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# REVIEW OF MOLECULAR TAXONOMY STUDIES ON COLEOPTERA AQUATIC INSECTS

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**A B S T R A C T**

With millions of species and their life-stage transformations, the animal kingdom makes taxonomy difficult. Insects are the most numerous group of animals, and its taxonomy is primarily based on morphological characters. However, molecular systems have been developed in recent years especially in order to discriminate closely related species and in order to identify the species which have not been distinguished by taxonomic methods currently employed. Over the last ten decades, the use of molecular methods, especially DNA sequence data has had a profound influence on taxonomy. The DNA sequences which are commonly used, occur either in the nucleus of the cell or in organelles such as mitochondria. This is a review article about molecular taxonomy studies on insects, especially Coleoptera aquatic ones.

**Keywords**: Aquatic insects, molecular taxonomy, Coleoptera, nucleus and mitochondrial genes, review.

## INTRODUCTION

Molecular systematic studies have been started in the 1970 for the first time using ribosomal RNA for the classification of bacteria (Fox *et al.,* 1980). In the last 20-25 years, molecular instruments in various organism groups are being used widely for this purpose (Baker and Palumbi, 1994; Sperling *et al.,* 1994; De Salle and Birstein, 1996). A final taxonomic system for the animal kingdom will probably include at least 10 million species partitioned among more than a million genera. Given such high diversity, there is a growing realization that it is critical to seek technological assistance for its initial description and its subsequent recognition (Godfray, 2002; Blaxter, 2003). There are 1,200,000 species of insects in the world and among all insects, less than 3 percent are aquatic beetles. Jäch and Balke. (2008) estimate that there are currently about 18,000 species of water beetle of which 70% have been described. About thirty families have aquatic representatives, 25 of them having at least half of them aquatic. The estimates for the dominant families are, from October 2005, Dytiscidae with 3,908 species, 5,000 being estimated, Hydraenidae (1,380/2,500), Hydrophilidae (1,800/2,320), Elmidae (1,330/1,850), Scirtidae (900/1,700) and Gyrinidae (750/1,000). The Palaearctic (3,350 named as opposed to 3,900 estimated), the Neotropical (2,510/3,900) and the Afrotropical (2,700/3,750) regions have the most species, followed by the Oriental (2,200/3,580) and the Australasian (1,300/2,100), the Nearctic (1,420/1,550) being by far the poorest in terms of diversity (Jäch and Balke, 2008). In this article, many molecular taxonomy studies, was revised up from the past to the present. In this context, not directly related to aquatic insects or some very specific evaluation studies were excluded. The advantages and disadvantages of some methodological differences to ensure a better understanding of the subject was discussed in detail.

The application of DNA data in taxonomy and species diagnosis has aroused a great deal of controversy, but there is general agreement that genetic information is useful for associating different developmental stages of organisms and for identifying partially preserved specimens unsuitable for morphological study (Vences *et al.,* 2005; Wheeler 2004; Will *et al.,* 2005). DNA data provide a character system universal to all life stages with the potential to overcome the problems of working with different semaphoronts. A DNA-based approach has

