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# Integrating Molecular Approaches in Taxonomic Study of Family Rhyparochromid (Hemiptera: Heteroptera): A Review

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***Authors' contributions***

*This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.*

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## ABSTRACT

The family Rhyparochromidae (Hemiptera: Heteroptera) commonly known as Dirt-colored seed bugs, holds over 2000 species globally and is one of the largest families in seed bugs. For a long time, traditional taxonomy has been based on physical traits like body size, wing shape and structure of external genitals or their color patterns. However, depending solely on these morphological features makes it challenging to identify and classify the species. Due to which cryptic species, convergence, and intraspecies variation, diversity and phylogenetic resolution were frequently inaccurate. For instance, the incorporation of molecular tools like DNA barcoding, phylogenetics analysis and CO1 gene as a mitochondrial gene, and ribosomal RNA genes like 16S rDNA and 18S rDNA genes has revolutionized taxonomy and provide insights in species

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identification and classification. This article summarizes the recent studies on molecular strategies for the taxonomy of Rhyparochromidae, which includes the applications of mitochondrial molecular markers for phylogenetic studies. It presents a more integrative view for analysis by molecular tools to facilitate species delimitation, clarify phylogenetic obscurities and provide information on the previously unnoticed biodiversity. Furthermore, the study highlights the advantages, challenges and future prospective for taxonomy by the incorporation of molecular techniques which aids in classification and identification of species of family Rhyparochromidae.

*Keywords: Rhyparochromidae; molecular; markers; Taxonomy; Species; identification; cryptic; speciation; mitochondrial; DNA; barcoding.*

## 1. INTRODUCTION

The family Rhyparochromidae, commonly referred to as dirt-colored seed bugs, constitutes a diverse group within the suborder Heteroptera of order Hemiptera, with more than 2000 described species distributed worldwide. The term *Rhyparochromidae* is derived from Greek words, *rhyparos* means "dirt" and *chromus* means "color" (Mohamed et al., 2013). Mostly rhyparochromids are phytophagous and ground dwelling insects that they are found near ground. They find seeds under leaf litter beneath pants and sometimes in ant nests (Schuh & Slater, 1995). Rhyparochromidae exhibits remarkable morphological and ecological diversity. They are characterized by the segmented body which is divided into three regions -Head, thorax, and the abdomen, along with the jointed appendages and also an exoskeleton which is made up of chitin. The family Rhyparochromidae- commonly known as seed bugs. Biodiversity and distribution of lethaeine seed bugs from Argentina are given by (Dellapé et al., 2015). Rhyparochromids were first established as a sapragenic group, and were considered by many workers to be a subfamily within Lygaeoidea. (Henry, 1997) reclassified the Lygaeoidea and established the family Rhyparochromidae. Interestingly, some species of *Cleradin*, known for their blood-feeding habits, have been reported to occasionally bite humans (Harrington, B.J., 1980; Malipatil, 1981). Bugs of family Rhyparochromidae usually have flattened body shape with distinct color patterns. For example, *Rhyparochromus vulgaris* is distinguished by its black and orange coloration whereas *Raglius alboacuminatus* is characterized by elongated body (Henry, 2004). Adults of *Rhyparochromus saturnius* bugs often ranges from 5 to 10mm. The presence of specific genital organs is crucial for accurate species identification (Henry & Adamski, 1998a). The family Rhyparochromidae includes numerous species that share similar physical traits which makes the traditional methods of taxonomy difficult to identify species. It was based on the morphological features like body shape, color, genitalia, wing pattern for identification. However, presence of cryptic species and there's a lot of hidden diversity within species often leads to misidentification.

Due to the similar morphology and high genetic diversity between species poses a problem in phylogenetic studies of bugs. To solve the problem various molecular techniques, such as DNA barcoding, mitochondrial gene sequencing, and phylogenetics, have emerged as powerful tools that helps in accurate identification. Various mitochondrial markers have been used to identify species and also provide insights in evolutionary studies within species. Molecular markers such as 16S rRNA, 12S rRNA, ND, ATPase are used, among which the protein coding gene mitochondrial genes (CO1) have proven invaluable in recent years, offering insights into phylogenetic relationships (Ratnasingham & Hebert, 2007). Molecular markers are the DNA sequences that help scientists identify species, assess genetic diversity, and infer the evolutionary relationships between species. As these molecular markers provide a stable and consistent means of identification, also distinguish cryptic species. DNA based techniques makes it easier to identify insects at any stage of life (eggs, larva, pupae, adult) without any need for morphological analysis (Mani et al., 2022).

Mitochondrial DNA (mtDNA) are the DNA sequences that can be used in species identification due to the unique features as it contains multiple copies of their genome and easy to extract form degraded specimens. Also, mtDNA is inherited maternally it does not undergo recombination (Avise et al., 1987). mtDNA have both conserved (12S and 16S rRNA genes) and variable regions (CO1, CO11, ND genes) which makes it suitable for various genetic studies. The CO1 gene is widely recognized a standard DNA barcode used to

identify insect species. Mitochondrial DNA is widely used as a molecular marker which helps in study of phylogeny in animals, due to its simple genomic structure (Kaur & Singh, 2020). Through sequence profiling, CO1 gene is a common target gene used in DNA barcoding that allows for rapid and accurate identification of species. CO1 has the ability to assess genetic distance among species which shows the effectiveness of this gene is species identification (Naeem et al., 2020). This mitochondrial DNA molecule comprises 37 genes including 13 protein-coding genes, 22 tRNA genes and 2 rRNA genes, also a control region or AT- rich region is present which is a non-coding region (Wolstenholme, 1992); (Simon et al., 1994a).

