

DNA Barcoding and Taxonomic Classification of Black Flies in Southeast Asia

Programme:BIFA

Project ID: BIFA6_017

Project lead organization:Universiti Malaya

Project implementation period:1/9/2021 - 28/2/2023

Report approved: 24/3/2023

Narrative Final report

Executive Summary

A total of 359 occurrence records from 109 black fly species with corresponding COI sequences from Malaysia (57 sequences from 22 species), Indonesia (86 sequences from 29 species), Thailand (81 sequences from 13 species) and Vietnam (135 sequences from 45 species) were successfully deposited in the GBIF database.

For best practices, the type specimens or specimens collected from type localities are the most reliable sources for DNA barcoding because they have already been verified by experienced taxonomists. The black flies specimens used in this project comprised type specimens, collected from type locations, and all samples have been identified by the world-renowned black fly's taxonomist Prof. Dr. Hiroyuki Takaoka. This ensures the DNA barcodes generated from this study can be used globally for accurate identification and comparisons.

The COI-based DNA barcoding showed value in molecular identification of most black fly members in Southeast Asia, excluding a few species complexes. Additional effort was attempted to delimit these complex members such as the use of fast-evolving nuclear genes (BZF and ECP1 genes), but the successful identification rate was low. These complex members may require other options such as cytological analyses for more accurate delineation. Several species were also unavailable for in-depth inspection (eg. intraspecific analyses) due to limited sampling numbers. Population genetic studies with larger sample sizes from different biogeographic areas might be conducted to gain a better insight on the molecular diversity of black flies in Southeast Asia.

There are no post-project activities at the moment.

Progress against milestones

Has your project completed all planned activities?: Yes

Has your project produced all deliverables?: No

Rationale: Three manuscripts related to DNA barcoding of black flies will be submitted for publication by the end of this year. Currently the two draft manuscripts are being checking by the co-authors. One manuscript is pending for checking by the supervisors. In fact this deliverable will be delivered after the completion of this project, not within the project period. Hope this is acceptable.

Report on Activities

Activity implementation summary

All the activities have been completed successfully. A total of 359 occurrence records from 109 black fly species with corresponding COI sequences from Malaysia (57 sequences from 22 species), Indonesia (86 sequences from 29 species), Thailand (81 sequences from 13 species) and Vietnam

(135 sequences from 45 species) were successfully deposited in the GBIF database. PCR, sequencing and analysis of black fly species complexes such as members of Simulium asakaoe group based on multiple gene markers (BZF and ECP1 genes were also completed, though the performance is lower than the barcoding COI gene sequences.

No new activity was implemented and there will be no post-project activity.

Completed activities

Activity name: DNA barcoding and metabarcoding of black flies in Malaysia

Description: Perform DNA extraction, PCR, sequencing and digitization of black fly samples in Malaysia

Start Date - End Date: 1/9/2021 - 30/11/2021

Verification Sources: https://www.gbif.org/occurrence/search?publishing_org=c676862d-3f47-4cc1-96a0-479ed1f65370

Activity name: DNA barcoding and metabarcoding of black flies in Indonesia

Description: Perform DNA extraction, PCR, sequencing and digitization of black fly samples in Indonesia

Start Date - End Date: 1/12/2021 - 28/2/2022

Verification Sources: https://www.gbif.org/occurrence/search?publishing_org=c676862d-3f47-4cc1-96a0-479ed1f65370

Activity name: DNA barcoding and metabarcoding of black flies in Vietnam

Description: Perform DNA extraction, PCR, sequencing and digitization of black fly samples in Vietnam

Start Date - End Date: 1/3/2022 - 30/4/2022

Verification Sources: https://www.gbif.org/occurrence/search?publishing_org=c676862d-3f47-4cc1-96a0-479ed1f65370

Activity name: DNA barcoding and metabarcoding of black flies in Thailand

Description: Perform DNA extraction, PCR, sequencing and digitization of black fly samples in Thailand

Start Date - End Date: 1/5/2022 - 31/7/2022

Verification Sources: https://www.gbif.org/occurrence/search?publishing_org=c676862d-3f47-4cc1-96a0-479ed1f65370

Activity name: Taxonomic classification of common black fly species in Southeast Asia

Description: Perform PCR, sequencing and analysis of black fly samples in various countries based on multiple gene markers

Start Date - End Date: 1/2/2022 - 31/8/2022

Verification Sources: https://www.gbif.org/occurrence/search?publishing_org=c676862d-3f47-4cc1-96a0-479ed1f65370

Activity name: Standardization of datasets from Malaysia and Indonesia

Description: Standardize the datasets mobilised by the project and ensure their readiness to be published through GBIF

Start Date - End Date: 1/3/2022 - 30/6/2022

Verification Sources: https://www.gbif.org/occurrence/search?publishing_org=c676862d-3f47-4cc1-96a0-479ed1f65370

Activity name: Publication of datasets from Malaysia and Indonesia through GBIF

Description: Publish all the datasets through GBIF

Start Date - End Date: 1/5/2023 - 31/8/2022

Verification Sources: https://www.gbif.org/occurrence/search?publishing_org=c676862d-3f47-4cc1-96a0-479ed1f65370

