Updated checklist of the mosquitoes (Diptera: Culicidae) of Belgium

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ABSTRACT: Most information about the systematics and bioecology of Belgian mosquitoes dates back from before 1950, and only scattered information was produced during the last decades. In this paper we review and update the list of mosquito species recorded in Belgium, from first report (1908) to 2015. Six genera and 31 species were recorded so far, including 28 autochthonous species and three invasive alien species recently recorded in Belgium: *Aedes albopictus* (Skuse 1894), *Ae. japonicus japonicus* (Theobald 1901), and *Ae. koreicus* (Edwards 1917). The six genera are *Anopheles* (five species), *Aedes* (sixteen species), *Coquillettidia* (one species), *Culex* (four species), *Culiseta* (four species), and *Orthopodomyia* (one species). ***Journal of Vector Ecology* 40 (2): 398-407. 2015.**

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INTRODUCTION

Many insects in the Family Culicidae (Diptera: Nematocera) are vectors of pathogens that cause infectious diseases affecting both humans and animals, such as malaria, dengue fever, Chikungunya disease, West Nile fever, and filariasis. In addition, mosquitoes can be abundant nuisance biters that can further affect human health (Ponçon et al. 2007). Despite their medical interest, Culicidae were little investigated in regards to the Belgian fauna. The first systematic study of Belgian mosquito species was only done at the beginning of the 20th century. The entomologist Goetghebuer (1910a) has published a first preliminary list of nine mosquito species collected in Belgium from 1903 to 1910. In 1925, he also published his updated list of mosquitoes in Belgium with 19 species recorded (Goetghebuer 1908, 1909, 1910a, 1910b, 1921, 1925, 1930, 1934, 1943). Species of the genus *Anopheles* have been previously studied, in particular by Rodhain and Van Hoof (1942, 1943). The latter attributed the role of major vectors of *Plasmodium* in the local context to *An. atroparvus* Van Thiel 1927. However, Goetghebuer (1925) considered that *An. claviger* (Meigen 1804) (at that time named *An. bifurcatus*) was implicated in the malaria fever cases that occurred in the country. Rodhain and Van Hoof (1942, 1943) and Rodhain and Van Mechelen (1944) devoted their work on sibling species of the *An. maculipennis* complex. They noted the presence of *An. atroparvus*, *An. messeae* Falleroni 1926, and *An. maculipennis sensu stricto* Meigen 1818 (under the name *typicus*). More specific work on the Belgian mosquitoes was performed by Wanson (1952) in the Antwerp region and by Van Aken (1961) on chaetotaxic study of pupal stages of certain Belgian Culicidae species. A few decades later, Gosseries and Goddeeris (1991) published a checklist of 24 species of Culicidae. The majority of those species were recorded between 1910 and 1960 and specimens have been stored at the Royal Belgian Institute of Natural Sciences (RBINS). In their electronic identification key, mainly based on literature information, Schaffner et al. (2001) recorded 26 species in Belgium (with two additional species to the last list: *Aedes albopictus* [*Stegomyia albopicta*, *sensu* Reinert et al. (2006)] and *Cx. torrentium* Martini 1925). Thanks to a specific survey at possible points of entry, the invasive mosquito *Ae. albopictus* was identified for the first time in Belgium at a used tire storage facility in 2000 (Schaffner et al. 2004). The species was collected in a company importing used tires from Japan and the U.S.A. located in northern Belgium (Oost-Vlaanderen Province) and, after several years of absence, was found reintroduced very recently at the same site (Boukraa et al. 2013).

Another eastern Asian mosquito, *Ae. japonicus japonicus* [*Hulecoeteomyia japonica sensu* Reinert et al. (2009)], has been recorded in the country. It was observed in southern Belgium (Natoye, Namur Province) first in 2002 (Versteirt et al. 2009). Along with this species, *Cx. hortensis hortensis* Ficalbi 1889 was also recorded and added to the Belgian mosquito fauna (Versteirt et al. 2009). After a new investigation and revision of the Belgium Culicidae collection from the RBINS, Dekoninck et al. (2011a) discovered more specimens of this species [collected by Bequaert in Aywaille, Nonceveux on 6/7/1947] as well as one male and one female of *Culiseta subochrea* (Edwards 1921) [collected at Destelbergen, Heusden (Oost-Vlaanderen Province), 2/8/1944 and in Blankenberge (West-Vlaanderen Province), 6/11/1955, respectively], a species not yet identified and listed among the Belgian fauna. This investigation of RBINS was done in the framework of the MODIRISK project (an inventory of

