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Culicidae fauna from Canton Ticino and report of three new species for Switzerland

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In The Canton of Ticino, a Swiss border region located on the Southern side of the Alps, knowledge on the mosquito fauna (Diptera: Culicidae) is both dated and incomplete. Therefore, we gathered data from different studies conducted between 2003 and 2011 on the mosquito fauna found in this area. Immature stages were sampled using a standard pint dipper (model 1132, BioQuip Products, USA). Adults were sampled using heavy duty EVS CO_2-baited mosquito traps (model 2801A, BioQuip Products, USA) with dry ice (CO_2) as an attractant, BG-sentinel traps (Biogents® AG, Regensburg, Germany) with BG-Lure (Biogents® AG) and CO_2 as attractants or Mosquito Magnet® Liberty plus with either Octenol or Lurex™ or Lurex3™ as attractant. A total of 27'688 mosquitoes (both juvenile and adult stages) were sampled in urban areas and major wetlands, with a focus on the Bolle di Magadino. Mosquitoes were morphologically identified to the species level. A total of 23 mosquito species were recorded, among which, three had never been found in Switzerland (*Aedes caspius*, *Coquillettidia buxtoni*, *Culex modestus*) and a further three which were new for the Canton of Ticino (*Ae. cataphylla*, *Cq. richiardii*, *Culex martinii*). Mosquito species causing major nuisance in settlements around wetlands are *Ae. sticticus*, *Ae. vexans* and *Cq. richiardii*, and in urban areas *Cx. pipiens / torrentium* and *Ae. albopictus*. Natural areas can act as a refuge for adult *Ae. albopictus* away from control measures in urban area, but they are unsuitable for reproduction. Wetlands in the Italian Province of Varese were sampled in order to compare mosquito fauna and anticipate introductions. No difference was detected except for the species *Uranotaenia unguiculata* that was not observed in the Canton of Ticino. Information on the mosquito fauna is important from a conservation perspective and also for our understanding of disease vector ecology

Keywords: Culicidae, surveillance, Bolle di Magadino, new records, *Aedes albopictus*, *Aedes caspius*, *Aedes cataphylla*, *Coquillettidia buxtoni*, *Coquillettidia richiardii*, *Culex martinii*, *Culex modestus*.

INTRODUCTION

Public attention on Culicidae (Diptera, Nematocera) is due to their nuisance and importance as vectors of pathogens. The possibility that allochthonous species are introduced into new areas due to climate change and worldwide trade, together with the discovery of their competence for new pathogens, underscore the need to improve our knowledge on mosquito distribution and diversity in specific regions. The climatic and geographic features of Ticino (Cotti *et al.* 1990), located south of the Alps in Switzerland, make this region of particular interest for studying the diversity of the mosquito fauna. The first published works on mosquitoes of Ticino were concerned mainly with malaria vectors, when, at the beginning of the last century, the

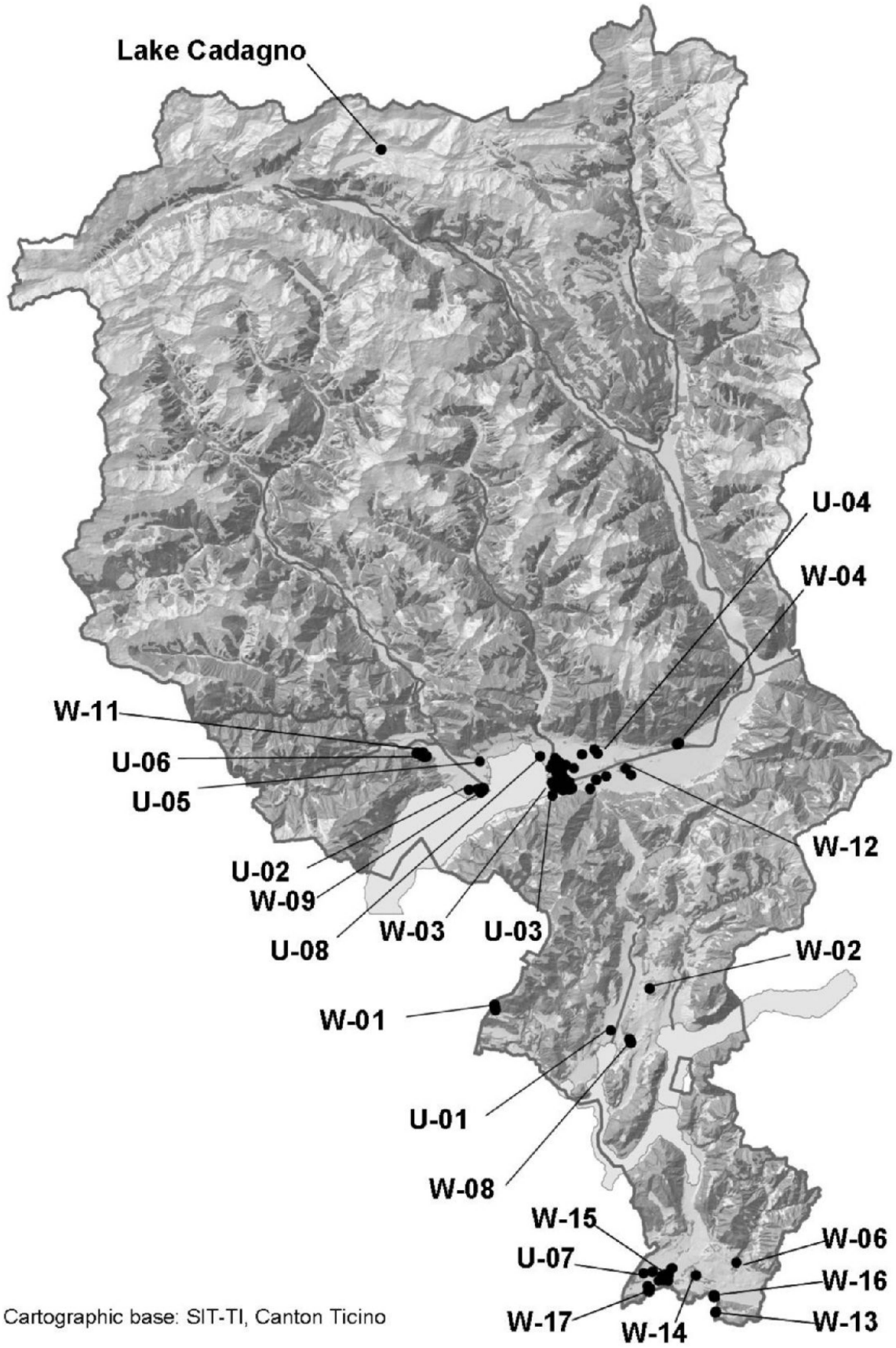

Fig. 1. Sampling locations of mosquitoes in canton Ticino (details are presented in Tab. 1).

