Applied Zoology
Research Article

ACTA ZOOLOGICA BULGARICA
Acta zool. bulg., 70 (2), 2018: 247-258

Diversity, Seasonal Abundance and Potential Vector Status of the Cave-dwelling Mosquitoes (Diptera: Culicidae) in the Bakony-Balaton Region

Attila Trájer[1,2*], Judit Schoffhauzer[3] & Judit Padisák[1,4]

[1]University of Pannonia, Department of Limnology, H-8200, Veszprém, Egyetem utca 10.
[2]Institute of Environmental Engineering, University of Pannonia, H-8200, Veszprém, Egyetem utca 10.
[3]Independent author, H-8227, Felsőörs, József Attila utca 14. E-mail: sofijudit@gmail.com
[4]MTA-PE, Limnoecology Research Group, H-8200, Veszprém, Egyetem utca 10.

Abstract: Caves are one of the potential natural shelters of overwintering mosquitoes. The aim of this study was to explore the diversity, seasonal abundance, gender ratio and the vector status of mosquito imagoes collected from 19 caves if the Bakony-Balaton Region, Hungary, based on data of a monograph covering nearly 30 years of sample collections. *Culex pipiens pipiens* and *Culiseta annulata* were the most abundant trogloxene mosquitoes; *Anopheles maculipennis*, *Anopheles messeae*, *Culex hortensis*, *Culex pipiens molestus* and *Culex territans* were also collected in caves from July to April. The total mosquito number showed an approximately continuous increasing trend from July to November and a decreasing trend from December to April. Most of the mosquito specimens were collected in autumn and winter. *Culex pipiens pipiens* individuals were present in the caves from August to April. Male mosquitoes were collected only in the second half of the year, while female individuals – both in the first and the second half of the year. Both the relative abundance of males and females exhibited an increasing trend between August and November. The infected *Culex pipiens pipiens* mosquitoes, which start their diapause in the autumn, can be the potential vectors of the West Nile virus during the next year. It can be concluded that caves are important habitats of mosquitoes that are vectors of non-human pathogens in Hungary.

Keywords: trogloxene mosquitoes; overwintering; diapause; West Nile virus

Introduction

The global dispersion of the Zika-virus drew attention to the necessity of assessment of the ecology and seasonal activity of mosquito species. Swamps, puddles, techno- and dendrotelmata and other aquatic habitats are the most frequently studied mosquito breeding sites. In contrast, the knowledge about their shelters and overwintering habitats is limited. Caves represent a potential natural shelter of mosquito species. Mosquitoes are trogloxenes (or accidentals) in caves, since they only use them as shelters but cannot complete their life cycle in this environment. Kjærandsen (1993) concluded that most of the trogloxene species of Diptera, including mosquitoes, are habitual trogloxenes inhabiting caves and cave systems only for hibernation. Mosquitoes in caves are prey for chiropterans and arachnid species. Trogloxene fauna consists of several relatively small, poikilotherm animals, while female mosquitoes must feed on homoeothermic (warm-blooded) birds and mammals. The only frequent trogloxene warm-blooded mammals in the temperate region belong to the order Chiroptera but they are the predators of mosquitoes in caves.

In contrast, the balanced temperature and permanently humid air and wall of caves provide the opportunity of successful overwintering for the imagoes. Mosquitoes enter caves to escape the outside unfavour-

*Corresponding author: attilatrajer@gmail.com

able conditions as low air temperature or dryness and the great predation pressure by birds during daylight (Ives 1938). Mosquitoes inhabit the caves periodically in a certain part of the year. Whang (1961) found that the largest number of *Culex pipiens* L., 1758 mosquitoes was collected from culverts and caves where the temperature and humidity were higher than in the surrounding environment. Whang (1961) found no larvae between November and April around the hibernating sites of this species and concluded that the individuals hibernate as adults where shelters are available. The mean air temperature of caves corresponds to the mean annual temperature of a given area. In wide areas of the temperate zone, ambient temperature can be too low for the full development of mosquito species. Whang (1961) found that while in the coldest months the average temperature was below the freezing point, temperatures in the main hibernating sites (including caves) were usually above 0°C. The difference between the temperatures of cave habitats and the ambient environment could be even 12-13°C.

Mosquitoes, in general, can be found in the parahypogean region ('twilight zone') of caves, but according to the ambient conditions, they can change their position. Buffington (1972) observed an extensive movement of *Cx. pipiens* populations even during midwinter, concluding that the changes of air current directions may have a significant effect on the movement of mosquitoes on the cave wall. The females of *Culex pipiens pallens* Coquillett, 1898 can be found only near the entrance in summer but in the deeper parts of the cave in winter (Shimogama & Takatsuki 1967). Küchlein & Ringelberg (1964) observed that *Cx. pipiens* mosquitoes can be found in the twilight zone in the cold period of January, mainly on the side opposite to the entrance of the cave. They found that the zone maxima in the distribution of the mosquitoes coincided with the decreasing light intensity in the twilight zone. The twilight zone is characterised by minor temperature fluctuations in conjunction with weather changes of the ambient environment, minimal plant life (mainly green algae and cyanobacteria) and less light.

Our aim was to study the diversity, seasonal abundance, gender ratio and the vector status of mosquito imagoes collected from 19 caves in the Bakony-Balaton Region, Hungary.

Materials and Methods

Geological and Geographical Setting

The Bakony Mountains and the Balaton Uplands are the parts of the Transdanubian Mountain Range situated on the ALCAPA megaunit which has African origin (Csontos & Nagymarosy 1998, Márton & Fodor 2003). These mountain ranges are mainly built-up Mesozoic carbonates, although Tertiary volcanites and non-volcanic Miocene sediments can also be found in the area (Németh & Martin 1999). Several types of opened and covered karst structures occur in the Transdanubian Range, including paleokarst structures that have been mainly formed on Mesozoic carbonates (Bolner-Takács 1999, Trájer *et al.* 2015, Veress 2009). Basalt-covered karst is also known in the Bakony Mountains (Móga & Németh 2005).

Mosquito Data

The mosquito data were obtained from the monography of Tóth (2006), the most detailed collection of mosqito occurrence in the region. Mosquito imagoes were collected between 1973 and 1999 by several authors. In some caves trappings were performed more than once. The number of the collected individuals were sorted by 1) species, 2) the time of collection and 3) the gender of the collected individuals.

The definition of the term 'cave' in Hungary is the following: 'a natural cavity whose longitudinal axis exceeds 2 meters in length, allowing the penetration of a man, and it has been formed in the firm rock of the Earth's crust.' The trogloxene mosquito fauna of 19 caves was studied. The synonym names used in the monograph and the official names were also indicated in the following list: Alba Regia Cave (Isztimér; 1), Bújó lik Cave (Ajka; 2), Forrás Cave=Forrás Cave of Tihany (Tihany; 3), Hajszabarnai Cave=Hajszabarnai Pénz lik Cave (Bakonyjákó; 4), Kecske lyuk Cave=Csesszneki sziklaodú Cave (Csesznek; 5), Kőpince Cave=Cuha valley No. 2. Cave (Bakonyszentlászló=Bakonyszentkirály; 6), Kőmosó Cave=Csesznek Cave (Csesznek; 7), Lóczy Cave (Balatonfüred; 8), Macska lik Cave (Nagyvázsony; 9), Magos hegy Cave (Dudar; 10), Nagy Pénz lik Cave (Bakonybél; 11), Odvas Kő Cave (Bakonyszücs; 12), Ördög lik Cave=Ördög lik Cave of Kőris Mt. (Bakonyszücs; 13), Pokol lik Cave (Ajka; 14), Pörgöl Cave=Száraz Gerence Cave (Bakonyszücs; 15), Pula Basalt Cave (Pula; 16), Lake Cave of Tapolca (Tapolca; 17), Tilos erdő Cave (Pénzesgyőr; 18) and Tűzköves hegy Cave (Szentgál; 19). The numbers after the settlement names are the sequence numbers of the caves used in the study. Hereinafter, the recently used officinal names of the caves are used (Fig. 1).