native and invading mosquito species in Belgium in relation to global change and modelling in 2007-2011). During this project, another exotic species, *Ae. koreicus* [*Hulecoeteomyia koreica*, *sensu* Reinert et al. (2009)] has been identified from Maasmechelen, where it successfully established (Dekoninck et al. 2011a, Versteirt et al. 2012a,b). The MODIRISK project has highlighted, after two years of intensive sampling at 971 locations across Belgium, a total of 23 mosquito species (Versteirt et al. 2013). Our checklist includes all species of mosquitoes that have been recorded and confirmed to occur in Belgium to date. A total of 6 genera and 31 species (28 native species and three invasive alien species) were recorded. It should be noted, however, that some other species are likely to be found in Belgium, including some that are indigenous to neighboring countries, such as *Cs. longiareolata* (Macquart 1838), *Cs. alaskaensis* (Ludlow 1906), *Cs. glaphyroptera* (Schiner 1864), and *Ae. geminus* Peus 1970. Two species have recently been mentioned as found in Belgium, *Cx. modestus* Ficalbi 1889 and *Cs. ochroptera* (Peus 1935) (Dekoninck et al. 2011a), but their presence could not be substantiated and first identifications were not accurate (F. Schaffner, unpublished data). Thus, these species are not included in the current list (see notes). Species are listed according to *A Catalog of the Mosquitoes of the World* and its supplements (Knight and Stone 1977, Knight 1978, Ward 1984, Gaffigan and Ward 1985, Ward 1992), and also the classification of the subgenus *Culex* and *Anopheles* proposed by Harbach (1988, 2004). Regarding the species of the tribe Aedini, and their reclassification suggested by Reinert et al. (2000, 2004, 2006, 2009), we use the new classification suggested by Wilkerson et al (2015), maintaining usage of the traditional genera names (correspondences are given in Table 1).

The species list

Based on a literature review of the Belgian mosquito fauna (Goetghebuer 1910a, 1925, Wanson 1952, Gosseries and Goddeeris 1991, Schaffner et al. 2001), the recent revision of the Belgium Culicidae collection from the Royal Belgian Institute of Natural Sciences (RBINS) by Dekoninck et al. (2011a), the systematic Culicidae monitoring in Belgian agricultural environments (Boukraa et al. 2011, 2012), and the nationwide inventory of mosquitoes in the framework of the MODIRISK project (Versteirt et al. 2013), we construct the revised checklist of mosquitoes of Belgium (Table 2). A total of 31 mosquito species have been recorded in all of Belgian territory, belonging to six genera: *Anopheles* (n=5), *Aedes* (n=16), *Coquillettidia* (n=1), *Culex* (n=4), *Culiseta* (n=4), and *Orthopodomyia* (n=1). Table 2 presents a list of species recorded chronologically by Goetghebuer (1925), Gosseries and Goddeeris (1991), Schaffner et al. (2001), Dekoninck et al. (2011a) and the current updated checklist.

CHECKLIST OF BELGIUM MOSQUITOES

Family CULICIDAE
Sub-family ANOPHELINAE
I) Genus *Anopheles* Meigen 1818
Subgenus *Anopheles* Meigen 1818
An. (Ano.) claviger s.l.:
1-*An. (Ano.) claviger s.s.* (Meigen 1804) [Note 1]
An. (Ano.) maculipennis s.l.: [Note 2]
2-*An. (Ano.) atroparvus* van Thiel 1927
3-*An. (Ano.) maculipennis s.s.* Meigen 1818
4-*An. (Ano.) messeae* Falleroni 1926
5- *An. (Ano.) plumbeus* Stephens 1828 [Note 3]

Sub-family CULICINAE
Tribe Aedini (see also Table 1)
II) Genus *Aedes* Meigen 1818
Sub-genus *Aedes* Meigen 1818
6-*Aedes (Aed.) cinereus s.l.* Meigen 1818 [Note 4]

Subgenus *Aedimorphus* Theobald 1903
7-*Ae. (Adm.) vexans* (Meigen 1830) [Note 5]

Subgenus *Dahliana* Reinert, Harbach & Kitching 2006
8-*Ae. (Dah.) geniculatus* (Olivier 1791)

Subgenus *Hulecoeteomyia* Theobald 1904
9-*Ae. (Hul.) japonicus* (Theobald 1901) [Note 6]
10-*Ae. (Hul.) koreicus* (Edwards 1917) [Note 7]

