ecdc
EUROPEAN CENTRE FOR DISEASE PREVENTION AND CONTROL

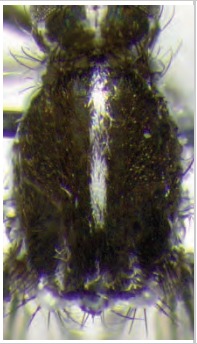
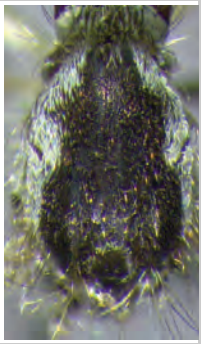
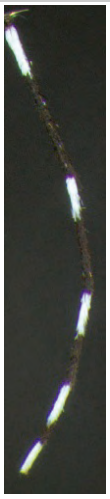
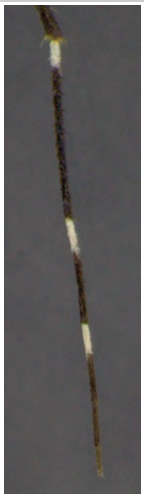
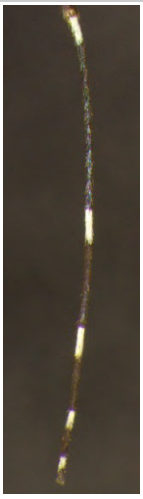
TECHNICAL REPORT

Guidelines for the surveillance of invasive mosquitoes in Europe

www.ecdc.europa.eu

Figure 9: Main diagnostic morphological characters for adults of IMS

Figure 9: Main diagnostic morphological characters for adults of IMS. A. Thorax (scutum, dorsal side); B. Abdomen (dorsal side); C. Hind tarsus (last segments of the third leg). Males have a more hairy and slender abdomen. A. Thorax (scutum, dorsal side); B. Abdomen (dorsal side); C. Hind tarsus (last segments of the third leg).
Males have a more hairy and slender abdomen

Ae. aegypti	*Ae. albopictus*	*Ae. atropalpus*	*Ae. j. japonicus*	*Ae. koreicus*	*Ae. triseriatus*
					
A. Silver scales in a shape of a lyre	A. Median silver-scale line	A. Mixed yellow and dark scales, dark median stripe	A. 5 stripes of golden scales, submedians long	A. 5 stripes of golden scales, submedians short	A. Broad median dark-scale band, sides with silver scales
					
B. Pale lateral marks and thin basal bands	B. Thin basal pale bands, laterally enlarged	B. Pale basal bands	B. Pale lateral and median patches	B. Pale lateral and median patches	B. Pale lateral patches
					
C. Segment V entirely white	C. Segment V entirely white	C. Inter-articular pale bands S. V white	C. Segments IV & V black	C. Segments IV and V with basal pale bands	C. Tarsi entirely dark

4 A key for the rapid identification of European container-breeding Aedine mosquitoes (larvae and adult females)

There is no identification tool available which includes all six IMS reported in Europe (cf. Table A). The available identification keys – which also include some IMS – cover a large spectrum of other species; consequently, the identification process can be rather time-consuming, unless it is multi-access (as in a computer-aided system). It is therefore suggested that a specific tool should be developed to rapidly and easily distinguish IMS from other container-breeding mosquitoes; this tool should also be specifically adapted to the surveillance of IMS.

The 37 species and sub-species reported in Europe as breeding in natural or artificial containers are listed in Table B. As a first step, a written dichotomous key is provided here for (1) sorting mosquito genera; and (2) identifying all European container-breeding Aedine species, for both larvae and adult females.

