] raised them to family level which has been followed by

most subsequent authors (Leaton, 1955). This study based on morphology and molecular studies on C. chinensis also provides separate family status for Dinidoridae. The recent assessment of the systematic position of the family Dinidoridae within the superfamily Pentatomoidea revealed by the Bayesian phylogenetic analysis of the mitochondrial 12S and 16S rDNA sequences (Lis et al., 2012) showed that the family formed a highly supported monophylum. Result from our study is also shows that Coridius chinensis forms monophyletic relationship with other dinidorid family members. The phylogenetic tree that we constructed Identifies C. chinensis (Dinidoridae) as a sister group of the clade consisting of all other pentatomoid taxa (including N. *viridula* of Pentatomidae). And family Dinidoridae shows paraphyletic relationship with Pentatomidae. This result is compatible with the result put forward by Garzia et al. in 2008 (Garzia et al., 2008) based on morphology as well as molecular studies of this group.

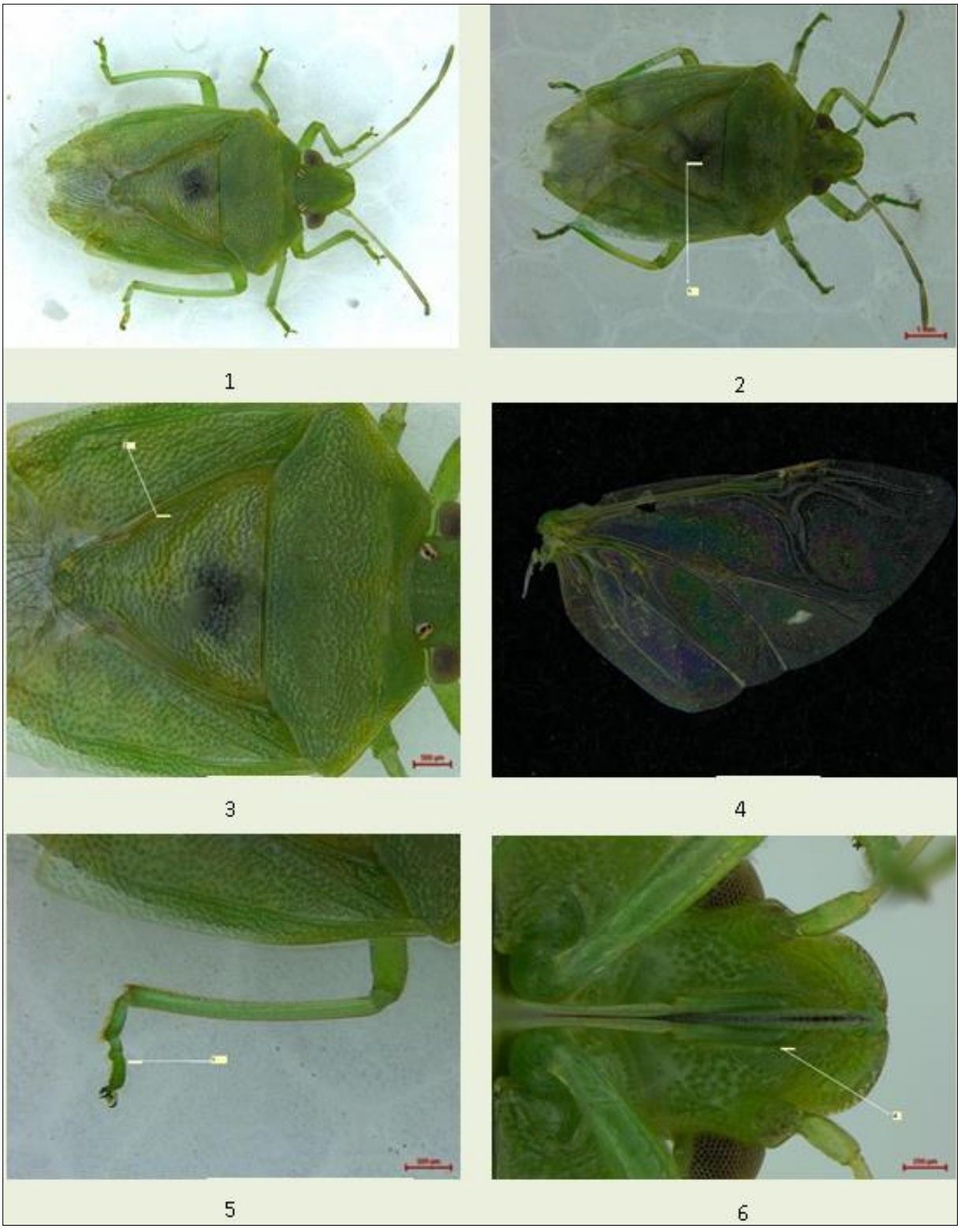

Fig 1-6: Distinguishing characters of Pentatomidae. 1, Whole organism; 2, Scutellum triangular and not completely covering abdomen dorsally; 3, Arrow showing scutellum with frena; 4, Hind wing without spur vein; 5, Tarsus with three segments; 6, Baccula not lobe like and open posteriorly.