Subgenus *Ochlerotatus* Lynch Arribalzaga 1891
11- *Ae. (Och.) annulipes* (Meigen 1830)
12- *Ae. (Och.) cantans* (Meigen 1818)
13- *Ae. (Och.) caspius* (Pallas 1771) [Note 8]
14- *Ae. (Och.) communis* (De Geer 1776) [Note 9]
15- *Ae. (Och.) detritus* (Haliday 1833) [Note 10]
16- *Ae. (Och.) dorsalis* (Meigen 1830) [Note 11]
17- *Ae. (Och.) flavescens* (Müller 1764)
18- *Ae. (Och.) punctor* (Kirby 1837) [Note 12]
19- *Ae. (Och.) rusticus* (Rossi 1790)
20- *Ae. (Och.) sticticus* (Meigen 1838)

Subgenus *Stegomyia* Theobald 1901
21- *Ae. (Stg.) albopictus* (Skuse 1894) [Note 13]

Tribe Culicini
III) Genus *Culex* Linnaeus 1758
Subgenus *Culex* Linnaeus 1758
Cx. (Cux.) pipiens s.l.:
22-*Cx. (Cux.) pipiens pipiens* Linnaeus 1758 [Note 14]
Cx. (Cux.) pipiens pipiens biotype *pipiens* Linnaeus 1758
Cx. (Cux.) pipiens pipiens biotype *molestus* Forskal 1775
23-*Cx. (Cux.) torrentium* Martini 1925 [Note 15]

Subgenus *Maillotia* Theobald 1907
24-*Cx. (Mai.) hortensis hortensis* Ficalbi 1889 [Note 16]
Subgenus *Neoculex* Dyar 1905
25-*Cx. (Ncx.) territans* Walker 1856

Table 1. Correspondence of species names of tribe Aedini according to the traditional [*sensu auctorum*], revised [*sensu* Reinert et al. (2009)], and new [*sensu* Wilkerson et al. 2015] classifications.

Traditional nomenclature *sensu auctorum*	Revised nomenclature *sensu* Reinert et al. (2009)	New nomenclature *sensu* Wilkerson et al. (2015)
Aedes (Aedes) cinereus	*Aedes cinereus**	*Aedes (Aedes) cinereus*
Aedes (Aedimorphus) vexans	*Aedimorphus vexans**	*Aedes (Aedimorphus) vexans*
Aedes (Finlaya) geniculatus	*Dahliana geniculata**	*Aedes (Dahliana) geniculatus*
Aedes (Finlaya) japonicus	*Hulecoeteomyia japonica**	*Aedes (Hulecoeteomyia) japonicus*
Aedes (Finlaya) koreicus	*Hulecoeteomyia koreica**	*Aedes (Hulecoeteomyia) koreicus*
Aedes (Ochlerotatus) annulipes	*Ochlerotatus annulipes**	*Aedes (Ochlerotatus) annulipes*
Aedes (Ochlerotatus) cantans	*Ochlerotatus cantans**	*Aedes (Ochlerotatus) cantans*
Aedes (Ochlerotatus) caspius	*Ochlerotatus caspius**	*Aedes (Ochlerotatus) caspius*
Aedes (Ochlerotatus) detritus	*Ochlerotatus detritus**	*Aedes (Ochlerotatus) detritus*
Aedes (Ochlerotatus) dorsalis	*Ochlerotatus dorsalis**	*Aedes (Ochlerotatus) dorsalis*
Aedes (Ochlerotatus) communis	*Ochlerotatus communis**	*Aedes (Ochlerotatus) communis*
Aedes (Ochlerotatus) flavescens	*Ochlerotatus flavescens**	*Aedes (Ochlerotatus) flavescens*
Aedes (Ochlerotatus) punctor	*Ochlerotatus punctor**	*Aedes (Ochlerotatus) punctor*
Aedes (Rusticoidus) rusticus	*Ochlerotatus (Rusticoidus) rusticus*	*Aedes (Ochlerotatus) rusticus*
Aedes (Ochlerotatus) sticticus	*Ochlerotatus sticticus**	*Aedes (Ochlerotatus) sticticus*
Aedes (Stegomyia) albopictus	*Stegomyia albopicta**	*Aedes (Stegomyia) albopictus*

*Subgenus unassigned.

