Supplementary Material S1

Molecular identification of flower-visiting insects and construction of the neighbor joining (NJ) tree

For each of the collected Hymenoptera and Diptera species, genomic DNA was extracted from the legs of specimens using the Bioline extraction kit (Bioline, Heidelberg, Germany) following the manufacturer's protocol. The DNA extracts were stored at -20 ºC. The amplification of the standard DNA barcode fragment (approx. 658 bp) of the mitochondrial cytochrome *c* oxidase subunit I (COI) was performed in a solution of 50 µl reaction mix volume containing 10 µl of 5× MyTaq Reaction Buffer (Bioline, Germany), 1 µl *Taq*DNA polymerase (Bioline, Heidelberg, Germany), 0.5 µl of forward primer 5′-GGTCAACAAATCATAAAGATATTGG-3′ (LCO1490), 0.5 µl of reverse primer 5′-TAAACTTCAGGGTGACCAAAAAATCA-3′ (HCO2198), 35 µl of double distilled water and 2 µl DNA template. The PCR protocol comprised an initial denaturation of 1 min at 95 ºC, then 35 cycles of 15 s at 95 ºC, 15 s at 46 ºC and 10 s at 72 ºC each, followed by a final extension step of 5 min at 72ºC. Bioline PCR purification kits (Bioline, Germany) were used to clean up the amplicons according to the manufacturer's instructions. The PCR primers were then used to sequence the amplicons in both directions at Macrogen Inc. (Amsterdam, Netherlands).

Sequences were assembled and cleaned in Bioedit version 7.2.5. No stop codons were observed. The DNA barcodes were aligned and trimmed in MEGAX version 10.2.4 (Kumar et al. 2018). Each unique DNA barcode was blasted in GenBank using the NCBI Blast programme (Johnson et al. 2008; Hall et al. 2011), and the most similar sequences (best matches) were downloaded and added to the DNA barcode dataset. The final DNA barcode dataset was then used to construct a neighbour-joining (NJ) tree using uncorrected p-distances to visualise similarities in the DNA barcodes and similarities with the best matches retrieved from GenBank. We considered the DNA barcode as identifiable if the p-distance between the barcode and its best match is less than 2% and then scored identifications as reliable or unreliable in cases where the taxon names matched or did not match, respectively.

References:

Hall T, Biosciences I, Carlsbad C (2011) BioEdit: an important software for molecular biology. GERF Bull Biosci 2: 60 – 61.

Johnson M, Zaretskaya I, Raytselis Y, Merezhuk Y, McGinnis S, Madden TL (2008) NCBI BLAST: a better web interface. Nucleic Acids Res 36: W5–W9.

Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol 35:1547.

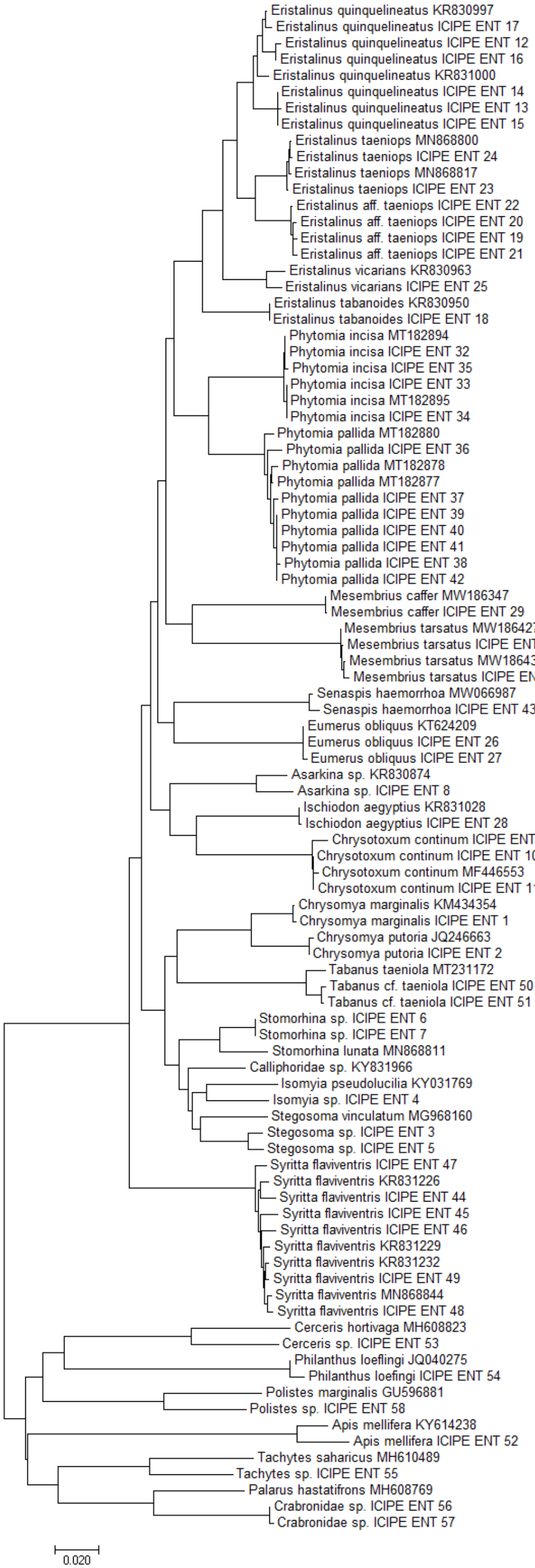

Figure S1: The neighbor joining (NJ) tree showing similarities between DNA barcodes and the best matches retrieved from GenBank.