Plane of Magadino and particularly the wetland reserve of the Bolle di Magadino, remained the only Swiss area still affected by the disease (Galli-Valerio 1905). Subsequently, malaria disappeared from the region in the 1930's (Borrani 1937). At the same time, another study on mosquitoes, not focused on malaria vectors, enlarged the knowledge of the mosquito fauna of the region (Vogel 1931).

Only at the end of the 1980's, when mosquito bites became a problem for residents and an economic threat for tourism for the region of Locarno, studies on mosquito fauna in the wetland area of Bolle di Magadino were reinitiated (Focarile 1987; Fouque *et al.* 1991) and mosquito control programmes were established (Fouque *et al.* 1998; Lüthy 1987).

Here we gathered data on mosquitoes from multiple sources covering a period between mid-April and the end of October 2003 to 2011. Monitoring at the Bolle di Magadino was intensified and additional wetlands of the Canton Ticino were investigated. To compare mosquito species and anticipate new introductions, wetlands in the Italian Province of Varese were also studied. In addition, mosquitoes were trapped to assess if they were a nuisance in urban areas and in villages bordering wetlands (Flacio 2004; Flacio & Rossi-Pedruzzi 2010; Rossi-Pedruzzi & Casati 2011). The recent urban spread of the tiger mosquito *Aedes albopictus*, which was observed for the first time in 2003 in Ticino (Flacio *et al.* 2004, 2010) pushed us to investigate if natural areas, surrounding colonized villages, could play a role

Tab. 1. Number of mosquito sampling sites and samplings according to the sampling locations in Canton Ticino (Switzerland) and Province of Varese (Italy) (2003 to 2011).
Area codes: W = wetland, U = urban zone.

Area ID	Sampling locations	N° sampling sites	N° of samplings					
			Project 1 2003	Project 2 2004	Project 2 2006	Project 3 2009	Project 3 2010	Project 4 2011
W-01	Astano	7				10	8	
W-02	Bolla di S. Martino (2512)	2				7	6	
W-03	Bolle di Magadino (2314, 2299)	122	118	49	31	75	65	22
W-04	Boschitt di Sementina (2302)	5				9	7	
W-05	Brabbia Swamp - Italy	19	36					
W-06	Gole della Breggia - Balerna	1					1	
W-07	Lake Biandronno - Italy	8	16					
W-08	Lake Muzzano (2323)	10				12	12	
W-09	Maggia river (2333)	12	4	7		9	7	2
W-10	Oasi della Bruschera - Italy	12	12					
W-11	Plane of Arbigo (2331, 3727)	17	15	24		8	13	
W-12	Plane of Magadino (2304, 2310)	7		5	3	2		
W-13	Swamps in Seseglio (2500)	7				12	9	
W-14	Valle della Motta - Coldrerio	1	1					
W-15	Wetlands of Genesterio (2503)	3	22	14		6	6	
W-16	Wetlands of Novazzano (2501)	5				6	7	
W-17	Wetlands of Stabio (2502, 2497)	31	20	43		11	12	
U-01	Agno	1		2				
U-02	Ascona	2	5			1		
U-03	Gambarogno	4		1		4	4	1
U-04	Lavertezzo	1		1				
U-05	Locarno	2	3			2		
U-06	Losone	2				1		
U-07	Stabio	2	7	5				
U-08	Tenero-Contra	2		1				

Note: The inventory number for wetlands of national or cantonal relevance is shown in parentheses.

in the development or the spread of the mosquito (Bernasconi 2010; Flacio & Rossi-Pedruzzi 2010). Gathering this information allows us to update the incomplete knowledge of the mosquito fauna in Ticino. This is important from a conservation perspective and for the assessment of risk hazards for human and animal health.

MATERIAL AND METHODS

Sampling locations

Data presented here originated from four projects conducted between 2003 and 2011. Project one (Flacio 2004) aimed at comparing the mosquito fauna in the major wetlands in Ticino and those in the Province of Varese (Italy) in order to anticipate new introductions and assess if mosquitoes breeding in wetlands could cause nuisance to inhabitants of surrounding villages. The second project continued the sampling in the Bolle di Magadino area as well as in the major wetlands in the south of Ticino: Stabio and Genesterio, in order to have a regular overview of the mosquito fauna in wetlands. The third project extended the survey to additional wetlands of the Canton that were closed to villages colonized by *Ae. albopictus* (Flacio *et al.* 2010) to see if wetlands could play a role in the geographic distribution of this species. The dispersal of *Ae. albopictus* in some wooded locations close to villages colonized by this species was also checked (Bernasconi 2010). The purpose of the fourth project (Rossi-Pedruzzi & Casati 2011) was to collect viruses in mosquitoes of some wetlands and urban zones, but faunistic information on the mosquito fauna of the wetland areas of the Bolle di Magadino and Maggia river was also considered. The sampling locations included in these four projects are listed in Tab. 1 and shown in Fig. 1.

In Ticino fourteen wetlands were controlled, among which eleven are protected (UFAM 2007; UNP 1993), as well as eight urban zones adjacent to the wetlands, i.e. residential locations outside the definite border of wetlands by the national or cantonal inventory. Urban zones were considered as close to wetland if the distance was <500m and distant if the distance was >500m (Tab. 2). A total of 246 sampling sites were investigated (Tab. 1), of which 122 were at Bolle di Magadino between mid-April and end of October 2003 to 2011. In all projects the sampling sites were visited three times in the season.

Additionally, mosquitoes were sampled in three protected wetland areas located in Italy in the bordering province of Varese, namely the Oasi della Bruschera (Protected area by regional laws n. 34934 of 19.07.1988), the Brabbia Ramsar swamp (Protection Area EUAP0323) and the lake Biandronno (Protection Area EUAP0308). Sampling (n=64) was conducted in the period from mid-June to the end of September 2003 (Tab. 1).

