

Affinities and Divergence of *Aedes* mosquitoes using bayesian relaxed clock and complex models of evolution

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AEDINI: MOSQUITOES OF BIOLOGICAL AND MEDICAL IMPORTANCE

Mosquitoes are the indirect cause of more morbidity and mortality among humans than any other group of organisms. Mosquito (Culicidae) are divided in two main clades Culicinae and Anophelinae.

Culicinae includes 3,559 species classified in two subfamilies and 113 genera. One third of these species belong to the tribe Aedini: they are notorious (and unfortunately excellent) vectors of a variety of viruses (arboviruses), filarial worms (helminths) and protozoans.

INCONGRUENCE BETWEEN NUCLEAR AND MITOCHONDRIAL DATA

We performed Bayesian phylogenetic and clock analysis using separately nuclear and mitochondrial genes (Fig. 1). We detected various topological incongruence which may reflect ancient introgressions. The mitochondrial tree is unresolved at many nodes, therefore incongruences are poorly supported: whole mitochondrial genome is needed to confirm ancient hybridisations/introgression events.

Although the age of Culicidae, Culicinae, and Aedini is similar among the two trees, the split between *A. aegypti* and *A. albopictus* ranges from circa 80 my using mitochondrial data to circa 30 my using nuclear data. Some of these incongruence can be explained by a biased sampling of genes in the datasets, but also by peculiar inheritance and genetic differences between nuclear and mitochondrial DNA.

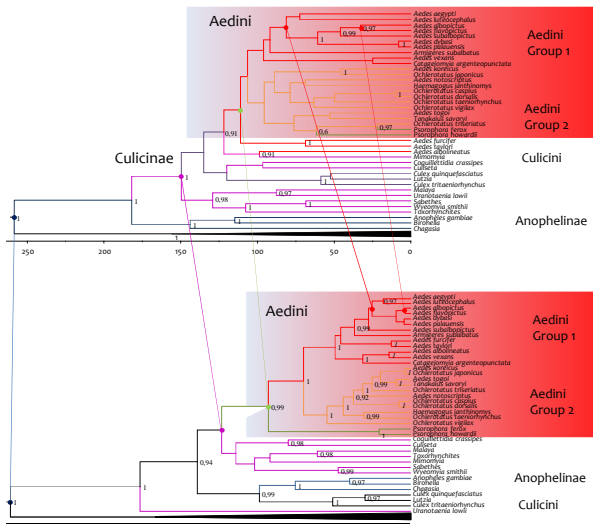


Fig1. Mitochondrial (tree above) and Nuclear (tree below) time trees. Lines connect the same node in the two dated phylogenies, only posterior probability higher than 0.9 are shown.

Brief methods

We analysed two different concatenate dataset Nuclear (28S, 18S, Enolase, Arginine-Kinase) and mitochondrial (16S, CoxI, CoxII, NAD4). The analysis were carried out using Beast2, RaxML and PhyloBayes using GTR+G and CAT+G replacement models. Model test revealed the Yule+ relaxed lognormal clock as the best fitting tree and clock model for our dataset in BEAST2. For calibration, we used two minimum fossil calibration point within Anopheles and Culex, plus a root prior for the split between Mosquito and Drosophilinae (as in Benton et al., 2006).

Reference

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POOR KNOWLEDGE OF AEDINAE DIVERGENCE TIME IMPAIR BIOLOGICAL STUDIES.

Knowledge of Aedini genetic diversity is still scattered and incomplete (only two genomes sequenced so far, (Gasperi et al., 2015; Nane et al., 2007)). In particular, the divergence time of Aedini has yet to be inferred using molecules.