Frequency of reports of container-breeding mosquito species according to their preferences for larval breeding sites, in their respective distribution area	**Tyre**	**Road drains**	**Other man-made containers**	**Phytotelms**	**Soft water rock pool**	**Salt water rock pool**	**Natural (non-container)**
Ae. aegypti	+++	++	+++	++	++	-	-
Ae. albopictus	+++	++	+++	++	++	-	-
Ae. atropalpus	+++	-	+++	+	+++	-	-
Ae. berlandi	-	-	-	+++	-	-	-
Ae. cretinus	+	-	+	+++	-	-	-
Ae. eatoni	-	-	+	+++	-	-	-
Ae. echinus	-	-	-	+++	-	-	
Ae. geniculatus	++	-	+	+++	-	-	-
Ae. gilcolladoi	-	-	-	+++	-	-	-
Ae. j. japonicus	+++	+++	+++	++	+++	-	-
Ae. koreicus	+++	+	+++	+	++	-	+[2]
Ae. mariae[2]	-	-	-	-	-	+++	-
Ae. p. pulcritarsis	-	-	-	+++	-	-	-
Ae. triseriatus	++	-	+	+++	+	-	-
Ae. vittatus	-	-	-	-	++	-	-
Ae. zammitii[1]	-	-	-	-	-	+++	-
An. claviger	++	-	+	-	+	-	+++
An. maculipennis s.l.	+	-	+	-	-	-	+++
An. petragnani	+	-	+	-	+	-	+++
An. plumbeus	++	+	+	+++	-	-	-
Cs. annulata	++	-	++	-	-	-	+++
Cs. atlantica	-	-	-	-	+++	-	-
Cs. bergrothi	-	-	-	-	+	-	++
Cs. glaphyroptera	+	-	+	-	+	-	++
Cs. longiareolata	++	++	+++	-	++	-	++
Cs. subochrea	-	-	++	-	-	-	+++
Cx. h. hortensis	++	+	+++	-	+++	-	+++
Cx. h. maderensis	-	-	+	-	+++	-	+
Cx. impudicus	-	-	-	-	+	-	+++
Cx. laticinctus	-	-	++	-	++	-	+++
Cx. mimeticus	-	-	-	-	+	-	+++
Cx. perexiguus	-	-	+	-	-	-	+++
Cx. pipiens	+++	+++	+++	+	++	-	+++
Cx. territans	-	-	+	-	-	-	+++
Cx. theileri	-	-	++	-	+	-	+++
Cx. torrentium	+	+	+++	-	-	-	+++
Or. pulcripalpis	-	-	-	+++	-	-	-

[1] Also salt water containers [2] Road tracks, ditches, garden ponds

+++ frequently reported; ++ occasionally reported; + rarely reported; - not reported at all.

Key to larvae (4th instar) of container-breeding Aedine mosquitoes of Europe

1 Genera

1 Siphon absent ..... *Anopheles*

Siphon well developed 2

2 Pecten absent ..... *Orthopodomyia pulcripalpis*

Pecten present 3

3 Siphonal setae (1-S) consisting of three or more pairs ..... *Culex*

Siphonal setae (1-S) consisting of a single pair 4

4 Siphonal setae (1-S) inserted near base of siphon ..... *Culiseta*

Siphonal setae (1-S) inserted nearer to middle of siphon ..... *Aedes* (incl. *Ochlerotatus*)

2 Aedes (incl. Ochlerotatus)

1 Siphonal index < 4.0; pecten teeth long and spine-like 2

Siphonal index > 4.0; pecten teeth short and scale-like 14

2 Cephalic seta 5-C multiple (4–7) branched and inserted far forwards on the front at level of 4- and 6-C and anterior to 7-C 3

Cephalic seta 5-C simple or double branched and inserted far posterior to 4- and 5-C and at level of or posterior to 7-C 5

3 Pecten spines all evenly spaced 4

Pecten with one or more distal spines more widely spaced ..... *Ae. japonicus*

4 Siphonal index > 2.5; saddle bearing developed spicules at its distal margin ..... *Ae. koreicus*

Siphonal index < 2.5; saddle bearing long spines at it distal margin [Madeira and Canary archipelagos] ... *Ae. eatoni*

5 Pecten with one or two distal spines more widely spaced 6

Pecten spines all evenly spaced 7

6 Comb of abdominal segment VIII developed in a triangular patch of more than 20 fringed scales ..... *Ae. atropalpus*

Comb of abdominal segment VIII developed in a single row of 6–9 large and pointed scales ..... *Ae. vittatus*

7 Antennal seta 1-A simple 8

Antennal seta 1-A with 6–9 branches 15

8 Abdominal setae 1 of segment I not longer than half of the length of the segment, with at most four branches and slightly stellate 9

Abdominal setae 1 of segment I as long or longer as the segment, with at least four branches and clearly stellate 12

