The composition, diversity, and distribution of mosquito fauna (Diptera: Culicidae) in Kosovo

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ABSTRACT: The aim of this study is to identify the mosquito species that currently exist and their distributions in Kosovo in order to determine current potential endemic zones and areas at a higher risk for future epidemics. These scientific data will be shared with public health authorities for implementing mosquito control programs. During a two-year period of monitoring in 48 localities in 23 provinces in Kosovo, a total of 1,604 mosquitoes representing 13 species and six genera were collected and morphologically identified. Members of species complexes were also classified to species using DNA barcoding. In total, 13 species were identified with *Culex pipiens s.l.*, the predominant species with an abundance rate of 39%. The remaining 12 species identified were grouped into five genera: *Anopheles*, *Aedes*, *Coquillettidia, Culiseta, Uranotaenia,* including species that are vectors of arboviruses in other parts of the world. ***Journal of Vector Ecology*** **44 (1): 94-104. 2019.**

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INTRODUCTION

Mosquitoes transmit medically important pathogens to human and animals worldwide (Service 1993). Globally, mosquito-borne viruses are being reported more often due to the movement of humans, animals, and goods on a global scale in combination with climate changes (Weaver and Reisen 2010). These conditions also create opportunities for invasive and often exotic vector species to establish in Europe (Schaffner et al. 2001). Their wind-borne long-distance dispersal over suitable habitats and ability to spread locally among human settlements emphasize the necessity of filling the information gaps on the distribution of vector species. One of the most important steps towards avoidance of mosquito-borne diseases is monitoring the species composition in a given country.

Surveillance and control of vector-borne diseases presents many challenges for public health authorities because of their complexity, and monitoring of human and/or animal cases alone is not enough to provide the information needed to mitigate or control the diseases. Collecting data on the distribution, abundance, and seasonality of vectors is one of the most essential steps in surveillance and control of vector-borne diseases. This is strongly linked with effective risk assessment and early warning systems (Braks et al. 2014). The distribution of a vector in a given area helps provide the first indications of potential risk. However, the simple presence or absence of a vector in a given area is unlikely to be a reliable indicator of how much infection exists. Measures of the number of vectors present and overall abundance are the next steps in the assessment of potential pathogen transmission and are more likely to be related to the pathogen transmission dynamics at a certain time and location (Ciota and Kramer 2013).

Although vectors do not recognize national borders, many health authorities prepare their own management for their geographical regions. Assessing the problem with a wider point of view has been an issue because necessary trans-boundary information has not been available. On the other hand, determining the species that occur in a region and their abundance in time and space are essential in a country where little is known about the vectors that are present and which pathogens they transmit. Gathering data on community composition often guides medical entomologists regarding potential introduction and disease dynamics.

Mosquitoes are widely distributed in the countries bordering the Balkans and a large number of arboviruses were recently identified in this region, but little research has addressed the indicators mentioned above in the Balkans and particularly in the Kosovo region. Historic interest in the mosquitoes of the former Yugoslavia has largely been based on their role as vectors of human or animal diseases, especially on malaria and its vectors. Endemic malaria used to be widespread in the lowlands and in some hilly areas of former Yugoslavia in prehistoric times (Popovic 1963). The overwhelming majority of the historical literature on mosquitoes found in the Balkans focused on malaria vectors that were restricted almost exclusively to the area where endemic malaria existed. (Lepes and Vitanovic 1962, Adamovic 1977, Karaman 1924, Jevtic et al. 1964, Litvinjenko

1981). Gruic (1932) and Simic (1934) reported the epidemiological situation of malaria around Prizren, a city in the south of Kosovo, and the importance of zoophilism of *Anopheles* species as a factor in the disappearance of cases in other parts of Yugoslavia. According to Kostitch (1937), this area, now called the Republic of Kosovo, has a longer malaria season and correspondingly, the number of anopheline mosquitoes was very high. Kostitch identified five *Anopheles* species in Yugoslavia, including the south of the country, and reported high numbers of malaria cases around Prizren and Skopje.

Geographical distribution patterns of anophelines are better documented than culicines in the former Yugoslavia. However in recent years, there have been numerous studies of mosquito fauna and distribution in neighboring countries such as Albania and Serbia, including the arrival of the invasive species *Aedes albopictus* into Europe and its establishment (Petric et al. 2011, 2014, Velo et al. 2013, 2016). Furthermore, in Kosovo, species diversity in all genera is largely undocumented. As the first monitoring of mosquitos in Kosovo, the aim of this paper is to assess broad patterns of mosquito composition, diversity, and distribution in the country.