In this context, molecular markers have emerged as a powerful tool to supplement morphological data. Molecular techniques used in taxonomy, such as DNA barcoding, Cytochrome oxidase 1 (CO1) gene as a mitochondrial gene and (16S rDNA, 18S rDNA) as ribosomal RNA genes, sequencing and phylogenetic studies, have proven effective tools for resolving problems in taxonomy, distinguish cryptic species, and constructing phylogenetic trees that gives insights in understanding the evolutionary history of species. The first mitochondrial genome for Rhyparochromidae was described as: a complete mitochondrial genome of *Panaorus albomaculatus* (Scott, 1874). This mitochondrial genome is made up of 16,345 base pairs, also consists of 37 genes along with the control regions. Most of the control regions is made up of a large tandem repeat region, and these control regions form a completely different and new patterns that hasn't been observed in other insects (T. Li et al., 2016a). In *p. albomaculatus,* Tandem repeat regions consist of 53 repeating sequences, this represents the highest number of tandem repeats that have been found in any other insect mitochondrial genomes. *Rhyparochromus vulgaris* and *Rhyparochromus saturnius* are distinguished by specific features that facilitate their identification. Rhyparochromus vulgaris is known as ground bug, has a large and elongated body with long legs and a dark wing membrane. *Rhyparochromus saturnius*, named as Palearctic seed bug, was first identified in North America on the basis of collections from 17 counties in California (Scudder, 2016) (Henry & Adamski, 1998b) Thomas J. Henry provides in depth analysis of biodiversity within the suborder Heteroptera, which is commonly known as true bugs. Their work represents an overview of seven infraorders and 91 families, includes their morphological characters, ecological roles, and also provide their role as plant feeders, predators, and indicators of environment health (Henry, 2017). Rhyparochromidae bugs can be identified by using the keys provided by Slater and Baranowski (1990). The paper reports *Pseudopachybrachius vinctus* as a new record for Arkansas and Oklahoma, representing the need for accurate identification methods in these states. Molecular methods provide an in-depth understanding to the similarities and variations among species and even provide the evolutionary relationships (Chordas et al., 2017).

## 1.1 Traditional methods in Taxonomy of Rhyparochromidae

Traditionally, the classification of Rhyparochromidae has relied on external features like body shape, coloration, pattern found on their wings and legs. Tribal classification within subfamily Rhyparochromidae proposes a more refined system to classify species based on morphological analysis. Rhyparochromidae was previously classified as subfamily under Lygaeidae but now it is considered as a separate family (Sweet, 1967). (Kondorosy, 2013), re-examine the taxonomic status and the nomenclature of the east Asian bug *Metochus abbreviates,* they confirm the synonymy of dieuches kreyenbergi Breddin 1906 with M. abbreviarrus through detailed morphological analysis of type specimen.

*Rhyparoclava pyrrhocoroides,* a newly identified genus and species of family Rhyparochromidae discovered in the Montagne de Francais Reserve in northern Madagascar. due to unique morphological characters, including brachyptery (reduced wing development), absence of ocelli, clavate antenna, mostly observed in Rhyparochromidae, (Kment et al., 2016). *Lanchnophorus webbi kondprosy*, sp. Nov., from Tamilnadu, India enhances the taxonomic clarity of Lanchnophorus and also provides valuable insights into diversity and distribution of bugs of family Rhyparochromidae.

Morphological identification is a crucial method for species recognition, especially in case where cryptic species are involved. Traditional methods rely on the physical features such as size, shape and colour of insect's body. Traditional taxonomy also uses identification keys as important tool in identification and classification of species. Identification keys like dichotomous keys helps in taxonomic study of species of insects by using a sequence based on the morphological traits such

as shape and size of body, coloration and other features also. In the taxonomic study of *Cimex hemipterus,* measurement of ratio between length and breadth of their pronotum evaluated to identify and classify the bug. This approach in traditional taxonomy helps to distinguish between closely related species (Zhang et al., 2021).

Morphometrics is another valuable tool in traditional taxonomy that is used for species identification and classification. It involves measuring and analysing the variations in shape and size between species by providing the statistical basis which is helpful in distinguishing between closely related species. Morphological identification of species develops various techniques which enhance the accuracy and efficiency of species identification. Methods for bugs identification and classification ranges from traditional measurements to advanced geometric morphometry and other automated systems are used where digital image processor are used. Geometric morphometrics quantifies the variation in shapes of insects and identifies the major differences among those species which cannot be easily identified. The Encyrtus sasakii complex was analysed with the help of morphometric data and reveals the differences among closely related species (Rudoy et al., 2022). Taxonomic analysis of Torrenticolidae mites combined the morphological characters alongside morphometrics to distinguish between closely related species (Gu et al., 2022). These methods of taxonomy are combined with molecular data to obtain the accurate identification of species and also resolve the uncertainties in species identification. Molecular data when combined with morphometrics can clarify the evolutionary relationships, as in Nearctic ambush bugs, where phylogenetic studies revealed the taxonomic ambiguities in the species (Masonick & Weirauch, 2020). Both morphological and molecular methods were integrated in study of aquatic bugs in Cameroon, revealing 62 identified species (Meyin A Ebong et al., 2016).

Various morphological characters are used in identification of species of family Rhyparochromidae which includes; Segmented Antennal structure and distribution of setae on the body of bugs. The shape and structure of pronotum and scutellum on the dorsal side of body. Structure of wings and legs are also helpful in identification of species. Male and female gentilic structures were also consider in taxonomy of bugs within family Rhyparochromidae. The genus *Paracholula,* consisted of two species*, Paracholula picta* and *Paracholula thoracica,* both recorded form Mexico. By Examining the morphological characteristics, particularly male genitalia, the evolutionary relationship between species were clarified (Peredo & Santacruz, 2014). (Cagatay, 1985) studies on male genitalia of bugs of family Rhyparochromidae from Turkey, including *Plinthisus hungaricus*, *Camptocera glaberrima, Lethaeus cribratisimus, Tropistethus holosericus, Gastrodes grossipes,* and *Scolopostethus thomsoni.* In this study, detailed description of pygophore and paramere and phallus are provided.

