



EVALUATION OF ABUNDANCE AND COMPOSITION OF *CULICOIDES* SPECIES IN DIFFERENT AREAS OF ALBANIA

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Summary

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Culicoides are responsible for a number of diseases transmitted to the animals causing severe health conditions and great economic losses. The outbreak of bluetongue virus in Albania in 2014 and lumpy skin disease in 2016 strongly indicated the establishment of a sentinel herd surveillance in the country ruminant farms. Considering all evidence for Albania, investigation of species composition and abundance of *Culicoides* on livestock farms was performed in 10 sentinel sites. Ultraviolet-light traps (OVI-traps) were placed in selected sites for collection of *Culicoides* starting from April 15 to July 30, 2021. A total of 37,111 *Culicoides* midges from 18 different species were collected during the investigation. The most abundant species identified were *C. newsteadi* and *C. obsoletus*. Moreover, three species were described for the first time in Albania (*C. collessi*, *C. chiopterus* and *C. immaculatus*). Presence and abundance of the collected *Culicoides* spp. are associated with potential outbreak of diseases (i.e. bluetongue, ephemeral fever) linked with their transmission from *Culicoides*.

Key words: Albania, bluetongue, *Culicoides*, entomological surveillance, vector-borne disease

INTRODUCTION

Culicoides spp. have an important role in the transmission of many vector borne diseases worldwide with a high impact on livestock (Yanase *et al.*, 2005; Carpenter *et al.*, 2008; 2009; Veronesi *et al.*, 2013). Among all disease agents transmitted by *Culicoides* spp, the most important ones

are bluetongue virus, Schmallenberg virus, African horse sickness virus and potentially bovine ephemeral fever virus (Magnon & Hagan, 1988; Gerry *et al.*, 2001; Walker, 2005; Elbers *et al.*, 2012; Fall *et al.*, 2015). Since the major arboviruses affecting humans are considered to

be transmitted by mosquitos, *Culicoides* are often a neglected group of species (Sick *et al.*, 2019). Despite having no zoonotic implication, they are responsible for significant economic impact as in outbreak events, attack rate, morbidity, mortality, and case fatality are high. To date, the only arbovirus identified as being primarily transmitted by *Culicoides* to and between humans is Oropouche virus (OROV), a member of the genus *Orthobunyavirus* that causes major epidemics of febrile illness in human populations of South and Central America and the Caribbean (Carpenter *et al.*, 2013).

Culicoides and arboviruses can have a major role in the public health sector in Europe considering their zoonotic potential. Moreover, *Culicoides* have an important role in general health status in wildlife causing dermatitis and other skin conditions. Since *Culicoides* larvae can develop in a great variety of breeding sites such as humid soil, meadows, wet decomposing vegetations, different types of dung, tree holes, lakes and rivers (Ulsu *et al.*, 2007; Werner *et al.*, 2020; Kameke *et al.*, 2021) their distribution is worldwide. *Culicoides* spp. are important biting vectors and play a pivotal role in the transmission of pathogens to warm-blooded animals and humans. They are very diverse; more than 1400 species are known, living in almost all physical conditions (Mellor *et al.*, 2000). A recent study provided extensive information on the distribution of *Culicoides* in Serbia, Kosovo, Bosnia and Hercegovina, Bulgaria, Croatia, North Macedonia and Montenegro (Pudar *et al.*, 2018). Data regarding Albania were missing in this study since traps were not installed in the country. Few studies have been performed in Albania to serologically identify the presence of bluetongue disease (Dedolli *et al.*, 2014)

which is the only known disease transmitted in Albania by *Culicoides*. Other studies have been performed in order to identify the *Culicoides* spp. in Albania (Goffredo *et al.*, 2004) however, data at the country level are not recent, dating more than fifteen years ago. Thus, the need of a new study evidencing distribution of *Culicoides* spp. is necessary to assess the risk of outbreak of different vector-borne diseases (bluetongue disease etc). The main objectives of this study were to identify the abundance, species diversity and dynamics of *Culicoides* spp. in Albania during a certain period of the year using dedicated OVI (Onderstepoort Veterinary Institute) traps. It was assumed that data would provide an important evidence for assessing the risk for the transmission of vector related diseases.

MATERIALS AND METHODS

Collection of Culicoides

Collection of *Culicoides* midges was done in multiple selected spots throughout the country. All spots included in the study were carefully selected in order to represent all the western lowlands of Albania.