Activity name: Standardization of datasets from Vietnam and Thailand

Description: Standardize the datasets mobilised by the project and ensure their readiness to be published through GBIF

Start Date - End Date: 1/9/2022 - 31/12/2022

Verification Sources: https://www.gbif.org/occurrence/search?publishing_org=c676862d-3f47-4cc1-96a0-479ed1f65370

Activity name: Publication of datasets from Vietnam and Thailand through GBIF

Description: Publish all the datasets through GBIF

Start Date - End Date: 1/11/2022 - 28/2/2023

Verification Sources: https://www.gbif.org/occurrence/search?publishing_org=c676862d-3f47-4cc1-96a0-479ed1f65370

Report on Deliverables

Production of Deliverables - Summary

A total of 359 occurrence records from 109 black fly species with corresponding COI sequences from Malaysia (57 sequences from 22 species), Indonesia (86 sequences from 29 species), Thailand (81 sequences from 13 species) and Vietnam (135 sequences from 45 species) were successfully deposited in the GBIF database.

Three manuscripts related to DNA barcoding of black flies will be submitted for publication by the end of this year. Currently the two draft manuscripts are being checked by the co-authors. One manuscript is pending for checking by the supervisors. In fact this deliverable will be delivered after the completion of this project, not within the project period. Hope this is acceptable.

Two master students (Master of Philosophy) Ms. Putt Qi Yan and Ms. Hew Yan Xin under this project have registered with the Universiti Malaya.

Production of deliverables

Title: Black flies in Southeast Asia

Type: Dataset

Status update: Completed

Dataset scope: Occurrence }

Expected number of records: 359

Data holder: Van Lun Low

Data host institution: Tropical Infectious Diseases Research and Education Centre (TIDREC), Universiti Malaya

Sampling method: Black fly samples collection was done at the accessible streams and rivers, whereby these are the breeding habitats for larvae and pupae of black flies. Pupae that attached on substrates such as leaves, stems, twigs, grasses and roots were collected using forceps. Then it will be kept alive individually in vials until adult emergence before being preserved in 80% ethanol for the identification process. The methods of collection and identification followed those of Takaoka (2003) and Adler et al. (2004).

% complete: 100

DOI: 10.15468/h5jh4z

Expected date of publication:

Title: Publication

Type: Other

Description: At least 3 ISI-indexed papers will be submitted for publication by the end of this year

Sources of verification: Pending

Title: Two master students

Type: Other

Description: Two master students under this project have registered with the Universiti Malaya

Sources of verification: Please refer attachment A1

Impact of COVID-19 pandemic on project implementation

Not applicable. The project is not affected by COVID-19 pandemic

Communications and visibility

Two papers were presented in International Conference of Medical Parasitology & Entomology 2022 (ICMPE 2022) to promote BIFA program and GBIF (see attachment A2).

Van Lun Low, as the Vice President of Malaysian Society of Parasitology and Tropical Medicine (MSPTM), communicated with MSPTM and introduced GBIF to the members of MSPTM. A seminar on the introduction to GBIF and data publishing will be organized by MSPTM on 22 February 2023 (see attachment A3).

Monitoring and evaluation

Final Evaluation

We successfully deposited a total of 359 occurrence records from 109 black fly species with corresponding COI sequences from Malaysia (57 sequences from 22 species), Indonesia (86 sequences from 29 species), Thailand (81 sequences from 13 species) and Vietnam (135 sequences from 45 species) in the GBIF database.

All activities were completed successfully within the timeframe by two research assistants financially supported by BIFA program and one research assistant from TIDREC. In short, COI-based DNA barcoding showed value in molecular identification of most black fly members in Southeast Asia, excluding a few species complexes. Sequencing of other genes was carried out to delineate those species complexes but there are still some members could not be resolved. Time and financial constraints have hindered further activities to be carried out at this moment.

There is no any feedback on the project's relevance from the partners and stakeholders, and no changes have been made to the project's original plans, and actions to follow-up. Big thanks to the GBIF Secretariat, especially the Asia Regional Support Team, Chihjen Ko who has been very helpful towards the completion of this project.

Best Practices and Lessons Learned

The best approach to building a DNA reference database is to barcode the type species or type locality specimen of each described species to avoid species misidentification, particularly when a vague or unverified reference specimens were used. This study established a comprehensive DNA barcode reference of black flies in Southeast Asia using samples from same habitat of first species described and named (type localities) or verified voucher specimens.

Post Project Activity(ies)

n/a

Sustainability plans

Three research assistants are trained through this project. The occurrence datasets will be updated when additional information is available, especially black flies that have not been included from other countries in Southeast Asia.

The future impact of this project can be seen in GenBank, Google Scholar, PubMed and World Black Flies Inventory. Additionally, with the increase of more sequences per species deposited in the barcode library, this surely will enhance the library's possible applications, particularly regarding correct species identification, monitoring possible vector species and species richness in biodiverse areas, such as tropical regions.

GBIF leads the Biodiversity Information Fund for Asia (BIFA), a programme funded by the Ministry of the Environment, Government of Japan. The programme provides supplementary support for activities addressing the needs of regional researchers and policymakers through mobilization and use of biodiversity data.

