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Molecular phylogenetics of Oestroidea (Diptera: Calyptratae) with emphasis on Calliphoridae: Insights into the inter-familial relationships and additional evidence for paraphyly among blowflies

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ABSTRACT

The superfamily Oestroidea, comprising ~15,000 species, is a large and ecologically diverse clade within the order Diptera. Among its six commonly recognized families, Calliphoridae seems to be crucial for understanding evolutionary relationships in the group, as it is recognized as a controversial paraphyletic grouping. To further investigate this matter, the ITS2, 28S, COI and 16S regions were used to infer phylogenetic relationships in Oestroidea with maximum-parsimony (MP), maximum-likelihood (ML) and Bayesian inference (BI) methods. For the BI analyses, a deep evaluation of different data partitioning strategies was conducted, including consideration of structural conformation (ITS2 and 16S) and codon position (COI) information. Results suggest the existence of two main clades in Oestroidea: (Tachinidae + Mesembrinellinae) and (Rhiniinae, (Sarcophagidae + Calliphoridae *sensu stricto*)). Oestridae was recovered as sister group of the remaining Oestroidea in the MP trees while it was placed closer to the (Rhiniinae + Sarcophagidae + Calliphoridae *sensu stricto*) group in the ML and BI trees. A paraphyletic Calliphoridae was recovered, confirming the exclusion of Rhiniinae, a clade recently promoted to family status and therefore already excluded. Mesembrinellinae could also be considered a distinct group apart from Calliphoridae, although further studies are required. Consideration of structural and codon position information led to a significant increase in the log-likelihoods of the analyses, which were accompanied by small changes in the inferred topologies, branch lengths and posterior probability support values. However, as model complexity increases, so does uncertainty across the estimated parameters, including tree topologies, and phylogenies inferred under very parameter-rich models may be less reliable even when possessing higher log-likelihoods.

1. Introduction

The family Calliphoridae (Diptera: Calyptratae: Oestroidea), whose members are commonly known as blow flies, is a very diverse and heterogeneous group comprising approximately 1500 species in a worldwide distribution (Pape et al., 2011). The family is better known for its saprophagous and myiasis-causing members in the subfamilies Chrysomyinae, Calliphorinae and Luciliinae (de Azeredo-Espin and Lessinger, 2006; Stevens and Wallman, 2006; Stevens et al., 2006), which have synanthropic habitats and great importance in forensic, veterinary, medical and economic issues (Zumpt, 1965; Guimarães et al., 1983; Hall and Wall, 1995; Amendt et al., 2004). However, the group encompasses an even greater diversity of feeding habits and breeding environments, including hematophagous parasitism of birds and mammals (e.g. species of *Protocalliphora*, *Trypocalliphora* and *Auchmeromyia*), parasitism of terrestrial gastropods (e.g. species of *Melanomya*, *Melinda* and many species in the subfamily Amenniinae) and earthworms (e.g. species of *Bellardia* and *Pollenia*) and close associations with termites' and ants' nests (e.g., species of *Bengalia*, *Tricyclea*, *Hemigymnochaeta* and *Termitocalliphora* and some species of Rhiniinae).

Historically, Calliphoridae has been a controversial group concerning both its composition and its monophyletic status. Regarding family monophyly, Lehrer (1970), Rognes (1991), McAlpine (1989) and Pape (1992) provided corroborating evidence (although recognizing its fragility), but both Hennig (1973) and Griffiths (1982) have noted lack of support for it. More recently, Rognes (1997) and Kutty et al. (2010) have provided evidence for

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non-monophyly based on analyses of morphological and molecular characters, respectively.

The classification into subfamilies and tribes is also controversial and the number of subfamilies attributed to Calliphoridae is variable, ranging from two (Shewell, 1987: Calliphorinae, with the tribes Calliphorini, Polleniini, Angioneurini and Luciliini; and Chrysomyinae, with the tribes Chrysomyiini, Rhiniini and Phormiini) to 13 (Rognes, 1986, 1991, 1997: Chrysomyinae, Calliphorinae, Luciliinae, Toxotarsinae, Melanomyinae, Auchmeromyinae, Bengaliinae, Polleniinae, Mesembrinellinae, Phumosiinae, Rhiniinae, Helicoboscinae and Ameniinae), with some intermediate schemes (e.g., Hennig, 1973: Calliphorinae, Chrysomyinae, Mesembrinellinae, Ameniinae and Rhiniinae).

The controversially monophyletic status of Calliphoridae, with recent evidence of paraphyly, allied with a currently poor understanding of Oestroidea interfamilial relationships, implies the possibility that the family, as traditionally considered, could be further divided into smaller groups with variable and uncertain placements among the remaining oestroid families. This makes Calliphoridae the key family for understanding the evolution and phylogeny of the Oestroidea (McAlpine, 1989; Rognes, 1997).

The Oestroidea (Diptera: Calyptratae) is a group commonly accepted to be monophyletic (Griffiths, 1972; Hennig, 1973; McAlpine, 1989; Pape, 1992; Rognes, 1997). It is formally recognized to contain five other families besides Calliphoridae (McAlpine, 1989; Pape and Thompson, 2010): (1) Mystacinobiidae, with a single described species, *Mystacinobia zelandica*, which lives in close association with the bat *Mystacina tuberculata*; (2) Rhinophoridae (~170 spp.), whose members are, for the species of known biology, parasitoids of woodlice; (3) Oestridae (~170 spp.), whose all known species are parasites of mammals in their larval stage; (4) Tachinidae (~9600 spp.), one of the largest families in Diptera whose larvae are parasitoid of other arthropods; and (5) Sarcophagidae (~3000 spp.), a group whose larvae are mostly known for their necrophagous habit, although some of them are parasites of mammals or parasitoids of other invertebrates.