MATERIALS AND METHODS

Study area

Mosquitoes for this study were collected in 23 municipalities in the territory of the Republic of Kosovo (Figure 1). Kosovo is located in the center of the Balkan Peninsula, in southeastern Europe. It is surrounded by high mountains that reach an altitude of 2,656 m. In the west are the Albanian Alps, the highest mountains in the country, that rise from the bottom of Dukagjini and reach altitudes over 2,000 m. The southern border of the country is surrounded by the Sharri Mountains, which together with the Albanian Alps, are the largest mountains and the most valued in terms of morphological, climatic, and biodiversity elements in Kosovo. In the eastern part, the mountains of Gollaku surround Kosovo, whereas in the north and northeast it is surrounded by the Kopaoniku Mountains.

Three large rivers run within the country: White Drini in the west, Ibri in the north, and Morava e Binçes in the southeast. The climate of Kosovo is predominantly continental. In the west there is a Mediterranean influence from the Adriatic Sea from Albania, in the south from the Aegean Sea, and in the north from the European continental landmass. The warmest areas in the country are the southern areas close to the border with Albania, whereas the coldest areas are the mountainous areas in the west and southeast of Kosovo, with the dominance of an alpine climate. Mean monthly temperatures ranges between 0° C (in January) and 37° C (in July). The average annual temperature in the country is 9.5° C, whereas the annual precipitation ranges from 600 to 1,300 mm per year.

Mosquito collection and identification

Mosquitoes were collected from June to September, 2016 and 2017 in 23 municipalities in Kosovo (Figure 1). In the first year of the research, adult mosquitoes were sampled from 30 localities in the southwestern part of the country (Dukagjini plane), which geographically belongs to three regions: 1) Prizreni (Dragash, Suhareka, and Prizren), 2) Gjakova (Gjakova, Malisheva, and Rahoveci), and 3) Peja (Peja, Klina, Deçani, Juniku, and Istogu). The following year of 2017, collection of mosquitoes continued in 18 localities in the northeastern part (Kosovo plane), in four regions: 1) Mitrovica (Vushtrri, Skenderaj, and Mitrovicë), 2) Prishtina (Fushë Kosovë, Obiliq, Podujevë, Prishtinë, and Lipjan), 3) Gjilan (Gjilan and Kamenicë), and 4) Ferizaj (Shtime, Viti, and Ferizaj).

The surveyed area lies at an altitude range of 320 to

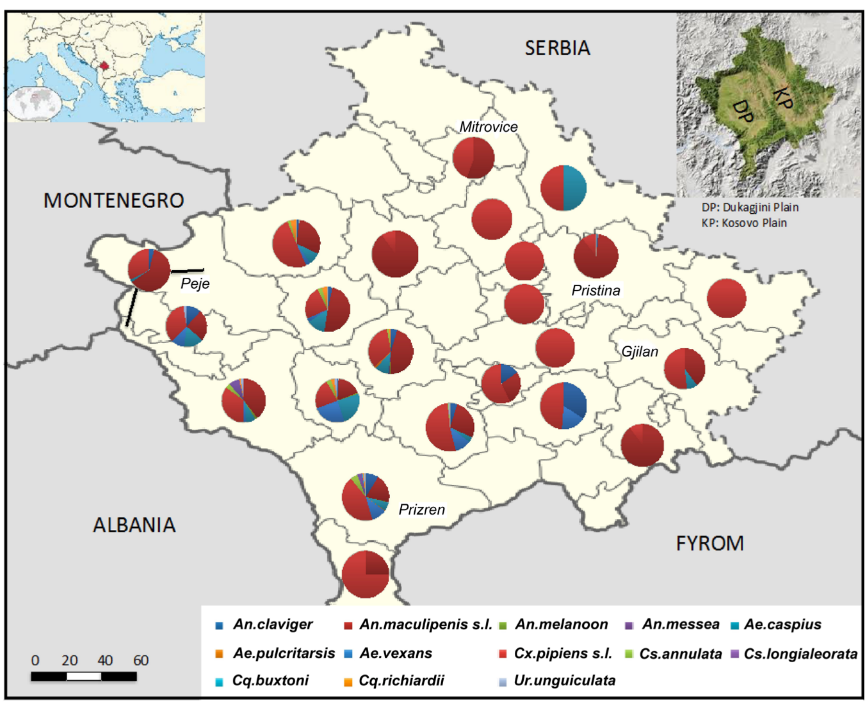

Figure 1. The occurrence of mosquito species in Kosovo. The maps were modified from Shutterstock.com.