Climate Data

Mean monthly ambient temperature and precipitation values of the period 1973-1999 were obtained from the 0.25° resolution E-OBS climate model

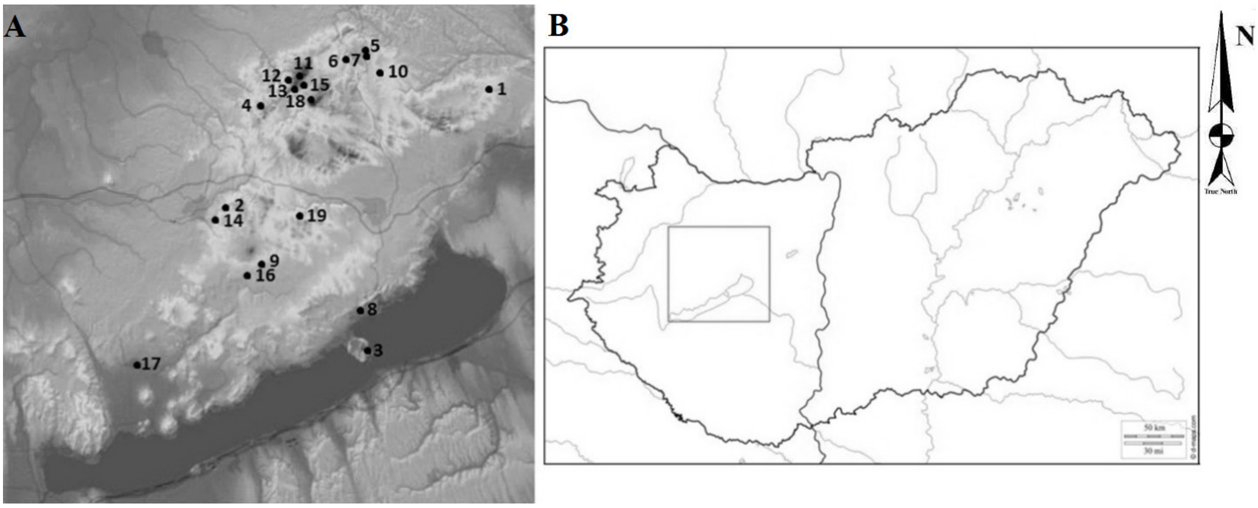


Fig. 1A. The geographical setting of the studied caves in the Bakony-Balaton Region. **Fig. 1B.** The area of the studied region in Hungary (for location and name of the caves, see Table 1).

Table 1. The presence/absence table of the collected mosquito material of the caves according to the data of Tóth (2006).

No.	Cave	*An. maculi-pennis*	*An. messeae*	*Cs. an-nulata*	*Cx. hort-ensis*	*Cx. pipiens pipiens*	*Cx. pipiens molestus*	*Cx. ter-ritans*	Number of species
1	Alba Regia Cave		x	x		x			3
2	Bújó lik Cave		x						1
3	Forrás Cave of Tihany			x		x			2
4	Hajszabarnai Pénz lik Cave			x	x	x		x	4
5	Cseszneki sziklaodú Cave					x			1
6	Cuha valley No. 2. Cave	x		x		x			3
7	Csesznek Cave			x		x			2
8	Lóczy Cave	x		x		x			3
9	Macska lik Cave		x	x		x			3
10	Magos hegy Cave			x		x			2
11	Nagy Pénz lyuk Cave					x			1
12	Odvas Kő Cave			x		x			2
13	Ördög lik Cave of Kőris Mt.			x		x			2
14	Pokol lik cave	x				x			2
15	Száraz Gerence Cave			x		x			2
16	Pula Basalt Cave			x		x			2
17	Lake Cave of Tapolca		x	x		x	x		4
18	Tilos erdő Cave			x	x	x			3
19	Tűzköves hegy Cave			x		x			2
	Number of positive sites	3	4	15	2	18	1	1	-

(Haylock et al. 2008) according to the covering grid of 17.25° to 18.25° and 46.75° to 47.50°. Mean monthly values were averaged.

West Nile Fever Data

The countrywide case number data of the West Nile fever were collated from the periodicals of the Hungarian National Epidemiological and Surveillance System (Epinfo 1-2). The monthly sums of the numbers of West-Nile-fever cases were converted into percentage of the total case number for the period.

Aridity Index

Aridity has a negative effect on the reproduction and activity of mosquito species. For the characterisation of the level of aridity, which is correspondent to both the air and soil aridity level, the Thornthwaite agro-meteorological index (*TA*I) was employed (Sábitz 2014):

$$TAI = 1.65 \cdot \left(\frac{P}{T + 12.2}\right)^{\frac{10}{9}}$$

P= monthly precipitation sum

T= monthly mean temperature

Cluster analysis

For the comparison of the caves' mosquito fauna, we used Hierarchical cluster-analysis with a Ward method and Principal component analysis run in XLSTAT 2016 statistical and forecasting software (Addinsoft 2016).

Results

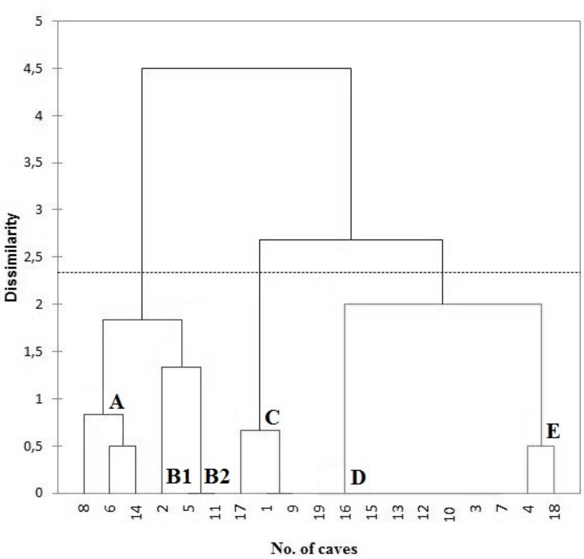
Species composition of the trogloxene mosquito fauna

