

Short communication

UDC 595.771(497.11)

<https://doi.org/10.46784/e-avm.v13i2.207>

## **BIODIVERSITY AND SEASONAL DISTRIBUTION OF *CULICOIDES* SPP. EXAMINED AT SCIENTIFIC VETERINARY INSTITUTE OF SERBIA DURING 2019**

Ivan Pavlović<sup>1\*</sup>, Slobodan Stanojević<sup>1</sup>, Nemanja Zdravković<sup>1</sup>,  
Oliver Radanović<sup>1</sup>

<sup>1</sup>Scientific Veterinary Institute of Serbia, Belgrade, Republic of Serbia

### **Abstract**

Continuous entomological monitoring of *Culicoides* spp. which is being conducted from 2014 has so far yielded significant results regarding biodiversity and seasonal dynamics of these insects in Serbia. The research we have carried out so far has contributed to mapping geographical distribution of the species we encounter as well as the variations in the number of populations in different years. As the monitoring continued, we were receiving new valuable data every year, which will help to predict the movement of these insects in the future on the basis of *climatograms* and enable preventative actions to be taken in order to counteract them. Unfortunately, during 2019, the monitoring was no longer done in one location but at three institutes. These include Belgrade Institute receiving samples from central Serbia and South Banat, Novi Sad which analysed the samples from Vojvodina and Kraljevo analysing the samples from South Serbia regions. This resulted in losing the ability to monitor biodiversity and other relevant data (sex ratio, etc.). In our work, therefore, we can only provide the results of testing biodiversity and seasonal dynamics of *Culicoides* spp. during 2019 in the epizootiology area of Scientific Veterinary Institute of Serbia in Belgrade, without Pomoravlje district since we did not receive samples from Jagodina Institute for the fourth consecutive year. *Culicoides* spp. from *Obsoletus* complex were established in 59.91% of samples, *Pulicaris* complex were established in 34.06% and other types of *culicoides* were established in less than 10% of the examined samples.

**Keywords:** *Culicoides* spp., epizootiology, Serbia

---

<sup>1\*</sup> Corresponding author: [dripavlovic58@gmail.com](mailto:dripavlovic58@gmail.com)

## BIODIVERZITET I SEZONSKA DISTRIBUCIJA *CULICOIDES* SPP. ISPITANIH U NAUČNOM INSTITUTU ZA VETERINARSTVO SRBIJE TOKOM 2019. GODINE

Ivan Pavlović<sup>1\*</sup>, Slobodan Stanojević<sup>1</sup>, Nemanja Zdravković<sup>1</sup>,  
Oliver Radanović<sup>1</sup>

<sup>1</sup> Naučni institut za veterinarstvo Srbije, Beograd, Republika Srbija

### Kratak sadržaj

Kontinuirani entomološki monitoring *Culicoides* spp. se vrši počevši od 2014. godine i dao je značajne rezultate vezane za biodiverzitet i sezonsku dinamiku pojavljivanja ovih insekata u Srbiji. Dosadašnja istraživanja pomogla su mapiranju geografske rasprostranjenosti vrsta i varijacije u brojnosti populacije u različitim godinama. Na osnovu njih i bioklimatograma moguće je predvideti kretanje njihove populacije i preventivno delovanje u cilju njihovog suzbijanja. Nažalost, počevši od 2019. godine monitoring je podeljen na tri instituta – u Beogradu za centralnu Srbiju i Južni Banat, u Novom Sadu za Vojvodinu i u Kraljevu za jug Srbije, tako da se ovim izgubio uvid u biodiverzitet i druge važne podatke (odnos polova i sl.). U našem radu zato su dati samo rezultati ispitivanja biodiverziteta i sezonske dinamike *Culicoides* spp. tokom 2019. godine na epizootiološkom području Naučnog instituta za veterinarstvo Srbije u Beogradu, bez podataka za Pomoravski region sa kojeg ne dobijamo uzorke četvrtu godinu zaredom. Pripadnici *Obsoletus* kompleksa su ustanovljeni u 59,91%, a iz *Pulicaris* kompleksa su ustanovljeni u 34,06% pozitivnih uzoraka, dok su ostale vrste kulikoida ustanovljene u manje od 10% pregledanih uzoraka.