### 1.2 Challenges Faced in Morphological Taxonomy

Various challenges are faced during morphological taxonomy that restricts the accurate identification and classification of species. Due to high variation within species, it causes difficultly in identification of the species. In the deep-sea environment, various challenges arise such as limited sampling and higher species diversity that results in numerous species remaining undescribed (Frutos et al., 2022). Because various species within family share same morphological characters. (Gao et al., 2013) reviews the taxonomic history of Rhyparochromidae family, focusing on the Drymini tribe. Various species are morphologically similar that poses challenges in identification, as in case of Tuber brumale, where molecular methods were used to identify cryptic species despite lacking distinct morphological differences (Merényi et al., 2017). This work highlights the challenges faced during the taxonomy of species based on morphology that makes it difficult to identify species. During the procedure of collection and preservation of insects, various specimens were degraded, which makes it challenging to study their morphology. To overcome the challenges, integration of molecular techniques with morphological analysis enhancing the accuracy of taxonomic work.

## 2. MOLECULAR METHODS USED IN SPECIES IDENTIFICATION

### 2.1 DNA-Barcoding for True bugs

Taxonomic ambiguities demonstrated in study titled “Building-up of a DNA Barcode Library for true bugs”, aimed to evaluate the effectiveness of DNA barcoding for the species identification in

true bugs in Germany (Raupach et al., 2014). In this study, researchers examined DNA barcodes from 1742 specimens, representing 457 species across 39 families of Heteroptera. Among these, 21 pairs of species (39 species) showed low nucleotide differences exhibits minimum pairwise kimura 2- parameter (K2P) less than 2.2% distance. Out of these 10 showed zero genetic distance which indicating high genetic similarity among species. Intraspecific divergence was also observed within species that are traditionally recognized as single taxonomic units. 91.5% of true bugs species identified successfully with the help of DNA barcoding. By using shirt and standardized gene regions as internal species tags, DNA barcoding provides rapid and accurate identification of species (Hebert & Gregory, 2005). The (CO1) gene has been integrated into DNA barcoding projects which aim to create a comprehensive library of species-specific sequences for rapid identification of species (Kurata et al., 2024).

In the study titled “Barcoding bugs: DNA-based identification of the True Bugs”, evaluated the effectiveness of DNA barcoding for the species identification in true bugs (Park et al., 2011). The researchers used specimens sourced from the Canadian National collection of insects for their studies from which they analyzed the 5’ region of CO1 gene from 344 species across 178 genera. Less than 2% genetic divergence within species was evaluated. In 77% of species pair belonging to the same genus the minimum genetic distance between them was greater than 3%. Some species show low interspecific divergence, indicating closely related species with less genetic differences.

DNA barcoding also differentiate several morphologically similar aphid species, also revealing cryptic species diversity that makes the CO1 based DNA barcoding is more reliable and essential tools for species identification (Rebijith et al., 2013).

DNA barcoding is an effective tool for identifying true bugs within the infraorder Pentatomorpha from Western Ghats of India includes mitochondrial cytochrome c oxidase 1 (mtCO1) gene sequencing, which serves as highly reliable molecular marker for species identification.

## 2.2 Protein-Coding Genes in Mitochondrial DNA as Molecular Markers

Various protein-coding genes of Mitochondria are used in evolutionary studies of insects. Insect mitochondria contain 13 protein-Coding genes. Based on studies by Zardoya and Meyer, (2000) mitochondrial protein-Coding genes can be classified into three groups: includes ND4, ND5, ND2, CO1 considered as “good”. Genes classified as “Medium” such as COB, ND1, ND6, these provide moderate phylogenetic results. While ATPase 6, ND3, ATPase 8 are considered as “Poor”, as these are less effective. Protein-coding genes have faster evolutionary rates as compared to rRNA genes, due to which these are known as powerful markers for understanding genetic diversity at families, genera and species levels (Mindell & Honeycutt, 1990).

### 2.2.1 CO1 gene; A mitochondrial DNA marker

The (CO1) gene, a mitochondrial DNA gene, widely used in taxonomy and phylogenetics to identify species and understand evolutionary relationships between species. CO1 gene, a protein-coding gene, is regarded as one of the most commonly used as mitochondrial DNA marker in the taxonomy and phylogenetics. It is found in the mitochondrial genome where it plays crucial role in electron transport chain (ETC). CO1 is the standard DNA barcode used in identifying and differentiating species due to its high conservation and species-specific variations (Sureshan et al., 2021), the complete mitochondrial genome of dusky cotton bug, *Oxycarenus laetus* sequenced and analyzed which was collected from two distinct regions in India; Bhatinda (north India) and Coimbatore (south India). Due to its smaller size, ease of amplification and high variability, CO1 is most commonly used. These features make it suitable to distinguish between those species which shares similar characters (Bergmann et al., 2013); (Mandal et al., 2014).