1,089 masl. The mosquito fauna was monitored in diverse ecosystems, including forests, streams, rivers, and residential areas. A total of 63 traps was set up in surveyed localities, with 48 indoor farms, stables, and cots with farm animals, without any vegetation, while 15 other traps were placed outdoors, in most cases in the vicinity of farms, mostly in gardens, with mixed vegetation (trees, shrubs, grasses, and other plants).

Data were recorded of the description of each trapping site (Table 1). Collections were made using an Insect Monitoring Trap (IMT) baited with light and dry ice. Collected specimens were stored in boxes with dry ice and stored in a -20° C freezer. At the end of the field study, a total of 1,604 adults was used in the subsequent analysis. Adult specimens were morphologically identified to species using interactive identification keys for mosquitoes of the Euro-Mediterranean region. Samples with uncertain morphological identification were identified molecularly.

Molecular identification

DNA extraction was performed following manufacturer's guidelines with a QiagenDNeasy Blood and Tissue Kit (Hilden, Germany). The DNA barcode region of mtDNA COI gene was amplified with universal LCO1490 and HCO2198 primers of Folmer et al. (1994) that consist of 658-bp after removing the primers. The PCR reactions comprised 3 ml DNA, 3 ml 10xNH4 Buffer, 1.5 ml dNTPs at 2.5 mM, 0.9 ml of each primer at 10 μM, 1.2 ml $MgCl_2$ at 50 mM, and 0.6 ml of Taqpolymerase (SNP HotStart, BIORON) made up to 30 ml with dd H2O. Temperature profile consisted of an initial denaturation at 94° C for 2 min, then 35 cycles of 94° C for 30 s, 50° C for 30 s, 72° C for 40 s, and a final extension of 72° C for 10 min. PCR products were visualized with RedSafe (INtRON Biotechnology) on 2% agarose gels and sequenced with amplification primers of both directions. Sequences were examined with Sequencer version 5.2.4 (Genes Codes Corporation, Ann Arbor, MI) and aligned with ClustalX V2 (Larkin et al. 2007). Invertebrate mitochondrial coded nucleotide sequences were also translated into amino acid sequences and no pseudogenes were found in the COI sequences (Clary and Wolstenholme 1985). All 1st+2nd+3rd+noncoding codon positions were included, and missing data and gaps were eliminated according to reference COI fragments, with a total of 459 positions used in the final dataset. The optimal neighbor-joining tree (Saitou and Nei 1987) was generated by MEGA software V7 (Kumar et al. 2016) calculation of pairwise distance parameters using Kimura's 2-parameter algorithm (Kimura 1980). Publicly available sequences were used to compare with the barcodes created by the Mosquito Barcoding Initiative (MBI) that are in the BOLD database and GenBank (http://blast.ncbi.nlm.nih.gov/).

Statistical analyses of the sampling results used PAST (Paleontological Statistics, ver.1.25). Species diversity was analyzed with calculations of Shannon-Wiener:

$$H' = s\Sigma(pi)(\log 2pi)\ i = 1$$

RESULTS

A total of 1,604 mosquitoes (62 males and 1,542 females) was collected from 23 municipalities in seven regions of the country (Table 2). Thirteen species were recorded: four *Anopheles*, three *Aedes*, one *Culex*, two *Culiseta*, two *Coquillettidia,* and one *Uranotaenia*. *Culex pipiens s.l.* was the most dominant species in the study area (n: 631; 39.3% of the total catch), followed by *Anopheles maculipennis s.l.* (540 specimens; 33.7% of the total catch) (Table 2). Environmental changes in the large-scale area may have affected species composition between localities. Overall, the region of Gjakova located in the southern part of the country yielded the largest number of specimens (452), which included 12 species and Mitrovica, which is located in north, had the lowest number of specimens (85) composed of two species, *Culex pipiens s.l.*, and *An. maculipennis s.l.* Two *Aedes* species, *Ae. vexans* and *Ae. caspius*, were also found widely dispersed in the study area.