In order to improve our understanding of their evolution, we present the first multigene estimate of Aedini divergences based on Bayesian inference and fossil calibrations. Results are helpful to provide a paleobiological background to understand present Aedini ecology and to ease genomic studies aimed at ameliorating their control

THE EVOLUTIONARY HISTORY OF AEDINAE AND OTHER MOSQUITOES

Our concatenated analysis of mitochondrial + nuclear data (spanning 8 genes and 7494 nucleotides) allows us to obtain a first picture of Aedini evolutionary history. Our tree indicate two monophyletic groups of Aedini, one of which likely indicating the proposed new genus *Ochlerotatus*. Mosquitoes (Culicidae) diverged approximately 180 million years ago, quite concomitant with the divergence of flowering plants (James A. Doyle 2012; Misof B. et al., 2014) consistent with the Culicidae habit of feeding on nectar. Aedini originated from other Culicinae circa 114 million years ago, concomitant with angiosperm diversification, and the first bird and mammals diversifications: this suggests interesting co-evolutionary scenarios.

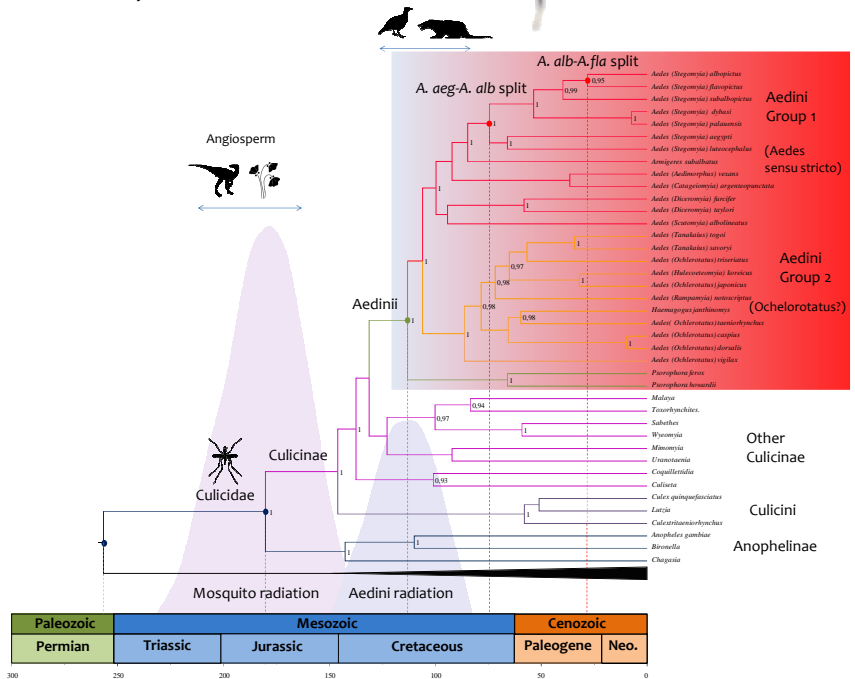


Fig2. Divergence estimates using beast2 and the concatenated dataset. The gray curves are the distribution of the 95% posterior probability limit for the age of the Culicidae and Aedini origin. Dotted lines indicate the split between *A. albopictus* and *A. aegypti* and *A. flavopictus*.

PERSECTIVES: A FRAMWEORK FOR EVOLUTIONARY AND MANAGEMNT STUDIES

Our estimates indicate that emerging models such as *A. albopictus* can be currently compared only against distantly related species (*A. aegypti*, circa 75 million years divergence). Our data indicate that comparative genomic of *A. albopictus* would provide much more detailed results if compared with closer related sister species *A. flavopictus* (20-5 million years ago depending on the marker). We advocate that sequencing the genome of the latter would allow a better interpretation of genomic features such as odorant receptors in *A. albopictus* (fig. 3).

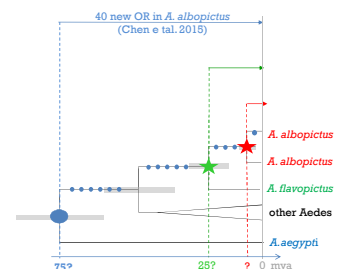


Fig3. Here we stress how the sister species genetic and genomics studies will help the understanding of the biological and ecological key innovation in *A. albopictus*, using a comparative genomics approach.

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