A total of 751 mosquito individuals of seven species were collected in the caves of the Bakony-Balaton Region in the period 1973-1999. That corresponded to only 15.2% of the mosquito fauna of the region (46). On average, 2.3 species were found per cave (minimum: 1, maximum: 4, median: 2.0, SD: 0.88): *Culex pipiens pipiens* L., 1758 specimens were found at 18 sites (94.7% of the caves), *Culiseta annulata* Schrank, 1776 at 15 sites (78.9%) and *Anopheles messeae* Falleroni, 1926 at 4 sites (21.1%; Table 1). The other species were found in the following number of caves: *Anopheles maculipenis* Meigen, 1818 at 3 sites (15.8%), *Culex hortensis* Ficalbi, 1890 at 2 sites (10.5%) and *Culex pipiens molestus* Forskal, 1775 and *Culex territans* Walker, 1856 known from only 1 site (5.3% each). None of the species of the genera *Aedes*, *Ochlerotatus*, *Coquillettidia*, *Orthopodomyia* or *Uranotaenia* were found in the caves of the region. *Culex pipiens pipiens* contributed 85.22% of the collected material, while the other six species altogether reached only 14.78% of the mosquito material (Table 2). *Cs. annulata* was the second most frequent trogloxene mosquito species of the Bakony-Balaton Region (11.19%). The other six species accounted for only 3.6% of the mosquito fauna. The frequency of the other collected mosquito species were as follows: *An. maculipennis* (1.46%)>*An. messeae* (1.20%)>*Cx. pipiens molestus* (0.53%)>*Cx. hortensis* (0.27%)>*Cx. territans* (0.13%; Table 3).

Zoogeography – cluster analysis and principal component analysis

The cluster analyses showed similarities between the mosquito fauna of Cuha Valley No. 2, Cave (6), Lóczy (8) and Pokol lik (14) Caves (group **A**); Bújó lik (2), Cseszneki sziklaodú (5) and Nagy Pénz lik (11) Caves (group **B**); Alba Regia (1), Macska lik (9) Caves and Lake Cave of Tapolca (17) (group **C**); Hajszabarnai Pénz lik (4) and Tilos erdő (18) Caves; Forrás Cave of Tihany (3) and Csesznek (7), Magos hegy (10), Odvas Kő (12), Ördög lik (13), Száraz Gerence Caves (15) (group **D**), Pula Basalt and Tűzköves hegy Caves (group **E**; Fig. 2A).

Based on the Principal component analysis, *An. maculipennis* was the most common element of the fauna of caves (6), (8) and (14) (group **A**). Only one



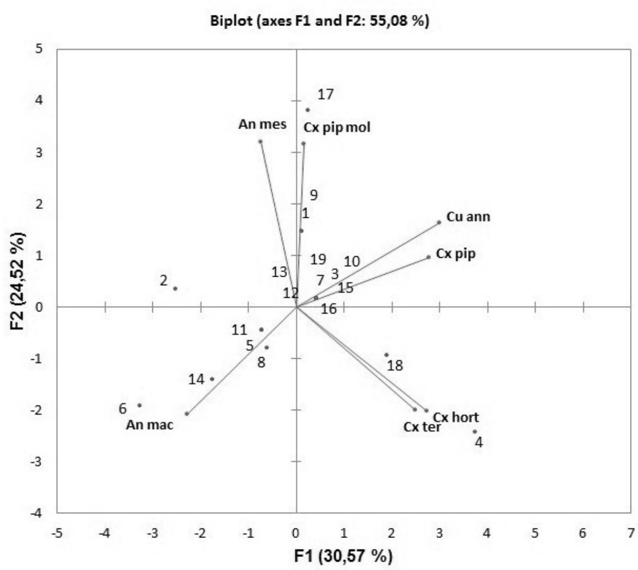


Fig. 2A. Cluster analysis for the caves of the Bakony-Balaton Region based on their trogloxene mosquito species (for location and name of the caves, see Table 1). **Fig. 2B.** Principal component analysis for the caves of the Bakony-Balaton Region based on their trogloxene mosquito species (for location and name of the caves, see Table 1). Dashed line indicates the level of significance.

mosquito species was collected in caves (2), (5) and (11) (group **B**). In Bújó lik Cave (2), only *An. messeae* (group **B1**), in the Cseszneki sziklaodú (5) and Nagy Pénz lik (11) caves solely *Cx. pipiens pipiens* formed the resident mosquito fauna (group **B2**). In caves (1), (9) and (17), *An. messeae*, *Cx. pipiens pipiens* and *Cs. annulata* were recorded (group **C**). The common mosquito fauna elements caves (4) and (18) were *Cx. hortensis*, *Cx. pipiens* and *Cs. annulata* (group **D**). Exclusively *Cs. annulata* and *Cx. pipiens pipiens* formed the resident mosquito fauna of caves (3), (7), (10), (12), (13), (15), Pula Basalt and Tűzköves hegy Caves (group **E**; Fig. 2B).

Seasonal Sex Ratio

Female imagoes formed the major part of the collected mosquitoes. The gender ratio was 0.056 (♂:♀). Only female individuals of *Cx. hortensis*, *Cx. pipiens molestus* and *Cx. teritans*, were collected, while for *An. maculipennis* (GR: 0.1), *An. messeae* (GR: 0.125), *Cs. annualata* (GR: 0.037) and *Cx. pipiens pipiens* (GR: 0.058) male and female individuals were trapped together. Male mosquitoes were collected only in the second half of the year, while female individuals were collected both in the first and the second half of the year. The relative abundance values of both males and females exhibited an increasing trend between August and November. The relative number of males of *Cx. pipiens pipiens* started to increase in July, reached its maximum in November and males were not found later. From July to November, the abundance of female mosquitoes showed a similar pattern, but female mosquitoes were continuously collected from July to April (Fig. 3A). , The relative number of males of *Cs. annulata* started to increase in October and reached a maximum in the same month. Fewer males were collected also in November. The abundance of females started to increase from August and reached its maximum in November. Female mosquitoes were continuously collected from July to April (Fig. 3B).

Seasonal Distribution

Trogloxene mosquitoes were collected from July to April. The total mosquito number showed an approximately continuous increasing trend from July to November and a decreasing trend from December to April. Most of the mosquito specimens were collected in autumn (61.51%), followed by winter (21.97%) and summer (12.11%); in spring were collected only 4.39% of all mosquitoes. The highest monthly abundance of *Cx. pipiens pipiens* (24.69%), *Cs. annulata* (40.48%) and *An. messeae* (88.89%) were observed in November (Fig. 4A). *Culex pipiens pipiens* had the highest relative monthly abundance values during the entire year. The highest species number of mosquitoes was collected in October (five species) and March (four species). At average, 2.4 mosquito species were collected per month and 2.9 species in those months when mosquitoes were present in the caves. No mosquito species were collected in May and June (0), the maximum was collected in October (five species). Four species were collected in both February and March. The relative abundance of *Cx. pipiens pipiens* continuously decreased from December to April. The

Table. 2. The absolute and relative numbers of the collected mosquitoes by species without the most frequent *Cx. pipiens pipiens*.

Species	Absolute numbers	Percentages of the total (%)
An. maculipennis	10	1.93
An. messeae	5	0.96
Cx. hortensis	2	0.39
Cx. pipiens molestus	4	0.77
Cx. territans	1	0.19
Cs. annulata	59	11.37

Table 3. The frequency of the mosquito species in the different cave groups (**A**: 6, 8 and 14; **B1**: 2; **B2**: 5, 11; **C**: 1, 9 and 17; **D**: 4, 18; **E**: 3, 7, 10, 12, 13, 15, 16 and 19).