### 2.2.1 Importance, advantages and limitations

**Importance:** Various species share similar morphological features but they are genetically distinct, to identify those closely related species CO1 gene is most commonly used. The complete mitochondrial genome of two closely related venerid species, *Ruditapes phillippinarum* and *R. variegatus* was sequenced by (Tang et al., 2022). CO1 gene is maternally inherited, which provides insight into maternal lineage and also provide insights in evolutionary history (Harrison, 1989). Due to high mutation rate of CO1 gene, it is suitable for study the recent evolutionary divergence among species. CO1 is commonly used as a genetic marker in insects at species level and also other higher levels (Simon

et al., 1994b). CO1 is also used for DNA barcoding across various species of insects. It allows for identification and classification of species, even among those species which possess similar physical traits.

**Advantages:** Due to higher amplification rates of CO1 across diverse taxa makes it useful in species identification. Also, CO1 with a distinctive character of maternal inheritance provides insights into maternal lineages. Mutation rates of CO1 gene is high which is sufficient to detect the recent divergence between species. By integrating CO1 gene with mitochondrial or nuclear markers enhances the reliability of species delimitation and phylogenetic analyses (Jin et al., 2018).

**Limitations:** Although CO1 is considered as best molecular marker for species-level identification, it may not always be effective in resolving deeper evolutionary relationships among species (e.g., family, order). In some cases, intraspecific variation can be higher than expected, which makes it difficult to identify species. For example, few species under genus Apolygus, shared identical CO1 sequences (Jung et al., 2011). To obtain the accurate phylogenetics status, CO1 gene should be combined with the other nuclear markers (e.g., 18S rDNA) also with other mitochondrial genes (e.g., 16S rDNA). Sampling methods can affect the accuracy of CO1 based phylogenetic analysis. Inadequate methods used in sampling of species can lead to incomplete and misleading outcomes (Funk, 1999).

Cytochrome Oxidase (CO1) is found to be best molecular marker for evolutionary studies among all protein-coding genes. (T. Li et al., 2016b), reported the first complete mitochondrial genome sequence for the Rhyparochromidae family, specifically for species *Panaorus albomaculatus.* The genome is 16,345 base pairs longer and consists of the standard set of 37 genes found in animal mitochondria, along with a control region. The control region contains unique large tandem-repeat. Researchers performed a comparative analysis using mitochondrial genomes form closely related species. Variations were observed in control regions, particularly in length and tandem repeats. CO1 sequences were also used to build a phylogenetic tree, which helped to determine the evolutionary relationship within Lygaeoidea superfamily. In these studies, the CO1 gene in Panaorus albomaculatus was highly conserved and a mitochondrial gene structure is observed. It showed AT bias, region which has higher proportion of adenine and thymine. Phylogenetic analysis of CO1 genes supported the monophyletic status of Rhyparochromidae, which determine the distinct evolutionary lineage within superfamily Lygaeoidea. It also revealed high similarity in sequences of DNA among closely related species. These findings further confirmed the effectiveness of CO1 as a molecular barcode for species identification in true bugs.

## 3. MITOCHONDRIAL RIBOSOMAL DNA AS GENETIC MARKERS

Molecular techniques used for classification and identification of bugs particularly involving 16S rDNA and 18S rDNA have become essential tools for identification, classification, and understanding the phylogeny within the species. In insect mitochondria, there are two ribosomal DNA genes: 18S rDNA and 16S rDNA. The large subunit 16S rDNA, is commonly used for studies at lower and intermediate levels. (Xie et al., 2005), investigates phylogenetic relationships within the Lygaeoidea superfamily, which includes Rhyparochromidae family. Nuclear ribosomal DNA sequences like 16S rDNA and 18S rDNA, can be used to understand the phylogeny and evolutionary relationships within species. These nuclear ribosomal DNA sequences are used to analyze the phylogeny of true water bugs (Heteroptera), makes it an effective source of data which helps to resolve the intraordinal phylogenetic problems at superfamily level within Heteroptera (Hua et al., 2009)., have become essential tools for understanding the phylogeny, taxonomy, and species identification within this family.

### 3.1 16S rDNA as Molecular Marker

16S rDNA is a mitochondrial ribosomal gene used for identification of species, as it is highly conserved in bugs. (Hsieh et al., 2020) design a universal DNA- mini barcode primer that targeting 120 bp of mt 16S rDNA gene which is a rapid and more efficient system for identification of insects 16S rDNA is also used for middle categorical levels such as families and genera. 16S rDNA is found in the mitochondrial as it evolves faster than other nuclear genes which makes it suitable to understand the evolutionary relationships among species, because it is composed of highly conserved as well as variable domains (Amit Roy, 2014). 16S rDNA is known for its high variability, it helps to distinguish between closely related (Liu et al., 2007).

### 3.2 18S rDNA as a Molecular Marker

18S rDNA nuclear ribosomal gene is highly conserved. But it evolves much slower than the other mitochondrial genes. It is used in understanding the evolutionary history as it is a part of small subunit of ribosome. Across the higher taxonomic ranks: family and superfamilies, it provides broad phylogenetic results (Campbell et al., 1995). 18S rDNA gene has also been used to understand the phylogenetic relationships between superfamilies, such as Lygaeoidea and Coreidea, (M. Li et al., 2012). 18S rDNA is used in combination with mitochondrial markers such as CO1 and 16S for integrative taxonomy.

## 4. CHALLENGES FACED IN USING MOLECULAR MARKERS

Molecular markers are used for identification and classification of species such as CO1, 16S rDNA and 18S rDNA. However various challenges arise that can affect the accuracy and reliability in results. Due to primer specificity and variation in genetic sequence, various challenges arise that can affect the results. Using CO1 as a molecular marker in species identification and classification often faces challenges with primer specificity. Due to variabilities in prime regions, it causes the difficulties in amplification of desired DNA fragments. Similarly, 16S rDNA and 18S rDNA markers can exhibit the variabilities in sequence, causes complications in DNA amplifications and sequencing, leads to inaccurate and biased data (Pitz et al., 2017). Presence of overlapping intra-specific and interspecific variability, molecular markers face challenges, such overlaps can lead to misidentification of species diversity (Smith et al., 2008).

