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Research article

Ecological setting of phlebotomine sand flies in the Republic of Kosovo

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ABSTRACT

Sand flies (Diptera, Psychodidae) are the principal vectors of *Leishmania* spp., the causative agents of leishmaniasis, as well as phleboviruses. In the Balkans, the endemicity and spreading of sand fly-borne diseases are evident, particularly in the Republic of Kosovo, a country with a predominantly humid continental climate. To date, understanding the drivers behind the spatial structure and diversity patterns of sand fly communities in humid continental regions remains limited. Therefore, elucidating the geographical and ecological factors contributing to the presence of potential vector species in the country is crucial. We aimed to enhance our understanding of factors influencing sand fly occurrence in cool and wet wintering humid continental areas, which could serve as a model for other countries with similar climatic conditions. Therefore, we assessed the currently known sand fly fauna through detailed environmental analyses, including Voronoi tessellation patterns, entropy calculations, Principal Coordinate and Component Analyses, Hierarchical Clustering, Random Trees, and climatic suitability patterns.

Notable differences in the ecological tolerance of the species were detected, and the most important climatic features limiting sand fly presence were wind speed and temperature seasonality. Sand flies were observed to prefer topographical environments with little roughness, and the modelled climatic suitability values indicated that, dominantly, the western plain regions of Kosovo harbour the most diverse sand fly fauna; and are the most threatened by sand fly-borne diseases. *Phlebotomus neglectus* and *P. perfiliewi*, both confirmed vectors for *L. infantum* and phleboviruses, were identified as two main species with vast distribution in Kosovo. Contrary to this, most other present species are relatively sparse and restricted to temperate rather than humid continental regions.

Our findings reveal a diverse potential sand fly fauna in Kosovo, indicating the need for tailored strategies to address varying risks across the country's western and eastern regions in relation to leishmaniasis control amidst changing environmental conditions.

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1. Introduction

Among the diverse array of arthropod vectors, sand flies (Diptera: Psychodidae: Phlebotominae) are associated with some of the most debilitating and neglected tropical diseases affecting human populations worldwide [1]. Firstly, they are the principal vectors of phleboviruses causing febrile illnesses that are also known as pappataci fever or sand fly fever. Following the distribution of their respective vector species, the most important Old World phleboviruses, which are the Sicilian virus (SFSV), Naples virus (SFNV), and Toscana virus (TOSV), are endemic in the Mediterranean, subtropical arid, and semi-arid climate regions of Europe, West and South Asia, and North Africa. Furthermore, pappataci fever also occurs in the monsoon-influenced humid subtropical climate territories of North India [2]. Along with phleboviruses, sand flies are also involved in the transmission of other viruses [3]. Even more importantly, sand flies are also the principal vectors of the protozoan parasites of the genus *Leishmania* (Kinetoplastida: Trypanosomatidae), the causative agents of human and animal leishmaniases, causing a complex of serious but still neglected infectious tropical diseases [4]. Cutaneous, mucocutaneous, and visceral forms are among their major clinical manifestations, and one billion people are estimated to be at risk of infection, affecting millions of people in more than 98 endemic countries, mostly in tropical and subtropical areas [5]. Beyond merely its immediate health-affecting effects, leishmaniasis has a significant socioeconomic impact that includes missed disability-adjusted life years (DALYs), financial hardship, and medical expenses [6].

Sand fly-borne diseases are not confined only to the hotspots of transmission, usually located in the tropical and subtropical regions (the Horn of Africa, Indian Subcontinent, tropical regions of South America, and Middle East), but they are also endemic in other parts of the world, including all Balkan countries [7–9], which are mainly located in temperate and continental climatic parts of western Eurasia. Visceral leishmaniasis due to *Leishmania infantum* is an emerging disease across the Balkan Peninsula and seems to exhibit a recent trend of geographical northward spreading facilitated by dog transport as well as migration of wild canids [8], with small foci or isolated autochthonous cases reaching as far as Slovenia [10] and southwestern Hungary [11]. Furthermore, human leishmaniasis is also re-emerging in Balkan countries and is most likely underreported [12]. In the Balkan Peninsula, human leishmaniasis is generally zoonotic visceral leishmaniasis caused by *Leishmania infantum* [7,13]. Noteworthy, a human case of *Leishmania tropica* infection has been reported in Serbia [13] and DNA of *L. tropica* has been detected in a *P. neglectus* specimen in Kosovo [14]. Anthroponotic transmission of either type currently is not the primary concern in this region; however, due to the relatively poor exploration of the area, the future appearance of new forms of human leishmaniasis in the Balkans cannot be ruled out [7].