Sampling methods

Juvenile stages were sampled using a standard pint dipper (model 1132, BioQuip Products, Rancho Dominguez, USA), consisting of a white plastic container of 11 cm in diameter with a capacity of 350 ml. For every sampling site water collection was repeated at least 3 times in order to cover a total water surface of 30 m^2. Larvae were stored in 70 % ethanol after sampling whereas pupae were kept in the laboratory where they emerged as adults (Projects 1–3). For adult sampling, heavy duty EVS CO_2-baited mosquito traps (model 2801A, BioQuip Products) were placed with the

mosquito entrance at 1.5 m above ground level (projects 1–4) and dry ice (CO_2) was used as attractant. In order to extend the number of species, BG-sentinel traps (Biogents® AG, Regensburg, Germany) with BG-Lure (Biogents® AG) and CO_2 as attractants were placed on the ground (project 3–4). In some cases we used Mosquito Magnet® Liberty plus with either Octenol or Lurex™ or Lurex3™ as an attractant (www.mosquitomagnet.com) (project 2). Traps were placed in the afternoon and removed the following day no later than midday. For instant catches an insect net was used (project 1). Adults were killed by exposure to dry ice and stored at -20 °C.

Identification

Mosquitoes were identified to the species level using morphological keys (Becker *et al.* 2010; Romi *et al.* 1997; Schaffner *et al.* 2001; Severini *et al.* 2009; Stojanovich & Scott 1997). Identifications of species new to Switzerland were verified and confirmed by Dr. F. Schaffner (Institute of Parasitology, University of Zurich, Switzerland). When examining adults, only females were used.

Since differentiation of species by morphological characters is nearly impossible and molecular methods were not applied for the identification (Proft *et al.* 1999; Romi *et al.* 2000), we named *Anopheles maculipennis*: *An. maculipennis* sensu lato (s.l.). For the same reason the two biotypes of *Culex pipiens*, i.e. *pipiens* and *molestus* (Rudolf *et al.* 2013; Kang & Sim 2013), together with its sibling species *Cx. torrentium*, could not be characterized, therefore they are indicated as *Cx. pipiens / torrentium*. Similarly, *Aedes cinereus* and *Ae. geminus* could not be sorted and were considered as belonging to the *cinereus* group, here indicated as *Ae. cinereus / geminus*. Recently, changes were published within the tribe Aedini (Reinert 2000; Reinert *et al.* 2004) that led to a scientific debate and confusion since many names are used for a single taxon. In this paper, the traditional names are used, i.e. *Aedes* is considered as the genus, and *Ochlerotatus* and *Rusticoidus* as subgenera for the *Ochlerotatus* spp. sensu Reinert (2000), the same with *Aedes (Finlaya) geniculatus* for *Ochlerotatus (Finlaya) geniculatus* sensu Reinert (2000), and with *Aedes (Stegomyia) albopictus* for *Stegomyia albopicta* sensu Reinert (2004).

RESULTS

A total of 27'688 individuals (9'293 larvae and 18'395 adults) were collected during 722/802 field samplings (Tab. 1). Three groups and twenty mosquito species were identified, six of which were new to Switzerland and/or Ticino.

New mosquito species for Switzerland and Ticino.

Three mosquito species new to Switzerland (*Aedes caspius*, *Coquillettidia buxtoni*, and *Cx. modestus*) and three to Ticino (*Ae. cataphylla*, *Cq. richiardii*, and *Cx. martinii*) are reported. Samples of these mosquito species are deposited in the collections of the Museo Cantonale di Storia Naturale of Lugano. Sampling locations and environment for these species in reference to the Swiss checklist (Briegel 1998; Merz *et al.* 2006) are compiled in Tab. 2, whereas detailed information including location, date, stage and sampling method are listed in Tab. 3. *Aedes (Ochlerotatus) caspius caspius* (Pallas, 1771) was collected at ten locations from the Italian border area to the region of the Plane of Magadino. This species was regularly recorded during the

Tab. 2. General overview and locations of mosquito species that occur in Canton Ticino (Switzerland) and Province of Varese (Italy).

Species	Reported from Switzerland literature	Reported from Canton Ticino literature	Sampling locations		Obesrved in		
			Canton Ticino (this study)	Province of Varese, Italy (this study)	wetlands	urban area close to wetland (<500 m)	urban area far from wetland (>500 m)
Anopheles (Anopheles) claviger (Meigen, 1804)	Briegel 1998	Borrani 1937; Briegel 1973; Fouque *et al.* 1991; Galli-Valerio 1905; Vogel 1931	W-01, W-04, W-13, W-16		x		
Anopheles (Anopheles) maculipennis Meigen, 1818	Merz et al. 2006	Borrani 1937; Briegel 1973; Focarile 1987; Fouque *et al.* 1991; Galli-Valerio 1905					
Anopheles (Anopheles) maculipennis s.l. Meigen, 1818	Briegel 1998		W-01, W-03-04, W-09, W-12-13, W-15-17, U-03, U-06-07	W-05, W-07, W-10	x	x	
Anopheles (Anopheles) messeae Falleroni, 1926	Merz *et al.* 2006	Briegel *et al.* 2002					
Anopheles (Anopheles) plumbeus Stephens, 1828	Briegel 1998	Borrani 1937; Briegel 1973; Focarile 1987; Vogel 1931	W-03-04, W-09, W-12, W-15-16		x		
Aedes (Aedes) cinereus Meigen, 1818	Briegel 1998	Focarile 1987; Fouque *et al.* 1991					
Aedes (Aedes) cinereus / geminus			W-01-03, W-08-09, W-11, W-15-17, U-06, U-08		x	x	
Aedes (Aedes) geminus Peus, 1970	dubious						
Aedes (Aedimorphus) vexans vexans (Meigen, 1830)	Briegel 1998	Fouque *et al.* 1991; Fouque *et al.* 1998; Lüthy 1987	W-02-04, W-06, W-09, W-11-13, W-15-17, U-01-04, U-08		x	x	
Aedes (Finlaya) geniculatus (Olivier, 1791)	Briegel 1998	Borrani 1937; Briegel 1973; Focarile 1987; Vogel 1931	W-01, W-03, W-11, W-16, U-08; surveillance on *Aedes albopictus*		x	x	x

