

Seasonal Dynamics, Parity Rate, and Composition of *Culicoides* (Diptera: Ceratopogonidae) Occurring in the Vicinity of Wild and Domestic Ruminants in the Czech Republic

Jana Rádová,^{1,2} Marie Mračková,¹ Zdeňka Galková,¹ Jiří Lamka,³ Karol Račka,⁴ Pavel Barták,⁴ and Jan Votýpka¹

¹Departement of Parasitology, Faculty of Sciences, Charles University, Viničná 7, 12844 Prague, Czech Republic (radova@natur.cuni.cz; MajkaThalie@seznam.cz; yahi@seznam.cz; vapid@natur.cuni.cz), ²Corresponding author, e-mail: radova@natur.cuni.cz,

³Department of Pharmacology and Toxicology, Faculty of Pharmacy, Charles University, Heyrovského 1203, 500 02 Hradec Králové, Czech Republic (lamka@faf.cuni.cz), and ⁴Departement of Virology, State Veterinary Institute Jihlava, Rantířovská 93, 58605 Jihlava, Czech Republic (racka@svujihlava.cz; bartak@svujihlava.cz)

Received 4 September 2015; Accepted 21 November 2015

Abstract

In the light of the emergence of bluetongue and Schmallenberg viruses in northern and central Europe, an extensive entomological survey within the framework of a bluetongue control program was undertaken from 2008 to 2013 in the Czech Republic to investigate *Culicoides* biting midges (Diptera: Ceratopogonidae) collected in close proximity of domestic livestock and semiwild ruminants. Insects were sampled using CDC black-light suction traps placed overnight near ruminants in farms or in forest game preserves to provide data on *Culicoides* fauna collected near these two groups of hosts inhabiting different environments. From almost a half million biting midge specimens collected at 41 sampling sites, 34 species were identified including three species newly recorded for the Czech Republic: *Culicoides* (*Oecacta*) *clastrieri* Callot, Kremer & Deduit, *Culicoides* (*Oecacta*) *odiatus* Austen, and *Culicoides* (*Pontoculicoides*) *saevus* Kieffer. The *Culicoides obsoletus* species group, incriminated as a bluetongue virus vector, was predominant in both domestic livestock (91%) and semiwild game (52%). A relatively high proportion (around 30%) of *C. obsoletus* Meigen females with pigmented abdomen (= more likely parous) was observed from spring till autumn. In contrast, adult biting midges were found to be largely absent during at least three winter months, approximately December till March, which could be considered as the biting midge vector-free period.

Key words: *Culicoides*, livestock, wild ruminant, dynamics, parity rate

Biting midges of the genus *Culicoides* (Diptera: Ceratopogonidae) are distributed worldwide, and include 1,355 extant species classified in 30 subgenera and almost 40 species complexes (Borkent 2015). Hematophagous *Culicoides* females feed on a range of vertebrate species, but often have a clear preferences for mammal or avian hosts (Martínez-de la Puente et al. 2015). Biting midges transmit many parasites including viruses, bacteria, protists, and filarial worms. Some play an important role as the vectors of pathogens responsible for diseases with veterinary and public health significance, especially two notable diseases of ruminants and equines, Bluetongue disease (BTV) and African horse sickness (Linley 1985, Mellor et al. 2000, Carpenter et al. 2013, Purse et al. 2015).

Bluetongue virus has an African origin, and before 1998, bluetongue disease was considered tropical with brief, sporadic invasions in the southern periphery of Europe (Purse et al. 2005).

However, the possible spread of the Afrotropical vector, *Culicoides imicola* Kieffer, over the Mediterranean basin and the introduction of BT in Europe transmitted secondarily by indigenous Palaearctic *Culicoides* species, have plunged Europe into a major sanitary crisis. The first case of BTV emerged in the Czech Republic in 2007 (www.svsr.cz, OIE). Similarly, Schmallenberg virus (SBV), a novel *Culicoides*-transmitted ruminant pathogen, has emerged and spread across Europe since 2011 (Hoffmann et al. 2012), and in 2012, the disease has been reported on the Czech territory (www.svsr.cz).

Culicoides species of veterinary importance usually feed on livestock and horses, and breed in associated habitats. Species suspected to play an active role in BTV and SBV transmissions, e.g., *Culicoides obsoletus*, *C. chiopterus* Meigen, *C. dewulfi* Goetghebuer, *C. pulicaris* L., *C. punctatus* Meigen, and *C. nubeculosus* Meigen (Caracappa et al. 2003, Mehlhorn et al. 2007,

Table 1. List of livestock farm catching sites

No.	Name	GPS-N	GPS-E	Altitude	No.	Name	GPS-N	GPS-E	Altitude
1	Dlouhý Újezd	49° 46'07"	12° 38'08"	520	18	Hradištko	49° 51'44"	14° 24'59"	286
2	Předslav	49° 26'25"	13° 21'09"	407	19	Střemy	50° 23'01"	14° 33'22"	250
3	Kralovice	49° 58'02"	13° 27'15"	465	20	Zákupy	50° 42'32"	14° 37'07"	316
4	Lučina	50° 13'54"	13° 00'47"	471	21	Olešná	49° 32'43"	16° 07'19"	608
5	Havlíčkův Brod	49° 36'40"	15° 33'09"	412	22	Markvarec	49° 5'2"	15° 21'15"	537
6	Dlouhá Ves	50° 25'56"	15° 47'18"	310	23	Rantířov	49° 24'33"	15° 30'50"	491
7	Pracejovice	49° 13'18"	13° 47'48"	627	24	Věcov	49° 36'50"	16° 10'17"	619
8	Křižanovice	49° 51'39"	15° 45'46"	487	25	Těšany	49° 2'56"	16° 46'3"	209
9	Dvůr Králové	50° 26'13"	15° 48'24"	304	26	Koroseky	48° 55'54"	14° 23'34"	438
10	Srbská Kamenice	50° 48'51"	14° 21'09"	220	27	Krčmaň	49° 31'13"	17° 19'53"	215
11	Zásmuky	49° 58'10"	15° 01'54"	307	28	Těšov	49° 1'33"	17° 40'50"	232
12	Úpořiny	50° 36'52"	13° 52'24"	224	29	Heřmanovice	50° 10'28"	17° 25'30"	568
13	Dmýšice	49° 31'30"	14° 19'54"	546	30	Břeží	48° 48'47"	16° 34'22"	193
14	Dolní Žandov	49° 54'52"	12° 40'40"	542	31	Skorošice	50° 19'1"	17° 01'1"	537
15	Struhařov	49° 45'52"	14° 45'22"	422	32	Trnava u Zlína	49° 16'51"	17° 49'20"	346
16	Roudný	50° 33'02"	15° 16'12"	326	33	Skalice	49° 39'38"	18° 23'46"	345
17	Ruda	50° 08'27"	13° 52'32"	418	34	Osík u Litomyšle	49° 50'16"	16° 16'18"	385

