New Culicidae species records for Belgium validated using DNA-based techniques

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INTRODUCTION

The most recent revised Belgian culicid fauna checklist reported the occurrence of 28 native species, belonging to six genera (Boukraa *et al.*, 2015). In addition, three invasive species were found to be introduced or established. This species list was built based on a literature review, on the revision of the Royal Belgian Institute of Natural Sciences culicid collection, and on a nationwide inventory of mosquitoes performed in the framework of the MODIRISK project (2007-2011). During two following monitoring projects (EXOSURV and FASFC), aiming to detect the occurrence of exotic mosquito species (EMS), no new species were detected. Since 2017, a fourth EMS monitoring project is running (MEMO). Apart from monitoring EMS, the study also provides valuable information concerning the overall mosquito fauna and its distribution. To discriminate between species, both morphology and DNA-based identifications are performed.

MATERIAL, METHODS AND RESULTS

Culex modestus

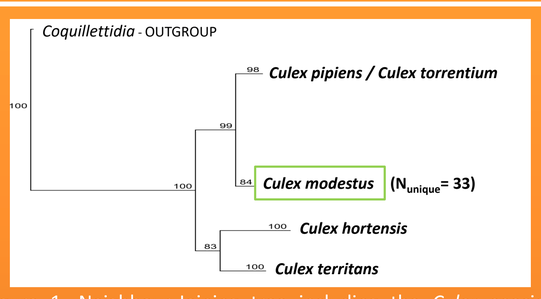


Figure 1. Neighbour-Joining tree including the *Culex* species found in Belgium (COI DNA marker; K2P; 658 bp; 425 unique sequences; BS; 500 replicates).

Distribution: **Albania,** Algeria, China, Croatia, Czech Republic, Denmark, Greece, Hungary, Iran, Iraq, Israel, Italy, Moldova, Mongolia, Morocco, Netherlands, Poland, Romania, Russia, Slovakia, Spain, Sweden, Tajikistan, Turkey, and the United Kingdom.

One specimen (larva) found in East Flanders

Culiseta longiareolata

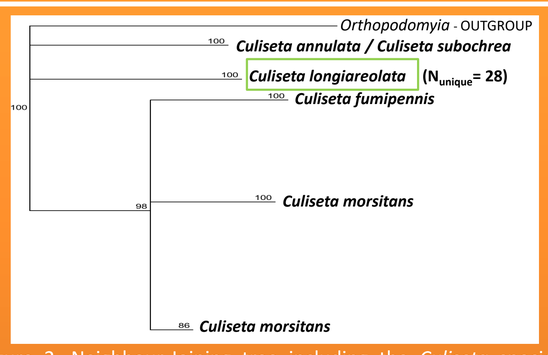


Figure 2. Neighbour-Joining tree including the *Culiseta* species found in Belgium (COI DNA marker; K2P; 658 bp; 120 unique sequences; BS; 500 replicates).

Distribution: Albania, Austria, Botswana, Bulgaria, Cyprus, Djibouti, Egypt, Ethiopia, France, Greece, Hungary, India, Iran, Iraq, Israel, Italy, Jordan, Lebanon, Lesotho, Mauritania, Moldova, Morocco, Namibia, Pakistan, Portugal, Romania, Russia, Slovakia, Somalia, South Africa, Spain, Sudan, Syria, Tajikistan, Tunisia, Turkey, Ukraine, Yemen.

Seven specimens (5 larvae, 1 adult female, 1 adult male) found in Limburg, Hainaut and Namur

Anopheles maculipennis s.l.

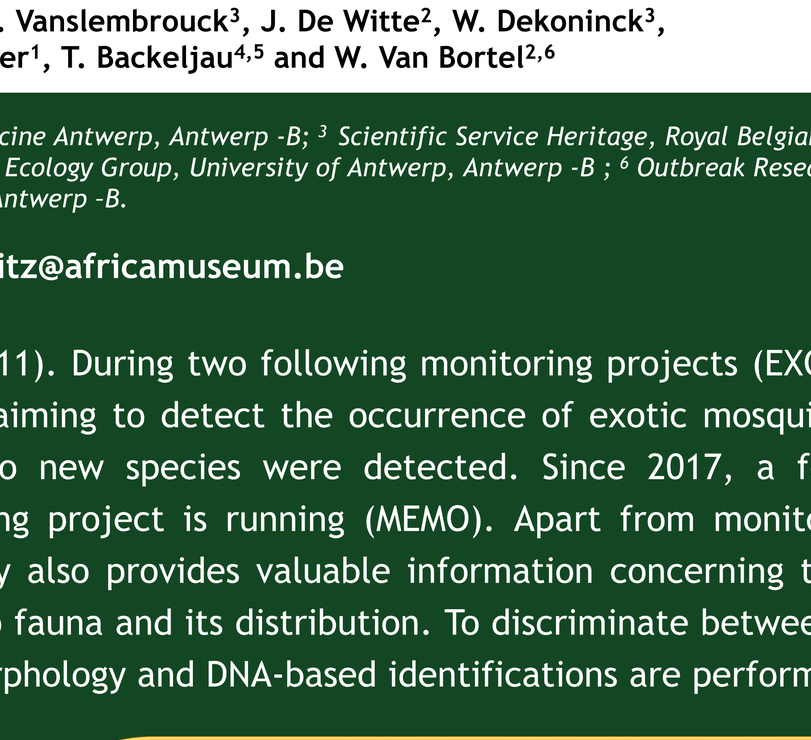
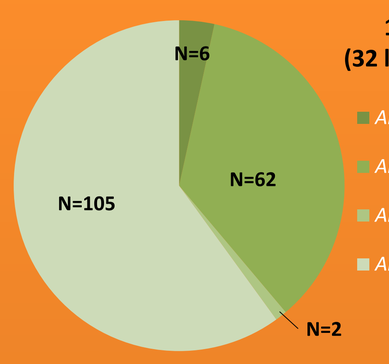