Species	Reported from Switzerland literature	Reported from Canton Ticino literature	Sampling locations		Obesrved in		
			Canton Ticino (this study)	Province of Varese, Italy (this study)	wetlands	urban area close to wetland (<500 m)	urban area far from wetland (>500 m)
Aedes (Ochlerotatus) annulipes (Meigen, 1830)	Briegel 1998	Fouque *et al.* 1991	W-03, W-17		x		
Aedes (Ochlerotatus) cantans (Meigen, 1818)	Briegel 1998	Focarile 1987; Fouque *et al.* 1991	W-01-03, W-08-09, W-11, W-15-17		x		
Aedes (Ochlerotatus) caspius caspius (Pallas, 1771)			W-03, W-08-09, W-11-12, W-15-17, U-02-03, U-07	W-05	x	x	x
Aedes (Ochlerotatus) cataphylla (Dyar, 1916)	Briegel 1998		Lake Cadagno				
Aedes (Ochlerotatus) communis (De Geer, 1776)	Briegel 1998	Briegel 1973					
Aedes (Ochlerotatus) dorsalis (Meigen, 1830)	Briegel 1998						
Aedes (Ochlerotatus) excrucians (Walker, 1856)	Briegel 1998						
Aedes (Ochlerotatus) flavescens (Müller, 1764)	Briegel 1998						
Aedes (Ochlerotatus) intrudens Dyar, 1919	Briegel 1998	Fouque *et al.* 1991	W-03-04, W-13, W-17		x		
Aedes (Ochlerotatus) pullatus (Coquillett, 1904)	Briegel 1998	Borrani 1937					
Aedes (Ochlerotatus) punctor (Kirby, 1837)	Briegel 1998	Fouque *et al.* 1991					
Aedes (Ochlerotatus) riparius Dyar et Knab, 1907	dubious						
Aedes (Ochlerotatus) sticticus (Meigen, 1838)	Briegel 1998	Borrani 1937; Fouque *et al.* 1991	W-01-03, W-09, W-11-12, W-15, W-17, U-03,-04, U-06-07		x	x	
Aedes (Rusticoidus) refiki (Medschid, 1928)	Briegel 1998						
Aedes (Rusticoidus) rusticus (Rossi, 1790)	Briegel 1998	Focarile 1987	W-09, W-15, U-02		x	x	
Aedes (Stegomyia) albopictus (Skuse, 1894)	Merz *et al.* 2006		W-16-17 and (Flacio *et al.* 2004)		x	x	x
Coquillettidia (Coquillettidia) buxtoni (Edwards, 1923)			W-03, W-08, W-11		x	x	
Coquillettidia (Coquillettidia) richiardii (Ficalbi, 1889)	Briegel 1998		W-03, W-08, W-11, U-06		x	x	

Species	Reported from Switzerland literature	Reported from Canton Ticino literature	Sampling locations		Obesrved in		
			Canton Ticino (this study)	Province of Varese, Italy (this study)	wetlands	urban area close to wetland (<500 m)	urban area far from wetland (>500 m)
Culex (Barraudius) modestus Ficalbi, 1890			W-09, W-13, W-15-17	W-05, W-10	x		
Culex (Culex) pipiens Linnaeus, 1758	Briegel 1998	Borrani 1937; Briegel 1973; Fouque *et al.* 1991; Lüthy 1987; Vogel 1931					
Culex (Culex) pipiens / torrentium			W-01-04, W-08-09, W-11-13, W-15-17, U-01-05, U-07, surveillance on *Aedes albopictus*	W-05, W-07, W-10	x	x	x
Culex (Culex) Theobald, 1903	dubious	Fouque *et al.* 1991					
Culex (Culex) torrentium Martini, 1925	Briegel 1998	Fouque *et al.* 1991					
Culex (Maillotia) hortensis hortensis Ficalbi, 1890	Briegel 1998	Fouque *et al.* 1991; Vogel 1931	W-01, W-03-04, W-08, W-11, W-13, W-15-17, surveillance on *Aedes albopictus*		x		x
Culex (Neoculex) martinii Medschid, 1930	dubious		W-02-03, W-13, W-15,	W-05, W-07, W-10	x		
Culex (Neoculex) territans Walker, 1856	Briegel 1998	Focarile 1987; Fouque *et al.* 1991	W-02-04, W-08-09, W-11, W-13-16	W-05, W-07, W-10	x		
Culiseta (Culicella) fumipennis (Stephens, 1825)	Briegel 1998						
Culiseta (Culicella) morsitans (Theobald, 1901)	Briegel 1998						
Culiseta (Culiseta) annulata (Schrank, 1776)	Briegel 1998	Borrani 1937; Fouque *et al.* 1991	W-02-03, W-08-09, W-12-13, W-15-17, surveillance on *Aedes albopictus*	W-05, W-07, W-10	x	x	x
Culiseta (Culiseta)glaphyroptera (Schiner, 1864)	dubious						
Orthopodomyia pulchripalpis (Rondani, 1872)	dubious	Fouque *et al.* 1991					
Uranotaenia (Pseudoficalbia) unguiculata Edwards, 1913				W-10			

survey from 2003 to 2011, with a total of 79 females and 31 larvae (representing 0.4 % of all mosquitoes recorded). This species was found as adults in both wetlands and urban environments. *Culex (Culex) modestus* Ficalbi, 1890 was observed in four different wetlands in Southern Ticino and in one close to Lake Maggiore. A total of 51 females and 5 larvae (0.2 % of the collected mosquitoes) were identified. This species was also recorded in the Province of Varese (Italy), with four *Cx. modestus* larvae collected at the Oasi della Bruschera and four additional larvae in the Brabbia swamp, representing 0.8 % of the total number of mosquitoes sampled in Italy. Finally, a total of 40 females belonging to the species *Cq. (Coquillettidia) buxtoni* (Edwards, 1923) (1.5 % of the collected mosquitoes) were recorded in three wetland ecosystems and in one urban location close to wetland.