Saegerman et al. 2008, Hoffmann et al. 2009, De Regge et al. 2012, Elbers et al. 2013, Veronesi et al. 2013), are present on the territory of the Czech Republic. However, most entomological studies on biting midges in this country were carried out decades ago and did not specifically target species in the proximity of the wild and domestic ruminants, whose health could potentially be impacted. The biting midge fauna was well described by Országh (1980) who enumerated 63 *Culicoides* species for the area of the former Czechoslovakia. After this study, several papers appeared on diversity, biology, and abundance changes of biting midges, or describing new species for the Czech Republic; however, the majority of these studies focused only on limited parts of the country as south Moravia (Knoz and Michálek 1987, Knoz and Vaňhara 1991, Knoz 1998), west and south Bohemia (Országh and Minar 1986, Knoz et al. 2004), or north Bohemia (Tóthová et al. 2004, Knoz and Tóthová 2008). The last and the most up-to-date checklist of *Culicoides* species reported 49 species for the Czech lands of Bohemia and Moravia (Tóthová and Knoz 2009).

The present study is based on a countrywide long-term sampling survey, aimed to describe the biting midge fauna, seasonal dynamics, and the occurrence of parous (with pigmented abdomens) and nulliparous females of the dominant *Culicoides* species present in the proximity of livestock (bovids) and semiwild ruminants (red and fallow deer, mouflon etc.), which may be potentially involved in the life cycle and transmission of BTV and SBV in the Czech Republic.

Materials and Methods

Sampling Sites and Catching Procedures

The survey to collect biting midges was conducted at 34 sampling sites with livestock (Table 1) located throughout the Czech Republic (monitored from 2008 to 2013; usually from April to the beginning of December), and eight localities with semiwild ruminants (monitored in 2010 and 2011; from June to August; Table 2). Biting midges were collected in the immediate surroundings of domestic animals within the cattle farms located in agricultural landscapes or in the close proximity of feed troughs regularly visited by red and fallow deer, mouflon, and other semiwild ruminants situated in forest games parks and preserves. For sampling, CDC black-light suction traps (New Standard Miniature Black-light (UV) Trap,

model 1212, John W. Hock Company, Gainesville, FL) designated for collecting biting midge, and powered by a 6-V battery, were used. In this setup, a suction fan transfers attracted insects to a collection jar with ~50 ml of 50–70% ethanol. Traps equipped with photosensors operated for two consecutive nights (48 h) every two weeks during the sampling seasons. For four selected livestock sampling sites (numbers 15, 20, 26, and 30; see Table 1), we operated traps throughout the whole year. All traps were affixed outside sheds (for livestock) or near feed troughs (for semiwild ruminants), with the lower part of the trap between 1 to 1.5 m above the ground level.

Culicoides Identification

After transferring to the laboratory, biting midges were kept in 70% alcohol until subsequent analyses. All specimens were identified to species level based on wing patterns evaluated by a stereomicroscope and based on the microscopic examination of other characters (e.g., spermathecae, sensilla coeloconica, abundance of mactrotrichie etc.). Doubtful species and species without a wing pattern were mounted into CMCP-9 medium (Polysciences, Warrington, PA) and identified under a light microscope. Several dichotomous keys or species descriptions were used to identify the collected specimens (Országh 1980, Delecolle 1985, www.culicoides.net). Species of the *Culicoides obsoletus* group were classified using multiplex polymerase chain reaction (PCR; Nolan et al. 2007): randomly selected 450 females (150 individually and 300 in six pools; 50 specimens per pool) from different livestock localities were tested. The identification of species with unspotted wings and the species detected for the first time in the Czech Republic were confirmed using barcoding analysis of the mitochondrial cytochrome c oxidase subunit I gene (COI) with LCO 1490 and HCO 2198 primers (Folmer et al. 1994) and compared with the Barcode of Life Data Systems. The total DNA was extracted from individual midges using High pure PCR template preparation kit (Roche, Mannheim, Germany) according to the manufacturer's instructions, with DNA elution into 80 µl of PCR water. PCR amplification was done using PPP Master Mix (Top-Bio, Czech Republic) in volume of 25 µl according to Folmer et al. (1994). To determine the percentage of females with a pigmented abdomen, the feature which most likely corresponds with their parity status, all *Culicoides obsoletus* females obtained from

Table 2. List of forest game preserve sampling sites with semiwild ruminants

No	Name	GPS-N	GPS-E	Altitude	Semiwild ruminants
J1	Bělččko	50° 09'10"	15° 57'41"	287	mouflon, fallow deer
J2	Břevnice	49° 39'12"	15° 36'59"	471	red deer, fallow deer
J3	Lázně Bohdaneč	50° 05'20"	15° 39'52"	222	mouflon, fallow deer
J4	Vřísek	50° 36'44"	14° 30'29"	304	mouflon, bezoar goat
J5	Žleby	49° 53'20"	15° 29'50"	245	red deer, fallow deer, sika deer, Cameroon goat, mouflon
J6	Bystrá	50° 36'40"	15° 24'30"	474	fallow deer
J7A	Vrchlabí	50° 37'32"	15° 37'46"	504	red deer, fallow deer, mouflon
J7B	KRNAP	50° 41'24"	15° 36'26"	974	red deer

semiwild ruminants collections and from the five selected livestock collecting sites with constantly highest number of midges (numbers 2, 11, 18, 20, and 23; see Table 1) were sorted according their physiological stage using the method of abdomen pigmentation (Dyce 1969).

Data Analysis

Original counts (number of individuals) of biting midges were normalized using a Log10 (n + 1) transformation; the normality of data distribution was tested using the analysis of variance (two-way ANOVA main effects) and regression models (STATISTICA 6.0, StatSoft, Inc., Tulsa, OK), using collection sites and year as main effects.

