

Comparative genetic analysis of invasive mosquito species in Belgium supports diverse introduction pathways



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INTRODUCTION

Early detection of exotic mosquito species (EMS) is of vital importance to prevent transmissions of mosquito-borne diseases, such as dengue and Zika, which pose significant public health risks. Since 2007, the introduction of EMS in Belgium, especially *Aedes* species, has been studied during successive nationwide entomological active monitoring at Points of Entries (PoEs), such as used tire import companies. In 2022, passive monitoring through citizen science was implemented to increase the spatio-temporal coverage for detecting invasive species, especially *Aedes albopictus* and *Aedes japonicus*, both at PoEs and in residential areas. *Ae. albopictus* has been entering Belgium increasingly over the last decade through various pathways, with twice as many positive locations and a notable rise in detections in 2023, including instances of overwintering. *Ae. japonicus* has also expanded despite eradication efforts. To delay the species' establishment and spread, accurate and reliable species-level identification of all life stages, as well as geographical origin assessment, are essential so that appropriate control measures can be taken.



MATERIAL & METHODS



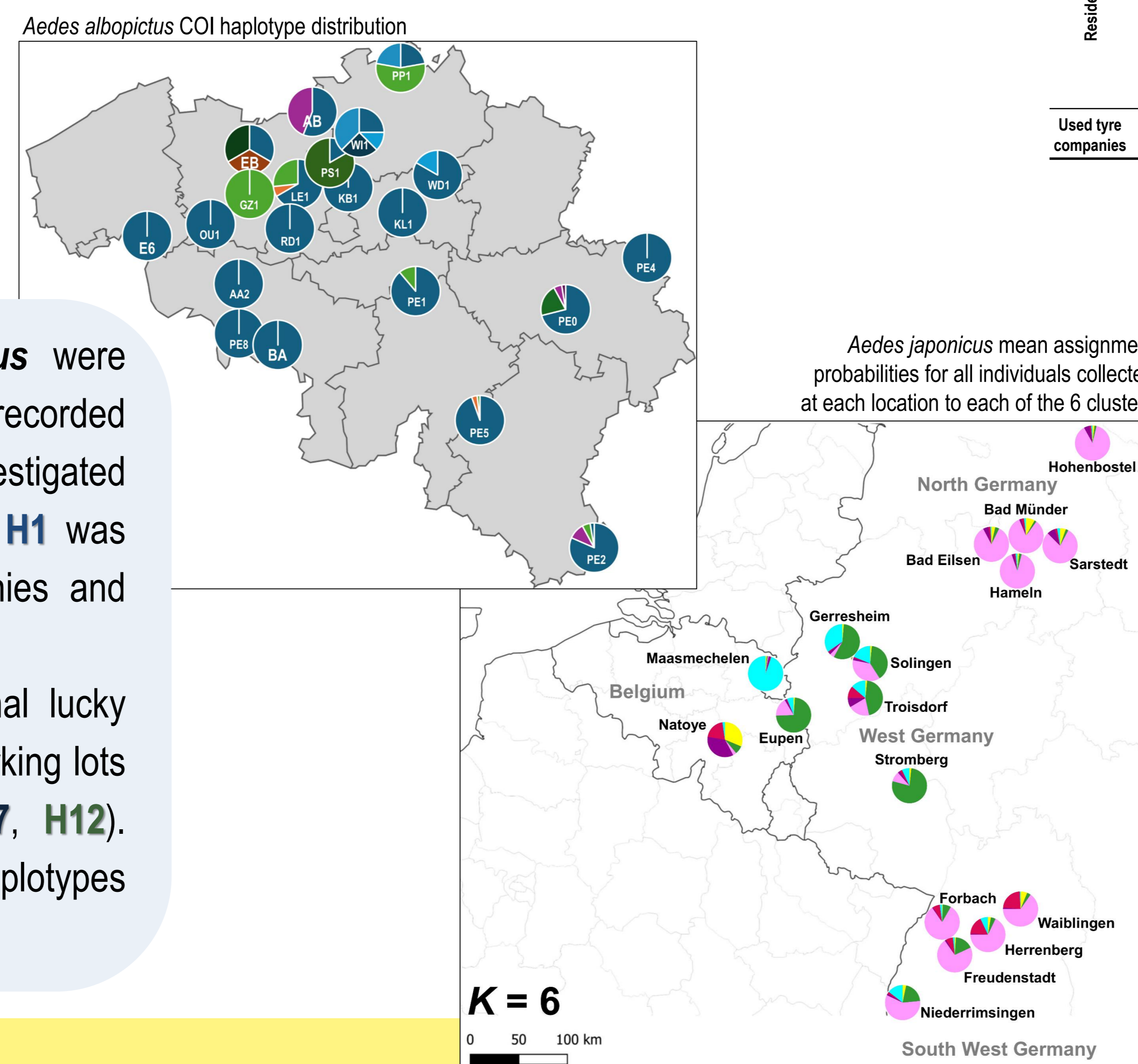
DNA-barcoding was applied to validate the morphological identifications – especially useful in the case of eggs. The genetic diversity at the cytochrome *c* oxidase subunit I was screened within 254 *Ae. albopictus*. The 292 *Ae. japonicus* specimens from Belgium and Germany were genotyped at 7 microsatellites to investigate the origin of recent (re-) introductions along the border with Germany and at Natoye.