The collection phase was from April 2021 to July 2021. Collection of samples was made at a frequency of 1 sampling/2 weeks. In all sites, OVI traps were used to collect *Culicoides*. Prior to the placement of light traps the solution needed for the collection of biting midges consisting basically in water and few drops of detergent, was prepared according to the producer's manual. After assemblage, the light traps were checked for the correct placement of all parts. In some cases, light traps were reinforced with an extra elastic to prevent the detachment of the baker from the other part due to its weight, since in some zones the wind during the night

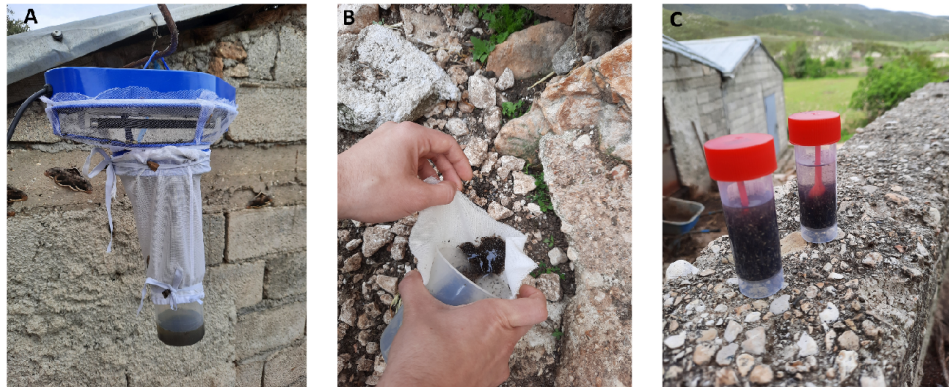


Fig. 1. Light trap after a night in function (A), collection (B) and storage of the samples (C).

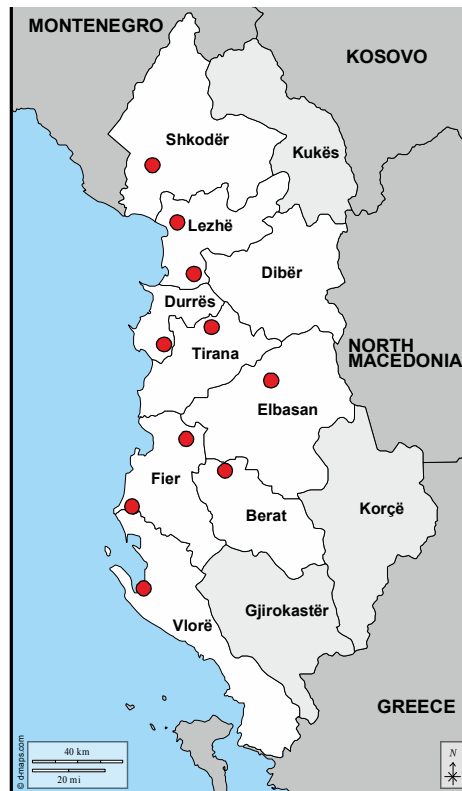


Fig. 2. Location sites of *Culicoides* spp. collection. Red circles indicate the place of the ten selected farms where ultraviolet-light traps (OVI-traps) were placed.

was relatively strong (Vlora and Durrësi region, Fig. 1A).

All light OVI traps were turned on 2 hours before sunset and removed 2 hours after sunrise. In all cases OVI traps were placed attached to the stable of animals in a well-defined distance from ground; 1.5 m and moreover checked for their proper functioning for at least 20 minutes after the traps were turned on. All samples collected were placed in 70% ethyl alcohol and transported in the laboratory (Fig. 1B,C).

Analyses of specimens were performed within 48 h after each sampling. After each sampling OVI traps were carefully cleaned to avoid the *Culicoides* to pass from one sampling to another. Data about humidity, temperature and the geographical position were collected for all selected sites and presented as supplementary material ([http://uni-sz.bg/truni6/wp-content/uploads/vmf/file/Supplementary material.pdf](http://uni-sz.bg/truni6/wp-content/uploads/vmf/file/Supplementary%20material.pdf)).

Identification of *Culicoides*

Morphological identification at species level has been carried out based on keys and taxonomic characters using interactive identification key for *Culicoides* IIC (http://www.iikculicoides.net). The pro-

Table 1. Data of selected sites (coordinates, livestock and altitude)