With the exception of Calliphoridae, all currently-recognized families of Oestroidea have well corroborated monophyletic status (Oestridae: Wood, 1987; Pape, 1992, 2001; Tachinidae: Rognes, 1986; Wood, 1987; Pape, 1992; Tschorsnig and Richter, 1998; Stireman et al., 2006; Sarcophagidae: Pape, 1992, 1996; Kutty et al., 2010), although the monophyly of Rhinophoridae has currently been corroborated only by larval characters (Pape, 1986, 1992; Pape and Arnaud, 2001).

The number of families in Oestroidea was recently increased by the promotion of Rhiniidae, formerly a subfamily of Calliphoridae with ~370 described species (Pape et al., 2011), which is now accorded valid family status in the Biosystematic Database of World Diptera (Pape and Thompson, 2010). Other calliphorid subfamilies had already been proposed to be elevated to family status, such as the Mesembrinellinae (Guimarães, 1977) and the Bengaliinae (Lehrer, 2005), although there is still much controversy with these classifications and further studies are required.

The works of Rognes (1997) and Kutty et al. (2010), the later combining the information of a broad range of molecular markers from a very large dataset of Calyptratae species, have provided evidence for non-monophyly of the Calliphoridae and proposed different placements for the para/polyphyletic groups. However, there is still a large number of different phylogenetic hypotheses for relationships between oestroid families and calliphorid subfamilies, some of them poorly supported, highlighting the need for further studies.

Additionally, as the work of Kutty et al. (2010) – the largest study available so far on this subject – could not comprise an extensive evaluation of the effects of different phylogenetic inference methods in the inferred trees, in particular the influence of different data partitioning strategies in a Bayesian framework, due to the prohibitive nature of the very large assembled dataset, a more diverse investigation on this subject is still lacking.

In this context, this work provides a molecular phylogenetic analysis of interfamilial relationships in the Oestroidea, with emphasis on the placement of some calliphorid subfamilies, based on both nuclear and mitochondrial molecular markers, using different approaches to phylogenetic inference. Taking advantage of the robustness of the Bayesian framework regarding the use of complex and parameter-rich models, different data partition strategies were evaluated using both sequence- and secondary structure-based substitution models, and their impacts on the estimated topologies and overall support of the trees were assessed. Implications for the accuracy of the phylogenetic inference process when using parameter-rich models were then discussed.

Results described here complement the work of Kutty et al. (2010), giving an independent evaluation of some of their results and, therefore, contributing to a deeper and more confident understanding of the phylogeny of Oestroidea.

2. Materials and methods

2.1. Specimens and DNA extraction

The 56 specimens used in the molecular phylogenetic analyses are listed in Table 1. Calliphorid subfamilies were classified according to Rognes (1986, 1991, 1997), including the Rhiniinae as a subfamily, which has now family status, recognizing its phylogenetic independence. The term Calliphoridae *sensu stricto* (Calliphoridae *s.s.*) was used here to designate the Calliphoridae excluding Rhiniinae and Mesembrinellinae, whereas Calliphoridae *sensu lato* (Calliphoridae *s.l.*) was used to designate the traditional clade composed by (Calliphoridae *s.s.* + Rhiniinae + Mesembrinellinae).

Taxon sampling included 8 of the 13 calliphorid subfamilies recognized by Rognes (1997) and 4 of the 6 oestroid families currently recognized (here excluding Rhiniidae) (McAlpine, 1989). Species of the superfamilies Muscoidea and Hippoboscoidea were used as outgroups.

DNA extraction for frozen specimens was carried out using a phenol/chlorophorm protocol (Infante and Azeredo-Espin, 1995), whereas for dried and ethanol-preserved specimens both the DNAzol reagent (Invitrogen) and the Spin Tissue Mini-Kit (Invitek) were used.

2.2. PCR amplification, DNA cloning and sequencing

Four DNA regions were amplified by PCR and sequenced: (1) the complete region of the internal transcribed spacer 2 (ITS2), (2) a portion of the 5′ region of the 28S ribosomal subunit, both from the nuclear ribosomal DNA (rDNA) cluster, (3) the 5′ region of the cytochrome c oxidase subunit I (COI) and (4) the 3′ portion of the 16S rDNA, both from the mitochondrial genome.

PCR reactions for the ITS2 region were conducted with 10 mM Tris–HCl (pH 8.8), 50 mM KCl, 2 mM $MgCl_2$, 80 μM dNTPs, 0.4 μM 5.8S primer (5′-ATCACTCGGCTCGTGGGATTCGAT-3′), 0.4 μM 28S primer (5′-GTTAGTTTCTTTTCCTCCCCT-3′), 2.5U *Taq* DNA polymerase (Fermentas) and 1–2 μg of extracted DNA for a 25 μL reaction. PCR reactions were performed with an initial denaturation step of 95 °C for 3 min, followed by 35 cycles of 95 °C for 1 min, 55 °C for 45 s and 72 °C for 2 min, and a final elongation step of 72 °C for 3 min.

PCR amplifications for the remaining regions were conducted with 10 mM Tris–HCl (pH 8.8), 50 mM KCl, 1 mM $MgCl_2$, 80 μM dNTPs, 0.4 μM forward primer, 0.4 μM reverse primer, 2.5U *Taq* DNA polymerase (Fermentas) and 1–2 μg of extracted DNA for a