By the integration of multiple molecular markers like CO1, 16S and 18S rDNA poses significant challenges, because each marker target different organisms leads to difficulty in species detection.

**PCR-RFLP Markers**: These PCR-RFLP Polymerase chain reaction-restriction fragment length polymorphism markers has been used to distinguish closely related species within family. This method involves amplifying the 16S rDNA region and then uses restriction enzymes for digestion of sequence to produce species-specific fragments (Yang et al., 2016). PCR-RFLP offers a quick and precise way to differentiate between two closely related species, which is essential for effective pest management strategies.

**Phylogenetic Analysis:** Molecular data plays important role in understanding the evolutionary relationships between species. Phylogenetic studies use methods such as Maximum likelihood (ML) and Bayesian Inference (BI) to build phylogenetic trees, offering insights into evolutionary relationship between species. These molecular methods of taxonomy help to understand the classifications of species and also reveal the evolutionary lineages within the family. Species identification and classification becomes more accurate by using these methods as compared to the traditional taxonomy. All the families within Lygaeoidea, except for Rhyparochromidae, are monophyletic. Which means that these families have a common ancestor as compared to family Rhyparochromidae, Lygaeoidae are more closely related (Carapelli et al., 2021). Phylogenetic analysis of family Lygaeidae within order Hemiptera is done by utilizing the molecular data to understand the valuable insights into evolutionary history of the family.

*Henryaria*, a newly identified genus within tribe Myodochini of the family Rhyparochromidae, along with description of two new species from Bolivia and Peru (Dellapé et al., 2016). Morphological similarities were described, particularly male genitalia focused, and also compare the newly identified genus *Henryaria* with closely related genera within tribe.

## 5. CONCLUSION

Due to variation within species, traditional methods of taxonomy are challenging. To reduce the complications in species identification, molecular techniques are used in the taxonomy of Rhyparochromidae family. It has significantly improved the identification and classification of species, also helpful in phylogenetic studies. To identify the species various molecular tools used such as, mitochondrial gene (CO1) and nuclear gene sequencing (16S rDNA, 18S rDNA), DNA barcoding and phylogenetic analysis provide deeper understanding of species diversity, which makes easy to differentiate between closely related species and cryptic species. Molecular approaches in taxonomy not only enhance traditional methods but also, they resolve challenges such as morphologically similar species and variations within species. By describing genetic databases, refining standardized markers, and integrating computational tools with traditional taxonomy will be crucial in understanding the species

taxonomy and phylogenetics. Researchers can achieve a deeper understanding in the diversity of Rhyparochromid bugs by combining the morphological and molecular techniques, ultimately contributing in classification of bugs and evolutionary studies would be more accurate.

## 6. FUTURE PERSPECTIVE

In Future research, molecular techniques used in species identification and classification family should focus on sequencing a wider range of specimens, which expands the genetic databases from diverse geographical regions. Molecular data is combined with ecological and behavioral studies that makes it easy to understand the classification of species. Next-generation sequencing methods utilized to explore the evolutionary relationships within species. By combining molecular tools with morphological methods of taxonomy, makes it easier to identify the cryptic species and also provide insights in phylogenetic studies.

## DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

## REFERENCES


Amit Roy, S. R. (2014). Molecular Markers in Phylogenetic Studies-A Review. *Journal of Phylogenetics & Evolutionary Biology*, *02*(02). https://doi.org/10.4172/2329-9002.1000131

Avise, J. C., Arnold, J., Ball, R. M., Bermingham, E., Lamb, T., Neigel, J. E., Reeb, C. A., & Saunders, N. C. (1987). INTRASPECIFIC PHYLOGEOGRAPHY: The Mitochondrial DNA Bridge Between Population Genetics and Systematics. *Annual Review of Ecology and Systematics*, *18*(1), 489–522. https://doi.org/10.1146/annurev.es.18.110187.002421

Bergmann, T., Rach, J., Damm, S., DeSalle, R., Schierwater, B., & Hadrys, H. (2013). The potential of distance-based thresholds and character-based DNA barcoding for defining problematic taxonomic entities by CO 1 and ND 1. *Molecular Ecology Resources*, *13*(6), 1069–1081. https://doi.org/10.1111/1755-0998.12125

Cagatay, N. (1985). Studies on the taxonomy and the morphology of male genitalia of Rhyparochrominae (Heteroptera-Lygaeidae) from Turkey. *Plant Protection Bulletin (Turkey)*, *25*(3).

Campbell, B. C., Steffen-Campbell, J. D., Sorensen, J. T., & Gill, R. J. (1995). Paraphyly of Homoptera and Auchenorrhyncha inferred from 18S rDNA nucleotide sequences. *Systematic Entomology*, *20*(3), 175–194. https://doi.org/10.1111/j.1365-3113.1995.tb00090.x

Carapelli, A., Brunetti, C., Cucini, C., Cardaioli, E., Soltani, A., Amri, M., Mediouni Ben Jemâa, J., Fanciulli, P. P., & Nardi, F. (2021). The mitogenome of the true bug *Nysius cymoides* (Insecta, Heteroptera) and the phylogeny of Lygaeoidea. *Mitochondrial DNA Part B*, *6*(8), 2366–2368. https://doi.org/10.1080/23802359.2021.1951139

Chordas, S. W., Tumlison, R., & McAllister, C. T. (2017). First Report of the True Bug Pseudopachybrachius vinctus (Hemiptera: Rhyparochromidae) for Arkansas and Oklahoma, USA. *Entomological News*, *127*(3), 269–272.