District	Location	Coordinates	Type of livestock	Approximate altitude (m)
Vlorë	Orikum	40°18'17.8"N 19°26'56.0"E	Sheep, cattle	40
Fier	Pishporo	40°39'44.9"N 19°22'52.3"E	Cattle	50
Lushnjë	Tre urat	40°59'34.4"N 19°36'52.4"E	Cattle	50
Durrës	Gjepalaj	41°19'14.3"N 19°34'44.7"E	Sheep, cattle	50
Berat	Kozarë	40°49'53.7"N 19°54'03.8"E	Cattle	70
Elbasan	Fushë Mbret	41°05'57.6"N 20°04'22.0"E	Cattle	50
Tiranë	Kamëz	41°21'55.0"N 19°46'03.1"E	Cattle, sheep, pigs, horses	100
Krujë	Mamurras	41°35'43.4"N 19°36'51.1"E	Cattle, sheep, pigs, horses	30
Lezhë	Balldren	41°49'13.4"N 19°37'43.2"E	Cattle	60
Shkodër	Livadhe	42°03'49.8"N 19°29'58.6"E	Cattle	20

cess of the morphological identification was conducted by the supervision of two experts in the field.

RESULTS

Ten sites were selected for the collection of *Culicoides* spp as follows; Mamurras, Tirana, Fushë Mbret, Pishporo, Shkodër, Grabian, Balldren, Durrës, Kozare and Orikum (Fig. 2). Coordinates, type of livestock in the selected farms and approximate altitude are presented in Table 1.

Eighty samples of *Culicoides* were collected during this investigation. The majority of *Culicoides* collection sites included in the study were situated near the sea level having a mean altitude of 65 meters above the sea level.

From all 80 collected samples, 37,111 *Culicoides* specimens were processed. Eighteen species were identified during the evaluation of specimens. On all 10 sites included in the study, two species were always present: *C. obsolotetus* and *C. newsteadi*. These species represents also the major number of *Culicoides* identified overall with a percentage of 35% and 30.2% respectively. Sites with the higher number of *Culicoides* collected were Orikum with 8,883 *Culicoides* midges (23.9%) and Kozarë with 7,679 midges (20.6%) while the site with the lower number of *Culicoides* was Mamurras with 1,113 (2.9%). Other sites had a variable number of *Culicoides* collected. All data about collected *Culicoides* midges are presented in Table 2 and Fig. 3.

Table 2. *Culicoides* species identified in each region

No	Culicoides species	Orkum	Grabian	Shkodër	Ballëren	Kamëz	Pishporo	Durrës	Kozare	Fushë Mbret	Mamurras	Total
1	<i>C. newsteadi</i>	2631	542	1143	1688	357	1179	1475	784	996	439	11234
2	<i>C. obseletus</i>	2282	1422	1261	1005	389	929	1920	2321	1021	447	12997
3	<i>C. species</i>	141	138	113	177	146	101	227	267	194	191	1695
4	<i>C. circumscriptus</i>	546	217	0	0	38	0	0	584	0	0	1385
5	<i>C. puncticollis</i>	1070	103	203	0	38	51	0	632	80	18	2195
6	<i>C. gefgelensi/</i>	470	193	0	0	0	0	0	0	0	0	663
7	<i>C. immaculatus</i>	452	211	0	0	0	0	0	0	0	0	663
8	<i>C. pulicaris</i>	637	0	0	0	0	0	423	0	0	0	1060
9	<i>C. univittatus</i>	0	167	0	0	25	0	0	264	0	0	456
10	<i>C. punctatus</i>	0	252	0	0	0	0	0	175	0	0	427
11	<i>C. stigma</i>	0	2	0	0	0	0	0	0	0	0	2
12	<i>C. chiapterus</i>	0	0	0	557	0	0	0	0	0	0	557
13	<i>C. seavus</i>	0	0	0	0	236	0	0	0	0	0	236
14	<i>C. collessi</i>	0	0	0	0	17	0	0	0	0	0	17
15	<i>C. cantaneii</i>	0	0	0	0	0	63	113	1424	24	18	1642
16	<i>C. alzenicus</i>	0	0	0	0	0	0	0	91	0	0	91
17	<i>C. duddingstoni</i>	0	0	0	0	0	0	0	456	0	0	456
18	<i>C. nubeculosus</i>	654	0	0	0	0	0	0	681	0	0	1335
Total		8883	3247	2720	3427	1246	2323	4158	7679	2315	1113	37111