Results

Culicoides Species Composition

During our 6-yr survey, CDC traps were run for 3,453 traps per night during sampling seasons from 2008 to 2013 in livestock farms, and 147 traps per night in 2010 and 2011 close to semiwild ruminants in forest game preserves. Altogether, 43.2% (1,551) traps per night contained no *Culicoides*; in contrast, 2.9% (104) traps captured >1,000 specimens per night, the maximum was 9,490. In total, 466,849 *Culicoides* females were collected. The number of *Culicoides* males in our samples was negligible, lower than 0.1% in total. It should be noted that *Culicoides* specimens made up a significant proportion of all insects captured by the CDC traps. On livestock farms, biting midges composed in total 36.3% of all captured insect specimens (from zero up to 100% of the samples; mean: 14.4%; median: 3.3%); in semiwild ruminant samples, *Culicoides* spp. represent 8.3% of the total number of captured insects (from 1% up to 60%; mean: 11.5%; median: 7.3%).

The morphological identification supplemented in selected cases by barcoding analysis revealed the presence of 34 species belonging to six subgenera: *Avaritia*, *Beltranmyia*, *Culicoides*, *Monoculicoides*, *Oecacta*, and *Pontoculicoides* (Table 3). The following three species were found for the first time in the Czech Republic: *Culicoides clastrieri*, *Culicoides odiatus*, and *Culicoides saevus*.

The two most widespread species found in our survey were *Culicoides obsoletus* and *Culicoides pulicaris*, recorded in all the sites investigated, followed by *C. punctatus*, absent at just one locality, and *C. pallidicornis* Kieffer, absent at three localities. Occurrences of *Culicoides* species at the studied localities are summarized in Table 4.

The species *C. obsoletus* s.s. is one of the most common species in Central and Northern Europe, and together with *C. scoticus* Downes & Kettle, *C. deuulfi*, and *C. chiopterus* forms the *C. obsoletus* group (Savini et al. 2005, Nolan et al. 2007, Werner et al.

Table 3. Biting midge species captured by CDC black-light traps situated in the proximity of livestock in farms and semiwild ruminants in forest game preserves

<i>Culicoides</i> Subgenera / species	Livestock		Game	
	No.	%	No.	%
<i>Oecacta</i>				
<i>achrayi</i>	36	0.01		
<i>clastrieri</i>	42	0.01	96	0.54
<i>pallidicornis</i>	4 525	1.10	3 336	18.79
<i>dzhafarovi</i>	172	0.04		
<i>fascipennis</i>	4	0.00	14	0.08
<i>festivipennis</i>	724	0.16	267	1.50
<i>furcillatus</i>	20	0.00	497	2.80
<i>heliophilus</i>	3 329	0.74	113	0.64
<i>jurensis</i>	8	0.00		
<i>cubitalis</i>	2	0.00	113	0.64
<i>pumilus</i>	23	0.01		
<i>odiatus</i>	1 406	0.31		
<i>pictipennis</i>	452	0.10	152	0.86
<i>pseudoheliophilus</i>	20	0.00		
<i>reconditus</i>	563	0.13	514	2.89
<i>segnis</i>	221	0.05	15	0.08
<i>simulator</i>	2	0.00		
<i>subfascipennis</i>	3	0.00	111	0.63
<i>vexans</i>	86	0.02	32	0.18
<i>Avaritia</i>				
<i>abchazicus</i>	193	0.04		
<i>obsoletus</i> group	408 856	91.04	9 287	52.30
<i>Beltranmyia</i>				
<i>circumsriptus</i>	736	0.16	256	1.44
<i>salinarius</i>	10	0.00		
<i>Culicoides</i>				
<i>griseus</i>	180	0.04		
<i>impunctatus</i>	204	0.05	35	0.20
<i>pulicaris</i>	14 955	3.33	1 312	7.39
<i>punctatus</i>	10 138	2.26	710	4.00
<i>Monoculicoides</i>				
<i>nubeculosus</i>	1 960	0.44	892	5.02
<i>riethi</i>	11	0.00		
<i>stigma</i>	6	0.00	2	0.01
<i>Pontoculicoides</i>				
<i>tauricus</i>	68	0.02		
<i>saevus</i>	138	0.03	2	0.01

2012). Species of this group could be easily distinguished by male morphology; nevertheless the number of collected males was not sufficient for further epidemiological analysis. Females of the *C. obsoletus* group are very similar, which makes their morphological differentiation almost impossible; however, their species identification is possible by using multiplex PCR (Nolan et al. 2007). As

[illegible]

In addition to the host species targeted (livestock vs. semiwild ruminants) and the surrounding environment (agriculture landscape vs. forest), sampling sites also differed in altitude. The highest species richness was found at locality number 12 (Úpořiny; 224 m a.s.l.)

Sampling sites within the cattle farms and semiwild ruminant game preserves resulted in 449,093 (mean: 130 per trap; median: 2 per trap) and 17,756 (mean: 120 per trap; median: 23 per trap) *Culicoides* females, respectively. The numbers of biting midges captured per trap (traps with <10 *Culicoides* specimens captured were excluded from the analysis) varied significantly among years, for both cattle farms (ANOVA; $F=6.61$, $df=3$, 1916; $P<0.001$; mean/median: 2008—548/47, 2009—209/74, 2010—294/69, and 2011—323/77; Fig. 1) and forest game preserves ($F=17.72$, $df=1$, 135; $P<0.001$; mean/median: 2010—239/83 and 2011—71/26). The proportion of the most abundant species, *C. obsoletus*, was similar among years for catches near cattle (2008—89.5%, 2009—92.6%, 2010—90.7%, and 2011—92.8%); on the other hand, we found differences in the proportions of the two main *Culicoides*

with 20 species (Table 4). The most species-poor site was locality number J7B, KRNP (The Krkonoše Mountains National Park) with elevation around 1,000 m and only three species found; however, because of the outstanding elevation, this site was excluded from the analysis. Our overall analysis, however, did not show any significant differences in the number of *Culicoides* species (regression model: $F=0.109$, $df=1$, 39; $P=0.74$) or number of captured biting midge specimens captured per trap per night ($F=0.564$, $df=1$, 812; $P=0.75$) depending on altitude.

Seasonal Dynamics of *C. obsoletus*

The seasonal abundance of the most abundant species, *Culicoides obsoletus*, collected at five livestock farms (numbers 2, 11, 18, 20, and 23) in years 2008 to 2011 is presented in Fig. 1. Numbers of *C. obsoletus* were highest during the spring, but several peaks appeared during the seasons in all four studied years. In all analyzed years, numbers of *C. obsoletus* significantly decreased in late October and the latest specimens were captured on November 27, 2009 and December 2, 2008. The earliest capture of *C. obsoletus* was on March 25, 2010. In 2009–2010, traps ran continuously during the whole winter at the four selected cattle farms (numbers 15, 20, 26, and 30), but no *Culicoides* were found in winter months.