In addition to these three mosquito species newly recorded in Switzerland, an additional three are new only to Ticino. One female of *Aedes (Ochlerotatus) cataphylla* Dyar, 1916 was collected at only one location, in the Saint Gotthard region, the Piora valley (close to the Lake Cadagno) at an altitude of 1960 meters above sea level. *Coquillettidia (Coq.) richiardii* (Ficalbi, 1889) specimens (620 adults; 2.25 % of the collected mosquitoes) were collected in four wetlands around the region of Magadino, in one near the Lake Muzzano and in an urbanized area close to a wetland ecosystem. Finally, 26 females and 88 larvae of *Culex (Neoculex) martinii* Medschid, 1930 (0.4 % of the collected mosquitoes) were identified in four wetlands from the Southern part of Ticino to the region of Magadino.

General overview of the mosquito fauna

Here, a total of 23 mosquito species were identified (Tab. 2). An overview of these mosquito species that occur in Ticino and in some wetlands in Italy in the bordering Province of Varese is provided in Tab. 2. All species that were recorded in Italy were also recorded in Ticino, except *Uranotaenia unguiculata*. In the following sections the mosquito fauna is presented according to the main environments.

a) Wetlands

The wetland area of Bolle di Magadino was an area of high sampling effort, because it is the major wetland area in Ticino and there is a fear amongst local people that mosquitoes could cause problems in residential areas. A total of 18 species could be recorded from this location (Tab. 4). In this ecosystem the mosquito density is influenced by the water level fluctuations of the Lago Maggiore that generate successive temporary flooded areas. The mosquito population is dominated by floodwater species like *Aedes vexans* and *Ae. sticticus*. In permanent water bodies, the most frequent species are *Anopheles maculipennis* s.l, *Ae. cinereus / geminus* and *Culex pipiens / torrentium*.

All species recorded in the other investigated wetland locations were present at Bolle di Magadino, except *Culex modestus* and *Aedes rusticus*, (Tab. 3). *Ae. rusticus* was recorded in the wetlands of Stabio and at the Maggia river.

b) Wetland border zones

A total of ten mosquito species whose main habitats are wetland areas were also collected in wetland border zones (Tab. 2). *Aedes albopictus*, the Asian tiger mosquito,

Tab. 3. Details on mosquito species newly identified in Canton Ticino and/or in Switzerland (part 1).

Species	Date	Sampling location	Method	Adults	Larvae
Aedes albopictus	28.7.2009	Ronchi - Muzzano	Insect net	4	
	1.9.2009	Wetlands of Novazzano	BG-Sentinel	1	
	20.7.2010	Wetlands of Novazzano	BG-Sentinel	1	
	28.8.2011	Val di Spinee - Vacallo	Insect net	10	
Aedes caspius	5.7.2003	Stabio	EVS CO_2	10	
	5.7.2003	Wetlands of Stabio	EVS CO_2	24	
	15.7.2003	Maggia river	EVS CO_2	3	
	18.7.2003	Ascona	Insect net	2	
	16.7.2004	Plane of Arbigo	EVS CO_2	8	
	3.8.2004	Gambarogno	EVS CO_2	3	
	17.8.2004	Plane of Magadino	EVS CO_2	2	
	27.8.2004	Bolle di Magadino	EVS CO_2	5	
	28.8.2004	Plane of Magadino	EVS CO_2	2	
	26.7.2006	Bolle di Magadino	EVS CO_2/Dipper	1	15
	3.8.2006	Bolle di Magadino	EVS CO_2/Dipper	1	15
	9.7.2009	Lake of Muzzano	EVS CO_2	2	
	11.7.2009	Wetlands of Stabio	EVS CO_2	6	
	21.7.2009	Wetlands of Novazzano	EVS CO_2	1	
	24.7.2009	Gambarogno	EVS CO_2	2	
	6.4.2010	Wetlands of Stabio	Dipper		1
	29.6.2010	Maggia river	EVS CO_2	1	
	29.6.2010	Maggia river	BG-Sentinel	1	
	29.7.2010	Wetlands of Stabio	EVS CO_2	1	
	6.7.2011	Bolle di Magadino	EVS CO_2	1	
	15.7.2011	Bolle di Magadino	EVS CO_2	3	
Aedes cataphylla	1.06.2007	Lake Cadagno	Insect net	1	
Coquillettidia buxtoni	16.7.2004	Plane of Arbigo	EVS CO_2	1	
	9.7.2009	Lake Muzzano	BG-Sentinel	3	
	9.7.2009	Lake Muzzano	EVS CO_2	13	
	25.6.2010	Lake Muzzano	EVS CO_2	1	
	25.6.2010	Lake Muzzano	BG-Sentinel	13	
	29.6.2010	Losone	BG-Sentinel	4	
	27.7.2010	Lake Muzzano	BG-Sentinel	3	
	15.7.2011	Bolle di Magadino	EVS CO_2	2	

Tab. 3. Details on mosquito species newly identified in Canton Ticino and/or in Switzerland (part 2).