Dellapé, P. M., Melo, M. C., & Donnell, J. E. O. (2015). Biodiversity and distribution of lethaeine seed bugs (Heteroptera, Rhyparochromidae, Lethaeini) from Argentina. *Zoological Studies*, *54*(1), 34. https://doi.org/10.1186/s40555-015-0114-y

Dellapé, P. M., Melo, M. C., & Henry, T. J. (2016). A phylogenetic revision of the true bug genus *Heraeus* (Hemiptera: Rhyparochromidae: Myodochini), with the description of two new genera and 30 new species: P. M. Dellapé *et al*. *Zoological Journal of the Linnean Society*, *177*(1), 29–134. https://doi.org/10.1111/zoj.12362

Frutos, I., Kaiser, S., Pułaski, Ł., Studzian, M., & Błażewicz, M. (2022). Challenges and Advances in the Taxonomy of Deep-Sea Peracarida: From Traditional to Modern Methods. *Frontiers in Marine Science*, *9*, 799191. https://doi.org/10.3389/fmars.2022.799191

Funk, D. J. (1999). Molecular systematics of cytochrome oxidase I and 16S from

Neochlamisus leaf beetles and the importance of sampling. *Molecular Biology and Evolution*, *16*(1), 67–82.

Gao, C., Kondorosy, E., & Bu, W. (2013). A REVIEW OF THE GENUS AROCATUS FROM PALAEARCTIC AND ORIENTAL REGIONS (HEMIPTERA: HETEROPTERA: LYGAEIDAE). *Raffles Bulletin of Zoology*, *61*(2).

Gu, X., Zheng, Y., Li, H., & Guo, J. (2022). A case of integrative taxonomy based on traditional morphology, molecular systematics and geometric morphometrics in the taxonomy of Torrenticolidae (Acari, Hydrachnidiae). *Systematic and Applied Acarology*. https://doi.org/10.11158/saa.27.5.6

Harrington, B.J. (1980). Slaterobius, a new genus of Myodochini from Central America (Hemiptera: Lygaeidae). *Journal of the Kansas Entomological Society*, *53*(3), 523–529.

Harrison, R. G. (1989). Animal mitochondrial DNA as a genetic marker in population and evolutionary biology. *Trends in Ecology & Evolution*, *4*(1), 6–11. https://doi.org/10.1016/0169-5347(89)90006-2

Hebert, P. D. N., & Gregory, T. R. (2005). The Promise of DNA Barcoding for Taxonomy. *Systematic Biology*, *54*(5), 852–859. https://doi.org/10.1080/10635150500354886

Henry, T. J. (1997). Phylogenetic Analysis of Family Groups within the Infraorder Pentatomomorpha (Hemiptera: Heteroptera), with Emphasis on the Lygaeoidea. *Annals of the Entomological Society of America*, *90*(3), 275–301. https://doi.org/10.1093/aesa/90.3.275

Henry, T. J. (2004). Raglius alboacuminatus (Goeze) and Rhyparochromus vulgaris (schilling) (Lygaeoidea: Rhyparochromidae): Two Palearctic bugs newly discovered in North America. *Proceedings-Entomological Society of Washington*, *106*(3), 513–513.

Henry, T. J. (2017). Biodiversity of heteroptera. *Insect Biodiversity: Science and Society*, 279–335.

Henry, T. J., & Adamski, D. (1998a). Rhyparochromus saturnius (Rossi) (Heteroptera: Lygaeoidea: Rhyparochromidae), a palearctic seed bug newly discovered in North America. *Journal of the New York Entomological Society*, 132–140.

Henry, T. J., & Adamski, D. (1998b). Rhyparochromus saturnius (Rossi) (Heteroptera: Lygaeoidea: Rhyparochromidae), a palearctic seed bug newly discovered in North America. *Journal of the New York Entomological Society*, 132–140.

Hsieh, C., Huang, C., Wu, W., & Wang, H. (2020). A rapid insect species identification system using mini-barcode pyrosequencing. *Pest Management Science*, *76*(4), 1222–1227. https://doi.org/10.1002/ps.5674

Hua, J., Li, M., Dong, P., Cui, Y., Xie, Q., & Bu, W. (2009). Phylogenetic analysis of the true water bugs (Insecta: Hemiptera: Heteroptera: Nepomorpha): evidence from mitochondrial genomes. *BMC Evolutionary Biology*, *9*(1), 134. https://doi.org/10.1186/1471-2148-9-134

Jin, Q., Hu, X.-M., Han, H.-L., Chen, F., Cai, W.-J., Ruan, Q.-Q., Liu, B., Luo, G.-J., Wang, H., & Liu, X. (2018). A two-step DNA barcoding approach for delimiting moth species: Moths of Dongling Mountain (Beijing, China) as a case study. *Scientific Reports*, *8*(1), 14256.

Jung, S., Duwal, R. K., & Lee, S. (2011). COI barcoding of true bugs (Insecta, Heteroptera). *Molecular Ecology Resources*, *11*(2), 266–270.

Kaur, R., & Singh, D. (2020). Molecular markers a valuable tool for species identification of insects: A review. *Annals of Entomology*, *38*(1).

Kment, P., Hemala, V., & Baňař, P. (2016). *Rhyparoclava pyrrhocoroides, a new genus and species of autapomorphic Rhyparochromidae with clavate antennae from Madagascar (Hemiptera: Heteroptera)*. https://doi.org/10.5281/ZENODO.5309044

Kondorosy, E. (2013). Taxonomic changes in some predominantly Palaearctic distributed genera of Drymini (Heteroptera, Rhyparochromidae). *ZooKeys*, *319*, 211–221. https://doi.org/10.3897/zookeys.319.4465

Kurata, S., Mano, S., Nakahama, N., Hirota, S. K., Suyama, Y., & Ito, M. (2024). Development of mitochondrial DNA cytochrome c oxidase subunit I primer sets to construct DNA barcoding library using next-generation sequencing. *Biodiversity Data Journal*, *12*, e117014.