Group	*An. maculipennis*	*An. messeae*	*Cs. annulata*	*Cx. hortensis*	*Cx. pipiens pipiens*	*Cx. pipiens molestus*	*Cx. territans*
A	1	0	0.33	0	0.66	0	0
B1	0	1	0	0	0	0	0
B2	0	0	0	0	1	0	0
C	0	1	1	0	1	0.33	0
D	0	0	1	1	1	0	0.5
E	0	0	1	0	1	0	0

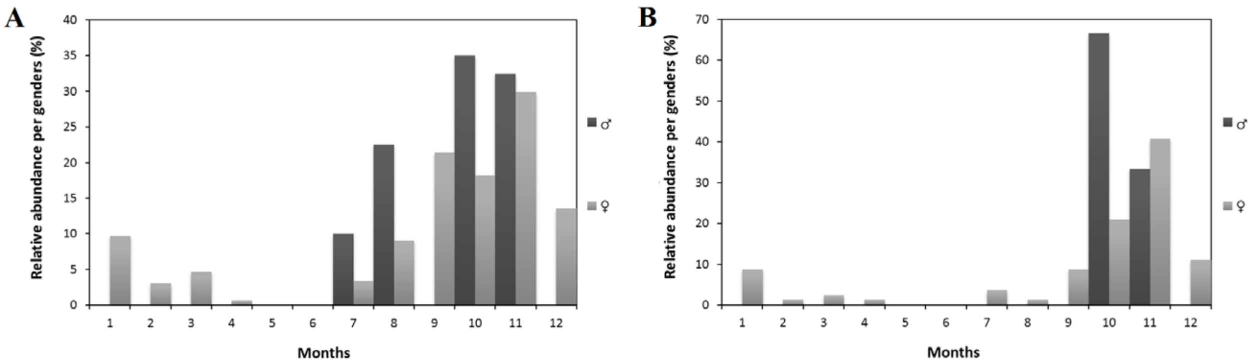


Fig. 3A. The relative monthly abundance values (%) of *Cx. pipiens pipiens* mosquitoes by genders. **Fig. 3B**. The relative monthly abundance values (%) of *Cs. annulata* mosquitoes by genders.

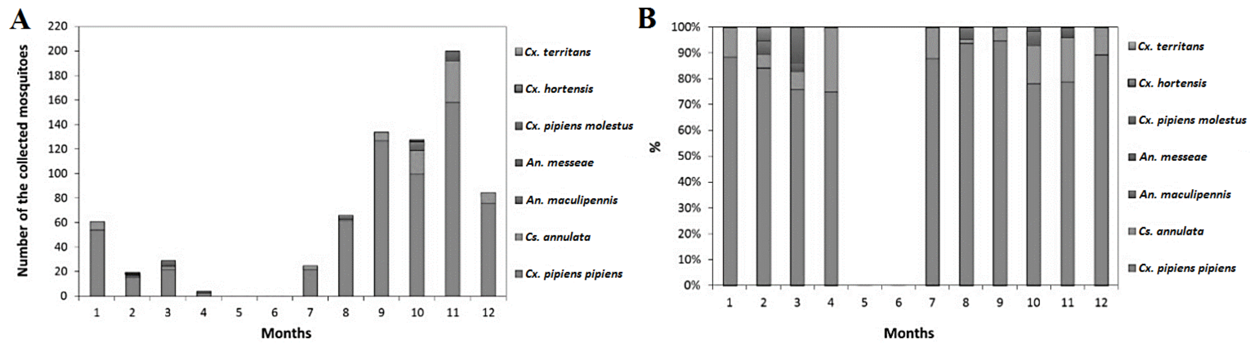


Fig. 4A. The absolute number of the collected mosquitoes by months. **Fig. 4B.** The relative abundance of the collected mosquito species by months.

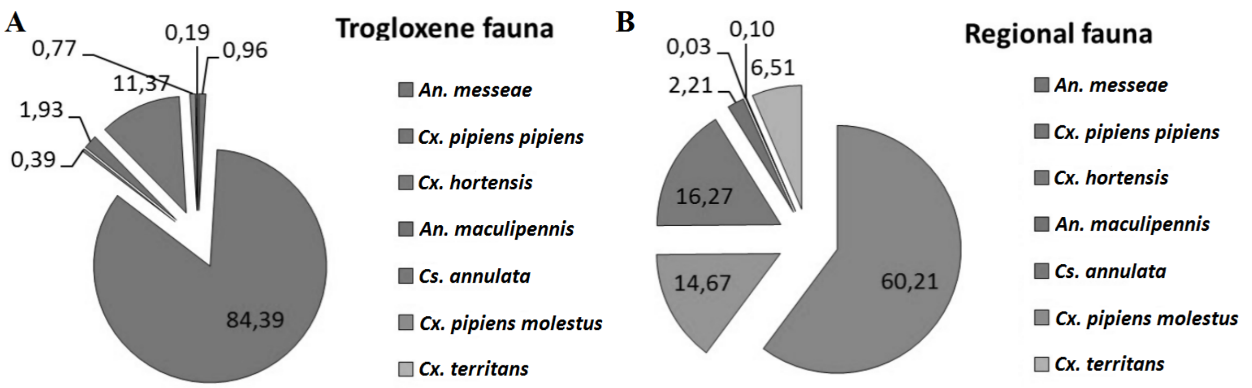


Fig. 5. The relative abundance values of the trogloxene fauna compared to the relative values of the same species in case of the Bakony-Balaton Region's according to the data of Tóth (2006).

relative abundance of *Cs. annulata* was the highest in April (Fig. 4B).

Comparison of Trogloxene and Regional Fauna

The relative abundance of species of the trogloxene mosquito fauna differs from the same species' frequency in the Bakony-Balaton Region. In both cases, *Cx. pipiens pipiens* was the most abundant mosquito, but this mosquito is less dominant in the collected material of the region (84.39 vs. 60.21%). The abundance of *Cs. annulata* of the region is similar to that in the trogloxene environment (14.66 vs. 11.37%). In contrast, *An. maculipennis* and *Cx. territans* are more frequent in the region than in the trogloxene fauna (1.93 vs. 16.28% and 0.19% vs. 6.25%). Each *An. messeae* and *Cx. pipiens molestus* contributed less to both trogloxene and regional abundance (0.96 vs. 2.20 and 0.77 vs. 0.10%; Fig. 5).