**Ključne reči:** *Culicoides* spp., epizootiologija, Srbija

### INTRODUCTION

Continuous entomological monitoring of *Culicoides* spp., which started in 2014 in accordance with the Guidelines on how to conduct entomological examinations for the monitoring and control of bluetongue disease on the territory of the Republic of Serbia, has so far yielded significant results regarding biodiversity and seasonal dynamics of these insects in Serbia. The research we have done so far has contributed to mapping the geographical distribution of the species we encountered as well as the variations in the number of

populations in different years (Pavlović et al., 2016a; 2017, 2018, 2019; Vasić et al. 2019). As the monitoring continued, we were receiving new valuable data every year, which will help to predict the movement of these insects in the future on the basis of climate conditions and enable preventative actions to be taken to counteract them (Maksimović-Zorić et al., 2016).

Unfortunately, starting in 2019, the monitoring was divided between three institutes - Belgrade Institute receiving samples from central Serbia and South Banat, Novi Sad with samples from Vojvodina and Kraljevo analysing the samples from South Serbia regions, in accordance with new instructions by Veterinary Directorate, which resulted in losing an insight into biodiversity, sex ratio and most importantly age of the females that are primary vectors.

Therefore, we can only provide the results of testing biodiversity and seasonal dynamics of *Culicoides* spp. in our work during 2019 from the epizootiology areas which were examined at Scientific Veterinary Institute of Serbia in Belgrade.

## MATERIAL AND METHODS

Insect samples from the epizootology area of Belgrade, South Banat and central Serbia were submitted during 2019 for examination. We did not receive any samples from Pomoravlje districts for the fourth consecutive year (Fig. 1). During 2019 we examined a total of 793 samples of insects.

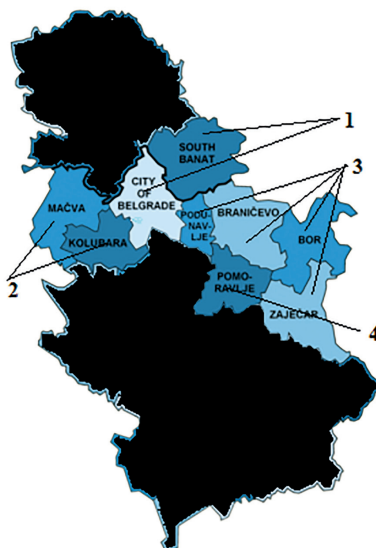


Figure 1. Epizootiology areas examined at Scientific Veterinary Institute of Serbia in Belgrade. 1. City of Belgrade and South Banat; 2. Western Serbia Districts; 3. Eastern Serbia Districts; 4. Pomoravlje district

Determination of *Culicoides* spp insects was performed by morphometric method recommended by the Italian National Reference Centre for Exotic Diseases (National Reference Centre for the study of Exotic Animal Diseases (CESME) Reference Laboratory for Bluetongue OIE, Istituto Sperimentale Zooprofilattico dell'Abruzzo e del Molise "G. Caporale" (IZSAM) from Teramo, Italy. Species definition of *Culicoides* spp. has traditionally been based on the morphology of adult insects. Adult individuals of *Culicoides* spp. are notable for their characteristic wing pigmentation pattern and distribution of wing microtrichia, which in certain species can be used as the principal diagnostic feature. Then, we observed the antennal XI/X ratio (length of segment XI divided by length of segment X), and the shape and size of the 3<sup>rd</sup> palpal segment. Finally, we compared all the observed traits with IIKC (interactive identification key for *Culicoides*) database pictures. In practice, however, the requirement is that specimens should be slide mounted, image-captured, measured and analysed which is time-consuming and therefore the use of morphometries for identification purposes in high-throughput systems such as surveillance programs is recommended (Weeks et al., 1999, Mathieu et al., 2012).

## RESULT AND DISCUSSION

The occurrence of bluetongue disease during 2006 induced the need to begin with these entomological studies which were performed at parasitology laboratories of Scientific Veterinary Institute of Serbia. The research carried out during 2006-2007 period confirmed the presence of *Culicoides* spp. and later the research performed during 2011-2012 allowed us to gradually gain an insight into the fauna of these species (Pavlović et al., 2009, 2014).

During our long-term research on the whole territory of Serbia we have identified thirty-three *Culicoides* species. During examinations performed in the above epizootiological areas during 2019, the following types of *Culicoides* were found: *C. circumscriptus*, *C. deltus*, *C. fasciipennis*, *C. furcillatus*, *C. griseidorsum*, *C. lupicaris*, *C. nubeculosus*, *C. obsoletus*, *C. pallidicornis*, *C. parotti*, *C. picturatus*, *C. pulicaris*, *C. punctatus*, *C. scoticus* and *C. subfasciipenni*.

