

## 1. PHD PROJECT DESCRIPTION (4000 characters max., including the aims and work plan)

### Project title: The phylogenetic relationships within Muscidae (Diptera): a perspective illuminated by phylogenomic and immature stages morphology data

#### 1.1. Project goals

The main goals of the project are to 1) resolve relationships at shallow, intermediate and deep taxonomic levels within the family Muscidae by a large-scale phylogeny reconstruction with the application of phylogenomic approaches; 2) describe for the first time and/or revise immature stages morphology of Muscidae representing major evolutionary lineages; and 3) incorporate data from immature stages to find morphological corroboration for specific nodes on phylogenetic trees, especially those at odds with adult morphology based classification.

#### 1.2. Outline

The fly family Muscidae, with c. 6 000 species, is an excellent model to test various evolutionary hypotheses because of their extraordinary variety of morphologies, life histories and behaviour in both the immature and adult stages, the strong selective pressure on larval stages and a likely rapid Tertiary radiation. However, a vital first step in exploring evolutionary issues is the establishment of a robust phylogenetic hypotheses. The interpretation of Muscidae as a family and relationships within the group, particularly their classification into higher-level taxa, has been a matter of debate for many years [1,2]. Although significant progress in muscid classification has been made thanks to the incorporation of molecular data, many parts of the resultant trees still remain unresolved. This is because: 1) the traditionally recognized subfamilies were either not monophyletic or their monophyly was strongly affected by different approaches to phylogenetic reconstruction; and 2) significant differences in statistical support for relationships obtained from different analytical approaches. One way to overcome these problems is to generate genomic datasets by means of Next Generation Sequencing (NGS) and employ various phylogenetic approaches [3]. Although details of the morphology of the immature stages of insects have been recognised as valuable sources of phylogenetic information, most studies have incorporated solely adult characters [4], and this is also the case for the Muscidae. Thus, it may prove rewarding for muscid classification to exploit data from immature stages in order to assess conflicting topologies.

#### 1.3. Work plan

- 1) Collecting material for molecular and morphological analyses: use of newly collected/reared samples and/or specimens originating from Museum collections (up to 300 terminal taxa).
- 2) Application of the state-of-the-art phylogenomic methods for phylogeny reconstruction: ultra-conserved elements (UCE) to sample conserved elements of genome together with flanking variable regions to reconstruct higher-level relationships, and restriction-site associated DNA sequencing (RAD-seq) for intrageneric relationships.

Construction of family-specific probes set UCE based on publicly available genomes of muscid species and newly sequenced genomes.

- 3) Phylogeny reconstruction by means of concatenation and coalescence approaches.
- 4) Examination of larval morphology with use of a combination of light microscopy, confocal laser scanning microscopy (CLSM) and scanning electron microscopy (SEM) methods.
- 5) Comparison of newly obtained phylogenetic hypotheses with those based on traditional morphological taxonomy.
- 6) Preparation of manuscripts and publication of obtained results.

#### **1.4. Literature**

1. Kutty, S.N.; Pont, A.C.; Meier, R.; Pape, T. Complete tribal sampling reveals basal split in Muscidae (Diptera), confirms saprophagy as ancestral feeding mode, and reveals an evolutionary correlation between instar numbers and carnivory. *Mol. Phylogenet. Evol.* **2014**, *78*, 349–364, doi:10.1016/j.ympev.2014.05.027.
2. Haseyama, K.L.F.; Wiegmann, B.M.; Almeida, E.A.B.; de Carvalho, C.J.B. Say goodbye to tribes in the new house fly classification: A new molecular phylogenetic analysis and an updated biogeographical narrative for the Muscidae (Diptera). *Mol. Phylogenet. Evol.* **2015**, *89*, 1–12, doi:10.1016/j.ympev.2015.04.006.
3. Kjer, K.; Borowiec, M.L.; Frandsen, P.B.; Ware, J. Advances using molecular data in insect systematics. *Curr. Opin. Insect Sci.* **2016**, *18*, 40–47, doi:10.1016/J.COIS.2016.09.006.
4. Meier, R.; Lim, G.S. Conflict, convergent evolution, and the relative importance of immature and adult characters in Endopterygote phylogenetics. *Annu. Rev. Entomol.* **2009**, *54*, 85–104, doi:10.1146/annurev.ento.54.110807.090459.

#### **1.5. Required initial knowledge and skills of the PhD candidate**

Master degree in biology or biotechnology and fluency in English.

Experience in various microscopic methods.

Experience in molecular methods: DNA isolation, PCR, NGS library preparation will be an advantage.

Bioinformatics skills: data analysis in R.

Enthusiasm for biological science, particularly entomology.

#### **1.6. Expected development of the PhD candidate's knowledge and skills**

PhD candidate will obtain necessary taxonomic skills allowing to work with various species of Muscidae. Student will learn and practice laboratory protocols of the state-of-the-art phylogenomic methods. Will develop skills in bioinformatics, such as *de novo* assembly of whole genomes, genome-scale data analysis, preparation of custom probes set for UCE approach, analysis of phylogenomic data and performing phylogeny reconstructions with concatenation and coalescence approaches. Those skills will allow PhD candidate to undertake in the future phylogenetic studies on various groups of animals and plants. Student will increase also manual skills in preparation of

entomological material and examination with application of various microscopic approaches. Preparation of scientific articles and publication of obtained results will provide necessary skills to become independent scientist.