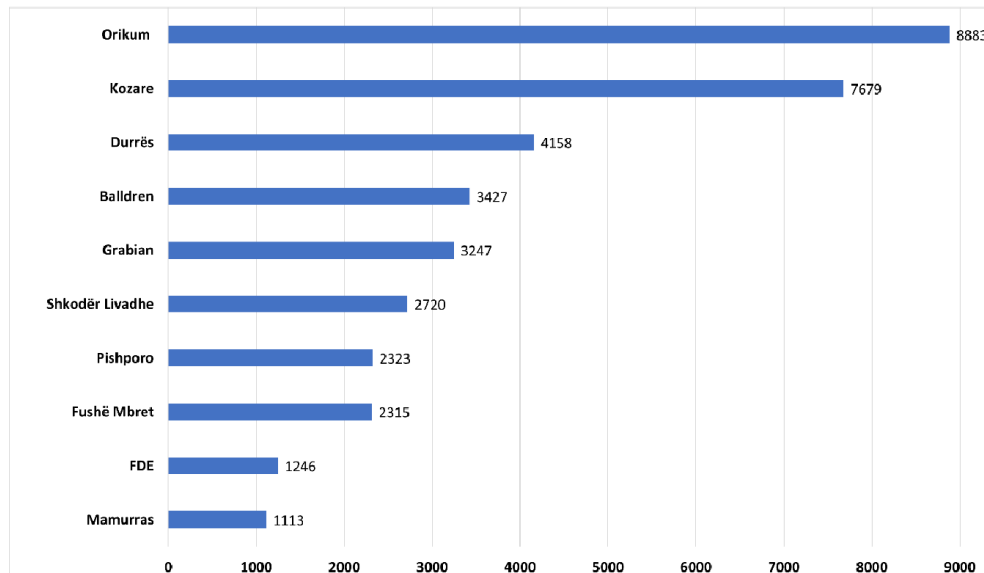


Fig. 3. Number of *Culicoides* spp. collected in each region.

DISCUSSION

Despite the high probability of presence of *Culicoides* spp. in Albania, studies focused on their collection and identification are lacking. In this study we provide valuable data in terms of number and species of *Culicoides* identified. Previous reports on the presence of *Culicoides* in Albania are rare (Goffredo *et al.*, 2004) and there is no operating programme to monitor vectors and vector-borne diseases transmitted by *Culicoides* midges. Moreover, the majority of locations in the study conducted by Goffredo and colleagues were different compared to ours. All traps used in this study were specific for *Culicoides* sampling explaining the high purity of the sample composition. Different species of *Culicoides* were identified during our evaluations. Some species identified such as *C. newsteadi*, *C. punctatus*, *C.*

pulicaris, *C. obsoletus*, are directly linked to the transmission of bluetongue virus (BTV) (Foxi *et al.*, 2016). Thus these observations constitute a very important finding for the future decision making country strategy for the BTV control.

In our investigation the highest number of *Culicoides* belonged to the species *C. obsoletus* and *C. newsteadi* while the lowest number to *C. stigma*. Data regarding the most frequent species from our evaluation are in accordance with other studies conducted in Kosovo, Bosnia and Hercegovina and Croatia (Pudar *et al.*, 2018), except the presence of *C. punctatus* which was identified in low numbers in our study. The species *Culicoides imicola* was never identified by our experts in the collected samples. However, the fact that farms in our study were selected near the sea level having different environmental conditions, type of farm animals,

farm size, wind velocity, temperature, humidity, and altitude may have played a role in vector abundance and diversity.

Orikum situated in Vlora region was the site with the highest number of *Culicoides* collected (8,883) followed by Kozarë in the Berat district (7,679). In our opinion this phenomena can be due to the Orikum Lagoon and Devolli River in Kozarë which are found approximately 300 meters to the sites of collection, thus creating good conditions for *Culicoides* replication since watery region can enhance the possibility of *Culicoides* growth (Mullen & Murphree, 2019). The site with the lowest number of *Culicoides* was Mamurras in the district of Kruja. This can be due to the vector control programme implemented and applied by the owner one month before the start of the sampling in the site. In addition the farm size was small which allowed for better application of these measures. In best of authors' knowledge, *Culicoides* species such as *C. collessi*, *C. chiopterus* and *C. immaculatus* were described for the first time in Albania in our study. This information will serve to update the checklist of *Culicoides* in Albania and can be further used from the authorities for international trade regulation and national control programmes of arboviruses.

Authors are aware of the fact that data at country level coverage are not sufficient to compile a significant inference in general terms. Although our study was performed in most developed livestock area of the country, sites across the borders with Greece, North Macedonia and Kosovo still pose a challenge for the future. Collection of *Culicoides* in these sites would give valuable information on abundance and different species in these areas. Moreover additional studies are needed in the future to better understand the distri-

bution and abundance of *Culicoides* spp, especially *C. imicola* during all year periods, at different altitudes and in border countries.

CONCLUSIONS

Three different species of *Culicoides* were described for the first time in Albania in this study. The species identified are important vectors of a number of arboviruses in southern Europe. Based on all data from this investigation, outbreaks of new serovars of bluetongue and other diseases transmitted by *Culicoides* may occur in the future.

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