Dynamics of *C. obsoletus* Females With Pigmented Abdomen

At five selected livestock sampling sites (numbers 2, 11, 18, 20, and 23) and at all forest game preserve localities, the physiological status of *Culicoides obsoletus* females was analyzed according to (Dyce 1969) to determine the parity rate dynamics over the five studied years from 2008 to 2013. The rate of parity, represented in our study by females with pigmented abdomen, and the proportion of females with eggs were similar in both types of studied localities (Fig. 2). On livestock farms, where biting midges were captured throughout most of the year, the proportion of pigmented or parous females was lower in the beginning of the year and the first peak appeared at the end of May. Later, pigmented or parous females were present in a relatively high proportion till the end of the year, 31.7% in average (Fig. 2A). Overall, the highest percentage of parous females ever recorded was 78.3% in mid-July of 2013. Similarly as at livestock farms, at localities with semiwild ruminants, the proportion of pigmented or parous females was relatively high and stable throughout the year, 39% in average, and since we caught midges just during three months of the year, the proportion of pigmented (more likely parous) females only varied between 18.8 and 63.2% (Fig. 2B).

Discussion

Species Composition

Our study represents the first systematic and long-term (2008 to 2013) survey of *Culicoides* in the Czech Republic using light trapping. We have studied the occurrence, seasonal dynamics, and proportion of pigmented (most likely parous) females of *Culicoides* species at two types of localities, on livestock farms and in forest game preserves with semiwild ruminants. Previously, 63 species of *Culicoides* biting midges had been recorded from the former Czechoslovakia (Országh 1980), with 49 species in the Czech Republic and 55 species in Slovakia (Tóthová and Knoz 2009).

Our 6-yr study updates the number of *Culicoides* species in the Czech Republic to the current 52 species by the adding of three new species: *Culicoides clastrieri*, *C. odiatus*, and *C. saevus*. All these

three new species of biting midges have recently been found in Slovakia (Tóthová and Knoz 2009, Sarvašová et al. 2014a), and their occurrence in Czechia might be linked to global climate changes, as described previously for other hematophagous diptera (e.g., Wittmann et al. 2001, Votýpka et al. 2008) or to the lack of proper surveys as the former studies were limited in scope. Two of these species, *C. odiatus* and *C. saevus*, are present mainly in southern part of Europe, and the third species, *C. clastrieri*, is more likely in the western and northern part of Europe (Beuk and Pape 2013). In Bohemia, we found for the first time seven species that were previously known just from Moravia: *C. salinarius* Kieffer, *C. riethi* Kieffer, *C. dzhafarovi* Remm, *C. furcillatus*, *C. pseudoheliophilus* Callot et Kremer, *C. simulator* Edwards, and *C. tauricus* Gutsevich; and vice versa the species *C. abchazicus* Dzharfarov was found for the first time in Moravia.

In most studies published after 1980, the trapping sites were selected in wooded fields, meadows, and nearby waterways using different collecting methods. Our study focused on biting midges of veterinary and medical public health importance—potential vectors of bluetongue and/or Schmallenberg viruses—and the choice of localities and trapping methods were adapted to this focus. It is likely that our choice of a backlight trapping method (see Venter et al. 2009, De Regge et al. 2015) in combination with the presence of domestic or semiwild animals close to traps and dissimilar surrounding environments significantly influenced the spectra of *Culicoides* found. We assume that the most significant factor in the occurrence of biting midges at our monitored localities was notably the presence of host animals, large ungulates; on the other hand altitude had no significant effect on the incidence and abundance of biting midge species, which correspond with previous findings from Switzerland (Kaufmann et al. 2012).

The majority of *Culicoides* species caught during our study are considered mammaliophilic. The most abundant and widespread species was the *Culicoides obsoletus*, regularly representing about 80% of the catches. This species was also the most abundant in other central European countries where similar surveillances were carried out (Linto et al. 2002, De Liberato et al. 2003, Clausen et al. 2009, Ander et al. 2012, De Regge et al. 2015). The *Culicoides obsoletus* group consists of four species (Conte et al. 2003, Meiswinkel et al. 2004), out of which three, *C. obsoletus* s.s., *C. dewulfi*, and *C. chiopterus*, were found in our study. The presence of the fourth species, *C. scoticus*, was not detected, though this species was previously described from our territory (Tóthová and Knoz 2009). Different catching methods have been used in other studies, however, and this species was usually collected in very low numbers. As we used multiplex PCR for identification of the *C. obsoletus* group using only 450 specimens from livestock localities, the (apparent) absence of *C. scoticus* in our samples could be explained by its very low proportion and abundance.

Captures on Cattle Farms and in Forest Game Preserves

It is well known that causative agents of several diseases are shared between wildlife and livestock, among them the bluetongue virus (Gortázar et al. 2007), and that pathogens can be easily overlooked when only circulating within wild animals (García et al. 2009). In this study, we sampled biting midge faunas on two types of localities, farms with livestock in agriculture landscape surrounded by meadows and fields and mostly forest game preserves with semiwild ruminants. Both studied type of localities differ in many aspects including surrounding environment, host animals, and also dissimilar collecting seasons. We are fully aware of all these inadequacies

