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A first, local DNA barcode reference database of the forensically important flies (Diptera) of the island of La Réunion

ID 416

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ABSTRACT

Background: Forensic entomologists use fly larvae of the order Diptera to establish the time interval between death and body discovery. The identification of these flies is decisive in forensic casework but is hampered by difficulties in identification and the potential presence of fly larvae that have no forensic interest. The identification of forensically relevant fly species, and their discrimination from non-forensically important species is facilitated with DNA barcoding but only if a representative local reference barcode library is available. **Results:** We constructed a local reference library of 195 COI barcodes from 29 species of the families Calliphoridae, Fannidae, Muscidae, and Sarcophagidae from the island of La Réunion. Our results show that 1) the library contains most of the forensically relevant species of these families from the island, and 2) all fly species can be unambiguously identified with DNA barcoding using a variety of analytical methods. Two public libraries (GenBank and the Barcode of Life Data Systems (BOLD)) only allowed to identify half the number of species of these families present in La Réunion, showing that both libraries are not representative for this island fauna. Yet, nine out of the ten species with a forensic interest could be identified using both public libraries showing that, for forensic casework, the libraries prove helpful. **Significance:** This is the first DNA barcode reference database for the forensically important fly species of La Réunion. The database will contribute to the growing use of dipteran larval composition on corpses to estimate the post-mortem interval.

INTRODUCTION

Larvae of Diptera are among the first colonizers of corpses, and thus provide relevant information for estimating the post-mortem interval (PMI). Of extreme importance is the correct identification of these larvae, because species differ in their developmental time and misidentifications will cause inaccurate PMI estimates. DNA barcoding can match unknown insect specimens to reference barcodes given that a local, representative, and comprehensive reference library of DNA barcodes of the forensically relevant entomofauna is available. Here, we provide such a reference library for the Calliphoridae, Fannidae, Muscidae, and Sarcophagidae of the island of La Réunion.

MATERIAL & METHODS

337 adult flies were collected at 26 locations on La Réunion (Fig. 1) and identified based on morphology. Genomic DNA was extracted from legs of 211 specimens and the barcode fragment of COI was amplified following Folmer et al. (1994). Intra- and interspecific nucleotide sequence divergences (uncorrected p-distances) were calculated in MEGA v.6. Then, we considered each sequence as a query that was identified using the other sequences as a reference dataset with several criteria:

(1) the 'tree-based identification' in which identifications of queries were considered as correct if the query and all conspecific sequences formed a monophyletic group;
(2) the Best Match (BM) and Best Close Match (BCM) criteria;
(3) the application of haplotypes as queries to search for most similar sequences in GenBank and BOLD.

