Identification of Belgian mosquito species (Diptera: Culicidae) by DNA barcoding

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Introduction

Many mosquito species are potential vectors of pathogens. A correct identification is essential for effective mosquito monitoring and control. A recent country-wide inventory of the Culicidae in Belgium (2007-2008) recorded 23 morphologically distinct species, two of which are established alien species (*Aedes japonicus japonicus* and *Aedes koreicus*). This study aims to establish a DNA barcode reference database for the Belgian mosquito fauna and to evaluate its utility in species identification.

Material and Methods

Adult specimens (N_{total} = 26 000) were collected during a 2-year survey in Belgium (MODIRISK project) from 910 random sites (urban, rural and natural), using CO_2-baited Mosquito Magnet Liberty Plus traps. Samples were kept dry or frozen at -20 degrees Celsius. Morphological identification was performed using the identification keys of Schaffner (1993, 2001) and Becker (2010). The DNA of a subset of 260 specimens (Table 1) was extracted, using one to four legs. The genetic diversity of mosquitoes was assessed using a 658bp fragment of the mitochondrial cytochrome oxidase I (COI) gene (Folmer region). Neighbor-joining tree was reconstructed based on Kimura 2-parameter distances (MEGA 6). The proportion of correctly identified specimens was estimated with SpeciesIdentifier v1.5.

Species	Number of specimens	Average K2P distance (%)	Maximum observed K2P difference between conspecific specimens (%)
Aedes annulipes	12	0.92	2.06
Aedes cantans	12	0.28	0.63
Aedes caspius	14	1.32	3.03
Aedes cinereus	13	0.61	1.42
Aedes communis	13	0.10	0.31
Aedes detritus	9	0.24	0.94
Aedes geniculatus	15	0.25	0.63
Aedes japonicus japonicus	4	0.00	0.00
Aedes koreicus	2	6.22	6.22
Aedes punctor	19	1.21	4.03
Aedes rusticus	10	0.12	0.31
Aedes sticticus	8	1.33	2.88
Aedes vexans	16	1.25	4.86
Anopheles claviger	8	0.88	1.58
Anopheles maculipennis s.s.	11	1.64	2.72
Anopheles messeae	6	1.00	1.90
Anopheles plumbeus	5	0.00	0.00
Coquillettidia richiardii	15	0.10	0.63
Culex pipiens	34	0.67	3.19
Culex territans	4	2.52	3.99
Culex torrentium	5	0.25	0.47
Culiseta annulata	16	0.04	0.31
Culiseta fumipennis	3	0.52	0.63
Culiseta morsitans	6	0.31	0.63

Table 1. Sampled species, sample size and maximum observed intraspecific Kimura K2P distances among COI sequences.

Results

Intraspecific Kimura 2-parameter (K2P) distances averaged 0.7%, and the maximum observed intraspecific K2P distance was 6.2% for *Aedes koreicus* (N=2). A small overlap between intra- and interspecific K2P distances for congeneric sequences was observed (Fig. 1). Overall, the identification success using best match and the best close match criteria were high, above 98% (Table 2). Concerning *Aedes annulipes* and *Aedes cantans*, two closely related species morphologically indistinguishable, no clear genetic division was found (Fig. 2). The members of the *Anopheles maculipennis* complex (*Anopheles maculipennis* s.s. and *An. Messeae)*, were weakly supported by bootstrap values.

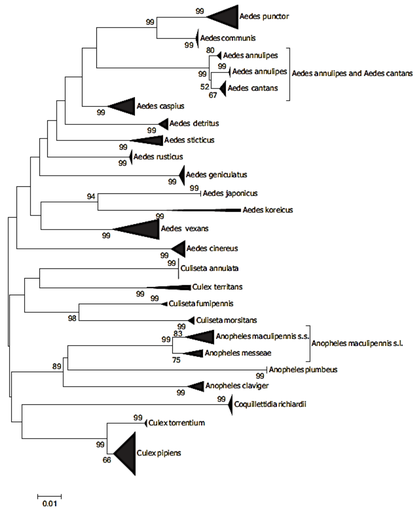

Fig. 2. NJ tree based on the K2P distances among COI sequences (658bp fragment) of 24 Culicidae species. Bootstrap values are shown at the branch nodes (below 50%: not shown)

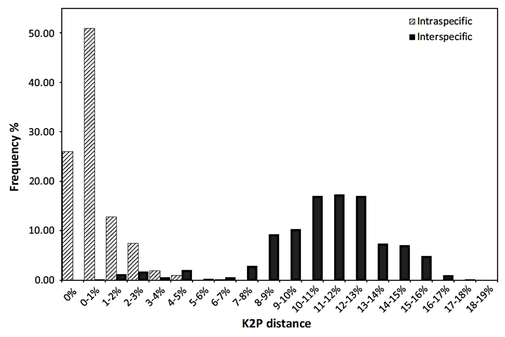

Fig. 1. Frequency distribution of the intra- and interspecific K2P distances for congeneric sequences.

Criterion	Correct identification (%)	Ambiguous identification (%)	Incorrect identification (%)	No match (%)
Best Match	100.00	0.0	0.0	0.0
Best Close Match	98.46	0.0	0.0	1.53
All Species Barcodes	78.46	20.1	0.0	1.53

Table 2. Identification success using the Kimura two-parameter distances with three different criteria: Best Match, Best Close Match and All Species Barcodes based on the threshold of 2.6 calculated by SpeciesIdentifier (*a priori* threshold of 3% yielded the same results).

Conclusion

Most species appeared as well-supported clusters. This study showed that DNA barcoding offers a reliable framework for mosquito species identification in Belgium except for two pairs of closely related species that gave ambiguous identification (same BIN cluster in BOLD and identified as one Molecular Operational Taxonomic Unit (MOTU)).

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