Tribe Culisetini
IV) Genus *Culiseta* Felt 1904
Subgenus *Culicella* Felt 1904
26-*Cs. (Cuc.) fumipennis* (Stephens 1825)
27- *Cs. (Cuc.) morsitans* (Theobald 1901)

Subgenus *Culiseta* Felt 1904
28- *Cs. (Cus.) annulata* (Schrank 1776)
29- *Cs. (Cus.) subochrea* (Edwards 1921) [Note 17]

Tribe Mansoniini
V) Genus *Coquillettidia* Dyar 1905
Subgenus *Coquillettidia* Dyar 1905
30- *Cq. (Coq.) richiardii* (Ficalbi 1889)

Tribe Orthopodomyiini
VI) Genus *Orthopodomyia* Theobald 1904
31- *Or. pulcripalpis* (Rondani 1872) [Note 18]

NOTES

Note 1. The Claviger complex includes two species, designated *An. claviger s. s.* and *An. petragnani* Del Vecchio 1939 (Coluzzi 1962). These two species can be distinguished morphologically by some characters of the immature stages (Coluzzi et al. 1965, Schaffner at al. 2001) and genetically by protein electrophoresis (Schaffner et al. 2000, 2003) and by a polymerase chain reaction (PCR) amplification of the second internal transcribed spacer of ribosomal DNA (ITS2) region (Kampen et al. 2003). *Anopheles claviger* was first reported in Belgium under the name *An. bifurcatus* L. by Goetghebuer (1908). The species is present and common throughout the territory of Belgium. It has been reported particularly along the littoral, the woods in Flanders, the Lower Scheldt, around Ghent, and in the extreme south in Virton (Goetghebuer 1925, 1930, 1934, 1943, Van Aken 1961). In the nationwide inventory of MODIRISK project, Versteirt et al. (2013) captured *An. claviger s.s.* at over 100 study sites. It was ubiquitous in different environments prospected, including urban/ rural/ import risk areas industry. All populations from the Claviger complex collected investigated by morphology or molecular tools were identified as belonging to *An. claviger s.s.* (Versteirt et al. 2013), which is not surprising as *An. petragnani* has a circum-Mediterranean distribution. *Anopheles claviger* probably played a role as a vector in the transmission of malaria plasmodia in Belgium (Goetghebuer 1925) and in some other parts of Europe (Jetten and Taskken 1994).

Note 2. The Maculipennis subgroup contains nine Palearctic species, of which seven are reported in Europe (Harbach 2004). *Anopheles subalpinus* Hackett & Lewis 1935 was removed and placed in synonymy to *An. melanoon* Hackett 1934 (Linton et al. 2002). Due to the similarity of the members of Maculipennis subgroup and overlapping morphological characters, species identification within immature and adult stages is very challenging and usually impossible (Jetten and Takken 1994, Schaffner et al. 2001). It has been suggested they can be sorted on the basis of eggshell patterns, such as markings of the dorsal exochorion, presence of floats and their size, position, and texture (Becker

Table 2. Recorded mosquito species from 1908 to 2015 in Belgium (* = uncertain; + = present; - = absent; [ ] = not counted in the species lists; # recorded by Wanson (1952); *Th. = Theobaldia; Ta. = Taeniorhynchus*).

Species	Goetghebuer (1925)	Gosseries & Goddeeris (1991)	Schaffner et al. (2001)	Dekoninck et al. (2011)	Status of species in Belgium (2015)
An. claviger	+ (Syn.: *An. bifurcatus*)	+	+	+	Native
An. maculipennis s.l.	+	+	[+]	+	[Native]
An. maculipennis s.s.	-	-	+	-	Native
An. messeae	-	+	+	+	Native
An. atroparvus	-	+	+	+	Native
An. plumbeus	-	+	+	+	Native
Ae. cinereus	+	+	+	+	Native
Ae. vexans	+	+	+	+	Native
Ae. geniculatus	+	+	+	+	Native
Ae. japonicus	-	-	-	+	Established
Ae. koreicus	-	-	-	+	Established
Ae. annulipes	+	+	+	+	Native
Ae. cantans	+	+	+	+	Native
Ae. caspius	+ (Syn.: *Ae. punctatus*)	+	+	+	Native
Ae. communis	+	+	+	+	Native
Ae. detritus	+	+	+	+	Native
Ae. dorsalis	-	+	+	+	Native
Ae. flavescens	-	+	+	+	Native
Ae. punctor	+	+	+	+	Native
Ae. rusticus	+	+	+	+	Native
Ae. sticticus	+	+	+	+	Native
Ae. albopictus	-	-	+	+	Introduced
Cx. modestus	-	-	-	+	[Not confirmed]
Cx. pipiens s.l.	+	+	+	+	Native
Cx. p. biotype *pipiens*	-	-	-	-	[Native]
Cx. p. biotype *molestus*	-	-	-	-	[Native]
Cx. torrentium	-	-	+	+	Native
Cx. hortensis	-	-	-	+	Native
Cx. territans	+ (Syn.: *Cx. pyrenaicus*)	+	+	+	Native
Cs. fumipennis	+ (Syn.: *Th. fumipennis*)	+	+	+	Native
Cs. morsitans	+ (Syn.: *Th. morsitans*)	+	+	+	Native
Cs. annulata	+ (Syn.: *Th. annulata*)	+	+	+	Native
Cs. subochrea	-	-	-	+	Native
Cs. ochroptera	-	-	-	+	[Not confirmed]
Cq. richiardii	+ (Syn.: *Ta. richiardii*)	+	+	+	Native
Or. pulcripalpis #,*	-	-	-	-	Native
Number of species	**19**	**24**	**26**	**32**	**31**