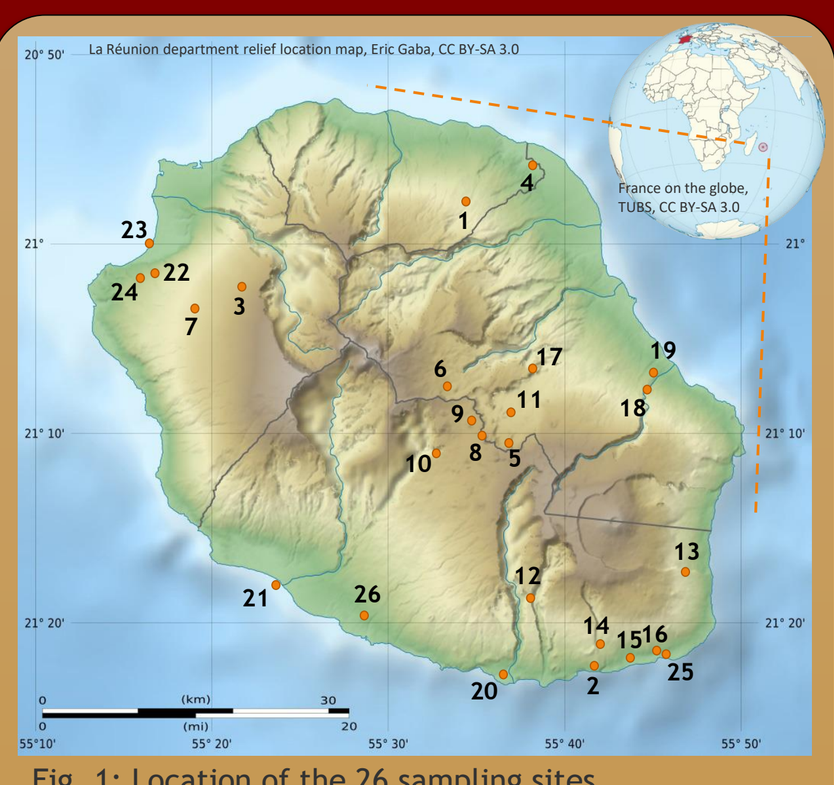

Fig. 1: Location of the 26 sampling sites.

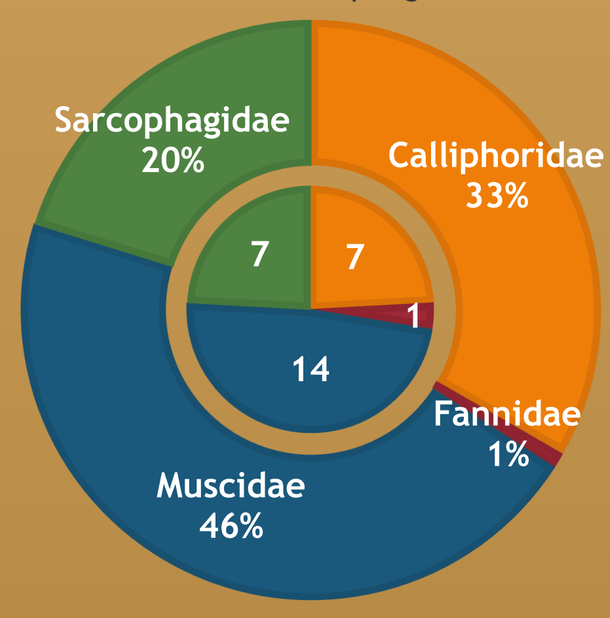

Fig. 2: Number of species (inner circle) and proportion of specimens (total 337; outer circle) collected for each of the four forensically important fly families.