Species	Date	Sampling location	Method	Adults	Larvae
Coquillettidia richiardii	16.7.2004	Plane of Arbigo	EVS CO_2	57	
	28.7.2004	Plane of Magadino	EVS CO_2	22	
	9.7.2009	Lake Muzzano	BG-Sentinel	4	
	9.7.2009	Lake Muzzano	EVS CO_2	4	
	17.7.2009	Losone	EVS CO_2	61	
	17.7.2009	Losone	BG-Sentinel	85	
	17.7.2009	Maggia river	EVS CO_2	2	
	23.7.2009	Bolle di Magadino	EVS CO_2	1	
	25.8.2009	Bolle di Magadino	EVS CO_2	6	
	28.8.2009	Plane of Arbigo	EVS CO_2	2	
	28.8.2009	Losone	BG-Sentinel	6	
	5.8.2009	Plane of Magadino	EVS CO_2	1	
	20.8.2009	Lake Muzzano	BG-Sentinel	1	
	16.7.2010	Bolle di Magadino	BG-Sentinel	2	
	29.6.2010	Plane of Arbigo	EVS CO_2	13	
	29.6.2010	Losone	BG-Sentinel	238	
	29.6.2010	Maggia river	EVS CO_2	1	
	14.7.2010	Plane of Magadino	EVS CO_2	3	
	14.7.2010	Plane of Magadino	BG-Sentinel	1	
	16.7.2010	Plane of Magadino	EVS CO_2	2	
	27.7.2010	Lake Muzzano	EVS CO_2	1	
	27.7.2010	Lake Muzzano	BG-Sentinel	11	
	20.8.2010	Plane di Arbigo	EVS CO_2	13	
	20.8.2010	Losone	BG-Sentinel	36	
	3.9.2010	Plane of Magadino	EVS CO_2	2	
	3.9.2010	Bolle di Magadino	EVS CO_2	4	
	6.7.2011	Bolle di Magadino	EVS CO_2	1	
	6.7.2011	Bolle di Magadino	EVS CO_2	2	
	15.7.2011	Bolle di Magadino	EVS CO_2	12	
	5.8.2011	Bolle di Magadino	EVS CO_2	5	
	19.8.2011	Bolle di Magadino	EVS CO_2	7	
	26.8.2011	Bolle di Magadino	EVS CO_2	13	
	9.9.2011	Bolle di Magadino	BG-Sentinel	1	
Culex martinii	25.6.2003	Wetlands of Genesterio	EVS CO_2/Dipper	15	40
	26.9.2003	Wetlands of Genesterio	Dipper		20
	16.7.2009	Bolla di S. Martino	BG-Sentinel	3	
	21.7.2009	Swamps in Seseglio	EVS CO_2	1	
	23.7.2009	Bolle di Magadino	Dipper		2
	23.7.2009	Bolle di Magadino	Dipper		4
	23.7.2009	Bolle di Magadino	BG-Sentinel	7	
	27.7.2009	Bolle di Magadino	Dipper		16
	31.8.2009	Swamps in Seseglio	Dipper		1
	6.10.2009	Bolle di Magadino	Dipper		5
Culex modestus	5.7.2003	Wetlands of Genesterio	EVS CO_2	1	
	15.7.2003	Maggia river	EVS CO_2	13	
	7.8.2003	Wetlands of Stabio	Dipper		5
	11.7.2009	Wetlands of Stabio	BG-Sentinel	2	
	11.7.2009	Wetlands of Stabio	EVS CO_2	5	
	21.7.2009	Swamps in Seseglio	BG-Sentinel	1	
	21.7.2009	Swamps in Seseglio	EVS CO_2	27	
	20.7.2010	Wetlands of Novazzano	EVS CO_2	1	
	29.7.2010	Wetlands of Genesterio	EVS CO_2	1	

was the only urban species encountered in these wetland border zones. Adults of this species were recorded at low densities in some natural locations bordering urban areas. This included a wooded area in Muzzano connecting the hard shoulder of the highway to the town, in the wood of the Val de Spinee crossing Morbio Inferiore and Vacallo, and in the wood of the wetland Prà Coltello, which is close to the industrial area of Novazzano (Tab. 2). No evidence of reproduction in larval habitats was observed in these areas and no *Ae. albopictus* were found at Bolle di Magadino. For the malaria vector species *Anopheles maculippennis* s.l., only two adults were collected in an urban area of the Plane of Arbigo and three close to the Bolle di Magadino, while 74 larvae were sampled in a pound in the town of Stabio.

c) Urban areas

In urban areas, six mosquito species were recorded (Tab. 2). Thanks to a surveillance system on *Aedes albopictus* (Flacio *et al.* 2013) it is known that *Culex pipiens / torrentium* and *Ae. albopictus* were the most abundant. Both species are container-inhabiting species but the first was the most frequently found, in particular in catch basins and water storage containers. *Ae. geniculatus* eggs are frequently sampled together with those of *Ae. albopictus* in the framework of the surveillance system of this species that is based on ovitraps placed in urban environments.

Seasonal dynamic of mosquitoes

An overview of the seasonal dynamics of the mosquito species in the surveyed locations is presented in Tab. 5. Early in the season, from April till the end of May, when the water temperature is still low, larvae belonging to the species *Aedes cantans*, *Ae. caspius*, *Ae. cinereus / geminus*, *Ae. sticticus*, *Ae. vexans*, *Anopheles maculipennis* s.l., *Culex hortensis*, *Cx. pipiens / torrentium*, *Cx. territans* and *Culiseta annulata* were found. All species were collected during summertime. In autumn, only adults belonging to the species *Aedes cinereus / geminus*, *Ae. intrudens*, *Ae. sticticus*, *Ae. vexans*, *Culex pipiens / torrentium*, *Anopheles plumbeus* and *An. claviger* could be collected.

DISCUSSION

During the different surveys, we collected mosquitoes belonging to 23 species among which six were new to Switzerland and/or the Canton of Ticino. In doing so, we have provided valuable contributions to our knowledge on the mosquito fauna of the region. The invasive species *Aedes albopictus*, spreading from Italy to Mediterranean countries, is now established in Ticino and the population density is continuously increasing (Flacio *et al.* 2013). Our results show that *Ae. caspius*, a species newly reported for Ticino, is well established in the area. It is a Palaearctic species, known all over Europe (Snow & Ramsdale 1999) and considered to be one of the most nuisance causing mosquito species in northern Italy. It is considered as a brackish water species but can develop in fresh water areas such as rice fields, ponds, marshes and ditches. The female bites all warm-blooded animals and humans. They bite during the day, at dusk and dawn, usually outside the home. Adults can fly long distances, sometimes over 20 km from their breeding sites. The species is considered as a vector for West Nile virus (WNV), Tahyna virus, the bacterium *Francisella tularensis* (the agent of tularaemia) and agents of animal filariasis (Becker 2010). In

Tab. 4. Number of mosquito species collected at Bolle di Magadino (2003-2011) according to their developmental stage (larvae and adults) and number of samplings.

Species	Adults	Samplings	Larvae	Samplings
Aedes annulipes	3	3	0	0
Aedes cantans	366	31	44	3
Aedes caspius caspius	10	7	15	2
Aedes cinereus / geminus	313	84	150	28
Aedes geniculatus	2	2	0	0
Aedes intrudens	25	16	0	0
Aedes sticticus	1139	64	668	38
Aedes vexans vexans	1503	108	1353	67
Anopheles claviger	16	5	0	0
Anopheles maculipennis s.l.	207	53	571	79
Anopheles plumbeus	32	20	65	3
Coquillettidia buxtoni	2	1	0	0
Coquillettidia richiardii	54	25	0	0
Culex hortensis hortensis	0	0	15	4
Culex martinii	7	1	27	4
Culex pipiens / torrentium	392	90	358	31
Culex territans	14	2	1052	73
Culiseta annulata	15	8	91	10

2011 Schaffner & Mathis (2011) also reported the presence of this species in Switzerland, although the location was not specified. However, it is surprising that *Ae. caspius* has not been described earlier in this country since it occurs all over Europe. This species is well known in Italy (Veronesi *et al.* 2012) mainly in wetlands such as rice growing areas. Here, we observed *Ae. caspius* development in one Italian wetland, the Brabbia swamp, where 100 larvae were collected. Even if *Ae. caspius* was detected both in wetlands and urban areas, the observed densities of this mosquito species limit the risk of disease transmission and disturbance of local residents.