PoE type	Code	Location	<i>Ae. albopictus</i> haplotypes													Total	
			H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12	H13		
Lucky bamboo	EB	Lochristi	1						1	1							3
	PE1	Aische-en-Refail	8					1									9
	PE2	Hondelange	31				4	2				1					13
	E6	Kortrijk	7														7
	PP1	Minderhout	2					5								2	9
	PE4	Raeren	4														4
Residential areas	PE8	Saint-Ghislain	6														6
	PE0	Sprimont	27					8		2					1		38
	PE5	Wanlin	52	2					1								26
	AA2	Ath	2														2
	GZ1	Gijzenzele	1						1								1
	KB1	Kapelle-op-den-bos	1														1
	KL1	Kessel-Lo	3														3
	LE1	Lebbeke	20	2					8								30
	OU1	Oudenaarde	1														1
	PS1	Puurs-Sint-Amands	1													5	6
Used tyre companies	RD1	Roosdaal	1														1
	WI1	Wilrijk	2							2							3
	WD1	Wolfsdonk	5			1											8
BA	Frumeries	1														1	
AB	Kallo	14					11										25

RESULTS

Thirteen distinct COI haplotypes of *Ae. albopictus* were identified with one dominant haplotype (H1) that was recorded within more than 189 specimens collected over all investigated locations (Hd: 0.245±0.001 (SD: 0.035)). Haplotype H1 was largely shared between international import companies and parking lots / residential areas.

Unique haplotypes were identified at the international lucky bamboo import company (EB: H8, H9), at highway parking lots (H3, H10, H11), and in residential areas (H4, H7, H12). Highway parking lots and residential areas shared 3 haplotypes (H6, H2, H13).



Finally, haplotype H5 was shared between the used tyre import company AB and two parking lots (PE0, PE2).

Microsatellite investigation of *Ae. japonicus* revealed disparities between specimens collected over years at Natoye (pre and post elimination campaign), suggesting ongoing introductions. Also, clustering analyses showed a link between Belgian specimens of *Ae. japonicus* and those from the western German cluster.

CONCLUSION

The slight difference in haplotype composition at import company PoEs (long-distance introduction) and parking lots / residential areas (medium-distance introduction) in Belgium supports field observations wherein tiger mosquitoes are believed to hitchhike from established populations in neighboring countries via passive ground transport. Both types of sampling site (long-distance (AB) vs. medium-distance (PE0 & PE2)) sharing haplotype H5 may be related to the ongoing import of used tires by a company located in the French department of Aisne (Hauts-de-France region), where *Ae. albopictus* is established. Additionally, for *Aedes japonicus* collected at the border, clustering results suggest a relation with the western German population, suggesting cross-border movements that may complicate eradication efforts. In the future, more detailed geographical origin assessments can be obtained by studying the genetic composition of close-by established populations and using finer-scale genetic markers.



Funding



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