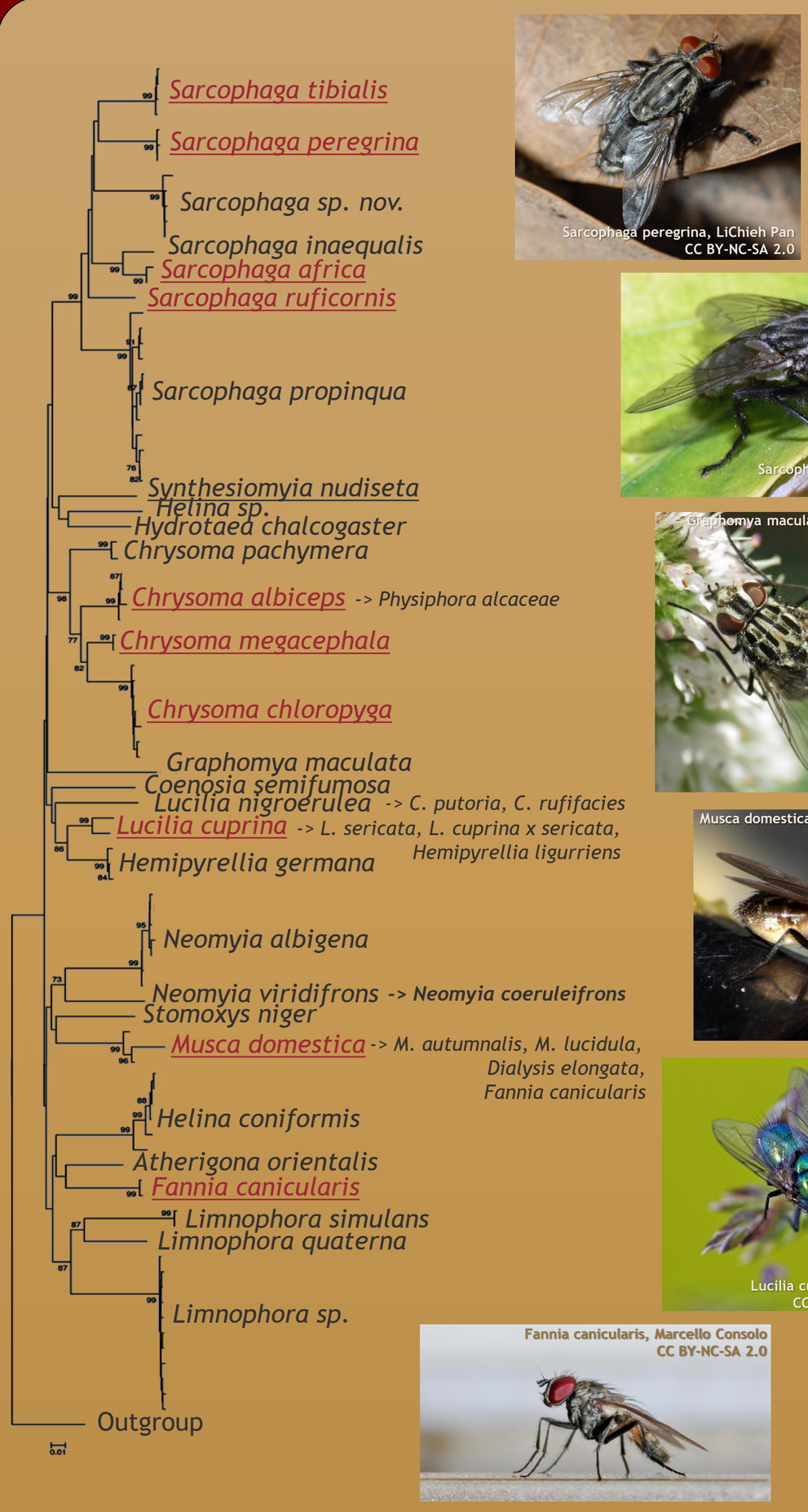

Fig. 3: NJ and ML tree of 89 COI haplotypes from 29 species of the families Calliphoridae, Fannidae, Muscidae, and Sarcophagidae from La Réunion. Bootstrap values ≥ 70% are shown at the nodes as NJ/ML. The species for which a conspecific match of ≥ 98 % similarity was found on GenBank or BOLD are indicated with an underscore, the additional heterospecific best matches (≥ 98 %) are added after the respective species (->), while the species name in bold was the **only best match** (≠ target). The species indicated in red are of known to be of forensic interest.

RESULTS & DISCUSSION

Fig. 2 shows the distribution of the specimen and species numbers of the 337 collected flies over the four families. 195 barcodes were generated for 29 species of which at least 10 have a forensic relevance (Fig. 3); all those with >2 barcodes formed monophyletic groups. There was a barcode gap in the Calliphoridae, Muscidae, and Sarcophagidae (Fannidae contained only one species) and all species were ≥ 3.5 % divergent from their nearest neighbor. Consequently, identification success was 100% for both the BM and BCM methods. Highly similar matches were found for 11 of the 29 species in GenBank and BOLD (Fig. 3), due to the absence of barcodes for the other 18 species. Heterospecific matches found are most probably misidentifications in GenBank, except for *L. cuprina* and *L. sericata* which are known to hybridize.

The Diptera fauna of La Réunion remains poorly studied but for the time being DNA barcoding allows to unambiguously identify all of the 29 species included in this study.

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