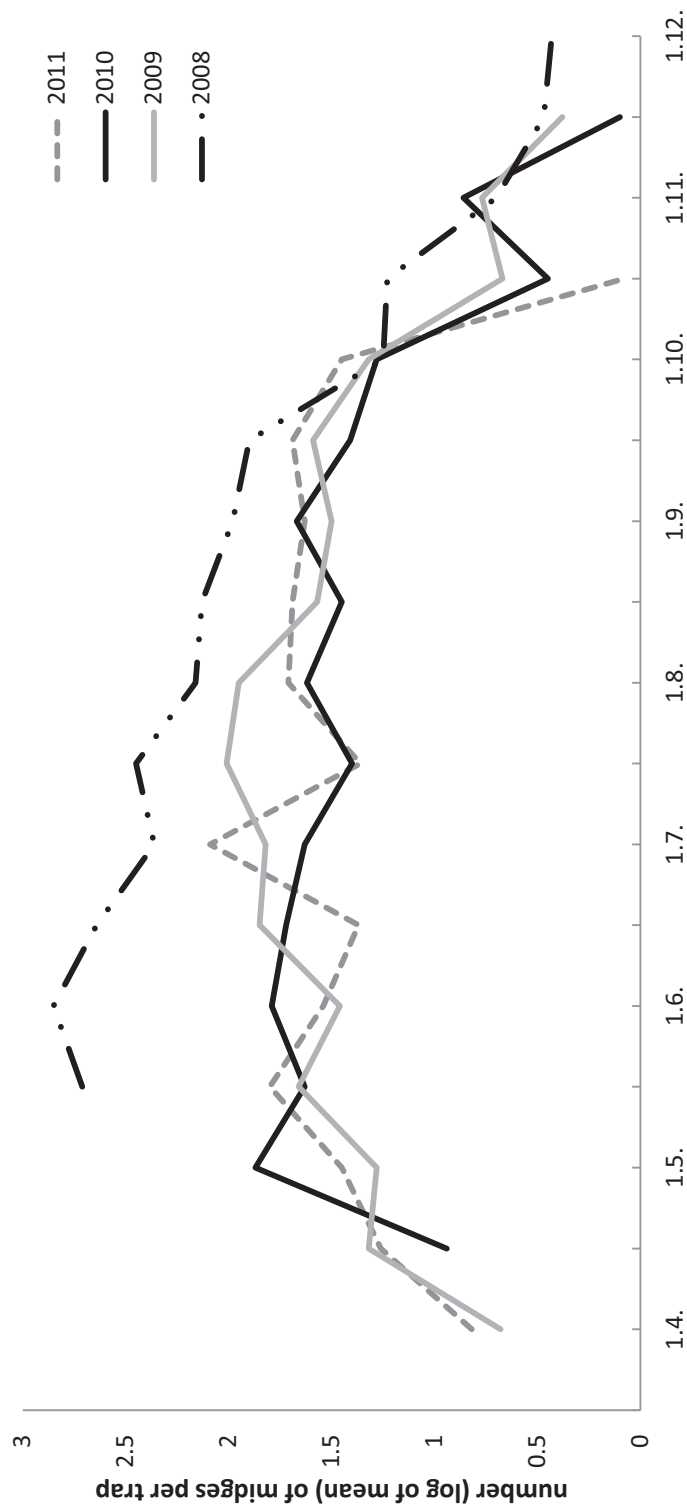


Fig. 1. Seasonal dynamics (expressed as the mean of the log10 of captured biting midges per trap) of *C. obsolitus* collected on livestock farms within four consecutive years, 2008 to 2011.

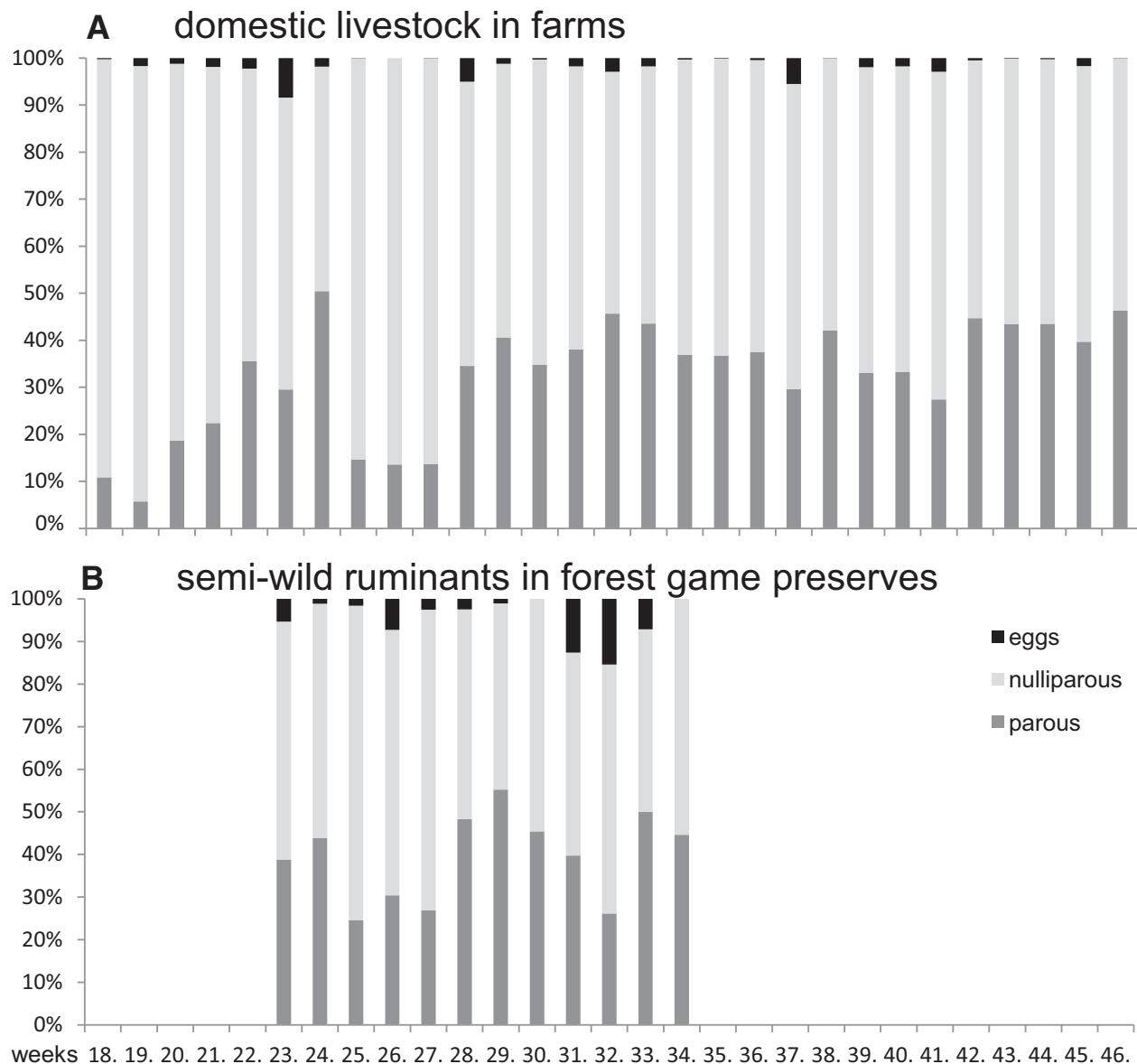


Fig. 2. Combined percentage of parous or pigmented, nulliparous or nonpigmented, and gravid *C. obsoletus* females captured in five (numbers 2, 11, 18, 20, and 23) selected livestock farms (A) and forest game preserves with semiwild ruminants (B).

and discrepancies caused by logistic problems, and it is therefore necessary to consider our results rather tentative. In addition to this, our data are hardly comparable as the number of traps per night was significantly lower for games (147) compared to livestock farms (3,443). Nevertheless some tendencies are obvious. Several *Culicoides* species or groups were common for both types of localities, including *C. obsoletus*, a proven vector of the bluetongue and Schmallenberg viruses. Difference was found in the total species or groups number caught at cattle farm localities (26 species or groups) versus games (16 species or groups). Species composition at sites with semiwild ruminants could be influenced by the surrounding environment (e.g., forest) and only occasional presence of host animals in contrast to farms where livestock were present in stables almost continuously in the close proximity of traps. For example, the higher proportion of ornithophilic species, e.g., *C. circumscriptus*, *C. reconditus*, and *C. festiviipennis* (Országh 1980), in semiwild ruminants traps was most probably influenced by the surrounding

forest environment that accommodates additional hosts. Similarly, the relatively high proportion (2.8%) of *Culicoides furcillatus* in semiwild ruminants traps is in accordance with previous findings of this sylvatic species (Országh 1980) in a forest habitat in Spain collected by Monteys et al. (2009).