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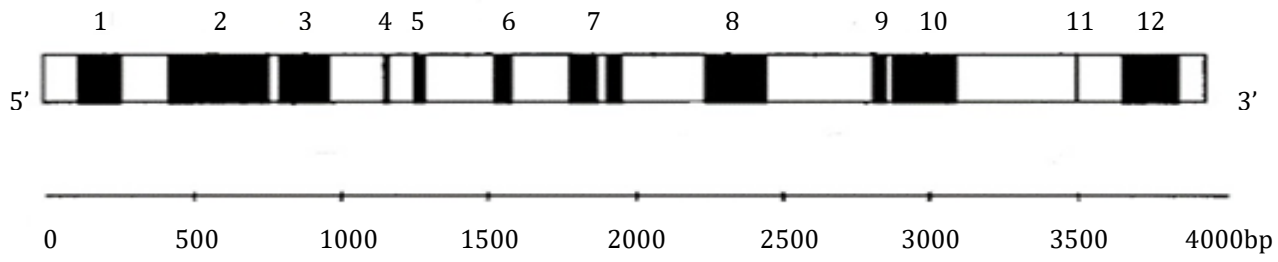
already been used to associate different developmental stages in order to identify agricultural pests and invasive species (Ball and Armstrong, 2006; Harper *et al.,* 2005; Miller *et al.,* 1999; Rao *et al.,* 2006; Scheffer *et al.,* 2006), forensically important insects (Wells and Sperling, 2001), larval parasitoids (Agusti *et al.,* 2005) and endangered species in their early life stages (DeSalle and Birstein, 1996). Initial attempts have also been made to survey larval or mixed larval and adult assemblages with DNA methods (Barber and Boyce, 2006; Paquin and Hedin, 2004). The increasing taxonomic content of DNA databases and rapid sequencing technology now permit tree construction at ever larger scales (Hibbett *et al.,* 2005; Kallersjo *et al.,* 1998; McMahon and Sanderson, 2006; Soltis *et al.,* 1999). However, traditional phylogenetic methodologies struggle to accommodate these huge data sets, whilst newly developed techniques, more capable of coping with largescale analyses, have not become generally established. In the last decade, technical progresses in molecular biology (e.g. Saiki *et al.,* 1985; Mullis *et al.,* 1986) have allowed evolutionary biologists to collect large DNA sequence data sets in a reasonably short amount of time. This has opened the way for extensive studies on the pattern of evolution of several mitochondrial and nuclear genes (Simon *et al.,* 1994) and for using DNA sequences to reconstruct phylogenetic relationships at different taxonomic levels (Thomas *et al.,* 1989; Swofford *et al.,* 1996).

Molecular markers can be divided into DNA markers and protein markers. DNA markers have been widely used due to the disadvantages of allozymes and isozymes which can be referred as protein markers. The thousands of protein-coding genes in the eukaryotic nuclear genome present the richest untapped source of genetic data for phylogenetic research. These genes show a number of favorable properties for phylogenetic analysis (Wiegmann *et al.,* 2000). They evolve more slowly and are less prone to base-composition bias than mitochondrial markers (Lin and Danforth, 2004), and they typically present fewer alignment issues than ribosomal genes (Danforth *et al.,* 2005). On the other hand, these genes do not always contain reliable priming sites, they can be present in multiple paralogous copies, and they may contain lengthy introns that complicate amplification, alignment, and sequencing (Sanderson and Shaffer, 2002). Wild and Maddison. (2008) used the nuclear protein-coding genes for beetle systematics. After screening 24 genes for phylogenetic potential, they selected eight of these for sequencing across 31 test taxa of coleoptera.

Eukaryotic nuclear genes encoding for ribosomal RNA subunits are organized intandemly repeated units which consist of the genes for the 18S, the 5.8S and the 28SrRNA subunits, separated by transcribed (ETS, ITS1 and ITS2) and non-transcribed (IGS) spacers. The 28S subunit is the largest one and it has been shown to be a mosaic of core regions and hypervariable ''expansion segments'' (Clark *et al.,* 1984), also called ''Divergent Domains'' (Hassouna *et al.,* 1984). Core segments have precise counterparts in prokaryotic rRNAs. They are thought to play an essential role in the ribosome function and have very conserved nucleotide sequences even among distantly related taxa. Divergent Domains do not have precise counterparts in prokaryotic rRNAs and they vary considerably across taxa in both primary sequence and length. Traditionally, 12 divergent domains are recognized in metazoan 28S rRNA which have been named D1 to D12 (Hassouna *et al.,* 1984; Michot *et al.,* 1984). The D7 domain can be furtherly divided into the domains D7a and D7b (Hassouna *et al.,* 1984). Single-stranded rRNA sequences have the property of folding, bending and pairing within themselves using stretches of complementary sequence. Therefore they assume a secondary structure consisting of paired regions, called stems, interrupted with unpaired regions (loops and bulges). Map of 28S rDNA of Drosophila melanogaster (Hancock *et al.,* 1988) with divergent domains indicated in black (Figure 1.).

Figure 1. Map of 28S rDNA of Drosophila melanogaster.