### ***City of Belgrade and South Banat***

In Belgrade and South Banat we have detected 17 *Culicoides* species. The most abundant species were *C. scoticus*, *C. nubeculosus*, *C. obsoletus*, *C. parotti*, *C. circumscriptus* and *C. subfasciipenni*. Similar species were also found by Oprescu et al. (2008) and Tilibaşa et al. (2014) in Romania (Timisoara region) that borders with this area.

### ***Western Serbia (Mačva and Kolubara Districts)***

In the West Serbia we have found 23 species. The dominant species near Drina and Sava River in North-western Serbia (Mačva and Kolubara District) were the following: *C. circumscriptus*, *C. griseidorsum*, *C. fasciipennis*, *C. pulicaris* and *C. scoticus*. Similar species were also found in Bosnia and Herzegovina, Croatia and Montenegro (Omeragić et al., 2009, Bosnić, 2011, Pudar et al., 2018).

### ***Eastern Serbia Districts***

On the other side, in the Northeast part of Serbia we have determined 21 *Culicoides* species. In Podunavlje and Braničevo District the predominate species found were *C. circumscriptus*, *C. obsoletus*, *C. fasciipennis*, *C. nubeculosus*, *C. parotti*, *C. pulicaris* and *C. scoticus*. At same time, in Bor and Zaječar districts the most abundant were *C. pulicaris* and *C. scoticus*. *C. fasciipennis* and *C. obsoletus*. Similar species were also found in Bulgaria and Romania which border those districts (Ioniță et al., 2009; Ilie et al., 2013; Bobeva et al., 2013; Pudar et al., 2018).

### ***Pomoravlje district***

Based on the data collected earlier, until the period when the samples from the territory of Jagodina stopped coming, the most abundant *Culicoides* species were *C. obsoletus*, *C. fasciipennis*, *C. nubeculosus*, *C. pulicaris* and *C. scoticus*. Unfortunately, in the past four years we did not receive any samples from this epizootic area, so we do not have the data about the current situation regarding that district.

*Culicoides* spp. from *Obsoletus* complex were detected in 59.91% of the samples. Males were found in 20.54%, unpigmented (young) females in 68.91%, females which feed on the blood in 7.17%, and 3.38% were gravid females (Fig. 2).

*Culicoides* spp. from the *Pulicaris* complex were found in 34.06%. Males were found in 19.76%, unpigmented (young) females in 65.66%, females which feed on the blood in 11.01%, and 3.57% were gravid females (Fig. 2).

Other types of *Culicoides* spp. have been detected in less than 10% of the examined samples.

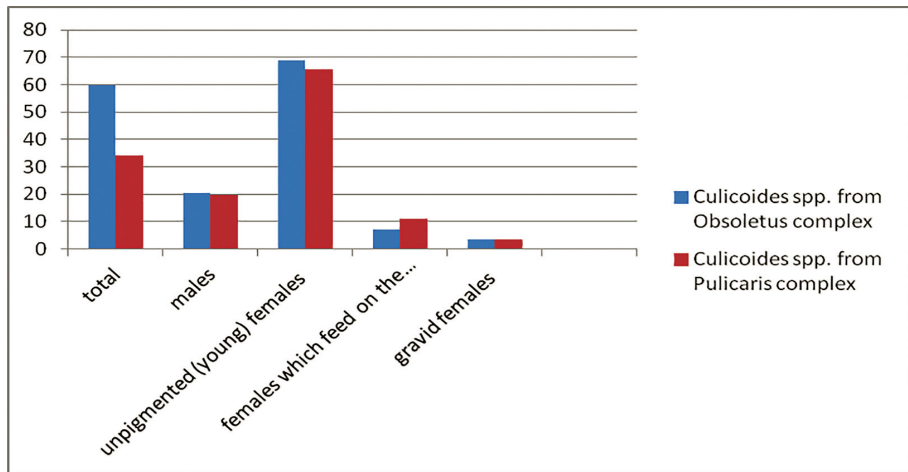


Figure 2. Prevalence, sex ratio and female structure of *Culicoides* spp. from the *Obsoletus* and *Pulicaris* complex