9 Thoracic setae 10-12-M and 10-12-T inserted near a small tooth; comb scales with a strong central tooth and several minute basal denticles or without denticles 10

Thoracic setae 10-12-M and 10-12-T inserted near a stout spine; comb scales with well developed basal denticles each side of central tooth ..... *Ae. aegypti*

10 Comb of segment VIII in a single regular row of scales with a strong central tooth and several minute basal denticles; saddle lateral seta 1-X simple or double branched 11

Comb of segment VIII in a single or partly double irregular row of scales with a strong central tooth and without denticles; saddle lateral seta 1-X multiple (5–7) branched ..... *Ae. triseriatus*

11 Outer cephalic seta 7-C with 2–3 branches; additional seta on the siphon ..... *Ae. albopictus*

Outer cephalic seta 7-C simple; in addition to the siphonal tuft 1-S, a simple seta is inserted laterally within the apical third of the siphon ..... *Ae. cretinus*

12 Branches of stellate seta 1 on abdominal segment I about the same length as the segment; pecten 1/4–2/5 as long as the siphon 13

Branches of stellate seta 1 on abdominal segment I longer than the length of the segment; pecten at least half as long as the siphon ..... *Ae. echinus*

13 Comb scale number usually less than 16; cephalic setae 6-C with 1–2 branches ..... *Ae. geniculatus*

Comb scale number usually more than 17; cephalic setae 6-C with 3 or more branches ..... *Ae. gilcolladoi*

14 Siphon extremely long and slender, siphonal index > 5.5; comb of segment VIII developed in a triangular patch of 13–24 scales ..... *Ae. berlandi*

Siphon shorter, siphonal index 4.0–5.0; comb of segment VIII developed in a more or less irregular row of 6–10 scales ..... *Ae. pulcritarsis*

15 Antenna with numerous spicules; pecten teeth with less than 4 lateral denticles at the base; comb of segment VIII with usually less than 16 scales ..... *Ae. zammitii*

Antenna with less numerous spicules; pecten teeth with 4 or more lateral denticles at the base; comb of segment VIII with usually more than 16 scales ..... *Ae. mariae*

Key to adult females of container-breeding Aedine mosquitoes of Europe

1 Genera

1 Maxillary palps as long as proboscis; scutellum evenly rounded and uniformly setose ..... *Anopheles*

Maxillary palps distinctly shorter than proboscis; scutellum trilobed, setae arranged in 3 sets 2

2 Prespiracular setae present ..... *Culiseta*

Prespiracular setae absent 3

3 Tarsomere I of fore legs longer than tarsomeres II to V together; tarsomere IV of fore legs reduced, not longer than broad ..... *Orthopodomyia pulcripalpis*

Tarsomere I of fore legs usually shorter than tarsomeres II to V together; tarsomere IV of fore legs not reduced, distinctly longer than broad 4

4 Postspiracular setae present; abdomen tapering apically, cerci long easily visible ... *Aedes* (incl. *Ochlerotatus*)

Postspiracular setae absent; abdomen rounded apically, cerci short, hardly visible ..... *Culex*

2 Aedes (incl. Ochlerotatus)

1 Tarsomeres I–III of hind leg with pale rings 2

Tarsomeres I–III of hind leg without pale rings 8

2 Pale rings present only at base of tarsomeres 3

Each pale ring embraces two tarsomeres, the apex of one and the base of the following tarsomere 11

3 Scutum with white longitudinal stripes; palps with an apical white scale patch 4

Scutum with longitudinal yellowish stripes or bands; palps entirely dark or with a few white scales 7

4 Scutum with one or more longitudinal white stripes; tibia of hind leg without a median white ring 5

Scutum with two or three pairs of small white spots, distributed along the dorsocentral area; tibia of hind leg with a median white rings ..... *Ae. vittatus*

5 Scutum with two narrow medio-dorsal white stripes and 2 broad lateral white stripes, lyre shaped *Ae. aegypti*

Scutum with one acrostrichal white stripe; if lateral stripes are present, they are narrow and do not continue over transverse suture, never lyre-shaped 6

6 Acrostrichal white stripe broad; two posterior medio-dorsal white stripes narrow, short, not reaching the middle of scutum; metameron bare ..... *Ae. albopictus*

Acrostrichal white stripe narrow; two posterior medio-dorsal white stripes narrow, long, reaching the middle of scutum; metameron with a patch of white scales ..... *Ae. cretinus*