Fig 7

Fig 8

Fig 9

Fig 7-9: Distinguishing characters of Dinidoridae. 7, Whole organism; 8, Head ventral view (arrow showing buccula lobe like and closed posteriorly); 9, First segment of labium longer than buccula.

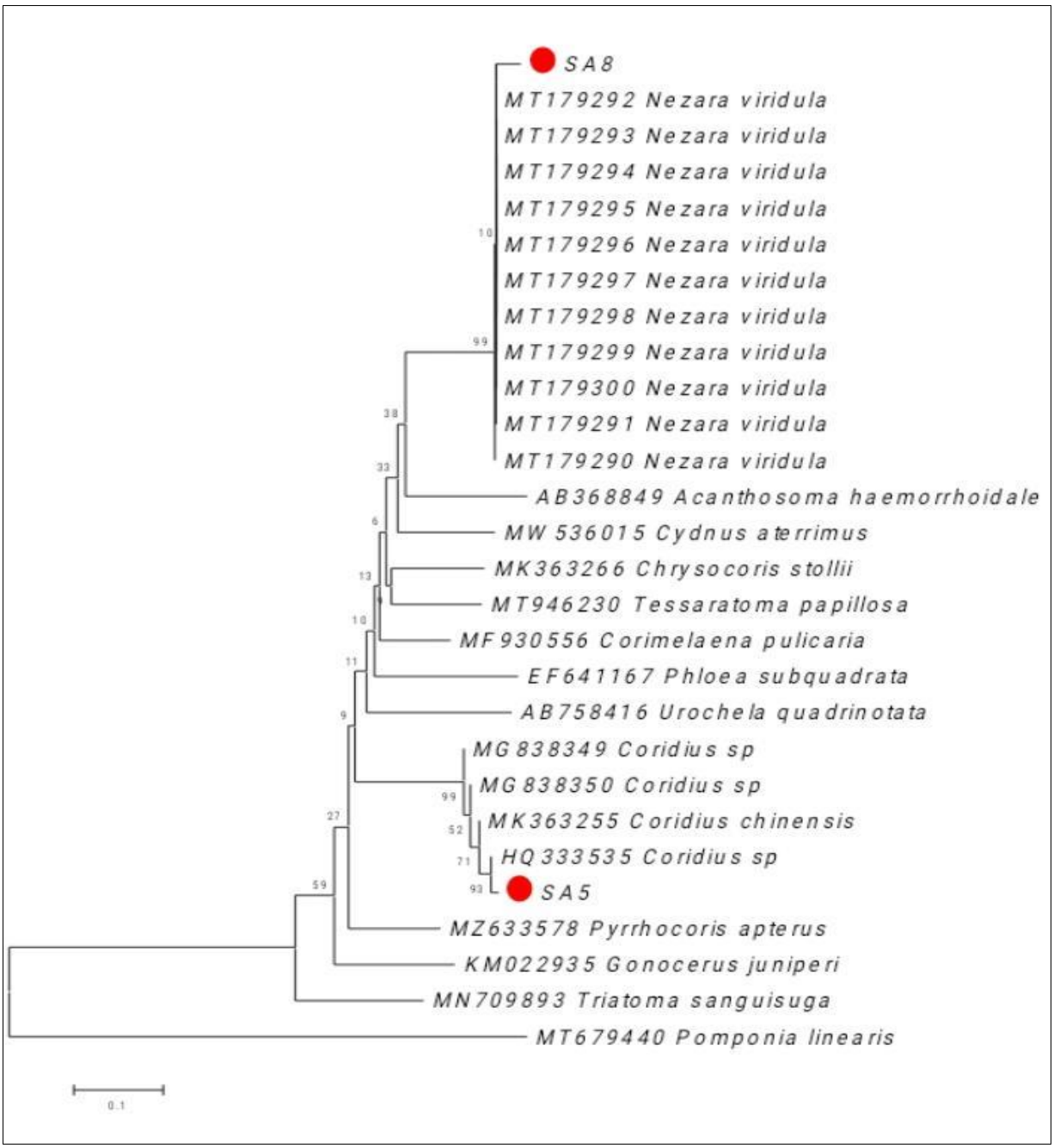

Fig 10: Evolutionary relationship of Pentatomidae (SA8- *Nezara viridula*) and Dinidoridae (SA5- *Coridius chinensis*)

Conclusion

Plant bugs cause harm to various plants by feeding on their nutrients. For instance, *Nezara viridula* is highly polyphagous and has been reported from over 30 plant families (Hoffmann, 1991) [2], whereas *Coridius chinensis* is considered a damaging pest of cucurbit crops. Although both these bugs belong to different families, they have certain similarity in their morphology. This study deals with the DNA barcoding and phylogenetic analysis of two morphologically similar Pentatomoid bugs (SA5 and SA8) of different families; Dinidoridae and Pentatomidae. SA8 is identified as Nezara viridula (family Pentatomidae) and SA5 is identified as Coridius chinensis (family Dinidoridae) based on analysis of mitochondrial COX I gene. Thus, the present study confirms that DNA barcoding is extremely helpful for quick and accurate identification of pests. The phylogenetic analysis shows that the *C. chinensis* is monophyletic with other members of the family Dinidoridae and the family Dinidoridae have paraphyletic relationship with Pentatomidae.

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