Naturally, dogs and other carnivores attached to human settlements are the most important reservoir hosts of *Leishmania infantum*. In the Republic of Kosovo, notable levels of seroprevalence of canine leishmaniosis were found in asymptomatic dogs, and the presence of *Leishmania infantum* was also recorded based on DNA screening [15,16]. *Leishmania infantum* in Kosovo was detected in *P. neglectus* and *P. perfiliewi* [16]. The role of wildlife reservoirs, some apparently invasive and spreading to new regions, shall not be underestimated in leishmaniasis epidemiology either. Golden jackals (*Canis aureus*) are prevalent hosts susceptible to *Leishmania* parasites [8]. These indigenous wild canids in Southeast Europe have been rapidly spreading since the Yugoslavian Conflict and in the following decades, representing an invasive species in lowland and mid-altitude territories [17]. As evident from historical records as well as recent field surveys, sand flies, principal vectors of leishmaniases, also widely occur in the Balkan, with diverse local sand fly faunae comprising species of several subgenera, including a number of proven or suspected vectors of leishmaniases [18].

Recently, 13 sand fly species have been recorded in the Balkan countries, with 9 of these in Kosovo [14,16,18], and abundance as well as diversity underline a future potential to spread northwards to the adjacent Central European countries due to ongoing climatic changes [19,20]. In the northern occurrence borders, sand fly species can efficiently use the advantages of natural or anthropogenic shelters to cope with the relatively harsh winters and establish small but permanent populations [21]. Without a better understanding of the current epidemiological and ecological status of sand flies and their associated diseases in Balkan countries, the Central European countries might not correctly assess the near-future risk of the emergence of sand fly-borne diseases [20]. The key point to establish fitting prevention activities is the multi-scale evaluation of the ecology of leishmaniasis in regions where it is currently endemic. To develop such strategies, a broader view of the phenomenon is essential, which is best embodied by the Sustainable Development Goals (SDG) agenda in a modern thematic way [22]. Beyond health and well-being (SDG 3), other SDGs that may contribute to the establishment of the socio-environmental basis of the persistence and spread of sand fly-transmitted diseases, such as, e.g., climate change (SDG 13), and protecting terrestrial ecosystems (SDG 15), are closely linked to the amelioration of the health and economic burden of sand fly-transmitted diseases [23].

The abovementioned issues are particularly relevant within humid continental zones. However, a significant research gap exists concerning sand fly species in humid continental climatic conditions, with studies primarily focusing on tropical, subtropical, and milder temperate (Mediterranean) regions [24–26]. This research gap is not only significant but also emergent, as humid continental regions are expected to undergo the most radical potential changes in climatic classes by the end of the 21st century [27]. Addressing this gap is crucial for understanding the ecological dynamics and potential disease transmission risks in these climatic zones.

The above-mentioned facts indicate that neither the present nor the potential future occurrence of sand fly-borne diseases can be understood and predicted without a deep knowledge of the ecological background of sand fly species involved in transmission cycles. For this purpose, this study aimed to investigate the spatial, biological, and ecological factors determining the occurrence of sand fly species in Kosovo, where several sand fly species exist and *Leishmania infantum*-linked seropositivity was observed in dogs and humans.

Kosovo was selected as a research focus due to its predominantly humid continental climate, which provides an ideal setting for ongoing investigations. Despite this climatic classification, recent studies have unveiled a diverse sand fly fauna within the country. Furthermore, Kosovo's varied topography and the juxtaposition of maritime and continental influences, which can rapidly change even within a small geographical area, render it an exceptional research location. This unique combination of factors makes Kosovo well-suited for studying ecological dynamics and the impacts of climatic variations on sand fly populations.

2. Material and methods

2.1. Logical framework and data acquisition

The study region is presented in Fig. 1, showing the geographical position of the Republic of Kosovo in the Balkan Peninsula (Fig. 1 A), the districts of the country, and the larger cities (Fig. 1 B). The names of the districts are concordant with the names of the indicated capitals.