Within beetles sequences of 18S rDNA were used to reconstruct phylogeny of Adephaga (Shull *et al.,* 2001), Hydradephaga (Ribera *et al.,* 2002) and Carabidae (Maddison *et al.,* 1998), and to clarify the interrelationships between the suborders of Coleoptera (Caterino *et al.,* 2002). It contains both slow- and fast-evolving sections and is potentially useful for resolving relationships over a wide hierarchical range (Ribera *et al.,* 2002). This gene also yielded a good resolution within Histeridae, as more than the ′normal′ sequence variation was found within this taxon (Caterino and Vogler, 2002). However, it should also be mentioned that in some studies the 18S rDNA could not resolve phylogenetic relationships sufficiently, e.g. basal relationships within carabids (Maddison *et al.,* 1999) or subfamilial relationships in Curculionidae (Marvaldi *et al.,* 2002). The 5′ region of the 28S rDNA was used to resolve the phylogenetic relationships within subgroups of Carabidae (Kim *et al.,* 2000; Cryan *et al.,* 2001) and Curculionoidea (Sequeira *et al.,* 2000). Ribosomal RNA genes remain among the most widely used phylogenetic markers and therefore techniques for their analysis at this scale are particularly important. In insects, the small subunit (SSU) rRNA gene has been the dominant marker (Chalwatzis *et al.,* 1996; Kjer, 2004; Pashley *et al.,* 1993; Wheeler *et al.,* 2001; Whiting *et al.,* 1997), but this gene is affected by great length variability and high variation in molecular rates, exacerbating the difficulty of finding optimal trees when numbers of taxa increase.

Metazoan mitochondrial DNA (mtDNA) occurs as a double-strand, circular molecule, ranging in size from approximately 14–39 kb that encodes 13 protein-coding genes (COI-III, Cytb, ND1-6, ND4L, ATP6, and ATP8), 2 rRNA genes (16S and 12S rRNA), and 22 tRNA genes (Wolstenholme, 1992) (Figure 3.). Additionally, it contains the adenine (A) + thymine (T)-rich region, which serves as the origin of heavy-strand mtDNA replication in vertebrates (Brown, 1985). The complete nucleotide sequences of insect mtDNA have been determined in 19 species including two coleopterans so far. Also, thousands of partial mitochondrial (mt) gene sequences from insects are found in GenBank and sequence variations of various mt regions have been used to gain information on the population genetic structure and/or evolutionary relationships of diverse insect species (Bae *et al.,* 2001; Besansky *et al.,* 1997; Kim *et al.,* 2000a; Zhang *et al.,* 1995). The number of complete mtgenomes has steadily been on the rise with the technical feasibility of sequencing their entirety (Hwang *et al.,* 2001; Yamauchi *et al.,* 2004). This increasing availability of mtgenome data invites comparative study. In addition to the large amount of nucleotide data that is useful for deep-level phylogenetic studies (Gray *et al.,* 1999; Nardi *et al.,* 2003; Cameron *et al.,* 2004; Cameron *et al.,* 2006; Cameron *et al.,* 2007), mtgenomes possess a number of evolutionarily interesting features such as length variation (Boyce *et al.,* 1989), altered tRNA anticodons or secondary structures (Steinberg and Cedergren, 1994; Eddy, 2002), atypical start codons (e.g., Lavrov *et al.,* 2000), base compositional bias (Gibson *et al.,* 2004; Gowri-Shankar and Rattray, 2006), codon usage (Jia and Higgs, 2007), and gene rearrangement (Zhang and Hewitt, 1997; Shao and Barker, 2003 ; Mueller and Boore, 2005). Some of these features appear to be lineage specific (Dowton *et al.,* 2002); however, this insight can only be obtained from comparative analysis at various taxonomic levels. The analysis of full mitochondrial genomes has been established as a powerful approach to elucidate deeper-level relationships among vertebrates (e.g., Zardoya and Meyer, 1996; Meyer and Zardoya, 2003; Murataa *et al.,* 2003) and also among Arthropods (e.g., Nardi *et al.,* 2003; Masta *et al.,* 2009).