et al. 2010). However this last character has shown to be of limited reliability (Proft et al. 1999). Currently, members of the Maculipennis subgroup can be identified to species level using molecular methods, particularly the second internal transcribed spacer of ribosomal DNA (rDNA-ITS2) sequences (Proft et al. 1999, Linton et al. 2003, Djadid et al. 2007) and protein spectra determined by MALDI-TOF Mass Spectrometry (F. Schaffner, unpublished data). Among the seven European species, three were found in Belgium: *An. atroparvus, An. messeae*, and *An. maculipennis s.s.* (Rodhain et al. 1943, Wanson 1952, Gosseries and Goddeeris 1991, Schaffner et al. 2001, Dekoninck et al. 2011a, Versteirt et al. 2013). *Anopheles messeae* and *An. maculipennis s.s.* were considered to be vectors in case their densities were high enough to maintain transmission (Jetten and Takken 1994). In Belgium, *An. atroparvus* was the main malaria vector. It was found not only in the coastal regions of West Flanders and polders of the Province of Antwerp, but also inland in fresh water, including Genck (Limburg Province) and Bierwart (Namur Province). *Anopheles messeae* and *An. maculipennis s.s.* seemed much more rare (Rodhain and Van Hoof 1942, 1943). More recently, results of molecular identification confirmed the presence of *An. maculipennis s.s.* and *An. messeae* throughout the national territory (Versteirt et al. 2013), whereas *An. atroparvus* which was considered the most widespread vector in Belgium (Rodhain and Van Hoof 1942, 1943, Rodhain and Van Mechelen 1944) was not found. The absence of this species is probably due to the scarcity of brackish wetlands currently and their destruction by agricultural and livestock activities. Molecular analysis of ITS2 sequence of Belgian *An. messeae* did not show a clear separation between *An. messeae s.s.* and *An. daciae* Linton, Nicolescu & Harbach 2004 (Nicolescu et al. 2004, Versteirt et al. 2013). Moreover, rDNA-ITS2 variants in *An. messeae* suggest the existence of intraspecific polymorphism and the species status of *An. daciae* is questionable (Bezzhonova and Coryacheva 2008).

Note 3. This tree-hole species was reported for the first time in 1952 by Wanson in Antwerp. Currently, *An. plumbeus* appears to have exploited novel breeding sites and adapted to man-made artificial breeding sites in Belgium especially manure collecting pits of abandoned and unclean pig stables (Dekoninck et al. 2011b). In a recent entomological survey, Boukraa et al. (2013) found it in used tires in Vrasene (Oost-Vlaanderen Province), where *An. plumbeus* was the most abundant adult species captured. Other recent data indicate a strong population expansion of this species all over Belgium (Versteirt et al. 2013) and confirm the aggressive biting and daytime activity of this species which is a highly competent vector of *Plasmodium falciparum* Welch 1897 (Schaffner et al. 2012) and a potential vector of West Nile virus (Dekoninck et al. 2011b).