DNA barcodes consisting of 658-bp of 21 specimens were used to build a neighbor-joining tree (Figure 2). The result shows the evolutionary distances using the Kimura 2 parameter method (Kimura 1980), with a bootstrap test with 9,000 replicates (Felsenstein 1985). One specimen for *Cx. pipiens s.l.* has shown fixed differences of *Cx. pipiens s.s.* (GenBank accession MH643756). Genetic differentiation of eight specimens thought to be *An. maculipennis s.l.* revealed the presence of *An. maculipennis s.s* (n:1), *An. melanoon* (n:6), and *An. messeae* (n:1) (GenBank Accession MH643757, MH643744, MH643746, MH643750, MH643754, MH643755, MH643758, MH643745). Morphological identifications of *Ae.caspius* (n: 147), *Ae. vexans* (n: 116), *An. claviger* (n:76), and *Cq. buxtoni* (n:1) were also confirmed with COI sequences (GenBank accession MH643759, MH643753, MH643762, MH643748, MH643749, MH643751, MH643752, MH643760, MH643761, MH643763, MH643764, MH643747). *Culiseta annulata* (n:32), *Cq.richiardii* (n:19), *Cs. longialeorata* (n:17), *Ur. unguiculata* (n:15), and *Ae. pulcritarsis*(n:3) specimens were identified morphologically without any complications.

Table 3 shows the abundance of all species according to altitudinal gradients. While a majority (92.7%) of the collection was collected at the range of 300 to 700 masl, 61.6% of the mosquito populations were distributed within 300 to 500 m and only 7.3% within 700 to 1,100 m. Twelve out of 13 species were collected at 300 to 400 m altitudinal range. Most important vectors of West Nile encephalitis and malaria, *Cx. pipiens s.l.* and *An.maculipennis s.l.*, were found at all altitudinal ranges. These species were registered at high densities within altitudes of 400 to 600 masl (total catch: 307 and 377, respectively). *Anopheles claviger* and *Ae. caspius* were also found up to 700 m altitude in the area. No mosquitoes were sampled above 1,100 masl in the study area.

The results show that there are significant differences between the southwestern and northeastern part of the country. Species numbers were found to be dramatically lower in municipalities in the north (4) compared to the south (13 species) (Table 4). Due to dominance of *An. maculipennis s.l.*, the lowest diversity was determined in both Rahoveci

Table 1. The geographical features of the sampling localities and mosquito trapping performed.

Region	Localities	Date of trapping	Area (km²)	Altitude of location (masl)	No. of sampling localities	No. of trapping nights	No. of + traps/total no. of traps	No. of sampling sites with domestic/ sylvatic animals	No. of sampling sites with rich/ poor vegetation*	No. of indoor/out-door traps
Prizreni	Dragash	26/8/2016	210.8	1050	2	1	2/2	2/0	1/1	1/1
	Prizren	10/6/2016	640	450	9	2	12/12	8/4	11/1	8/4
	Suhareka	24/6/2016	361	389	2	1	2/2	2/0	0/2	2/0
Gjakova	Rahoveci	1/7/2016	276	1020	3	1	3/3	3/0	0/3	3/0
	Gjakova	8/7/2016	587	460	3	1	3/3	3/0	0/3	3/0
	Malisheva	14/6/2016	306	538	2	1	2/2	2/0	0/2	2/0
Peja	Klina	17/7/2016	308	382	2	1	2/2	2/0	0/2	2/0
	Istogu	20/7/2016	454	480	2	1	2/2	2/0	0/2	2/0
	Deçan	19/8/2016	180	550	2	1	2/2	2/0	0/2	2/0
	Peja	9/8/2016	603	550	2	1	2/2	2/0	0/2	2/0
	Juniku	9/8/2016	86.2	517	1	1	1/1	1/0	0/1	1/0
Mitrovica	Mitrovica	7/6/2017	331	500	1	1	1/2	1/1	1/1	1/1
	Vushtrri	18/6/2017	344	944	1	1	1/1	1/0	0/1	1/0
	Skenderaj	26/6/2017	374	620	1	1	1/2	1/1	1/1	1/1
Prishtina	F. Kosovë	19/8/2017	83	543	1	1	1/1	1/0	0/1	
	Obiliq	19/8/2017	105	526	1	1	1/1	1/0	0/1	1/0
	Lipjan	13/8/2017	338	563	1	1	1/1	1/0	0/1	1/0
	Prishtinë	17/8/2017	572	652	3	1	3/6	3/3	2/4	4/2
	Podujevë	19/7/2017	643	670	1	1	1/1	1/0	0/1	1/0
Gjilani	Gjilani	9/8/2017	392	508	2	1	2/4	2/2	2/2	2/2
	Kamenicë	27/8/2017	424	470	1	1	1/1	1/0	0/1	1/0
Ferizaji	Shtime	21/8/2017	134	565	3	1	3/5	3/2	2/3	3/2
	Viti	15/8/2017	270	499	1	1	1/2	1/1	1/1	1/1
	Ferizaj	29/7/2017	345	500	1	1	1/2	1/1		1/1

Rich vegetation implies presence of trees and poor implies low levels or absence of vegetation.
Min Max -collection is done in only one locality, there is no difference in max-min elevation.