7 Hind leg tarsomere IV entirely dark or with a few pale scales at the base (no ring); subspiracular area usually without scale patch ..... *Ae. japonicus*

Hind leg tarsomere IV with a short basal pale ring; subspiracular area usually with a patch of pale scales..... ..... *Ae. koreicus*

8 Scutum with one medio-dorsal acrostrichal narrow stripe, and with two thin or broad lateral stripes..... 9

Scutum with a small anterior acrostrichal patch of white scales, not prolonged in a medio-dorsal stripe, and with two broad white lateral stripes ..... *Ae. triseriatus*

9 Proboscis entirely dark scaled; lateral stripes of scutum broad.....10

Proboscis with pale scales at the apex; lateral stripes of scutum narrow [Madeira and Canary archipelagos] .. *Ae. eatoni*

10 Metameron bare ..... *Ae. geniculatus, Ae. gilcolladoi*

Metameron with a patch of pale scales..... *Ae. echinus*

11 Tarsomeres V of all legs entirely pale scaled 12

Tarsomeres V of fore and mid legs dark scaled, of hind leg entirely pale scaled 13

12 Hind femur entirely dark; hind tibia only white at the apex; metameron with a patch of pale scales..... *Ae. berlandi*

Hind femur and hind tibia scattered with pale scales; metameron bare ..... *Ae. pulcritarsis*

13 Wing veins uniformly dark scaled or with at most a few isolated pale scales; palps entirely dark scaled ... *Ae. atropalpus*

Wing veins covered by mixed dark and pale scales; palps with dark and pale scales.....14

14 Palps with mixed dark and pale scales; metameron with a patch of pale scales..... *Ae. mariae*

Palps with dark scales at the basis and pale scales at the apex; metameron bare..... *Ae. zammitii*

5 Identification of eggs of invasive and indigenous European *Aedes* container-breeding species

Table C: References for egg description of invasive and indigenous container-breeding European *Aedes* species

Mosquito species	Egg description
Ae. aegypti	Matsuo et al. 1972; Linley 1989a
Ae. albopictus	Matsuo et al. 1972; Linley 1989a
Ae. atropalpus	Linley & Craig 1994
Ae. berlandi	Encinas-Grandes 1982
Ae. cretinus	-
Ae. eatoni	-
Ae. echinus	-
Ae. geniculatus	Encinas-Grandes 1982
Ae. gillcolladoi	-
Ae. japonicus	Matsuo et al. 1972; Haddow et al. 2009
Ae. koreicus	-
Ae. mariae	-
Ae. pulcritarsis	-
Ae. triseriatus	Zaim et al. 1977, Linley 1989b
Ae. vittatus	Hinton & Service 1969; Encinas-Grandes 1982
Ae. zammitii	-

6 Identification of mosquitoes by molecular tools

When morphological identification is not possible (e.g. when specimens are damaged), mosquitoes can be identified with genetic methods or protein profiling at any biological stage (from egg to adult), using only part of the insect (e.g. a single leg, a thorax).

Molecular, as morphological identification, can be provided as a service by properly equipped laboratories. The process may vary depending on the country, but mainly depends on the number of specimens to be analysed and the level of performance requested (i.e. whole process or only part of it). Indicative estimations can be given from cheapest to the most expensive process and per specimen, as follow: EUR 24 for MALDI-TOF MS, EUR 28 for morphological identification, 32 EUR for PCR, 56 EUR for PCR/sequencing.

Genetic identification

Mosquitoes can be identified with genetic methods at any biological stage (from egg to adults), using only part of the insect (e.g. a single leg, a thorax).

Genetic information of several loci is available for mosquitoes, particularly at the mtDNA CO1 barcode locus (Table D). Polymerase chain reaction (PCR) combined with sequencing of the amplicon can identify a specimen, providing that corresponding sequence data are deposited in the GenBank (http://www.ncbi.nlm.nih.gov/genbank/) data base. For mitochondrial genes, a sequence identity higher than 98% is needed to confirm identification.

For routine identification of mosquitoes, specific conventional real-time PCR assays have been developed in single or multiplexed formats. Unfortunately, no such assays are as yet available for the invasive and indigenous *Aedes* container-breeding species of concern in Europe, but such developments are in progress.