The COII is one of the most frequently used mitochondrial genes in phylogenetic analyses. A considerable amount of sequence information is available for this gene in several arthropods, and especially in insects (Liu and Beckenbach, 1992; Simon *et al.,* 1994). Extensive data have also been obtained in Collembola (Carapelli *et al.,* 1995; Frati *et al.,* 1997a) where the COII gene was found to be useful to reconstruct relationships between species and genera of Arthropleona. Species in a variety of animal groups have been discriminated reliably using different fragments of the mitochondrial gene, cytochrome c oxidase 1 (COI) (Hebert *et al.,* 2003a, 2004a, b; Hogg & Hebert 2004). Potential limitations of COI-based DNA barcoding: DNA barcoding using COI will be unable to provide accurate species identification in some cases. 1) COI is a mitochondrial gene, and mitochondrial genes typically are inherited maternally in animals. F1 hybrids would be indistinguishable from their maternal parent, but nuclear genes could be used to confirm hybrid status where hybridization is suspected. However, given the relative rarity of natural hybrids between animal species, COI should provide a reliable species

identification system for most species. 2) Very young species pairs might be difficult to identify using a COI-based system. This problem may be particularly noticeable if the species have ancestrally polymorphic mitochondrial haplotypes that do not sort according to subsequent speciation events (Funk and Omland, 2003). 3) Identifications using DNA barcodes (like identifications using morphology) will not work successfully for all species. However, the deep genetic divergences between most congeneric taxa suggest that such misidentifications will be relatively infrequent among the Ephemeroptera, and other studies have confirmed that this conclusion is probably general in the animal kingdom (Hebert *et al.,* 2003b; Hogg and Hebert, 2004). 4) The goal of profile-sequence databases is to include as much taxonomic coverage as possible. However, species identifications will not be possible if the specimen for which identification is sought is not represented in the profile- sequence database. In such cases, the COI profile should provide the next-highest level of identification (e.g., genus, subfamily, or family).

Given the success of the COI profile in identifying mayflies and other insect taxa (Lepidoptera: Hebert *et al.* 2003a, Collembola: Hogg and Hebert, 2004), the potential for successful identification of many other aquatic insect taxa using COI is extremely high. Taxonomic expertise is currently limited, and morphological identification is often fraught with difficulties (e.g., identifcation of eggs and early instar larvae, damaged specimens, or fragments of specimens). Thus, a DNA-based identification system would have significant benefits for aquatic research. In particular, a DNA-based system could provide an important tool for species identification in biomonitoring. The need for species-level identification in biomonitoring is contentious (see Bailey *et al.,* 2001; Lenat and Resh, 2001), but DNA barcoding could provide the option of species-level identification when taxonomic discrimination at the species level is warranted. It could also ensure uniform quality of taxonomic results in studies where the quality of taxonomic data might be compromised by the inability to identify early instars, damaged specimens, or fragments of specimens (Stribling *et al.,* 2003). Moreover, the increased taxonomic resolution delivered by DNA barcoding would provide more sensitive measures of the magnitudes and types of environmental impacts (Lenat and Resh, 2001). In summary, DNA barcoding can provide a powerful supplement to the traditional morphological approach to species identification. In some cases (e.g., aquatic biomonitoring), DNA barcoding systems (i.e., microarrays) may be developed to automate taxon identification as a means to provide rapid, efficient, and consistently accurate identifications. However, we stress that DNA barcoding is not meant to replace traditional taxonomic approaches. In fact, DNA barcoding cannot be accomplished without the involvement and expertise of taxonomists who can identify specimens from which reference sequences are obtained and who can deal with taxonomic issues resulting from the discovery of provisional species based on significant genetic divergences (Ball and Hebert, 2005). Several molecular systematic studies in arthropods showed that these genes (COI and COII) evolve at an appropriate speed for reconstruction of phylogenies at the generic level (e.g., Vogler and DeSalle, 1993; Brower, 1994; Brown *et al.,* 1994a; Funk *et al.,* 1995; Emerson and Wallis, 1995; Köpf *et al.,* 1998; Caterino and Sperling, 1999; Galia´n *et al.,* 1999; Gadau *et al.,* 1999; Ståhls and Nyblom, 2000) and sometimes even at the family level (Brown *et al.,* 1994b; Miura *et al.,* 1998; Dobler and Müller, 2000). Summary of recent phylogenetic studies on aquatic insects based on molecular markers is shown in Table 1.