Li, M., Tian, Y., Zhao, Y., & Bu, W. (2012). Higher Level Phylogeny and the First

Divergence Time Estimation of Heteroptera (Insecta: Hemiptera) Based on Multiple Genes. *Plos one*, *7*(2), e32152. https://doi.org/10.1371/journal.pone.0032152

Li, T., Yang, J., Li, Y., Cui, Y., Xie, Q., Bu, W., & Hillis, D. M. (2016a). A Mitochondrial Genome of Rhyparochromidae (Hemiptera: Heteroptera) and a Comparative Analysis of Related Mitochondrial Genomes. *Scientific Reports*, *6*(1), 35175. https://doi.org/10.1038/srep35175

Li, T., Yang, J., Li, Y., Cui, Y., Xie, Q., Bu, W., & Hillis, D. M. (2016b). A Mitochondrial Genome of Rhyparochromidae (Hemiptera: Heteroptera) and a Comparative Analysis of Related Mitochondrial Genomes. *Scientific Reports*, *6*(1), 35175. https://doi.org/10.1038/srep35175

Liu, H., Li, Z., Li, S., & Cai, W. (2007). Application of mitochondrial DNA sequences in molecular systematics of true bugs (Hemiptera: Heteroptera). *Entomotaxonomia*, *29*(4), 265–274.

Malipatil, M. (1981). Revision of Australian Cleradini (Heteroptera: Lygaeidae). *Australian Journal of Zoology*, *29*(5), 773. https://doi.org/10.1071/ZO9810773

Mandal, S. D., Chhakchhuak, L., Gurusubramanian, G., & Kumar, N. S. (2014). Mitochondrial markers for identification and phylogenetic studies in insects – A Review. *DNA Barcodes*, *2*(1). https://doi.org/10.2478/dna-2014-0001

Mani, M., Venkatesan, T., & Chethan, B. R. (2022). Molecular Identification of Insect Pests of Horticultural Crops. In M. Mani (Ed.), *Trends in Horticultural Entomology* (pp. 3–47). Springer Nature Singapore. https://doi.org/10.1007/978-981-19-0343-4_1

Masonick, P., & Weirauch, C. (2020). Integrative species delimitation in Nearctic ambush bugs (Heteroptera: Reduviidae: Phymatinae): insights from molecules, geometric morphometrics and ecological associations. *Systematic Entomology*, *45*(1), 205–223. https://doi.org/10.1111/syen.12388

Merényi, Z., Varga, T., Hubai, A. G., Pitlik, P., Erős, Á., Trappe, J. M., & Bratek, Z. (2017). Challenges in the delimitation of morphologically similar species: A case study of Tuber brumale agg. (Ascomycota, Pezizales). *Mycological Progress*, *16*(6), 613–624. https://doi.org/10.1007/s11557-017-1296-y

Meyin A Ebong, S., Petit, E., Le Gall, P., Chen, P.-P., Nieser, N., Guilbert, E., Njiokou, F., Marsollier, L., Guégan, J.-F., Pluot-Sigwalt, D., Eyangoh, S., & Harry, M. (2016). Molecular Species Delimitation and Morphology of Aquatic and Sub-Aquatic Bugs (Heteroptera) in Cameroon. *PLOS ONE*, *11*(5), e0154905. https://doi.org/10.1371/journal.pone.0154905

Mindell, D. P., & Honeycutt, R. L. (1990). Ribosomal RNA in Vertebrates: Evolution and Phylogenetic Applications. *Annual Review of Ecology and Systematics*, *21*(1), 541–566. https://doi.org/10.1146/annurev.es.21.110190.002545

Mohamed, S. K., Allah, S. M. G., Fadel, H. H., Badawy, R. M., & Sawaby, R. F. (2013). Taxonomic review of family Rhyparochromidae (Hemiptera: Lygaeoidea) from Egypt. *Egypt. J. Exp. Biol.(Zool.)*, *9*, 33–60.

Naeem, Z., Masud, S., Hassan, S., & Naeem, M. (2020). Molecular approach for identification of Catla catla using mitochondrial CO1 from Pakistan. *Mitochondrial DNA Part B*, *5*(3), 3000–3003.

Park, D.-S., Foottit, R., Maw, E., & Hebert, P. D. N. (2011). Barcoding Bugs: DNA-Based Identification of the True Bugs (Insecta: Hemiptera: Heteroptera). *PLoS ONE*, *6*(4), e18749. https://doi.org/10.1371/journal.pone.0018749

Peredo, L. C., & Santacruz, J. B. (2014). The genus Paracholula (Hemiptera: Heteroptera: Lygaeoidea: Rhyparochromidae: Rhyparochrominae: Myodochini). *Zootaxa*, *3753*(3). https://doi.org/10.11646/zootaxa.3753.3.5

Pitz, K., Closek, C., Djurhuus, A., Michisaki, R., Walz, K., Boehm, A., Breitbart, M., Kelly, R., & Chavez, F. (2017). Rewards and Challenges of eDNA Sequencing with Multiple Genetic Markers for Marine Observation Programs. *Proceedings of TDWG*, *1*, e20548. https://doi.org/10.3897/tdwgproceedings.1.20548