Note 4. Goetghebuer (1909) recorded *Ae. cinereus* for the first time in Belgium, in Destelbergen (Oost-Vlaanderen Province). After that, the species has been captured in several localities distributed over Belgium (Goetghebuer 1925, 1930, 1943). However, the description of *Ae. geminus* Peus 1970 as a sibling species of *Ae. cinereus* posed a problem about the species that exists in Belgium. The two species are morphologically similar and without the male genitalia the differentiation is difficult or even impossible (Becker et al. 2010). Hence, the records of *Ae. cinereus* in the recent studies in Belgium, including Versteirt et al. (2013) and Dekoninck et al. (2011a) were reported as *Ae. cinereus/geminus*; thus, we report it here as *Ae. cinereus sensu lato*. However, considering the presence of *Ae. geminus* in all neighboring countries (except Luxembourg), it is likely that the species occurs also in Belgium.

Note 5. The *Ae. vexans* taxon contains three subspecies: *Ae. vexans arabiensis* Patton 1905 [found in Africa and Middle east], *Ae. vexans nipponii* (Theobald 1907) [found in East Asia], and *Ae. vexans vexans* (Knight and Stone 1977, Schaffner et al. 2001, Reinert et al. 2004). The latter is the only representative of the subgenus *Aedimorphus* in Europe (Becker et al. 2010). In Belgium, it was first recorded by Goetghebuer (1909) under the old name, *Cx. vexans*. The species was found and listed in the majority of the recent publications on Belgian Culicidae (Gosseries and Goddeeris 1991, Dekoninck et al. 2011a, Versteirt et al. 2013).

Note 6. The *Ae. japonicus* complex is composed of four subspecies described in southeastern Asia: *Ae. j. japonicus*, *Ae. j. shintienensis* Tsai & Lien 1950, *Ae. j. yaeyamensis* Tanaka, Mizusawa & Saugstad 1979 and *Ae. j. amamiensis* Tanaka, Mizusawa & Saugstad 1979 (Tanaka et al. 1979, Cameron et al. 2010). Only *Ae. j. japonicus* was reported outside its original distribution area and it is currently considered one of the most important invasive mosquito species globally (Medlock et al. 2012, 2015). It was already recorded in 2002 in Belgium (Natoye, Namur Province), where it seemed to be well established (Versteirt et al. 2009). Deblauwe et al. (2014) noted that following implementation of the first control measures in 2012, the population had drastically reduced but the species was still present.

Note 7. *Aedes koreicus* is an invasive mosquito species originating from Korea that was recently recorded in Belgium (Versteirt et al. 2012b). Capelli et al. (2011) also found this species in Italy, the second European country that recorded *Ae. koreicus* outside its native region. In Belgium, it was found in Maasmechelen where it was successfully established (Versteirt et al. 2012a, 2012b, Deblauwe et al. 2014). However, its introduction pathways in Belgium remains unclear to date as it was found in a forest near an industrial zone, without a direct link to an international commerce itinerary (Versteirt et al. 2013).

Note 8. The *Ae. caspius* complex includes two subspecies: *Ae. caspius meira* Ribeiro, Ramos, Capela & Pires 1980 [endemic to the Cape Verde archipelago] and *Ae. caspius caspius* (Schaffner et al. 2001). Some authors admitted the presence of a variety in this complex, which is *Ae. caspius* var. *hargreavesi* Edwards (Knight and Stone 1977, Reinert and Harbach 2005). Within the *caspius* subspecies, two genetic forms A and B have been described (Cianchi et al. 1980). In Belgium, *Ae. caspius* was recorded (as *Ae. punctatus*) by Goetghebuer (1925), found in the dunes of Blankenberge (West Flanders Province). There is no indication that different forms of this species exist in Belgium.

Note 9. The taxon *Ae. communis* belongs to a complex with Holarctic distribution. Using DNA barcoding and polymerase chain reaction-restriction fragment length polymorphism, Hooman et al. (2014) noted that this complex contains four cryptic species in North America, namely *Ae. communis*, *Ae. churchillensis* Ellis & Brust 1973, *Ae. nevadensis* Chapman & Barr 1964, and *Ae. tahoensis* Dyar 1916. Only *Ae. communis s.s.* is present in Europe (Schaffner et al. 2001). Goetghebuer (1909) reported *Ae. communis* (as *Oc. nemorosus*) in Belgium (Goetghebuer 1925).

Note 10. The taxon *Ae. detritus* is a Palaearctic complex consists of two species, which are morphologically similar but genetically distinct, including *Ae. detritus* and *Ae. coluzzii* Rioux, Guilvard & Pasteur 1998 (Rioux et al. 1998). In Belgium, Goetghebuer (1921) discovered the presence of *Ae. detritus* in lowlands of Knokke (West Vlaanderen Province). This species was found recently in several rural and natural areas (Versteirt et al. 2013). There is no information about the presence of *Ae. coluzzii* in Belgium.