**CONCLUSION**

Among all living organisms, insects are the most numerous group in terms of the number of species. Despite this, the number of taxonomists working with classical methods is decreasing day by day (Hammond, 1992; Hawksworth and Kalin-Arroyo, 1995). One of the biggest challenges faced taxonomists who working with insects are type of the species witch need to control of stored insects in the museum in comparison with the private collections materials. This is often not possible due to various difficulties or takes a long time. In particular, these disadvantages preclude making effective results where time is important, such as agricultural works. All these obstacles to be overcome before, a researcher is to go beyond problematic taxonomic groups as a more difficult obstacles. For example, diagnosis of all life stages of insects, especially the immature stages (eggs, larvae, nymphs and pupae) often is not possible. In addition, sex differences, sibling species and etc. are the frequently encountered problems. On the other hand, varying degrees of variation, it is quite difficult to diagnose insect species (Traugott *et al.,* 2008; Baer *et al.,* 2004; Desneux *et al.,*

2009a). In front of all these difficulties, molecular taxonomy studies with advancing technology, continues to increase every day (Brown *et al.,* 1979; Bucklin *et al.,* 2007; Hebert *et al.,* 2003a). Besides solving many of the problems mentioned above, molecular systematics also provides some additional conveniences related insect groups that are worked by researchers. In these cases, many researchers have begun to use the remedy of molecular systematics (van Veen *et al.,* 2003; Chen *et al.,* 2006; Walton *et al.,* 1991; Gariepy *et al.,* 2008).

In the developed countries to uses of these techniques not only diagnose the target organism or insects phylogeny, but also the DNA library is the creation of natural living resources. However, molecular taxonomy studies are not particularly planed and so does not go beyond the information pollution. Molecular systematics methods are growing and being used rapidly around the World. Apparently, similar investigations going to be gradually increase continuously not only for aquatic insects but also for entire vivid groups in the near future.

Table 1. Several recent molecular systematic studies on aquatic insects.

| | | | | |
|---|---|---|---|---|
| Whiting *et al.,* | 1997 | Holometabolous Insect (involves aquatic insects) | Molecular and morphology | 18S and 28S |
| Caterino *et al.,* | 2002 | Coleoptera (include aquatic and terrestrial families) | Molecular | 18s rDNA |
| Ribera *et al.,* | 2002 | Hydradephagan | Molecular | 18S rRNA |
| Jordan *et al.,* | 2003 | Damselfly | Molecular | mitochondrial protein-coding genes (cytochrome oxidase II, A6, A8) and two mitochondrial tRNAgenes (lysine and aspartic acid) |
| Korte *et al.,* | 2004 | Staphyliniform (involves aquatic families) | Molecular | 18S and 28S rDNA |
| Caterino *et al.,* | 2005 | Staphyliniformia (Hydrophiloidea and Staphylinoidea) | molecular and morphological | 18S rDNA |
| Jordan *et al.,* | 2005 | Odonata: Coenagrionidae | Molecular | EF-1α Gene and COII |
| Bernhard *et al.,* | 2006 | Hydrophiloidea | Molecular | SSU rDNA and LSU rDNA, 12S rDNA, 16S rDNA, COI, COII |
| Çiampor & Ribera | 2006 | Elminae ( Coleoptera) | Molecular and morphology | 18S rRNA and (ribosomal unit + tRNAleu + 5' end of the NADH dehydrogenase1) cytochrome b and COI |
| Hayashi & Sota | 2008 | Coleoptera: Psephenidae | Molecular and morphology | COI |
| Bernhard *et al.,* | 2009 | Hydrophiloidea | molecular data and morphological characters of adults and immature stages | Nuclear SSU and LSU mitochondrial rrnS, rrnL, cox1 and cox2 genes. |
| Hayashi & Sota | 2010 | Coleoptera: Elmidae | Molecular and morphology | cox1 |
| Mađarıç *et al.,* | 2013 | Hydrophilidae | Molecular | 28S rRNA, 18S rRNA, 16S rRNA, 12S rRNA, COI and COII |
| Short & Fık´A C˘ek | 2013 | Hydrophilidae | Molecular | COI , COII and 16S 18S, 28S and arginine kinase |

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