(the region of Gjakova) in the west plain and in Vitia (the region of Gjilani) in the east plain. Table 4 shows the results of species number and diversity in sampling localities. These values were found to be highest, S:12; H': 1.822, between 300 to 400 m and the range of 400 to 500 m (S:9; H':1.355). At altitudes of 600 to 700 m, both diversity and species number were lower (S: 4; H':1.241).

Higher elevations, such as 700 to 800 m (H':0. 676; S: 2) and 1,000 to 1,100 m (H':0. 680; S: 2), showed a sharp decrease in species diversity and species number. No specimens were collected above 1,100 m. Table 4 also shows the results of the analysis of species numbers and diversity among the mountain ranges. Indeed, two species, *An. maculipennis s.l.* and *Cx. pipiens s.l.,* were found in all three mountain ranges at sampling locations at 1,089 m in the Sharri mountains in the south, 510 m in the Caraleva in the center, and 716 m in the Kopaonik mountains in the north. Accordingly, diversity was also found to be low at these elevations, with the lowest value of H': 0.325 in the Caraleva mountains in the center.

Out of 13 recorded species, twelve were captured indoors and seven of these were also captured outdoors. *Anopheles melanoon, An. messeae, Cq. buxtoni,* and *Ur. unguiculata* were only captured indoors, and the one species that was captured outdoors only was *Ae. pulcritarsis.* Species captured both indoors and outdoors were *An. claviger, An. maculipenis s.l.***,** *Ae. caspius, Ae. vexans, Cx. pipiens s.l.***,** *Cs. annulata,* and *Cq. richiardii* (Table 5.)

DISCUSSION

We identified at least thirteen mosquito species from Kosovo, some being widespread and abundant. Thirteen species and six genera were collected from the western region (DP) and only four species and three genera in the eastern

Table 2. Species, number, and abundance (%) of species in the study area presented per region.

Taxon	Region	Prizreni		Gjakova		Peja		Mitrovica		Prishtina		Gjilani		Ferizaj		Total	
	No. of localities	13		8		9		3		7		4		4		48	
Genus	Species	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
Anopheles	*claviger*	29	7.06	8	1.77	17	4.91	0	0.00	1	0.80	0	0.00	21	23.86	76	4.74
	maculipennis s.l.	93	22.63	143	31.64	139	40.17	53	62.35	58	46.40	41	42.27	13	14.77	540	33.67
	melanoon	1	0.24	5	1.11	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	6	0.37
	messeae	0	0.00	1	0.22	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	1	0.06
Aedes	*caspius*	20	4.87	72	15.93	37	10.69	0	0.00	13	10.40	5	5.15	0	0.00	147	9.16
	pulcritarsis	1	0.24	2	0.44	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	3	0.19
	vexans	43	10.46	52	11.50	14	4.05	0	0.00	0	0.00	0	0.00	7	7.95	116	7.23
Culex	*pipiens s.l.*	195	47.45	131	28.98	122	35.26	32	37.65	53	42.40	51	52.58	47	53.41	631	39.34
Culiseta	*annulata*	13	3.16	13	2.88	6	1.73	0	0.00	0	0.00	0	0.00	0	0.00	32	2.00
	longiareolata	8	1.95	9	1.99	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	17	1.06
Coquillettidia	*buxtoni*	1	0.24	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	1	0.06
	richiardii	2	0.49	8	1.77	9	2.60	0	0.00	0	0.00	0	0.00	0	0.00	19	1.18
Uranotaenia	*unguiculata*	5	1.22	8	1.77	2	0.58	0	0.00	0	0.00	0	0.00	0	0.00	15	0.94
Total number of specimens		411	100	452	100	346	100	85	100	125	100	97	100	88	100	1,604	100
		Dukagjini Plane						Kosovo Plane									
Number of species in each Plane		13						4									
Total number of species		13															

Table 3. Relation of species distribution (%) with altitude in the study area.