The temperature and relative humidity of the air have the most important impact on the short-term fluctuations of *Culicoides* (sudden increase in number) and then on their long-term spread (Conte et al., 2007). This enabled them to spread rapidly across Europe (Mehlhorn et al., 2007; Patakakis et al., 2009; Moř et al., 2018). *Culicoides* are active only at temperatures between 13°C and 35°C and they feed on the animals only at night (Wilson and Mellor, 2008). Moderately high temperatures favour their development, and very high temperatures can reduce the survival of adult insects. In temperate climates, like Serbia, they have a seasonal character and evolve especially towards the end of summer, when the density of *Culicoides* reaches the maximum (Pavlović, 2016a). We have determined this correlation in monitoring the seasonal dynamics of *Culicoides* species occurrence in Serbia in 2006-2007 and 2011-2012 periods and from 2014 to 2018 (Pavlović et al., 2009, 2014, 2016b, 2019). In our country, the average season of these insects is from March to October, depending on the examined area. Seasonal dynamics of the presence of *Culicoides* spp. was monitored for a year.

Usually, we have an average seasonal distribution of these insects from April to October, depending on the examined area. In the North (Vojvodina province), this period is from April to October. In the Northeast part of Serbia it is from July to October and in the Northwest from May to October. This is also the case in central Serbia, while in the South of Serbia this period is from late March or early April to October (Pavlović et al., 2017, 2018).

During 2019, *Culicoides* spp. were not found in any samples during January, November and December. The first occurrence we detected was in February and their prevalence was 0.22% while in March it was 1.02%, 22.33% during April, in May it was 31.21%, in June 65.90%, in July 71.95%, in August 43.74%, in September 67.66%, and in October it was 31.71% (Fig. 3)

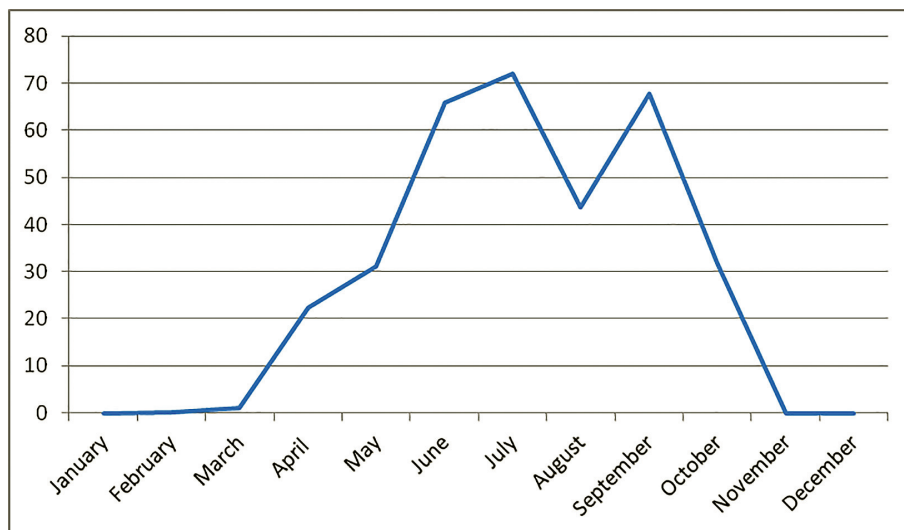


Figure 3. Season distribution of *Culicoides* spp. during 2019

## CONCLUSION

During entomological examination performed in 2019 in the epizootiology area of Scientific Veterinary Institute of Serbia, Belgrade, we identified fifteen *Culicoides* species. *Culicoides* from *Obsoletus* complex were dominant during the whole study period and were detected in 59.91% of samples. *Culicoides* spp. from the *Pulicaris* complex were found in 34.06% and other types of *Culicoides* spp. were identified in less than 10% of the examined samples. Seasonal distribution of these insects was from February to October, depending on the examined area. Considering that from 2019 this monitoring was carried out at three institutions in different regions of Serbia, we lost an insight into the biodiversity, sex ratio and most importantly the age of the females that are the primary vectors.

## ACKNOWLEDGEMENTS

This study was supported by the Ministry of Education, Science and Technological Development, Republic of Serbia (Contract for research funding No. 451-03-68/2020-14/200030), like part of project BT 31053.

## Authors' contributions

IP made insect collecting, determination of insects, epizootiology evaluation and wrote the manuscript, SS were involved in epizootiological processing and assessment, and in insect collection, and NZ an OR collect the insects and be involved in insect determination. All authors read and approved the final manuscript.

## Competing Interests

The authors declare that they have no competing interests.