Coincidence of Climatic and Solar Factors with the Seasonal Mosquito Abundance

Mosquitoes disappeared from caves when the monthly mean temperature reached the annual mean temperature of the region (about 10°C). The summer re-

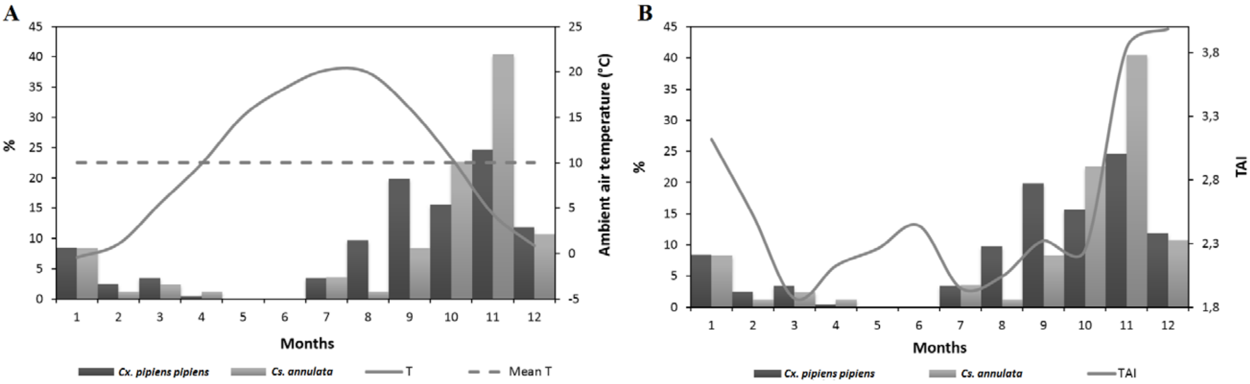


Fig. 6A. The annual relative abundance of *Cx. pipiens pipiens* and *Cs. annulata*, the monthly mean temperature of 1973-1999 and the mean annual temperature. **Fig. 6B.** The annual relative abundance of *Cx. pipiens pipiens* and *Cs. annulata* and the monthly mean TAI values of 1973-1999.

Table 4. The potentially transmitted pathogens (**Pla**: non-av*ian Plasmodium* species, **BATV**: Batai virus, **TAHV**: Tahyna virus , **WNV**: West Nile fever virus, **Myx** : Myxoma virus, **Fil**: filiaria species, **Fra**: *Francisella tularensis*, **avPla**: avian *Plasmodium* secies, **Dir**: *Dirofilaria immitis,* **Sin**: Sindbis virus (Joubert et al. 1967, Jöst et al. 2011, Lvov et al. 2004, Hubálek 2008; Hubálek et al. 2008, Kenyeres & Tóth 2008, Tatlor et al. 1955, Zittra et al. 2015).

Species	Pla	BATV	TAHV	WNV	Myx	Fil	Fra	avPla	Dir	Sin
An. maculipennis	+	+	+	+	+	+	+	-	-	-
An. messeae	+	+	+	+	+	+	+	-	-	-
Cx. hortensis	-	-	-	-	-	-	-	-	-	-
Cx. pipiens pipiens	-	-	-	-	-	-	-	+	+	-
Cx. pipiens molestus	-	+	+	+	-	-	-	+	+	+
Cx. territans	-	-	-	-	-	-	-	-	-	-
Cs. annulata	-	-	-	-	+	-	-	+	-	-

appearance of *Cx. pipiens pipiens* and *Cs. annulata* in the caves occurred slightly before the summer thermal maximum (July and August). The relative abundance of the mosquitoes in the caves started to increase rapidly when the monthly mean temperature dropped below the annual mean (Fig. 6A). The Thornthwaite agrometeorological index (TAI) exhibited a roughly bimodal annual run, with a greater late autumn-early winter and a smaller early summer peak. The relative abundance of *Cx. pipiens pipiens* and *Cs. annulata* started to increase in July, when the TAI showed its annual second minimum. During the summer precipitation and the consequent TAI maximum, mosquitoes were not collected in the studied caves. From July to November, the increase of the mosquito abundance coincided with the parallel increase of TAI. Mosquito abundance reached its maximum in November when TAI was almost the highest (Fig. 6B).

Potential Vector Status of Cave-Dwelling Mosquitoes

Weighting the number of trapped trogloxene mosquitoes with their vector status (yes/no), the following results were found: potential vectors of the avian *Plasmodium* species - 96.94%; *Dirofilaria immitis* Leidy, 1856 - 85.75%; Myxoma virus - 13.85%; non-avian *Plasmodium* species, *Francisella tularensis* (McCoy & Chapin, 1912) Dorofe'ev, 1947 and the pathogens of filariasis - 2.66-2.66-2.66%; Batai, Tahyna and West Nile fever virus – 3.19-3.19-

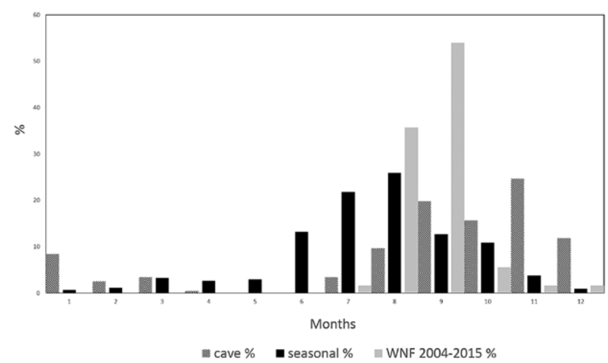


Fig. 7. Comparison of the abundance of active (sesonal%) and diapausing (cave%) *Cx. pipiens pipiens* mosquitoes in the Bakony-Balaton Region with the seasonal abundance of the species and the countrywide monthly percentage of observed WNF cases in Hungary in 2004-2015.

3.19%, and Sindbis virus – 0.53% of the collected mosquito individuals, respectively (Table 4).

The season of *Cx. pipiens pipiens* in the Bakony-Balaton Region, the most important vector of the West Nile fever in Hungary, starts about a month after the end of the winter diapause. The annual maximum of number of cases of the human West Nile fever was observed in September, one month after the annual maximum abundance of *Cx. pipiens pipiens* at a county level. Although humans are only the "dead-end"hosts of the virus, human cases indicate the main period when mosquitoes can transmit the virus. The infected mosquitoes, which started their diapause in the autumn months, could be the potential vectors of the West Nile fever in the next year (Fig. 7).

Discussion

It is somewhat surprising that only seven mosquito species were collected from October to March in caves of the Bakony-Balaton Region, since 19 mosquito species can overwinter in imago stage in Hungary: 9 *Culex*, 5 *Culiseta*, 4 *Anopheles* and 1 *Uranotaenia* species (Kenyeres & Tóth 2008). All these 19 species were collected from epigean habitats in the region. The four most abundant species were: *Cx. pipiens pipiens*, *Cs. annulata*, *An. maculipennis* and *An. messeae*. Minár & Ryba (1971) found similar trogloxene mosquito composition in Moravia describing the presence of *Cx. pipiens pipiens*, *Cs. annulata* and *An. messeae* from the studied caves. *Culex pipiens pipiens* and its close relatives are common trogloxene elements in the temperate areas of the world as it was observed in Japan (Shimogama & Takatsuki 1967), in the Czech Republic (Minár & Hájková 1966) or in the caves of South Korea (Whang 1961). It often co-occurs in caves with *Cs. annulata* (Minár & Hájková 1966).

None of the members of the genera *Aedes* and the diverse *Ochlerotatus*, occurring in the Bakony-Balaton Region, were found in the studied caves. This can be explained by the different overwintering strategies of the various mosquito genera. Most of the *Aedes* and *Ochlerotatus* species in the temperate zone hibernate in the egg-stage (Becker et al. 2010) and start their development in spring: after the eggs are flooded several times, hatching is induced. Only a few species of *Aedes* and *Ochlerotatus* overwinter in larval stage (Vinogradova 1969). Based on the above-mentioned conditions, adults of the species of these two genera are not expected to be recorded in the caves. Only Eszterhás (1996) has mentioned the presence of *Aedes vexans* Meigen, 1830 and of *Cx. pipiens* in the former Kádárta Spring Cave (Veszprém), however, without the number of the collected individuals and the exact date of collection. Unfortunately, the survey cannot be repeated because the cave has been crowded.