	Elevations (m)											
	300-400	%	401-500	%	501-600	%	601-700	%	701-800	%	1,000-1,100	%
No.of sampling sites	12		12		14		5		3	5		
No. of sampling night	12		12		14		5		3	5		
Species	n		n		n		N		N		n	
Anopheles claviger	33	5.95	4	0.92	32	7.86	7	7.6	0	0	0	0
Anopheles maculipennis s.l.	141	25.45	153	35.25	154	37.83	26	28.26	55	56.12	11	57.89
Anopheles melanoon	2	0.36	4	0.92	0	0	0	0	0	0	0	0
Anopheles messeae	0	0	0	0	1	0.24	0	0	0	0	0	0
Aedes caspius	87	15.7	20	4.6	17	4.17	19	20.65	5	5.1	0	0
Aedes pulcritarsis	1	0.18	0	0	2	0.49	0	0	0	0	0	0
Aedes vexans	68	12.27	33	7.6	14	3.43	0	0	0	0	0	0
Culex pipiens s.l.	168	30.32	198	45.62	179	43.98	40	43.47	38	38.77	8	42.1
Culiseta annulata	19	3.42	7	1.61	6	1.47	0	0	0	0	0	0
Culiseta longiareolata	8	1.44	9	2.07	0	0	0	0	0	0	0	0
Coquilettidia buxtoni	1	0.18	0	0	0	0	0	0	0	0	0	0
Coquillettidia richiardii	13	2.34	6	1.38	0	0	0	0	0	0	0	0
Uranotaenia unguiculata	13	2.34	0	0	2	0.49	0	0	0	0	0	0
Total	554	100	434	100	407	100	92	100	98	100	19	100
No. of Species	12		9		9		4		3		2	
%	61.6				31.1			7.3				

Table 4. Comparison of collecting localities in relation to species number (S) and diversity (H').

Localities	Species number	Diversity index (Shannon-Wiener)
	S	H'
Dragash	2	0.562
Prizren	12	1.721
Suhareka	7	1.281
Rahoveci	9	1.172
Gjakova	9	1.518
Malisheva	9	1.354
Klina	7	1.412
Istogu	7	1.310
Deçan	6	1.583
Peja&Junik	4	0.856
Mitrovica	2	0.687
Vushtrri	1	
Podujeva	2	0.693
Skenderaj	2	0.325
Prishtina	3	0.415
Fushe Kosova	1	
Obiliq	1	
Lipyan	1	
Kamenica	1	
Gjilani	3	0.919
Shtime	3	0.957
Ferizaji	3	1.018
Vitia	2	0.325
Total evaluation	13	1.551
Altitude (masl)	12	1.822
300-400	9	1.355
401-500	9	1.306
501-600	4	1.241
601-700	2	0.676
701-800	2	0.680
Plains		
Dukagjini (west)	13*	1.488
Kosovo (east)	4	0.393
Mountains		
Kopaonik (north)	2	0.687
Crnoljeva (center)	2	0.325
Sar (south)	2	0.562

*Min-Max.

region (KP). Of the thirteen species we reported, four species belong to the genus *Anopheles,* three species belong to *Aedes,* and one to *Culex,* which was *Culex pipiens s.l.*

More importantly, *Culex pipens s.l.,* a proven vector of WNV and other vector-borne viruses, and the suspected vector of malaria, *Anopheles maculipennis s.l.,* are both present in Kosovo and/or in Serbia. An important finding was that species distribution patterns and diversity were considerably different between two major plains of Kosovo, the Dukagjini Plain in the southwest, and the Kosovo Plain in the northeast. The Caraleva Hills between these two plains roughly bisect the western and eastern regions of Kosovo.

The distribution of species is very disjunctive, depending not only on temperature, humidity, and geographical barriers but also on the distribution and abundance of vertebrate hosts (Cross et al. 1996, Ghosh et al. 1999). Altitude creates a second-hand effect on environmental biotic and abiotic parameters and generates an indirect selective factor. We collected 13 species in the western region (DP) and only four species in the eastern region (KP), while diversity was 1.488 in DP in contrast to 0.393 in KP. The differences in species composition and distribution between the two plains may be due to the dominant effect of the Mediterranean climate, channeled from the Adriatic Sea along the geographical corridors located between Albania and DP, on habitat diversity of the DP region. Velo et al. (2013, 2016) published the result of a large-scale study on mosquito fauna and control strategies in Albania, indicating the importance of transition areas and possible corridors in terms of both climatic dynamisms and population dispersal of mosquitoes between Albania and Kosovo. In addition to this, the number of species in Caraleva Hills situated between DP and KP was to be two. Even though it was an unexpected result because of the patchy formation, some of the habitats, and the relatively lower elevation of the hills, the hills may be an allopatric barrier between the two plains in the center of Kosovo.