To address the aim of the study, the following sequence of investigations was performed.

1) Spatial data from published sand fly surveys was extracted from Vaselek et al. and Xhekaj et al. [14,16,28].
2) Sand fly occurrences were georeferenced in QGIS (Suppl. Fig. 1).
3) Topographical roughness values were calculated.
4) Different raster layers containing elevation, topographical roughness, and the data of 19 bioclimatic variables were sampled by the georeferenced sand fly occurrence sites.
5) The deep structure of the spatial patterns of sand fly occurrence was studied to reveal the hidden geographical patterns by Voronoi Tessellation and performing graph analysis based on the occurrence matrix of sand flies.
6) The regional structure of the diversity patterns was studied by the IDW interpolation of species numbers and sand fly species-individual number-based entropy values.
7) The importance of 20 climatic factors in the determination of successful collecting sites was studied using Random Forest algorithm.
8) Climatic suitability values and the potential number of sand fly species were modelled.

2.2. Environmental factors used for analyses

For further analysis, the study incorporated elevation, wind speed, and climate-related factors. Elevation and wind speed data at the 10-m level were taken from the Global Wind Atlas dataset [29]. Climate data was obtained from the WorldClim dataset, utilizing values from WorldClim version 2.1 for the period 1970–2000 [30]. In addition, the present Köppen-Geiger climatic map of Beck et al. [27] was utilized to characterize the overall climatic conditions of the sand fly collection sites.

2.3. Voronoi tessellation

In this study, Voronoi tessellation was utilized to delineate spatial boundaries around individual sand fly occurrence points [31], providing a comprehensive representation of the geographic distribution of sand fly populations. This method is well-suited for analysing multi-species distributions in spatially heterogeneous landscapes [32]. Voronoi tessellation is a useful spatial analysis

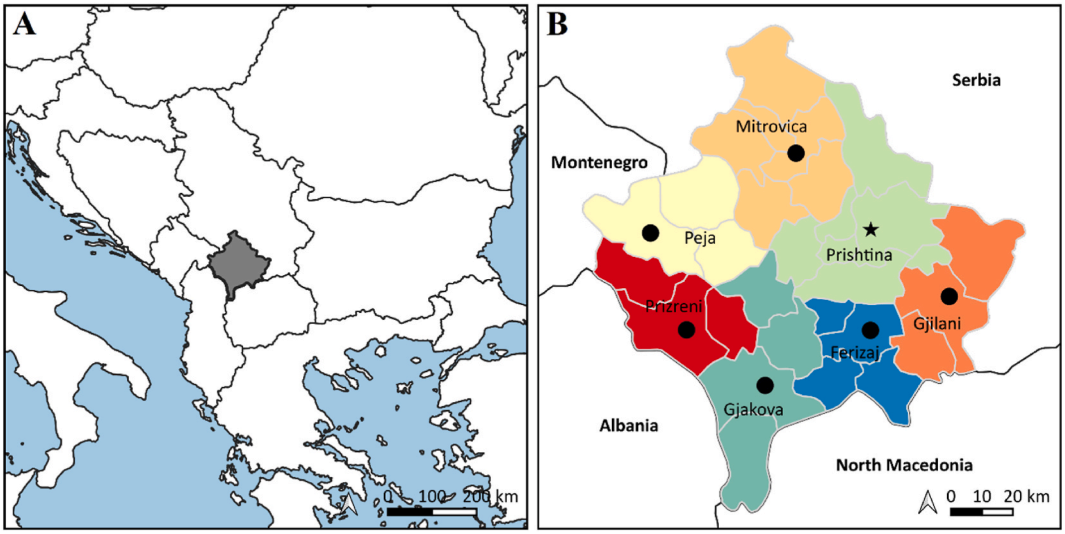

Fig. 1. Sampling region. Geographic position of the Republic of Kosovo (black) in the Balkan Peninsula (A). A map of Kosovo shows seven districts (coloured) and their respective major cities with corresponding names (B). The capital, Prishtina, is marked as a black star. The Mediterranean Sea is shown in blue on the left map. Country borders, first-level administrative divisions, and sea data were made with Natural Earth (naturalearthdata.com).

technique that partitions a space into a series of polygons based on proximity to a set of points (in this case, sand fly occurrence points). Each polygon represents the area closest to a particular point, and together they cover the entire study area. This method is valuable in ecological studies as it allows researchers to quantify and visualize spatial patterns and relationships in species distributions, facilitating the identification of key habitats, potential dispersal routes, and areas of high species richness or abundance. Voronoi tessellation was conducted in QGIS 3.34.3 with GRASS GIS 8.3.1.