For DNA-based identification, specimens can be used fresh, kept dry (in tubes with silica gel), in 70% or absolute ethanol, or frozen at -20 °C.

Table D: Molecular identification tools applied on invasive and indigenous *Aedes* container-breeding species

Mosquito spp.	Described gene sequences*						PCR assays	MALDI-TOF MS profile
	rRNA ITS1	rRNA ITS2	rDNA 18s	mtDNA CO1	mtDNA CO2	mtDNA ND4		
Ae. aegypti	✓	✓	✓	✓	✓	✓	✓	
Ae. albopictus	✓	✓	✓	✓	✓		✓	
Ae. atropalpus				✓	✓			
Ae. berlandi								
Ae. cretinus		✓						
Ae. eatoni								
Ae. echinus								
Ae. geniculatus				✓				
Ae. gillcolladoi								
Ae. japonicus				✓	✓			✓
Ae. koreicus					✓	✓		
Ae. mariae				✓				
Ae. pulcritarsis								
Ae. triseriatus	✓	✓		✓				
Ae. vittatus			✓				✓	
Ae. zammitii								

** Only the most frequently used sequences are listed; rRNA/DNA: ribosomal RNA/DNA; mtDNA: mitochondrial DNA*

Protein profiling

Identification of mosquitoes by isoenzyme analyses has been described (Rioux et al 1998; Awono et al 2006), but this approach is time-consuming and expensive and has only been used for invasive species in one study; this study revealed diagnostic enzymes that exhibited species-specific patterns that correctly identify *Ae. cretinus* and *Ae. albopictus* (Taafe Gount et al. 2004). For such identifications, specimens can only be used fresh or stored frozen at -80 °C.

As an alternative, protein profiling by using matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS) was recently described for the characterisation of *Ae. japonicus*, and this approach was extended to include other mosquito species (Kaufmann et al. 2011) (Table D). MALDI-TOF MS, which is a

rapid, simple, reliable and cost-effective method, was demonstrated to be suitable for the routine species identification of *Culicoides* biting midges (Kaufmann et al. 2012). Reference spectra need to be determined separately for the different developmental stages, and removal of the abdomen is needed to avoid the interference effects of gut contents. The specimens can be analysed fresh or after storage in 70% ethanol for several months.

Key references

Awono-Ambene HP, Simard F, Antonio-Nkondjio C, Cohuet A, Kengne P, Fontenille D. Multilocus enzyme electrophoresis supports speciation within the *Anopheles nili* group of malaria vectors in Cameroon. J Trop Med Hyg. 2006;75(4):656–58.

Bargues MD, Morchón R, Latorre JM, Cancrini G, Mas-Coma S, Simón F. Ribosomal DNA second internal transcribed spacer sequence studies of Culicid vectors from an endemic area of *Dirofilaria immitis* in Spain. Parasitol Res. 2006;99:205-13.

Becker N, Petrić D, Zgomba M, Boase C, Dahl C, Lane J, et al. Mosquitoes and their control. Heidelberg, Dordrecht, New York: Kluwer Academic/Plenum Publishers; 2003.

Becker N, Petrić D, Zgomba M, Boase C, Madon M, Dahl C, et al. Mosquitoes and their control. Heidelberg, Dordrecht, New York: Springer; 2010.

Cameron EC, Wilkerson RC, Mogi M, Miyagi I, Toma T, Kim HC, et al. Molecular phylogenetics of *Aedes japonicus*, a disease vector that recently invaded Western Europe, North America, and the Hawaiian islands. J Med Entomol. 2010 Jul;47(4):527-35.

Cook S, Diallo M, Sall AA, Cooper A, Holmes EC. Mitochondrial markers for molecular identification of *Aedes* mosquitoes (Diptera: Culicidae) involved in transmission of arboviral disease in West Africa. J Med Entomol. 2005;42(1):19-28.

Cywinska A, Hunter FF, Hebert PD. Identifying Canadian mosquito species through DNA barcodes. Med Vet Entomol. 2006 Dec;20(4):413-24.

Darsie RE, Samanidou-Voyadjoglou A. Keys for the identification of the mosquitoes of Greece. J Am Mosq Control Assoc. 1997;13(3):247-54.