Culex pipiens was recorded as a common and widely distributed species in the monitored area in Kosovo with 39.3% (n: 631) of the entire collection. This species also showed widespread distribution throughout all altitudinal ranges from 300 to 1,100 m (45 out of 48 localities), even though there was a negative correlation between population size and increasing altitude. *Culex pipiens* was sampled in high numbers in the lowlands while it was rarely found in mountainous areas, which indicates potential preference for the wetlands with higher temperature and humidity. Interestingly, this species was collected in higher numbers in urban sampling stations than in rural areas. Harbach (2012) reported that this species generally has higher population densities in urban breeding sites compared to rural regions. Since *Cx. pipiens* s.*l.* has a large ecological plasticity and strong reaction norm to extreme climatic conditions (Gunay et al. 2010), we expected its widespread distribution in Kosovo. On the other hand, the high abundance of this species throughout the entire country may cause important public health problems in the near future as a proven vector of West Nile virus.

Anopheles maculipennis s.l. is also widespread in the

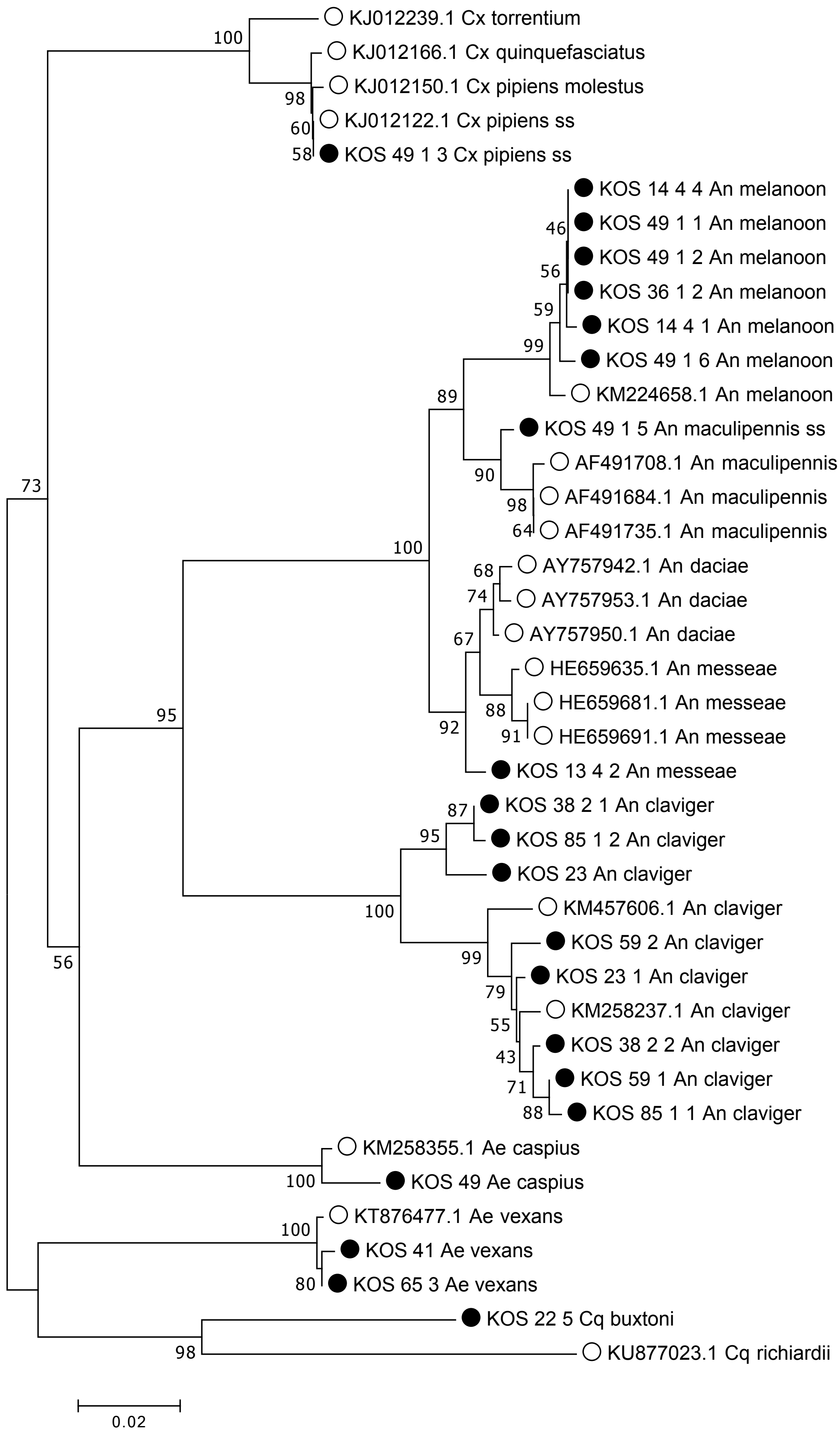

Figure 2. Neighbor-Joining analysis of DNA barcodes from Kosovo (dark colored circle) with reference nucleotide sequences from GenBank (light colored circles). Sum of branch length = 0.64000768 is shown.