A clear increasing trend of mosquito abundance has been observed in October and November, which is the main period of entry (Gazave 2001). Shimogama & Takatsuki (1967) have described somewhat similar seasonal patterns for the trogloxene *Cx. pipiens pallens,* which is a close Asian relative of *Cx. pipiens pipiens* and *Cx. pipiens molestus*. They found that the number of females of *Cx. pipiens pallens* was scarce in summer and increased rapidly in November, reaching its maximum in caves in December. In Japan, the abundance of this species decreased sharply in February. Theoretically, high predation pressure could be a potential reason of the low mosquito diversity. In the caves of the Bakony-Balaton Region, 14 Chiroptera species can be found. The most common bats are *Myotis myotis* Borkhausen, 1797 and *Myotis oxygnathus* Monticelli, 1885. We found that the mosquito abundance started to decrease rapidly after December and reached its absolute minimum in May and June. In this period of the year, mosquitos were not collected in the caves. Most of the Chiroptera species in the temperate regions retreat to caves in winter and hibernate for several months (Fenton & Brock 2001), imposing predation pressure during the prehibernation period in caves. On the other hand, arthropod predators, especially spiders also use caves as habitats.

Still, it is unlikely that bat foraging activity could explain the observed annual abundance patterns of mosquitoes in caves. Mosquitoes form a minor (or even negligible) part of the bat diet. A wide variety of nocturnal insects serve as food resources for bats, such as moths (Lepidoptera), beetles (Coleoptera), lacewings (Planipennia), caddisflies (Trichoptera), mayflies (Ephemeroptera) and midges (Chironomidae), with moths being the major part of the bats' diet (Becker et al. 2010, Gonsalves et al. 2013). The absence of the main mosquito species *Cx. pipiens pipiens* could be explained with its life cycle, rather than with predation. In all mosquito species, including *Cx. pipiens pipiens,* overwintering as hibernating females, the imagoes that manage to survive the winter abandon their shelters to lay eggs and die soon after. During the spring period with lower temperatures, it takes about a month for the larvae to complete their development and for the first generation of adults to emerge. For *Cx. pipiens pipiens* this happens approximately in the observed months.

The disappearance of the mosquitoes from the caves and the rising temperature in spring coincidenced. When the mean monthly temperature reached the annual mean temperature of the region in April, mosquitoes left the caves and started to quest blood meal in the ambient environment. According to Minár & Ryba (1971), the emergence of mosquitoes from overwintering sites can be observed when the temperature in the shelter is 8°C for *Cx. pipiens* and 8-12°C for *Cs. annulata*. They commonly occur together in the Czech Republic and Hungary, in the turn of March and April. The wet and warm weather in May and June favours the activity and reproduction of mosquitoes. After the summer solstice in mid-summer, the aridity index dropped below to a certain value (about 2.0 in TAI) and the mosquitoes visited the caves again in order to avoid the summer atmospheric drought. Parallel to the summer drought, the most important breeding sites of mosquitoes, such as small-water habitats (puddles, different natural- and technotelmata), started to dry out. We found that when the ambient monthly mean temperature dropped below 10°C, the mosquito abundance in the caves increased rapidly. Our results confirmed to the observations of Minár & Ryba (1971) who found that suitable overwintering sites for females of *Cx. pipiens* were cellars with temperatures of 0-8°C. For *Cs. annulata* the ideal air temperature during the winter diapause is between 7 to 8°C (Minár & Ryba 1971). It appears that male mosquitoes do not survive the winter solstice.

Male/female gender ratio was very low during the entire year. Male mosquitoes were not found after December and were absent in the first half of the year. It means that there were no overwintering male mosquitoes in the caves. This is a well-known fact for mosquitoes that overwinter in the adult stage. Male mosquitoes do not suck blood and are unable to form enough fatty reserves to survive the winter. Moreover, the lifespan of males is usually shorter than that of the females of the same generation. Shimogama & Takatsuki (1967) found that all the male *Cx. pipiens pallens* mosquitoes stayed close to the entrance in summer and none of them penetrated beyond the middle section of the caves. They also found that while the total number of males was small during the entire year, the abundance of males was very low from February to April and began to rise only in May. In contrast, during the cold period, females penetrated the deeper zones of the caves. They concluded that this behaviour could be the main cause of the very low survival rate of male mosquitoes in winter. In another study (Ives 1938), most individuals of *Culex* spp. were collected in the twilight zone in the caves, none having penetrated darker parts even in winter.

Anopheles maculipennis, An. messeae, *Cs. annulata* and *Cx. pipiens molestus* mainly feed on mammals, while *Cx. hortensis* and *Cx. pipiens pipiens* prefer the blood of birds. *Culex territans* prefers to feed mainly on blood of amphibians (Kenyeres & Tóth 2008). Although *An. maculipennis* and *An. messeae* are the front-line potential vectors of human *Plasmodium* species, infective malaria mosquitoes that survive the winter are no longer infective in the following spring. Sporozoites in their salivary glands are not able to survive for more than 40 days at low temperatures, therefore the females are free of parasites by the time they abandon their winter shelters (WHO 2010). The early onset of the malaria season in European countries, formerly epidemiologically endemic for malaria, was due to cases with long incubation period or relapses (Lysenko et al. 2003). The species of the *Anopheles maculipennis* complex, including *An. maculipennis* and *An. messeae*, can also transmit the West Nile fever virus, but these mosquitoes are not the chief vectors of the pathogen.

Persistence in overwintering *Culex* mosquitoes may be important in the maintenance of the West Nile fever virus because the virus was isolated from overwintering *Cx. pipiens* (Nasci et al. 2001). Farajollahi et al. (2005) detected the West Nile fever viral RNA from an overwintering pool of *Cx. pipens pipiens* material concluding that the mode of infection of overwintering females may have been due to transgenerational transmission. Reisen et al. (2006) hypothesised that the West Nile virus can survive winters by continued enzootic transmission, vertical transmission by *Culex* mosquitoes or due to the chronic infection of birds. Goddard et al. (2003) concluded that mosquitoes infected vertically during autumn, could potentially serve as a mechanism for the West Nile fever virus to overwinter and initiate horizontal transmission the following vegetation period.

Discussing the vector status of the mosquitoes, it can be concluded that caves are the major habitats of mosquitoes that are vectors of non-human pathogens or pathogens that rarely cause human infections like *D. immitis*. The winter transmission of infections to humans such as malaria is very unlikely. Mosquitoes preparing for hibernation digest the blood to form fatty reserves for the winter without producing eggs at the same time, which is known as "gonotrophic dissociation". Only females of some species within the *Anopheles maculipennis* complex that do not enter complete hibernation can take occasional blood meals during winter to withstand the prolonged periods of starvation (Becker et al. 2010). For example, in Korea, Whang (1961) found that *An. sinensis* Wiedemann, 1828 and *An. sineroides* Yamada, 1924 hibernate as adults in winter. Since these anophelines hibernating in cow stables were all fed, it was concluded that in caves they could feed in the cold period.