Seasonal Dynamics of *C. obsoletus*

The seasonal dynamics of the most abundant species, *C. obsoletus*, reaching >90% of all biting midges in some samples, was analyzed at five selected livestock farms. Several peaks in *C. obsoletus* abundance suggest several consecutive generations during the year and confirm that *C. obsoletus* is a multivoltine species (Országh 1980, Meiswinkel et al. 2014). The first and usually highest peak appeared in the second half of May, as the first generation emerged. Later in the year, several less evident and not well-synchronized peaks appear. The same tendency was also observed in semiwild ruminant

samples, even though we do not have data from most of the year. The earliest capture of *C. obsoletus* (and any *Culicoides* species in general) was on April 8 (2010) and the last on December 2 (2008). According to these data, the biting midge-free (vector-free) period lasts approximately from December till March. The observed between-years differences are probably connected with an average temperature, which was higher during all months of the year 2008; on the contrary, others three seasons were below the long-term average.

Dynamics of *C. obsoletus* Females With Pigmented Abdomen

If transovarial transmission is not considered, only parous females that already fed one or more times can be a vector and are able to transmit pathogens (and thus infections) to their vertebrate hosts. The less time consuming and widely used method of parity determination, the abdomen pigmentation method described by (Dyce 1969), was used in our study. It could be estimated that relatively high numbers of pigmented females presented on localities indicate a high vector potential for pathogens as BTV or SVB. In our study, we found the relatively high proportion of pigmented (more likely parous) females, fluctuating between 30 to 40% in average. Nevertheless in some studies was observed that part (up to 15%) of newly emerged midges show partial abdominal pigmentation (Harrup et al. 2013) and thus our result can be slightly overestimated.

Culicoides obsoletus is a multivoltine species with more generations per year; its parity rate decreased with the disappearance of the old generation and then increased again with feeding of the new generation. Just a few studies have focused on seasonal dynamics of pigmented or parous biting midge females. In California, *Culicoides variipennis* tend to have a highest proportion of nulliparous females in the early season and later the proportion fluctuated around 50% (Linhares and Anderson 1989) and also in Virginia the proportion of *C. obsoletus* parous females varied throughout the seasons (Zimmermann 1983). In the Slovakia, in the virological study *C. obsoletus* parous females ranged from 11 to 80% in May and June catches (Sarvašová et al. 2014b).

The overall percentage of pigmented females was higher in semiwild ruminant localities; however, comparison of the overlapping part of seasons, when both livestock and semiwild ruminant localities were sampled, the proportion of pigmented females is almost identical.

In conclusion, a relatively high number of *Culicoides* species occurs on livestock farms and likely feed on their inhabitants. Some of these species are also present at forest game preserve localities with semiwild ruminants and could serve as bridge vectors for pathogens shared by both groups of ungulates. Although we found three new biting midge species in the Czech Republic, our study yielded no evidence that African and South European BTV vector *C. imicola* midges occur in the country. Therefore, the transmission of bluetongue and Schmallenberg viruses is likely linked to local *Culicoides* species, as in other European countries (Meiswinkel et al. 2007, Dijkstra et al. 2008, Hoffmann et al. 2009). Further host preferences and virological analysis would be beneficial to determine vector species in the Czech Republic.

Acknowledgments

We are grateful to all farmers, game owners, and veterinarians involved in this surveillance program for their kind cooperation and help with biting midge catches. We would like to thank Helena Kulikova and Lenka Zitkova for their administrative support. This project was funded by the EurNegVec

COST Action TD1303 and COST-CZ LD14076 and by the grant SVV 260202 of the Grant Agency of Charles University.