Note 11. Morphological distinction between *Ae. dorsalis* and the *Ae. caspius* complex is very difficult, and *Ae. dorsalis* was long considered as a subspecies of *Ae. caspius* (Schaffner et al. 2001). Using artificial mating experiments and both morphological and genetically analyses, Lambert et al. (1990) confirmed the validity of *Ae. dorsalis* as a species segregated from *Ae. caspius*. In Belgium, *Ae. dorsalis* was initially mentioned in 1909 (as *Cx. dorsalis*) and confirmed (as *Grabhamia dorsalis*) in Belgium in 1910 (Goetghebuer 1910a). However, Goetghebuer (1925) did not mention this species in his catalog. More recently, Boukraa et al. (2013) found the species again although it was not found in the recent nationwide inventory of Belgian mosquitoes (Versteirt et al. 2013). Because of the difficulty of differentiating *Ae. caspius* and *Ae. dorsalis*, future research based on molecular analyses is needed to confirm the presence of *Ae. dorsalis* in Belgium.

Note 12. The Punctor complex consists of five cryptic species, of which three are present in Europe (Schaffner et al. 2001): *Ae. hexodontus* Dyar 1916, *Ae. punctodes* Dyar 1922, and *Ae. punctor*. In Europe, the first two species have a specific distribution limited to northern regions, unlike *Ae. Punctor,* for which the distribution range extends from Scandinavia to the Mediterranean (Becker et al. 2010). In Belgium, only *Ae. punctor* is present. It is mentioned since 1925 in all checklists of Belgian mosquitoes (Goetghebuer 1925, Gosseries and Goddeeris 1991, Schaffner et al. 2001, Dekoninck et al. 2011a). Versteirt et al. (2013) noted in their recent Belgian monitoring that *Ae. punctor* was found in all studied land cover classes.

Note 13. The Asian tiger mosquito (*Ae. albopictus*) was the first confirmed record of an exotic mosquito species in Belgium (Schaffner et al. 2004). Its discovery dates back to 2000 when larvae were found in a platform of imported used tires located in Vrasene (Oost-Vlaanderen Province) (Schaffner et al. 2004). In 2013, a reintroduction of *Ae. albopictus* in Vrasene was recorded, at the same used tire recycling company where it was observed in 2000 (Boukraa et al. 2013). In addition and more recently, Demeulemeester et al. (2014) found larvae of *Ae. albopictus* that were intercepted in lucky bamboo (*Dracaena braunii* Engl. 1892) shipments originating from the south coast of China. So far, no data demonstrated the establishment of *Ae. albopictus* in Belgium. Nevertheless, its repeated introductions in Belgium through the international used tire trade, the climatic conditions that would allow temperate strains to survive a Belgian winter by an egg diapause, and the highly adaptive ecology of this invasive vector makes it likely that *Ae. albopictus* could establish in Belgium (Schaffner et al. 2004, Fischer et al. 2011, Caminade et al. 2012, Boukraa et al. 2013).

Note 14. The ubiquitous species *Cx. pipiens* is the most abundant species found during the major entomological surveys in Belgium (Boukraa et al. 2012, Versteirt et al. 2013). Wanson (1952) reported the presence of two closely related species, identified as *Cx. pipiens* and *Cx. molestus* from the Antwerp area. However, Harbach et al. (1984) confirmed that in Europe, the *Cx. pipiens* complex contains two distinct forms (pipiens and molestus), which are morphologically indistinguishable but differ considerably in their physiology and behavior.

Note 15. Wanson (1952) recorded *Cx. torrentium* for the first time in Belgium. It can be differentiated from *Cx. pipiens s.l.* by the presence of prealar scales at the apex of mesepisternum and also by the structure of the male phallosome. Except for genitalia analyses, which were considered a good discriminatory character between *Cx. torrentium* and *Cx. pipiens s.l.*, morphological differentiation of larvae is very delicate and even impossible between females of these both species (Schaffner et al. 2001, Becker et al. 2010). Molecular-genetic tools based on various analyses of COI and ace-2 genes remain one of the most reliable methods to address the taxonomic problem between both species (Smith and Fonseca 2004, Fedorova and Shaikevich 2007). In parallel, Börstler et al. (2014) proposed the use of the morphometric wing characters, considered to be a reliable tool and a low-cost alternative in the absence of a molecular laboratory. Gosseries and Goddeeris (1991) did not list *Cx. torrentium* in their checklist. However, the majority of recent research confirmed that this species is one of the most abundant and omnipresent species in Belgium (Boukraa et al. 2012, Dekoninck et al. 2013, Versteirt et al. 2013).