Figure 3. 3% agarose gel picture of the RFLP patterns obtained after HhaI and Bsh1236I enzyme digestions of ITS2.



Species	ITS2 amplicon size
Anopheles daciae	485 bp
Anopheles messeae	485 bp
Anopheles maculipennis	472 bp
Anopheles atroparvus	487 bp

Figure 4. Pie chart displaying the proportion of the specimens identified in the *Anopheles maculipennis* s.l. species complex by RFLP analyses of ITS2.

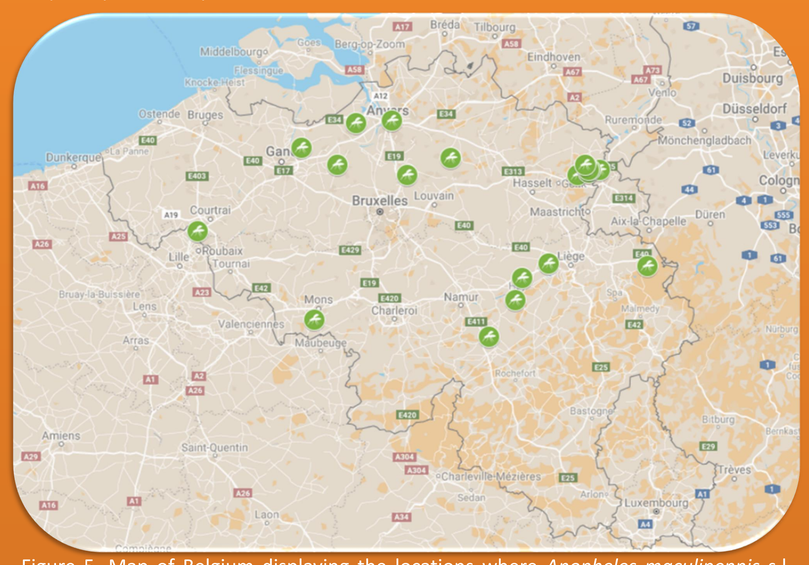


Figure 5. Map of Belgium displaying the locations where *Anopheles maculipennis* s.l. specimens were collected.

Genus	Species	Number of unique COI sequences included in the analysis	Average inter-K2P distance (%)	Max observed K2P distance between conspecific sequences (%)
Anopheles	*atroparvus*	18	2.49	1.51
	claviger	14	11.24	3.83
	daciae	0	na	na
	maculipennis	14	2.29	2.71
	messeae	75	2.73	2.82
	plumbeus	13	13.76	0.47
Aedes	*cinereus*	307	11.84	5.56
	vexans	437	0.97	4.62
	geniculatus	15	9.88	1.71
	japonicus	52	9.44	9.65
	koreicus	6	7.05	0.74
	annulipes	12	1.01	2.02
	cantans	10	0.33	1.30
	caspius	27	1.98	2.83
	communis	207	2.85	5.68
	detritus	4	7.74	1.11
	dorsalis	95	1.31	1.26
	flavescens	17	2.61	5.97
	punctor	31	4.61	5.85
	sticticus	250	5.86	10.77
	rusticus	4	6.97	0.27
	albopictus	215	11.09	12.38
Culex	*pipiens*	103	2.19	7.63
	modestus	33	5.45	4.74
	torrentium	99	1.82	0.90
	territans	181	9.43	6.63
	hortensis	9	8.87	0.85
Culiseta	*fumipennis*	5	4.16	9.04
	morsitans	76	6.83	9.99
	annulata	10	2.13	0.27
	subochrea	1	2.01	na
	longiareolata	28	9.67	0.53
Coquillettidia	*richiardii*	10	13.31	0.56
Orthopodomyia	*pulcripalpis*	1	12.05	na

Table 1. List of mosquito species occurring in Belgium, including the maximum observed intraspecific Kimura two-parameter (K2P) distances among COI sequences. The checklist of species was compiled from a literature review.

CONCLUSION

During the monitoring project, three species were found for the first time in Belgium: *Culiseta longiareolata*, *Anopheles daciae* and *Culex modestus*. *Cs. longiareolata* does not appear to have any epidemiological relevance for human or zoonotic diseases. However, mosquitoes of the *An. maculipennis* group are of public health concern since at least five of the 11 morphologically indistinct species have been historically considered as vectors of malaria in Europe. Also, *Cx. modestus* is an important vector of the West Nile virus (WNV) in continental Europe and, due to its preference for feeding on both avian and mammalian hosts, is considered to be a principal bridge vector of WNV (transmission from the reservoir host to mammals).

References

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