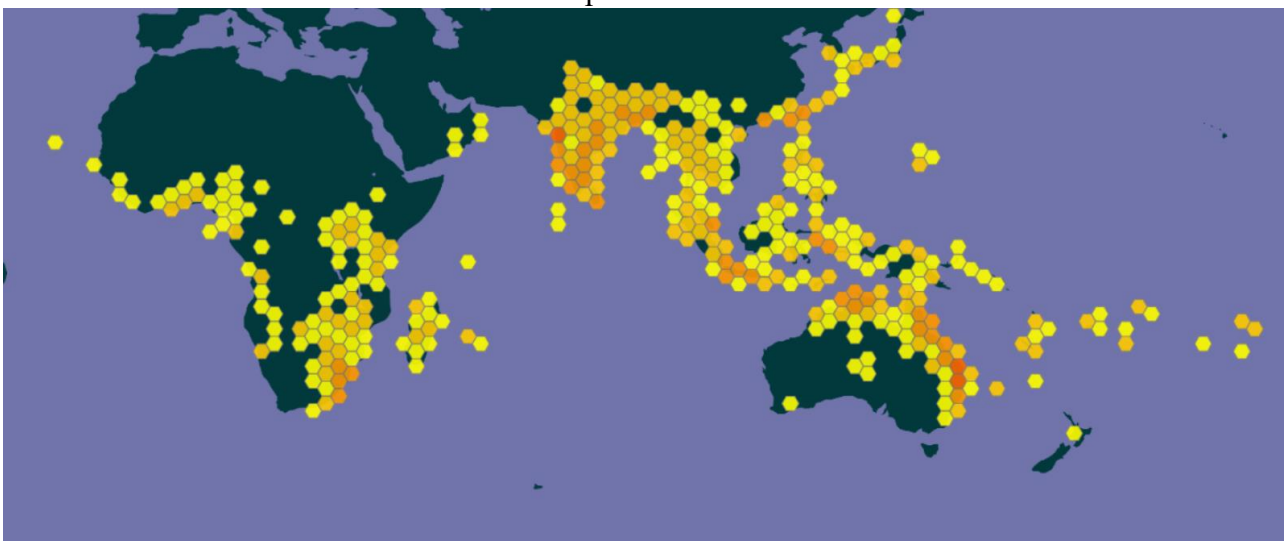
Protocol for genome samples – *Melanitis leda*

Part of a NCN-funded project by Freerk Molleman, Urszula Walczak, Ullasa Kodandaramaiah, and Vicencio Oostra. PhD student Tan Pham is in charge of genome work.

Samples and sites:

Melanitis leda is among the most widespread butterflies, distributed from Africa to Asia and Oceania, and the species can be found in habitats that range from dense forests to open grasslands, and at altitudes from lowlands to up to 2000 meters. This wide geographic range is probably facilitated by its ability to use a wide variety of grass species as host. It feeds on rotting fruit as adults, which makes them easy to collect using fruit-baited traps. They show seasonal dimorphism with wet- and dry-season forms, and especially dry season forms are highly variable. When collecting the sample, you should record, location, date, microhabitat (grassland/forest).

Distribution map of M.leda from Gbif



Preparation:

We would like to obtain up to 5 genome samples per site. After capturing the *M. leda* butterflies, you can keep them alive in a butterfly envelope without squeezing it for several days if needed. Ideally, the butterflies are kept cool (using a cool box with icepacks or a refrigerator) until they are removed from their envelopes. Only individuals that are still alive are then placed individually into vials with >95% ethanol. The wings need to be removed (cut with a fine scissors or forceps) before they are put into the vials. After handling a sample, you need to clean the equipment with ethanol at least two time to avoid cross-contamination. The wings are placed in a glassine envelope that is labeled with the same code as the genome sample. Note that ethanol can erase permanent markers so it is wise to use a pencil.

Storing:

Samples keep better at lower temperatures. However repeatedly freezing and thawing is bad for the DNA too. Therefore it is better to keep the samples in ethanol in a fridge in the field and send them as soon as possible to the final destination to be kept in a professional freezer at -80ºC. Ifyou need to keep them longer you can also keep them at -20 ºC for up to 4 months).

Sending:

Please send the samples to: Tan Pham (tan.pham@amu.edu.pl) /prof. Freerk Molleman, Department of Systematic Zoology, Institute of Environmental Biology, Faculty of Biology, Adam Mickiewicz University, Ul. Uniwersytetu Poznańskiego 6, PL-61-614 Poznań, Poland.

In India, please send the samples to: Indukala K/ prof. Ullasa Kodandaramaiah, Vanasiri Evolutionary Ecology Lab, ISER-TVM, Maruthamala P.O., Vithura, Thiruvananthapuram, 695551, Kerala, INDIA.

Samples can be send by normal mail, but the less time they spend in transit, the better. Let us know when you have sent something so that were are ready to receive it and put it in the freezer.