Note 16. *Culex hortensis* has two subspecies, *Cx. hortensis hortensis,* widely represented in the Mediterranean region and the rest of Europe, and *Cx. hortensis maderensis* Mattingly 1955, endemic to the Madeira Archipelago (Portugal) (Knight and Stone 1977, Schaffner et al. 2001). *Culex hortensis hortensis* was recently reported in Belgium where larvae were observed at the site of a second-hand tire company located in Natoye (Namur Province) (Versteirt et al. 2009). After revision of the Belgium Culicidae collection from the RBINS, Dekoninck et al. (2011a) discovered more specimens of this species (collected by Bequaert in Aywaille, Nonceveux on 6/7/1947). Boukraa et al. (2012) confirmed the presence and establishment of *Cx. hortensis* in two other localities, Chênée and Sprimont (Liège Province), where both the immature and adult stages were found. The larvae of this species were sampled in water troughs and ponds situated in

grasslands of equestrian farms (Boukraa et al. 2012). Larvae of *Cx. hortensis* usually occur in clear water and may develop in habitats with the presence of algae/vegetation, such as cemented drinking troughs, garden pots, puddles in torrent-beds, and small ponds (Schaffner et al. 2001, Becker et al. 2010).

Note 17. The presence of *Cs. subochrea* was already reported by Wanson (1952) during his study on mosquito nuisance in Antwerp. But no other field study has confirmed this species in Belgium. However, after a new investigation and revision of the Belgium Culicidae collection from the RBINS, Dekoninck et al. (2011a) found specimens belonging to *Cs. subochrea* that were collected by M. Bequaert at Destelbergen (Oost-Vlaanderen Province) in 1944 and in Blankenberge (West-Vlaanderen Province) in 1955. The current distribution of this species in Belgium remains unclear. More investigation and attention on identification of *Cs. Annulata,* especially for pupae, may be recommended to avoid confusion between the two species in Belgium.

Note 18. The distribution of *Or. pulcripalpis*, a Palearctic species, extends mainly from the Mediterranean region to the north of Belgium and southern England, Black Sea coast, and Transcaucasia (Ramsdale and Snow 2001, Becker et al. 2010). It is the only representative of the genus in Europe (Zavortink, 1968). Wanson (1952) recorded some larvae identified from tree holes of elm (*Ulmus spp.)* in Berchem (Antwerpen Province). However, the species was not mentioned in recent checklists and reports. Although it is not difficult to identify *Or. pulcripalpis*, because of the absence of preserved specimens from this single record, its presence in Belgium should be confirmed.

SPECIES NOT INCLUDED ON THE LIST

Culex modestus: The species was added to the most recent list of Belgian mosquitoes (Dekoninck et al. 2011a) based on morphological identification of larvae by Boukraa et al. (2011). However, unreliable characteristics were used and thus the identification could not be confirmed. Nevertheless, considering the occurrence of *Cx. modestus* in all countries surrounding Belgium (except Luxembourg) (Schaffner et al. 2001), an increase in records from England (Golding et al. 2012) and new findings in Denmark (Bodker et al. 2014), it is likely that the species may be present in Belgium.

Culiseta ochroptera: This species was orally recorded by Schaffner (personal communication) and added to the Belgian fauna (Dekoninck et al. 2011a). However, its identification could not be confirmed (by both in-depth morphological study and molecular investigation) and thus the species cannot be added to the Belgian fauna. But considering its occurrence in Germany, it cannot be excluded to find this species in the hilly area of western Belgium, where environmental conditions look the most suitable for that species.

CONCLUSION

The updated list of the Belgian mosquito fauna comprises 31 species. It is clear that the real number of mosquito species in Belgium exceeds the current number as the list may be still incomplete. Other species currently recorded in neighboring countries and under a similar environment and climate may also be present in Belgium. Further investigations targeting both larval stages and adults are required. In addition, increased trade, climate, and environmental changes are potential factors for the expansion and accidental introductions of new species in Belgium. Other more targeted surveys are recommended to study the bioecology and spatiotemporal distribution of the exotic species recorded in Belgium. These elements could be an important input in assessing the risk of re-emergence of some pathogens and parasites.

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