Ratnasingham, S., & Hebert, P. D. N. (2007). BOLD: The Barcode of Life Data System (http://www.barcodinglife.org). *Molecular Ecology Notes*, *7*(3), 355–364.

https://doi.org/10.1111/j.1471-8286.2007.01678.x

Raupach, M. J., Hendrich, L., Küchler, S. M., Deister, F., Morinière, J., & Gossner, M. M. (2014). Building-Up of a DNA Barcode Library for True Bugs (Insecta: Hemiptera: Heteroptera) of Germany Reveals Taxonomic Uncertainties and Surprises. *PLoS ONE*, *9*(9), e106940. https://doi.org/10.1371/journal.pone.0106940

Rebijith, K., Asokan, R., Kumar, N. K., Krishna, V., Chaitanya, B., & Ramamurthy, V. (2013). DNA barcoding and elucidation of cryptic aphid species (Hemiptera: Aphididae) in India. *Bulletin of Entomological Research*, *103*(5), 601–610.

Rudoy, A., Zhu, C.-D., Ferrari, R. R., & Zhang, Y.-Z. (2022). Integrative taxonomy based on morphometric and molecular data supports recognition of the three cryptic species within the Encyrtus sasakii complex (Hymenoptera, Encyrtidae). *Journal of Hymenoptera Research*, *90*, 129–152. https://doi.org/10.3897/jhr.90.75807

Schuh, R. T., & Slater, J. A. (1995). *True bugs of the world (Hemiptera: Heteroptera): Classification and natural history*. Cornell UNIVERSITY press.

Scott, J. (1874). XXXV.—*On a collection of Hemiptera Heteroptera from Japan. Descriptions of various new genera and species*. *Annals and Magazine of Natural History*, *14*(82), 289–304. https://doi.org/10.1080/00222937408680968

Scudder, G. G. (2016). Rhyparochromus vulgaris (Schilling) (Hemiptera: Heteroptera: Rhyparochromidae): Newly discovered in the interior of British Columbia. *Journal of the Entomological Society of British Columbia*, *113*, 90.

Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H., & Flook, P. (1994a). Evolution, Weighting, and Phylogenetic Utility of Mitochondrial Gene Sequences and a Compilation of Conserved Polymerase Chain Reaction Primers. *Annals of the Entomological Society of America*, *87*(6), 651–701. https://doi.org/10.1093/aesa/87.6.651

Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H., & Flook, P. (1994b). Evolution, Weighting, and Phylogenetic Utility of Mitochondrial Gene Sequences and a Compilation of Conserved Polymerase Chain Reaction Primers. *Annals of the Entomological Society of America*, *87*(6), 651–701. https://doi.org/10.1093/aesa/87.6.651

Slater, J. A., & Baranowski, R. M. (1990). *Lygaeidae of Florida (Hemiptera: Heteroptera)* (Vol. 14, p. 211). Division of Plant Industry, Florida Department of Agriculture and Consumer Services.

Smith, M. A., Poyarkov, N. A., & Hebert, P. D. N. (2008). DNA BARCODING: CO1 DNA barcoding amphibians: take the chance, meet the challenge. *Molecular Ecology Resources*, *8*(2), 235–246. https://doi.org/10.1111/j.1471-8286.2007.01964.x

Sureshan, S. C., Tanavade, R. V., Ghosh, S., Ghosh, S., Sella, R. N., & Mohideen, H. S. (2021). Complete mitochondrial genome sequencing of Oxycarenus laetus (Hemiptera: Lygaeidae) from two geographically distinct regions of India. *Scientific Reports*, *11*(1), 23738. https://doi.org/10.1038/s41598-021-02881-0

Sweet, M. H. (1967). The Tribal Classification of the Rhyparochrominae (Heteroptera: Lygaeidae)1. *Annals of the Entomological Society of America*, *60*(1), 208–226. https://doi.org/10.1093/aesa/60.1.208

Tang, Y., Huo, Z., Liu, Y., Wang, Y., Zuo, L., Fang, L., Zhao, W., Tan, Y., & Yan, X. (2022). Full Mitochondrial Genomes Reveal Species Differences between the Venerid Clams Ruditapes philippinarum and R. variegatus. *Genes*, *13*(11), 2157. https://doi.org/10.3390/genes13112157

Wolstenholme, D. R. (1992). Animal Mitochondrial DNA: Structure and Evolution. In *International Review of Cytology* (Vol. 141, pp. 173–216). Elsevier. https://doi.org/10.1016/S0074-7696(08)62066-5

Xie, Q., Bu, W., & Zheng, L. (2005). The Bayesian phylogenetic analysis of the 18S rRNA sequences from the main lineages of Trichophora (Insecta: Heteroptera: Pentatomomorpha). *Molecular Phylogenetics and Evolution*, *34*(2), 448–451. https://doi.org/10.1016/j.ympev.2004.10.015

Yang, C. Y., Kim, S.-J., Kwon, S.-J., & Ahn, S.-J. (2016). Molecular identification of closely related mirine plant bugs, Apolygus spinolae and A. lucorum (Heteroptera: Miridae) by PCR-RFLP markers. *Journal of*

*Asia-Pacific Entomology*, *19*(3), 729–734. https://doi.org/10.1016/j.aspen.2016.06.015

Zardoya, R., & Meyer, A. (2000). Mitochondrial evidence on the phylogenetic position of caecilians (Amphibia: Gymnophiona). *Genetics*, *155*(2), 765-775.

Zhang, J., Xia, Y., Wang, C., Han, D., Ren, D., Zheng, J., Xu, X., He, Y., & Wang, D. (2021). Morphological and molecular identification of tropical bed bugs from two cities of the Pearl River Delta in China. *Journal of Medical Entomology*, *58*(1), 471–474.



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