The habitat of *Culex modestus* in Ticino and Italy was restricted to wetland ecosystems. Only a limited number of specimens were collected. Its occurrence between July and August corresponds to the findings of a previous survey in Italy (Veronesi *et al.* 2012). This Palaearctic species can be observed all over Europe, except in the Northern countries of Scandinavia and the Baltic Sea (Schaffner *et al.* 2001). In Italy this species is particularly present along Northern coastal regions (Severini *et al.* 2009). The females are very aggressive toward humans and they usually bite at night. *Cx. modestus* is a known vector of WNV and suspected vector

Tab. 5. Seasonal dynamic of the collected mosquito species according to their developmental stages (larvae and adults)

Species	Week of sampling																																
	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44
Aedes annulipes										●		●			●			●													
Aedes cantans	O	O						●		●	●	●	●	●	●	●	O●	●	●												
Aedes caspius	O												●	●	●	●	O●	O●	●		●										
Aedes cinereus / gemnus	O	O				O	O	O●	O	●	O●	O●	●	●	●	●	O●	O●	●	●	●	●	O●	●	●		●	●	●	●	●
Aedes geniculatus											●		●			●		●													
Aedes intrudens												●	●		●		●	●	●	●	●	●	●				●				●
Aedes rusticus														●		●															
Aedes sticticus					O	O	O	O●	O●	●	O●	O●	●	●	●	●	O●	O●	●	●	●	●	O	●				●		●	
Aedes vexans		O				O	O	O●		●	O●	O●	●	●	O●	●	O●	O●	O●	O●	●	●	O●	●			●	●	●	●	●
Anopheles cleviger											●	●	●			O●	O	O●	●				O		O		●			●	●
Anopheles maculipennis s.l.					O	O		O	O	O	●	O●	O●	●	O●	O●	O●	O●	O●	O●	O●	O●	O		O	O					
Anopheles plumbeus										●		●	●		●	●	●	O●	O●	O●	●		●					●	●	●	
Coquillettidia buxtoni												●	●		●	●	●														
Coquillettidia richardii													●	●	●	●	●	●	●	●	●	●	●								
Culex hortensis					O		O	O	O			O				O	●	O			O		O●			O					
Culex martinii													O●			●	O●	O					O			O		O			
Culex modestus														●	●	●	●		O												
Culex pipiens / torrentium							O	●		●	O●	O●	O●	O●	O●	O●	O●	O●	O●	O●	O●	O●	O●	●	O●	O	●	O●	●	●	●
Culex territans								O●	O●	O		O●	O		O●	O●	O	O	O	O	O	O	O	O	O	O		O			
Culiseta annulata									O		●	O●	O●		O●	O●	O●	O●	●		O		O●	O		O		O			
Larvae	O																														
Adults	●																														

of other arboviruses, such as Sinbis, Tahyna, and Lednice (Lundström 1999; Balenghien *et al.* 2007). Although this species demonstrates a high vector competence in some contexts, it does not represent a major health threat in Ticino due to its rare occurrence.

In this study, adults of *Coquillettidia buxtoni*, observed in association with *Cq. richiardii*, were restricted to three wetland locations. Larvae and pupae of this Palaearctic-Mediterranean species develop in permanent waters where they are attached to the roots of the aquatic plants such as sweet flag (*Acorus* spp., very rare in Ticino) and bulrushes (*Typha* spp.) for breathing. They could not be collected in our projects. *Coquillettidia buxtoni* is reported in France, Italy, Romania and Spain (Ramsdale & Snow 2001). This species lives in association with *Cq. richiardii* (Severini *et al.* 2009), as observed in our study. Females bite humans and animals outdoors. There is no knowledge about its arboviral vector competence (Schaffner *et al.* 2001) and control measures against larvae are difficult to implement.

Although we recorded *Coquillettidia richiardii* for the first time in Ticino, this species seems to be widespread in all wetlands around the region of Locarno and the Lake of Muzzano. The absence of previous records in Ticino may be due to the fact that, in previous surveys, traps for adults were not used. Capture of *Cq. richiardii* adults is almost the only method to obtain indication of the presence of this species, and its nuisance was probably confused with one of the species considered the most abundant, i.e. *Aedes vexans*. Females are active between the end of June and the beginning of September and can be a problem for local residents. *Cq. richiardii* is a wetland inhabiting Euro-Siberian species, distributed all over Europe (Ramsdale & Snow 2001). Its ecology is similar to that of *Cq. buxtoni*. The females bite humans and are potential vectors of Batai, Tahyna and WNV. This species posed a nuisance to only one urban settlement, a campsite close to the plane of Arbigo, therefore we do not think there is the potential for arboviral transmission.

Although *Aedes cataphylla* has not been previously described in Ticino, the presence of one individual collected at high altitude is not surprising. This North-Holarctic species can be observed all over Europe and is usually found in cold climates (Schaffner *et al.* 2001). Larvae hatch in depressions filled by snow-melted water and the females bite humans. No pathogen transmission is reported for this species.

Finally, a few individuals of *Culex martinii*, a species newly reported for Ticino, were collected from the end of June to the beginning of October, mostly as larvae. Briegel (1998) in the Swiss checklist for Culicidae stated that the presence of this species in the country needed confirmation, but Schaffner & Mathis (2011) mentioned two observations as well (locations not specified by the authors). Our work confirms the presence of this species in Ticino. This Palaearctic-Mediterranean species is mostly distributed in Central and Southern Europe (Snow & Ramsdale 1999). *Cx. martinii* takes blood meals on batrachians and therefore easily entered the adult traps used. No role of transmission of infectious agents to humans has been reported.