## REFERENCE

1. Bobeva A., Zehtindjiev P, Bensch S, Radrova J. 2013. A survey of biting midges of the genus *Culicoides* Latreille, 1809 (Diptera: Ceratopogonidae) in NE Bulgaria, with respect to transmission of avian haemosporidians. *Acta Parasitologica*, 58, 4, 585-591. doi:10.2478/s11686-013-0185-z.
2. Bosnić S. 2011. Entomološka istraživanja insekata roda *Culicoides* vektora virusa bolesti plavog jezika u Hrvatskoj. Doktorska disertacija, Veterinarski fakultet Zagreb.
3. Conte A., Goffredo M., Ippoliti C., Meiswinkel R. 2007. Influence of biotic and abiotic factors on the distribution and abundance of *Culicoides imicola* and the *Obsoletus* Complex in Italy. *Veterinary Parasitology*, 150, 333-344. doi: 10.1016/j.vetpar.2007.09.021.
4. Ilie A., Serban C., Imre M., Sorescu D., Ilie M., Imre K., Degi J., Dărăbuș Gh., Morariu S., Munteanu P., Oprescu I. 2013. A survey (or presence, dynamics, prevalence) of *Culicoides* (Diptera: Ceratopogonidae) in Gorj county, Romania, preliminary results of entomological surveillance for bluetongue. *Lucrari Stiintifice medicina veterinara Timisoara*, 46, 3, 173-177.
5. Ioniță M, Mitrea I, Buzatu M, Dascălu L., Ionescu A. 2009. Seasonal dynamics of haematophag arthropod populations (ticks and *Culicoides* spp.) - vectors of pathogens in animals and humans, in different areas of Romania. *Lucrări Științifice Medicină Veterinară*, 52, 11, 629-636.



6. Maksimović Zorić J., Milićević V., Veljović Lj., Pavlović I., Radosavljević V., Valčić M., Glišić M. 2016. Bluetongue disease – epizootiology situation in Serbia in 2015, diagnosis and differential diagnosis. *Archives of Veterinary Medicine*, 9, 1, 13-22, doi: 10.46784/e-avm.v9i1.93.
7. Mathieu B., Cêtre-Sossah C., Garros C., Chavernac D., Balenghien T., Carpenter S., Setier-Rio M.L., Vignes-Lebbe R., Ung V., Candolfi E., Delécolle J.C. 2012. Development and validation of IIKC: an interactive identification key for *Culicoides* (Diptera: Ceratopogonidae) females from the Western Palaearctic region. *Parasites and Vectors*, 5, 137-139, doi: 10.1186/1756-3305-5-137.
8. Mehlhorn H, Walldorf V, Klimpel S, Jahn B, Jaeger F, Eschweiler J, Hoffmann B, Beer M. 2007. First occurrence of *Culicoides obsoletus*-transmitted bluetongue virus epidemic in Central Europa. *Parasitology Research*, 101, 219-228, doi: 10.1007/s00436-007-0519-6.
9. Moț D., Nichita I., Tîrziu E., Moț T. 2018. Bluetongue in Europe and Romania in the Last Years. *Scientific Papers Animal Science and Biotechnologies*, 5, 1, 203-213.
10. Omeragić J, Vejzagić N, Zuko A, Jazić A. 2009. *Culicoides obsoletus* (Diptera: Ceratopogonidae) in Bosnia and Herzegovina-first report. *Parasitology Research*, 105, 563-565, doi: 10.1007/s00436-009-1431-z.
11. Oprescu I, Dărăbuș G, Morariu S, Mederle N, Ilie M, Panici Z. 2008. The dynamics of *Culicoides* insect populations in didactical and experimental station Timișoara, between May and September 2005. *Lucrari Stiintifice medicina veterinara Timisoara*, 41, 460–72.
12. Patakakis M.J., Papazahariadou M., Wilson A., Mellor P.S., Frydas S., Papadopoulos O. 2009. Distribution of *Culicoides* in Greece. *Journal of Vector Ecology*, 34, 2, 234-251, doi: 10.1111/j.1948-7134.2009.00033.x.
13. Pavlović I., Rajković M., Kolarević M. 2009. Kontrola kulikoida – determinacija i suzbijanje. U: *Zbornik radova, XX Savetovanja dezinfekcija, dezinfekcija i deratizacija u zaštiti zdravlja životinja i ljudi sa međunarodnim učešćem*, Divčibare, Srbija, 89-92.
14. Pavlović I., Stanojević S., Rajković M., Šekler M., Plavšić B. 2014. Dosadašnja istraživanja *Culicoides* (*Insecta: Ceratopogonidae*) u Srbiji. U: *Zbornik kratkih sadržaja, XVI epizootiološki dani Srbije, Zrenjanin, Srbija*, 85-87.
15. Pavlović I. 2016a. Epidemiology of *Culicoides* species in Serbia - lessons learned from the national monitoring program after bluetongue outbreak, lecture 8. In: *Textbook of SCOPES International Partnership “Arbovirus Monitoring, Surveillance and Research–capacity building on mosquitoes and biting midges (AMSAR)” Summer School in Stara Planina and*