Darsie RF, Ward RA. Identification and geographical distribution of the mosquitoes of North America, north of Mexico. University of Florida Press, Gainesville: 2005

Das B, Swain S, Patra A, Das M, Tripathy HK, Mohapatra N, Kar SK, Hazra RK. Development and evaluation of a single-step multiplex PCR to differentiate the aquatic stages of morphologically similar *Aedes* (subgenus: *Stegomyia*) species. Trop Med International Health. 2012;17(2):235-43.

Encinas Grandes A. Taxonomía y biología de los mosquitos del área salmantina (Diptera, Culicidae). Salamanca, Spain Univ. de Salamanca: 1982.

Fonseca DM, Widdel AK, Hutchinson M, Spichiger SE, Kramer LD. Fine-scale spatial and temporal population genetics of *Aedes japonicus*, a new US mosquito, reveal multiple introductions. Molecular Ecology. 2010 Apr;19(8):1559-72.

Gaunt CM, Mutebi JP, Munstermann LE. Biochemical taxonomy and enzyme electrophoretic profiles during development, for three morphologically similar *Aedes* species (Diptera: Culicidae) of the subgenus *Stegomyia*. J Med Entomol. 2004;41(1):23-32.

Gutsevich AV, Monchadskii, Shtakel'berg AA. Fauna of the U.S.S.R. – Diptera: Mosquitoes. Jerusalem: 1974

Haddow AD, Moulton JK, Gerhardt RR, McCuiston LJ, Jones CJ. Description of the egg of *Ochlerotatus japonicus japonicus* (Diptera: Culicidae) using variable pressure scanning electron microscopy. J Med Entomol. 2009;46(1):9-14.

Higa Y, Toma T, Tsuda Y, Miyagi I. A multiplex PCR-based molecular identification of five morphologically related, medically important subgenus *Stegomyia* mosquitoes from the genus *Aedes* (Diptera: Culicidae) found in the Ryukyu Archipelago, Japan. Jpn J Infect Dis. 2010 Sep;63(5):312-6.

Hill LA, Davis JB, Hapgood G, Whelan PI, Smith GA, Ritchie SA, et al. Rapid identification of *Aedes albopictus*, *Aedes scutellaris*, and *Aedes aegypti* life stages using real-time polymerase chain reaction assays. Am J Trop Med Hyg. 2008;79(6):866-75.

Hinton HE, Service MW. The surface structure of aedine eggs as seen with the scanning electron microscope. Ann trop Med Parasitol. 1969; 63(4):409-11.

Ho CM, Liu YM, Wei YH, Hu ST. Gene for cytochrome c oxidase subunit II in the mitochondrial DNA of *Culex quinquefasciatus* and *Aedes aegypti* (Diptera: Culicidae). J Med Entomol. 1995 Mar;32(2):174-80.

Kamgang B, Brengues C, Fontenille D, Njiokou F, Simard F, et al. Genetic structure of the Tiger mosquito, *Aedes albopictus*, in Cameroon (Central Africa). PLoS ONE. 2011;6(5):e20257.

Kaufmann C, Schaffner F, Ziegler D, Pflüger V, Mathis A. Identification of field-caught Culicoides biting midges using matrix-assisted laser desorption/ionization time of flight mass spectrometry. Parasitology, 2012 Feb;139(2):248-58.

Kaufmann C, Ziegler D, Schaffner F, Carpenter S, Pflüger V, Mathis A. Evaluation of matrix-assisted laser desorption/ionization time of flight mass spectrometry for characterization of Culicoides nubeculosus biting midges. Medical and Veterinary Entomology, 2011, 25(1): 32-38

Latrofa MS, Dantas-Torres F, Annoscia G, Genchi M, Traversa D, Otranto D. A duplex real-time polymerase chain reaction assay for the detection of and differentiation between *Dirofilaria immitis* and *Dirofilaria repens* in dogs and mosquitoes. Vet Parasitol. 2012 Apr 30;185(2-4):181-5.

Linley JR, Craig JR. Morphology of long- and short-day eggs of *Aedes atropalpus* and *A. epactius* (Diptera: Culicidae). J Med Entomol. 1994;31(6):855-67.

Linley JR. Comparative fine structure of the eggs of *Aedes albopictus*, *Ae. aegypti*, and *Ae. bahamensis* (Diptera: Culicidae). J Med Entomol. 1989a;26(6):510-21.