Table 5. Species captured indoors and outdoors.

List of recorded mosquitoes species	Indoors	Outdoors	Only indoors	Only outdoors	Both indoors and outdoors
Anopheles claviger	+	+			+
Anopheles maculipenis s.l.	+	+			+
Anopheles melanoon	+	-	+		
Anopheles messea	+	-	+		
Aedes caspius	+	+			+
Aedes pulcritarsis	-	+		+	
Aedes vexans	+	+			+
Culex pipiens s.l.	+	+			+
Culiseta annulata	+	+			+
Culiseta longialeorata	+	-			
Coquillettidia buxtoni	+	-	+		
Coquillettidia richiardii	+	+			+
Uranotenia unguiculata	+	-	+		
No. of species	12	8	4	1	7

entire territory of Kosovo. It is a predominant species comprising 33.7% (n: 540) of the total population. The sub-populations of the *An. maculipennis* complex differ widely in their adaptation to environmental conditions. Therefore, the relative abundances of the members of this complex (*An. claviger, An. melanoon,* and *An. messeae*) vary conspicuously and characteristically from site to site in the examined area. According to Adamovic (1982), it was by far the most abundant and predominant species in the following areas of the eastern former Yugoslavia: the valley of the river Juzna Morava, the plain of Kosovo, the basin river Vardar, and the rift valleys of Macedonia, which is the closest malaria area to Kosovo. These studies correspond with our findings, as we recorded this species in the DP and KP with a high number of specimens, as well as in the hilly areas in the north and center of Kosovo (Figure 1, Table 2).

In this study, molecular results confirmed the presence of *An. maculipennis s.s., An. melanoon,* and *An. messeae* in Kosovo (Figure 2). Absence of the other members of this complex is not certain. Further studies are needed to reveal the true species richness of the complex, with a larger sample size and other gene regions, in order to detect the true distribution of the malaria vector in the country.

Our results are in close agreement with previous findings. *Anopheles messeae* species was considered a rare or extremely rare species in the plains of Kosovo, the basin of the river Juzna Morava in Serbia, and particularly in the lowlands of Macedonia (Adamovic 1983). This species was only identified in Malisheva at an altitude of 538 m as one specimen (0.06% of total specimens) in Kosovo. According to the molecular taxonomy results, this sample is closely related to both *An. daciae* and *An. messeae,* thus future studies including nuclear rDNA ITS2 gene region are essential for precise identification (Nicolescu et al. 2004). *Anopheles claviger* is also widely distributed in the former Yugoslavia (Adamovic 1983). It has been recorded as one of the common species in stables at the sampling stations of Prizren and Ferizaji with high genetic variation on the COI gene region (Figure 2).

Aedes caspius and *Ae. vexans* were also recorded in comparably high numbers (n: 147, 9.2%; n: 116, 7.2%, respectively) of the total catch, especially in the lowlands of DP and hilly areas of east and northeast Kosovo (Figure 1, Table 2). These species were recorded as two dominant species (n: 51, 25%; n: 50, 24%, respectively) in DP at an altitude of 433 m. Although we were not able to find recent studies on virus isolation from these species in Balkan countries, Adamovic (1982) isolated two strains of the Tahyna virus from the 13 pools of *Ae. vexans* and *Ae. caspius* in Serbia. The isolation of the two strains of the Tahyna virus from mixed populations of these species, and also two other pathological agents from mosquitoes of the *An. maculipennis* complex, warrants further examination of the mosquitoes inhabiting Serbia and Kosovo, particularly the virus-carrying species in the area.

The mosquito fauna of Kosovo, including 13 species belonging to six genera, have been documented and supported with molecular techniques for the first time. *Anopheles* and *Aedes* are well represented in our collections, but *Culex, Culiseta*, *Coquillettidia,* and *Uranotaenia* appeared to be under-represented. *Culex pipiens s.l.,* which is the only species collected from the genus *Culex,* was the predominant species in our samples, followed by *An. maculipennis s.l., Ae. caspius,* and *Ae. vexans.* Molecular results of this study also confirm the presence of *An. maculipennis s.s., An. melanoon,* and *An. messeae* in Kosovo (Figure 2).

This study provides a general understanding of mosquito populations in Kosovo despite limited time and resources. Future research is needed to make detailed investigations of mosquito fauna, distribution of mosquito species, ecology of the species transmitting possible pathogens, and to clarify pathogen transmission risk in the country by isolating and typing pathogens from vectors.

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