- Belgrade, Serbia: "Morphological identification and PCR screening of vectors transmitting Bluetongue, Schmallenberg and West Nile virus", SCOPES pub., 1-7.
16. Pavlović I., Bojkovski J., Silaghi C., Veronesi E., Vasić A., Simeunovic P., Oslobanu L., Anița D. 2016b. Impact of environmental factors on the biodiversity of *Culicoides* (Insecta: Ceratopogonidae) in Serbia. In *Book of Abstracts*, International Conference on Ecological Crisis: Technogenesis and Climate Change. Belgrade, Serbia, 71-72.
  17. Pavlović I., Veljović Lj., Milićević V., Maksimović-Zorić J., Stanojević S., Radanović O. 2017. Seasonal dynamics of the presence of *Culicoides* spp. in Serbia in the period 2015-2016. *Archives of Veterinary Medicine*, 10 (1), 3-12, doi: 10.46784/e-avm.v10i1.76.
  18. Pavlović I., Stanojević S., Veljović Lj., Maksimović-Zorić J., Radanović O., Plavšić B., Đurić B., Ostojić S. 2018. Study results of the presence of *Culicoides* spp. in Serbia during 2017. *Archives of Veterinary Medicine* 11, 1, 45-51, doi: 10.46784/e-avm.v11i1.16.
  19. Pavlović I., Stanojević S., Radanović O., Zdravković N., Veljović Lj., Đurić B., Maksimović-Zorić J. 2019. Biodiverzitet i sezonska distribucija *Culicoides* spp. u Srbiji tokom 2018.godine. U *Zbornik kratkih sadržaja*, XXI Simpozijum epizootiologa i epidemiologa - sa međunarodnim učešćem - (XXI Epizootiološki dani), Novi Sad, 186-187,
  20. Pudar D., Petrić D., Allène X., Alten B., Ayhan N., Cvetkovikj A., Garros C., Goletić T., Gunay F., Hlavackova K., Ignjatović Čupina A., Kavran M., Lestinova T., Mathieu B., Mikov O., Pajović I., Rakotoarivony I., Stefanovska J., Vaselek S., Zuko A., Balenghien T. 2018. An update of the *Culicoides* (Diptera: Ceratopogonidae) checklist for the Balkans. *Parasites & Vectors*, 11, 462, doi: 10.1186/s13071-018-3051-x.
  21. Tilibașa E.M., Badea C., Hora F.Ș., Dărăbuș G. 2014. A study on dynamics and prevalence in May-June 2013 of *Culicoides* spp., in Timiș county. *Lucrari Stiintifice medicina veterinara Timisoara*, 47, 3, 112-9.
  22. Vasić A., Zdravković N., Anița D., Bojkovski J., Marinov Mihai, M. Alexander Niculaua M., Oșlobanu Elena L., Pavlović I., Petrić D., Pflüger V., Pudar D., Savuța G., Simeunović P., Veronesi E., Silaghi C., the SCOPES AMSAR training group. 2019. Species diversity, host preference and arbovirus detection of *Culicoides* (Diptera: Ceratopogonidae) in south-eastern Serbia. *Parasites & Vectors* 2019, 12:61, 1-6. doi: 10.1186/s13071-019-3292-3.
  23. Wilson A., Mellor P. 2008. Bluetongue in Europe: vectors, epidemiology and climate change. *Parasitology Research*, 103 Supplement 1, 69-77, doi: 10.1007/s00436-008-1053-x.

24. Weeks P.J.D., O'Neill M.A., Gaston K.J., Gauld I.D. 1999. Automating insect identification: exploring the limitations of a prototype system. *Journal of Applied Entomology*, 123, 1–8, doi: 10.1046/j.1439-0418.1999.00307.x.

Received: 05.05.2020.

Accepted: 11.11.2020.