Mosquitoes found in the major wetland of Ticino: the Bolle di Magadino

Many of our study sites were located in the wetland of Bolle di Magadino. Some mosquito species identified here confirmed previous records from this wetland

(Focarile 1987; Fouque *et al.* 1991). This is the case for *Anopheles claviger, An. maculipennis* s.l., *An. plumbeus*, *Aedes cinereus / geminus*, *Ae. vexans*, *Ae. annulipes*, *Ae. cantans*, *Ae. geniculatus*, *Ae. intrudens*, *Ae. sticticus*, *Culex pipiens / torrentium*, *Cx. hortensis*, *Cx. territans* and *Culiseta annulata*. We could not confirm the presence of *Ae. rusticus* in this location, which was reported by Focarile (1987), despite the large number of specimens (17'256 adults and 6'317 larvae) collected. Interestingly, the currently most represented species, i.e. *Ae. vexans* and *Ae. sticticus*, were not mentioned in Focarile's study (1987). Similarly, Fouque *et al.* (1991) reported the presence of *Ae. punctor*, *Cx. theileri* and *Orthopodomyia pulchripalpis*, three species that we did not capture. Briegel (1998) considered that *Cx. theileri* and *Or. pulchripalpis* needed confirmation, which could not be provided here.

Fouque *et al.* (1998) assessed that *Aedes vexans* was responsible for most of the nuisance at the Bolle di Magadino, but we collected almost as many *Ae. sticticus* as *Ae. vexans* and both species frequently bite humans. The detection of *Ae. caspius*, *Culex martinii*, *Coquillettidia buxtoni* and *Cq. richiardii* for the first time at Bolle di Magadino may be due to the large amount of sampling and the first use of EVS CO_2-baited traps, which allow *Coquillettidia* species to be easily captured.

Environments of mosquitoes

All the mosquitoes collected in the wetlands are representative of this ecosystem. We observed that a few species such as *Anopheles maculipennis* s.l., *Aedes cinereus / geminus*, *Ae. geniculatus*, *Ae. caspius*, *Ae. vexans*, *Ae. sticticus*, *Culex pipiens / torrentium*, *Culiseta annulata*, *Coquillettidia buxtoni* and *Cq. richiardii* disperse from the wetlands to more residential areas causing some disturbances to the inhabitants. *Ae. vexans* and *Ae. sticticus* are the most disturbing species around the Bolle di Magadino and the level of nuisance is strongly related to the efficacy of the larval treatments that are used every year in the reserve (Guidi *et al.* 2011; Lüthy 2001; Lüthy & Patocchi 2013). Like Briegel *et al.* (2002), we observed that the presence of malaria vectors like *An. maculipennis* s.l. and *An. plumbeus* is too low to represent a risk for malaria transmission both in wetlands and in surrounding urban areas. *An. plumbeus* is confined to the wetlands and only 5 adults of *An. maculipennis* s.l. could be collected around the wetlands. The genus *Coquilletidia* causes nuisance in a camping area close to the Plane of Arbigo. It is difficult to reduce their numbers with larvicides, but further studies in this direction should be encouraged. *Cx. pipiens / torrentium* can be considered as a ubiquitous species. Currently, two forms (or biotypes) are recognized (Farajollahi *et al.* 2011) i.e. *Cx. pipiens* form *pipiens*, colonizing mostly natural environments, and *Cx. pipiens* form *molestus*, present generally in urban habitats. These two subspecies are characterized by different feeding behaviours: mostly birds for biotype *pipiens* and mammals for biotype *molestus*. *Cx. pipiens* s.l. is considered a bridge vector for several arboviruses, for example WNV (Andreadis 2012). Therefore the monitoring and the virus analysis of this species are included in an ongoing project conducted in Ticino (O. Engler, pers. comm.). The densities of the other mosquito species are too low to cause real nuisance.

Aedes albopictus, as a neobiota, represents a special case with its potential vectorial competence (Gjenero-Margan *et al.* 2011; Gould *et al.* 2010; Moutailler *et al.* 2009; Schmidt-Chanasit *et al.* 2010; Talbalaghi *et al.* 2010; Vazeille *et al.* 2008). This species was discovered in Ticino for the first time in 2003 (Flacio *et al.* 2004).

It is known to be a container-inhabiting urban mosquito colonizing the Mediterranean area of Europe. *Ae. albopictus* has colonized the majority of the urban locations surveyed with the monitoring system established in Ticino since 2000 (Flacio *et al.* 2013). There was no evidence that this species is reproducing in the forest areas of Ticino (Bernasconi 2010; Flacio & Rossi-Pedruzzi 2010). In the sampling locations no potential habitats like tree holes filled with water or neglected human containers were found. Therefore, we assume that adults use the fresh wooden areas to rest. As most efforts to control *Ae. albopictus* are implemented in urbanized parts, individuals can take refuge in surrounding natural areas. Data on the seasonal dynamics of *Ae. albopictus* show that the first hatching of overwintering eggs occurs around mid-April, while their population densities peak around mid-August and adults can be captured until mid-November (Flacio *et al.* 2013).

Concerning the seasonal dynamic of the other mosquito species, our observations fit to what is known. In some cases the number of individuals was so limited i.e. for *Aedes annulipes* (four adults) and *Ae. rusticus* (five adults) that we cannot comment on their seasonal dynamic.

The different projects here add new species to the known Swiss Culicidae fauna and improve our knowledge of the fauna of Ticino. In the future, additional exotic species could be introduced into Ticino, for instance *Aedes japonicus* coming from north of the Alps where it was recently recorded (Schaffner *et al.* 2009; Schaffner *et al.* 2011) or *Ae. koreicus* from Italy (Capelli *et al.* 2011). Other European species new to Switzerland could be detected such as *Culiseta subochrea* which was observed close to Geneva (Direction Générale de la Nature et du Paysage, Etat de Genève, Anya Rossi-Pedruzzi, pers. comm.). In Ticino most studies focus on sites located at low altitudes where human activity is concentrated and the risk of virus transmission to humans is highest. However, mosquito captures at higher altitudes have been reported by Schaffner & Mathis (2011). Observations reported here should encourage new ongoing studies on various aspects of mosquitoes not only in Ticino but also elsewhere in Switzerland.

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