References Cited

- Ander, M., R. Meiswinkel, and J. Chirico. 2012. Seasonal dynamics of biting midges (Diptera: Ceratopogonidae: *Culicoides*), the potential vectors of bluetongue virus, in Sweden. *Vet. Parasitol.* 184: 59–67.
- Beuk, P., and T. Pape. 2013. Fauna Europaea: Ceratopogonidae. Fauna Eur. version 2.6.2, (<http://www.faunaeur.org>)
- Borkent, A. 2015. World Species of Biting Midges (Diptera: Ceratopogonidae). (<http://www.inhs.illinois.edu/files/1114/2384/5200/CeratopogonidaeCatalog.pdf>)
- Caracappa, S., A. Torina, A. Guercio, F. Vitale, A. Calabro, G. Purpari, V. Ferrantelli, M. Vitale, and P. S. Mellor. 2003. Identification of a novel bluetongue virus vector species of *Culicoides* in Sicily. *Vet. Rec.* 153: 71–74.
- Carpenter, S., M. H. Groschup, C. Garros, M. L. Felipe-Bauer, and B. V. Purse. 2013. *Culicoides* biting midges, arboviruses and public health in Europe. *Antiviral Res.* 100: 102–13.
- Clausen, P. H., A. Stephan, S. Bartsch, A. Jandowsky, P. Hoffmann-Köhler, E. Schein, D. Mehlitz, and B. Bauer. 2009. Seasonal dynamics of biting midges (Diptera: Ceratopogonidae, *Culicoides* spp.) on dairy farms of Central Germany during the 2007/2008 epidemic of bluetongue. *Parasitol. Res.* 105: 381–386.
- Conte, A., A. Giovannini, L. Savini, M. Goffredo, P. Calistri, and R. Meiswinkel. 2003. The effect of climate on the presence of *Culicoides imicola* in Italy. *J. Vet. Med. B. Infect. Dis. Vet. Public Health* 50: 139–147.
- De Liberato, C., B. V. Purse, M. Goffredo, F. Scholl, and P. Scaramozzino. 2003. Geographical and seasonal distribution of the bluetongue virus vector, *Culicoides imicola*, in central Italy. *Med. Vet. Entomol.* 17: 388–394.
- De Regge, N., I. Deblauwe, R. De Deken, P. Vantieghem, M. Madder, D. Geysen, F. Smeets, B. Losson, T. van den Berg, and A. B. Cay. 2012. Detection of Schmallenberg virus in different *Culicoides* spp. by real-time RT-PCR. *Transbound. Emerg. Dis.* 59: 471–475.
- De Regge, N., R. De Deken, C. Fassotte, B. Losson, I. Deblauwe, M. Madder, P. Vantieghem, M. Tomme, F. Smeets, and A. B. Cay. 2015. *Culicoides* monitoring in Belgium in 2011: Analysis of spatiotemporal abundance, species diversity and Schmallenberg virus detection. *Med. Vet. Entomol.* 29: 263–275.
- Delecolle, J.-C. 1985. Nouvelle contribution à l'étude systématique et iconographique des espèces du genre *Culicoides* (Diptera: Ceratopogonidae) du Nord-Est de la France. M.S. thesis. Université Louis Pasteur de Strasbourg, France.
- Dijkstra, E., I. J. K. van der Ven, R. Meiswinkel, D. R. Hölzel, P. A. Van Rijn, and R. Meiswinkel. 2008. *Culicoides chiopterus* as a potential vector of bluetongue virus in Europe. *Vet. Rec.* 162: 422.
- Dyce, A. L. 1969. The recognition of nulliparous and parous *Culicoides* (Diptera: Ceratopogonidae) without dissection. *Aust. J. Entomol.* 8: 11–15.
- Elbers, A. R. W., R. Meiswinkel, E. van Weezep, M. M. S. van Oldruitenborgh-Oosterbaan, and E. A. Kooi. 2013. Schmallenberg virus in *Culicoides* spp. Biting midges, the Netherlands, 2011. *Emerg. Infect. Dis.* 19: 106–109.
- Folmer, O., M. Black, W. Hoeh, R. Lutz, and R. Vrijenhoek. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol. Mar. Biol. Biotechnol.* 3: 294–299.
- García, I., S. Napp, J. Casal, A. Perea, A. Allepuz, A. Alba, A. Carbonero, and A. Arenas. 2009. Bluetongue epidemiology in wild ruminants from Southern Spain. *Eur. J. Wildl. Res.* 55: 173–178.
- Gortázar, C., E. Ferroglio, U. Höfle, K. Frölich, and J. Vicente. 2007. Diseases shared between wildlife and livestock: A European perspective. *Eur. J. Wildl. Res.* 53: 241–256.
- Harrup, L. E., B. V. Purse, N. Golding, P. S. Mellor, and S. Carpenter. 2013. Larval development and emergence sites of farm-associated *Culicoides* in the United Kingdom. *Med. Vet. Entomol.* 27: 441–449.
- Hoffmann, B., B. Bauer, C. Bauer, H. J. Bätz, M. Beer, P. H. Clausen, M. Geier, J. M. Gethmann, E. Kiel, G. Liebisch, et al. 2009. Monitoring of