A wide overlapping period was found in the second half of the vegetation season when *Cx. pipiens pipiens* mosquitoes are both active and start their diapause in caves in the Bakony-Balaton region. Furthermore, from August to the end of autumn they can transmit the West Nile virus to humans in Hungary. It is assumed that the observed monthly abundance of *Cx. pipiens pipiens*, the bridge vector of the West Nile fever virus, in caves can be used as the diapause model of the species in artificial, non-heated environments as cellars, mines, stacks and basements. Although the Bakony-Balaton Region is only a part of Hungary, due to the relatively homogenous topographical and climatic circumstances the observed seasonal abundance patterns of the mosquito can be used in the modelling of the ecoregional seasonality of *Cx. pipiens pipiens*. In summer and autumn, the West Nile fever viruses circulate in bird and *Culex* populations. Humans and other mammals are only the "dead-end" hosts of the virus (Kilpatrick et al. 2007). The season of the human West Nile fever infections coincides with the re-appearance of diapausing mosquitoes in caves. In the case of the West Nile fever transmission, in addition to the exoanthropic cycle, a synanthropic cycle also exists (Savage et al. 1999, Tsai et al. 1998). In lowland areas (e.g. in major river basins) where large mosquito populations exist due to the lack of rocks, natural caves are missing. In contrast, cellars, unheated rooms, garages and outbuildings could provide somewhat similar conditions in winter as the natural caves in the mountainous areas. Since surviving female mosquitoes can be the hosts of the West Nile fever, the study of the seasonal coincidence of diapausing, host-seeker mosquitoes and the West Nile fever cases can provide some information about the probability of the survival of the virus in winter even in the lowland areas.

Conclusions

Culex pipiens pipiens and *Cs.annulata* are the most abundant trogloxene mosquito species in the Bakony-Balaton Region of Hungary. Caves are one of the potential overwintering habitats of their females. The presence of mosquitoes in the caves is the consequence of several factors as temperature, aridity and the length of the days. These environmental factors play key role in the determination of the presence of the mosquitoes in caves in the different seasons of the year. Based on the abundance and vector status of the mosquitoes, the recent trogloxene fauna have rather veterinary than human medical importance in Hungary. The seasonality in and out of the caves and the cave-like artificial environments of *Cx. pipiens pipiens* could allow the vertical transmission of the West Nile fever virus in overwintering mosquitoes in Hungary.

Acknowledgement: This study was supported by the National Research, Development and Innovation Office (NKFI-HK120595) and GINOP-2.3.2-15-2016-00016. We acknowledge the E-OBS dataset from the EU-FP6 project ENSEMBLES (http://ensembles-eu.metoffice.com) and the data providers in the ECA&D project (http://www.ecad).

References

Addinsoft S.A.R.L. 2012: Xlstat 2016: Leading data analysis and statistical solution for microsoft excel. Addinsoft SRL.

Becker N., Petrič D., Zgomba M., Boase C., Madon M., Dahl C. & Kaiser A. 2010. Mosquitoes and their control. 2nd ed. Heidelberg: Springer. 577 pp.

Bolner-Takács K. 1999: Paleokarst features and other climatic relics in Hungarian caves. Acta Carsologica. 28 (1): 27–37.

Buffington J. D. 1972: Hibernaculum choice in *Culex pipiens*. Journal of Medical Entomology 9 (2): 128-132. http://dx.doi.org/10.1093/jmedent/9.2.128 128−132.

Csontos L. & Nagymarosy A. 1998: The Mid-Hungarian line: a zone of repeated tectonic inversions. Tectonophysics 297 (1): 51−71. 10.1016/S0040-1951(98)00163-2.

Epinfo1: Nemzetközi/Hazai információ - Nyugat-Nílusi Láz Megbetegedések Magyarországon és Európában [Epinfo: International / national information about the West Nile Virus caused diseases in Hungary and Europe]. *Epinfo* 2010; *17:* 417–424 (2010). [updated 2010 April 25; cited 2016 November 1] Available from: http://epa.oszk.hu/00300/00398/00409/pdf/epinfo_EPA00398_2010_34.pdf. In Hungarian.

Epinfo2: Hazai információ-előzetes jelentés a Magyarországon előforduló, ízeltlábú vektorok által terjesztett zoonózisok évi járványügyi jellemzőiről [Epinfo: Domestic information report about the annual epidemiological characteristics of the arthropod vectors caused zoonotic diseases in Hungary]. *Epinfo* 2013; *21:* 137–148. [updated 2016 May 23; cited 2016 November 1] Available from: http://epa.oszk.hu/00300/00398/00578/pdf/EPA00398_epinfo_2014_13.pdf. In Hungarian.

Eszterhás I. 1996: The Kádárta Spring cave and its environment (A Kádártai-forrásbarlang és környéke). In: A természetvédelmi oltalom alatt nem álló területek természeti értékeinek feltárása és megóvása c. pályázat. Vulkánszpeleológiai Kollektíva. (In Hungarian). http://www.barlang.hu/pages/science/mkbt/vke93/13kadarta.htm [accessed: June 8, 2016; In Hungarian]

Fenton M. B. 2001. Bats, revised edition. New York: Checkmark Books. 224 p.

Farajollahi A., Crans W. J., Bryant P., Wolf B., Burkhalter K. L., Godsey M. S., Aspen S. E. & Nasci, R. S. 2005. Detection of West Nile viral RNA from an overwintering pool of *Culex pipens pipiens* (Diptera: Culicidae) in New Jersey, 2003. Journal of Medical Entomology 42 (3): 490−494.

Gazave É., Chevillon C., Lenormand T., Marquine M. & Raymond M. 2001. Dissecting the cost of insecticide resistance genes during the overwintering period of the mosquito *Culex pipiens*. Heredity 87 (4): 441−448. 10.1046/j.1365-2540.2001.00926.x.

Goddard L. B., Roth A. E., Reisen W. K. & Scott T. W. 2003. Vertical transmission of West Nile virus by three california *Culex* (Diptera: Culicidae) species. Journal of Medical Entomology 40 (6): 743-746.

Gonsalves L., Bicknell B., Law B., Webb C. & Monamy V. 2013. Mosquito Consumption by Insectivorous Bats: Does Size Matter? PLoS One 8 (10): e77183.

Haylock M. R., Hofstra N., Klein Tank A.M.G., Klok E. J., Jones P.D. & New M. 2008. A European daily high-resolution gridded dataset of surface temperature and precipitation for 1950-2006. Journal of Geophysical Research: Atmospheres 113 (D20): 119. 10.1029/2008JD010201.

Hubálek Z. 2008. Mosquito-borne viruses in Europe. Parasitology Research 103 (1): 29-43. 10.1007/s00436-008-1064-7.

Hubálek Z., Halouzka J., Juricova Z., Šikutová S., Rudolf I., Honza M., Janková J., Chytil J., Marec F. & Sitko J. 2008. Serologic survey of birds for West Nile flavivirus in southern Moravia (Czech Republic). Vector-Borne and Zoonotic Diseases 8 (5): 659-666. 10.1089/vbz.2007.0283.

Ives J. D. 1938. Cave hibernation of mosquitoes. Journal of the Tennessee Academy of Science 13 (1): 15-20.