Linley JR. Scanning electron microscopy of the egg of *Aedes (Protomacleaya) triseriatus* (Diptera: Culicidae). J Med Entomol. 1989b;26(5):474-8.

Matsuo K, Yoshida Y, Kunou I. The scanning electron microscopy of mosquitoes (Diptera: Culicidae). I. The egg surfaces of five species of *Aedes* and *Armigeres subalbatus*. J Kyoto pref Univ Med. 1972;81(7):358-63.

Medlock J, Hansford KM, Schaffner F, Versteirt V, Hendrickx G, Zeller H, et al. A review of the invasive mosquitoes in Europe: Ecology, public health risks, and control options. Vector Borne Zoonotic Dis. 2012 Mar 26;(12):xxx-xx

Morlais I, Severson DW. Complete mitochondrial DNA sequence and amino acid analysis of the cytochrome C oxidase subunit I (COI) from *Aedes aegypti*. DNA Seq. 2002 Apr;13(2):123-7.

Mousson L, Dauga C, Garrigues T, Schaffner F, Vazeille M, Failloux A-B. Phylogeography of *Aedes (Stegomyia) aegypti* (L.) and *Aedes (Stegomyia) albopictus* (Skuse) (Diptera: Culicidae) based on mitochondrial DNA variations. Genet Res. 2005;86:1-11.

Patsoula E, Samanidou-Voyadjoglou A, Spanakos G, Kremastinou J, Nasioulas G, Vakalis NC. Molecular and morphological characterization of *Aedes albopictus* in northwestern Greece and differentiation from *Aedes cretinus* and *Aedes aegypti*. J Med Entomol. 2006;43(1):40-54.

Reno HE, Vodkin MH, Novak RJ. Differentiation of *Aedes triseriatus* (Say) from *Aedes hendersoni* Cockerell (Diptera: Culicidae) by restriction fragment length polymorphisms of amplified ribosomal DNA. Am J Trop Med Hyg. 2000 Feb;62(2):193-9.

Rey D, Despres L, Schaffner F, Meyran JC. Mapping of resistance to vegetable polyphenols among *Aedes* taxa (Diptera, Culicidae) on a molecular phylogeny. Mol Phylog Evol. 2001;19(2):317-25.

Ribeiro H, Cunha RH. Identification keys of the mosquitoes (Diptera: Culicidae) of Continental Portugal, A cores and Madeira. Eur Mosq Bull. 1999;3:1-11.

Rioux JA, Guilvard E, Pasteur N. Description d'*Aedes (Ochlerotatus) coluzzii* n. sp. (Diptera, Culicidae), espèce jumelle A du complexe detritus. Parassitologia. 1998;40:353-60.

Schaffner F, Angel G, Geoffroy B, Hervy J-P, Rhaiem A, Brunhes J. The Mosquitoes of Europe/Les moustiques d'Europe. An identification and training programm [CD ROM software]/Logiciel d'identification et d'enseignement. Montpellier: IRD Editions & EID Méditerranée; 2001.

Schaffner F. Mosquitoes in used tyres in Europe: species list and larval key. Eur Mosq Bull. 2003;16:7-12.

Tanaka K, Mizusawa K, Saugstad ES. A revision of the adult and larval mosquitoes of Japan (including the Ryukyu Archipelago and the Ogasawara Islands) and Korea (Diptera: Culicidae). Contributions of the American Entomological Institute (Ann Arbor). 1979;16(1):1-987.

Wang J, Huang C. Molecular divergence of the mitochondrial cytochrome oxidase II gene in three mosquitoes. J Am Mosq Control Assoc. 2002;18(4):301-6.

Widdel AK, McCuiston LJ, Crans WJ, Kramer LD, Fonseca DM. Finding needles in the haystack: single copy microsatellite loci for *Aedes japonicus* (Diptera: Culicidae). Am J Trop Med Hyg. 2005 Oct;73(4):744-8.

Zaim M, Lewandowski HB, Newson HD, Hooper GR. Differentiation of *Aedes triseriatus* and *Ae. hendersoni* (Diptera: Culicidae) based on the surface structure of the egg shell. J Med Entomol. 1977;14(4):489-90.