- putative vectors of bluetongue virus serotype 8, Germany. *Emerg. Infect. Dis.* 15: 1481–1484.
- Hoffmann, B., M. Scheuch, D. Höper, R. Jungblut, M. Holsteg, H. Schirrmeier, M. Eschbaumer, K. V. Goller, K. Wernike, M. Fischer, et al. 2012. Novel orthobunyavirus in Cattle, Europe, 2011. *Emerg. Infect. Dis.* 18: 469–472.
- Kaufmann, C., I. Steinmann, D. Hegglin, F. Schaffner, and A. Mathis. 2012. Spatio-temporal occurrence of *Culicoides* biting midges in the climatic regions of Switzerland, along with large scale species identification by MALDI-TOF mass. *Parasit. Vectors.* 5: 246.
- Knoz, J. 1998. Ceratopogonidae, pp. 113–121. In R. Rozkošný and J. Vaňhara (eds.), *Diptera of the Pálava Biosphere Reserve of UNESCO*, I. Folia Fac. Sci. Nat. Univ. Masaryk. Brun. Biol.
- Knoz, J., and J. Michálek. 1987. The statistical evaluation of the climatic factors influence upon flying activity of the genus *Culicoides* (Ceratopogonidae, Diptera) on the South Moravia territory (Czechoslovakia). *Scr. Fac. Sci. Nat. Univ. Purkynianae Brun.* 17: 549–560.
- Knoz, J., and A. Tóthová. 2008. Pakomárcovití (Diptera: Ceratopogonidae) Jizerských hor a Frýdlantska, pp. 157–172. In P. Vonička and J. Preisler (eds.), *Results of the entomological survey in the Jizerské hory Mts and Frýdlant region I. Acta Musei Bohemiae Borealis*. National Science Library.
- Knoz, J., and J. Vaňhara. 1991. The effects of changes in moisture conditions on a community of haematophagous Diptera and ticks in a floodplain forest, pp. 469–504. In M. Penka, M. Vyskot, E. Klimo, and F. Vašíček (eds.), *Floodplain Forest Ecosystem after Water Management Measures*.
- Knoz, J., J. Olejníček, and I. Gelbič. 2004. Biting midges (Diptera, Ceratopogonidae) in the late domestic refuse dump Švábův Hrádek near České Budějovice. *Acta Musei Bohemiae Meridionalis, České Budějovice.* 44: 83–85.
- Linhares, A., and J. Anderson. 1989. *Culicoides variipennis* (Coquillett): seasonal abundance, voltinism, parity rates, and fecundity in northern California (Diptera: Ceratopogonidae). *Bull. Soc. Vector Ecol.* 14: 319–335.
- Linley, J. 1985. Biting midges (Diptera: Ceratopogonidae) as vectors of nonviral animal pathogens. *J. Med. Entomol.* 22: 589–599.
- Linto, Y. M., A. J. Mordue Luntz, R. H. Cruickshank, R. Meiswinkel, P. S. Mellor, and J. F. Dallas. 2002. Phylogenetic analysis of the mitochondrial cytochrome oxidase subunit I gene of five species of the *Culicoides imicola* species complex. *Med. Vet. Entomol.* 16: 139–146.
- Martínez-de la Puente, J., J. Figuerola, and R. Soriguer. 2015. Fur or feather? Feeding preferences of species of *Culicoides* biting midges in Europe. *Trends Parasitol.* 31: 16–22.
- Mehlhorn, H., V. Walldorf, S. Klimpel, B. Jahn, F. Jaeger, J. Eschweiler, B. Hoffmann, and M. Beer. 2007. First occurrence of *Culicoides obsoletus*-transmitted Bluetongue virus epidemic in Central Europe. *Parasitol. Res.* 101: 219–228.
- Meiswinkel, R., L. M. Gomulski, J.-C. Delécolle, M. Goffredo, and G. Gasperi. 2004. The taxonomy of *Culicoides* vector complexes - unfinished business. *Vet. Ital.* 40: 151–159.
- Meiswinkel, R., P. van Rijn, P. Leijss, and M. Goffredo. 2007. Potential new *Culicoides* vector of bluetongue virus in northern Europe. *Vet. Rec.* 161: 564–565.
- Meiswinkel, R., F. Scolamacchia, M. Dik, J. Mudde, E. Dijkstra, I. J. K. Van Der Ven, and A. R. W. Elbers. 2014. The Mondrian matrix: *Culicoides* biting midge abundance and seasonal incidence during the 2006–2008 epidemic of bluetongue in the Netherlands. *Med. Vet. Entomol.* 28: 10–20.
- Mellor, P. S., J. Boorman, and M. Baylis. 2000. *Culicoides* biting midges: their role as arbovirus vectors. *Annu. Rev. Entomol.* 45: 307–340.
- Monteys, V. S. I., J. C. Delécolle, J. O. Moreno-Vidal, and M. Pinna. 2009. New records of biting midges of the genus *Culicoides* latreille (diptera: ceratopogonidae) for Spain and Catalonia Autonomous Community (Ne Spain), with Notes on Their Biology and Veterinary Importance. *Proc. Entomol. Soc. Washingt.* 111: 714–733.
- Nolan, D. V., S. Carpenter, J. Barber, P. S. Mellor, J. F. Dallas, A. J. Mordue Luntz, and S. B. Pierrney. 2007. Rapid diagnostic PCR assays for members of the *Culicoides obsoletus* and *Culicoides pulicaris* species complexes, implicated vectors of bluetongue virus in Europe. *Vet. Microbiol.* 124: 82–94.
- Országh, I. 1980. Ceratopogonidae. In M. Chvála (ed.), *Krevsajáci mouchy a strecci – Diptera., Fauna CSSR 22*, Acad. Praha.
- Országh, I., and J. Minar. 1986. Pakomáriký rodu *Culicoides* (Diptera, Ceratopogonidae) jihozápadních Čech. *Dipterologica Bohemoslov.* 47–52.
- Purse, B. V., P. S. Mellor, D. J. Rogers, A. R. Samuel, P. P. C. Mertens, and M. Baylis. 2005. Climate change and the recent emergence of bluetongue in Europe. *Nat. Rev. Microbiol.* 3: 171–181.
- Purse, B. V., S. Carpenter, G. J. Venter, G. Bellis, and B. A. Mullens. 2015. Bionomics of temperate and tropical *Culicoides* midges: knowledge gaps and consequences for transmission of culicoides-borne viruses. *Annu. Rev. Entomol.* 60: 373–392.
- Saegerman, C., D. Berkvens, and P. S. Mellor. 2008. Bluetongue epidemiology in the European Union. *Emerg. Infect. Dis.* 14: 539–544.
- Sarvašová, A., M. Goffredo, I. Sopoliga, G. Savini, and A. Kočíšová. 2014a. *Culicoides* midges (Diptera: Ceratopogonidae) as vectors of orbiviruses in Slovakia. *Vet. Ital.* 50: 203–212.
- Sarvašová, A., A. Kočíšová, M. Halán, and J. Delécolle. 2014b. Morphological and molecular analysis of the genus *Culicoides* (Diptera: Ceratopogonidae) in Slovakia with five new records. *Zootaxa* 3872: 541–560.
- Savini, G., M. Goffredo, F. Monaco, A. Di Gennaro, M. A. Cafiero, L. Baldi, P. de Santis, R. Meiswinkel, and V. Caporale. 2005. Bluetongue virus isolations from midges belonging to the *Obsoletus* complex (*Culicoides*, Diptera: Ceratopogonidae) in Italy. *Vet. Rec.* 157: 133–139.
- Tóthová, A., and J. Knoz. 2009. Ceratopogonidae Newman, 1834. In L. Jedlička, M. Kúdela, and V. Sloukalová (eds.), *Checklist of Diptera of the Czech Republic and Slovakia. Electron. version 2.* (<http://zoology.fns.uniba.sk/diptera2009>)
- Tóthová, A., M. Barták, and J. Knoz. 2004. Ceratopogonidae of the Bílina and Duchcov environs, pp. 305–313. In Š. Kubík and M. Barták (eds.), *Dipterologica bohemoslovaca*, 11. Folia Fac. Sci. Nat. Univ. Masaryk. Brun. Biol.
- Venter, G. J., K. Labuschagne, K. G. Hermanides, S. N. B. Boikanyo, D. M. Majatlati, and L. Morey. 2009. Comparison of the efficiency of five suction light traps under field conditions in South Africa for the collection of *Culicoides* species. *Vet. Parasitol.* 166: 299–307.
- Veronesi, E., F. Antony, S. Gubbins, N. Golding, A. Blackwell, P. P. Mertens, J. Brownlie, K. E. Darpel, P. S. Mellor, and S. Carpenter. 2013. Measurement of the infection and dissemination of bluetongue virus in *Culicoides* biting midges using a semi-quantitative rt-PCR assay and isolation of infectious virus. *PLoS ONE* 8: e70800.
- Votýpka, J., V. Seblová, and J. Rádrová. 2008. Spread of the West Nile virus vector *Culex modestus* and the potential malaria vector *Anopheles hyrcanus* in central Europe. *J. Vector Ecol.* 33: 269–277.
- Werner, D., C. Bauer, C. Schulz, and H. Kampen. 2012. The breeding habitat preferences of *Obsoletus* complex *Culicoides* species (Diptera: Ceratopogonidae). *Mitt Dtsch Ges Allg Angew Entomol.* 18: 323–329.
- Wittmann, E. J., P. S. Mellor, and M. Baylis. 2001. Using climate data to map the potential distribution of *Culicoides imicola* (Diptera: Ceratopogonidae) in Europe. *Rev. Sci. Tech.* 20: 731–740.
- Zimmerman, R. H., and E. C. Turner. 1983. Seasonal abundance and parity of common *Culicoides* collected in Blacklight traps in Virginia pastures. *Mosq. News* 43: 63–69.