Joubert L., Oudar J., Mouchet J. & Hannoun C.1967. Transmission of myxomatosis by mosquitos in the Camargue. Pre-eminent part played by *Aedes caspius* and the *Anopheles maculipennis* group. Bulletin de l'Academie Veterinaire de France 40 (1): 315-322.

Jöst H., Bialonski A., Schmetz C., Günther S., Becker N. & Schmidt-Chanasit J. 2011. Isolation and phylogenetic analysis of Batai virus, Germany. The American Journal of Tropical Medicine and Hygiene 84 (2): 241-243. 10.4269/ajtmh.2011.10-0483.

Kenyeres Z. & Tóth S. 2008. Identification keys to Mosquitoes II (Imagoes) [Csípőszúnyog határozó II. In Hungarian]. Keszthely: Pannónia Centre Expert and Consuelling Coordinative Ltd. 96 pp.

Kilpatrick A. M., LaDeau S. L. & Marra P. P. 2007. "Ecology of West Nile virus transmission and its impact on birds in the western hemisphere". The Auk 124 (4): 1121-1136. doi:10.1642/0004-8038(2007)124[1121:eownvt]2.0.co;2.

Kjærandsen J. 1993. Diptera in mines and other cave systems in southern Norway. Entomologica Fennica 4 (3): 151-160. 10.1179/1743286315Y.

Küchlein J. & Ringelberg J. 1964. Further investigations on the distribution of hibernating *Culex pipiens* L. (Diptera, Culicidae) in artificial marl caves in South Limburg (Netherlands). Entomologia Experimentalis et Applicata 7 (1): 25-46. 10.1111/j.1570-7458.1960.tb00449.x.

Lysenko A., Kondrachin A. & Ejov M. Malariology. 2003. 2nd ed. Copenhagen: WHO, Regional office for Europe. 512 pp. WHO/MAL/03/1089 [in Russian]

Lvov D. K., Butenko A. M., Gromashevsky V. L., Kovtunov A. I., Prilipov A. G., Kinney R., Aristova V. A., Dzharkenov A. F., Samokhvalov E. I., Savage H. M., Schelkanov M. Y., Galkina I. V., Deryabin P. G., Gubler D. J., Kulikova L. N., Alkhovsky S. K., Moskvina T. M., Zlobina L. V., Sadykova G. K., Shatal A. G., Lvov D. N., Usachev V. E., Voronina A.G. 2004. West Nile virus and other zoonotic viruses in Russia: examples of emerging-reemerging situations. Vienna: Springer. pp. 85–96. 10.1007/978-3-7091-0572-6_7.

Márton E. & Fodor L. 2003. Tertiary paleomagnetic results and structural analysis from the Transdanubian Range (Hungary): rotational disintegration of the Alcapa unit. Tectonophysics 363 (3): 201-224. 10.1016/S0040-1951(02)00672-8.

Minár J. & Hájková Z. 1966. Knowledge on the hibernation of mosquitoes in our territory, especially in south Moravia. Folia Parasitologica 13 (3): 237-247.

Minár J. & Ryba J. 1971. Experimental studies on overwintering conditions of mosquitoes. Folia Parasitologica 18 (3): 255-259.

Móga J. & Németh R. 2005. The morphological research of the Basalt and Loess covered plateaus in the Bakony Mts. (Transdanubian Middle Mts.-Hungary). Acta Carsologica 34 (2): 397-414.

Nasci R. S., Savage H. M., White D. J., Miller J. R., Cropp B. C., Godsey M. S., Kerst A. J. & Lanciotti R. S. 2001. West Nile virus in overwintering *Culex mosquitoes*. New York City, 2000. Emerging Infectious Diseases 7 (4): 742.

Németh K. & Martin U. 1999. Late Miocene paleo-geomorphology of the Bakony-Balaton Highland Volcanic Field (Hungary) using physical volcanology data. Zeitschrift für Geomorphologie 43(4): 417-438.

Reisen W. K., Fang Y., Lothrop H. D., Martinez V. M., Wilson J., O'Connor P., Carney R., Cahoon-Young B., Shafii M. & Brault A. C. 2006. Overwintering of West Nile virus in southern California. Journal of Medical Entomology 43 (2): 344-355.

Sábitz J., Pongrácz R. & Bartholy J. 2014. Estimated changes of drought tendency in the Carpathian Basin. Hungarian Geographical Bulletin 63 (4): 365-378. 10.15201/hungeobull.63.4.1.

Savage H. M., Ceianu C., Nicolescu G., Karabatsos N., Lanciotti R., Vladimirescu A., Laiv L., Ungureanu A., Romanca C. & Tsai T. F. 1999. Entomologic and avian investigations of an epidemic of West Nile fever in Romania in 1996, with serologic and molecular characterization of a virus isolate from mosquitoes. The American Journal of Tropical Medicine and Hygiene 61 (4): 600-611.

Shimogama M. & Takatsuki Y. 1967. Seasonal changes in the distribution and abundance of mosquitoes especially of *Culex pipiens pattens* in a cave in Nagasaki City. Endemic Diseases Bulletin of Nagasaki University 8 (4): 159-165.

Tatlor R., Hublbut H., Work T. H., Kingston J. R. & Frothingham T. E. 1955. Sindbis virus: a newly recognized arthropod-transmitted virus. American Journal of Tropical Medicine and Hygiene 4 (5): 844-62.

Tóth S. 2006. Mosquito fauna (Diptera: Culicidae) of the Bakony Region. [A Bakonyvidék csípőszúnyog-faunája (Diptera: Culicidae) in Hungarian]. Debrecen: Acta Biologica Debrecina, Supplementum Oecologica Hungarica. 15: 240 pp.

Trájer A. J., Hammer T., Mlinárik L., Bede-Fazekas Á. &

Padisák J. 2015. The neogene-quaternary evolution of the karst landscape of the Veszprém plateau based on the study of Meggyespuszta paleodoline, Hungary. Acta Carsologica 44 (2): 177-190. http://dx.doi.org/10.3986/ac.v44i2.801

Tsai T. F., Popovici F., Cernescu C., Campbell G. L. & Nedelcu N. I. 1998. West Nile encephalitis epidemic in southeastern Romania. The Lancet 352 (9130): 767-771.

Veress M. 2009. Investigation of covered karst form development using geophysical measurements. Zeitschrift für Geomorphologie 53 (4): 469-486. 10.1127/0372-8854/2009/0053-0469.

Vinogradova A. 1969. Diapause in blood-sucking mosquitoes and its regulation. Leningrad: Nauka. 148 pp. [in Russian]

Whang C. H. 1961. Hibernation of mosquitoes in Korea. Mosquito News 21 (1): 17-20.

WHO. 2010. Guidelines on malaria elimination for the countries of the WHO European region. Copenhagen: WHO, Regional office for Europe. 110 pp. [in Russian]

Zittra C., Kocziha Zs., Pinnyei Sz., Harl J., Kieser K., Laciny A., Eigner B., Silbermayr K., Duscher G. G., Fok É. & Fuehrer H. P. 2015. Screening blood-fed mosquitoes for the diagnosis of filarioid helminths and avian malaria. Parasites & Vectors 8 (1): 1-6. 10.1186/s13071-015-0637-4.

Received: 10.02.2017

Accepted: 11.09.2017