2.4. Topographic roughness

Areas with high topographic roughness and varied slope angles may exhibit a diverse array of microhabitats, including crevices, rock outcrops, and steep slopes. Variations in roughness can create microclimatic gradients, leading to differences in temperature, humidity, and vegetation composition across the landscape [33]. These microclimate variations can influence sand fly behaviour, activity patterns, and breeding site selection. Furthermore, areas with complex terrain may offer a greater abundance and diversity of vertebrate hosts and reservoirs and their spatial distribution, supporting larger and more diverse sand fly populations. Conversely, steep slopes and rugged terrain may limit access to these resources, leading to spatial heterogeneity in sand fly abundance and diversity. Then, variation in topographic roughness and slope angle can impact the transmission dynamics of diseases [34] carried by sand flies, such as leishmaniasis.

Topographic roughness was quantified using QGIS software with GRASS GIS software [35] to assess its influence on the occurrence of sand fly species. This analysis involved computing the variation in elevation within a defined area, with higher values indicating greater terrain complexity and roughness. By integrating this topographic parameter with sand fly occurrence data, we aimed to elucidate the relationship between landscape heterogeneity and species distribution patterns. QGIS, a Geographic Information System (GIS) platform, provided the tools necessary to calculate topographic roughness from digital elevation models (DEM; [36]). This allowed for a spatially explicit assessment of terrain ruggedness across the study area. The subsequent analysis of its impact on sand fly occurrence provides valuable insights into the ecological preferences and habitat associations of these species. Calculations needed to create the topographic roughness model were conducted in the topographical roughness calculator of QGIS, which generates a single-band raster containing values derived from elevation computations. The highest inter-cell difference between a central pixel of DEM and its neighbouring cells determines topographical roughness, which represents the degree of surface irregularity. The following are the levels involved: 1) band number: the number of a band carrying elevation values; 2) elevation raster layer as the input layer; 3) Boolean algebra is used in the edge calculation process to create edges from the elevation raster. The process is documented in GDAL (https://gdal.org/programs/gdaldem.html).

2.5. Graph analysis

In addition, graph analysis was conducted to explore the interconnectivity and relative importance of sand fly species within their spatial network. Graph theory, or graph analysis, provides a powerful tool for examining complex ecological networks by representing species interactions as nodes and edges [37]. By analysing these networks, patterns of species co-occurrence can be uncovered by identifying keystone species and assessing community stability and resilience. Nodes in the graph represent individual species, with node size corresponding to their calculated importance. The thickness of the edges between nodes indicates the strength of connectivity between species. This approach enables the identification of key species, hubs of species interaction [38], and potential ecological roles within the sand fly populations, contributing to a deeper understanding of species dynamics and community structure. This method also enhances our understanding of ecological systems and aids conservation and management efforts. The graph analysis was performed in Scientific Python Environment (Spyder) v. 5.2.2 which is an adequate tool to evaluate environmental data science problems [39].

2.6. Calculation of sand fly diversity-related entropy patterns

At first, the primary diversity was plotted and Inverese Distance Weighting (IDW) interpolated using the IDW interpolating tool of QGIS. IDW is a valuable tool for mapping species richness, among other purposes [40]. Then, Shannon's entropy was calculated, which value considers not only the absolute relative number of species in a collecting site but produces values that take both the proportion of a species in a collected assemblage and the number of the collected species into account, and due to this, makes the fauna of different sites better comparable. By incorporating information about species abundance and distribution, entropy measures provide a more comprehensive understanding of diversity within a community [41]. Shannon's entropy values were calculated using the Scientific Python Environment (Spyder) v. 5.2.2. program.

The diversity entropy of sand fly species captured at a given site was calculated as follows (Eq. (1)):

$$H(X) = -\sum_{i=1}^{n} P(x_i) \times log_2(P(x_i)) \tag{1}$$

Where $H(X)$ is the entropy of the diversity of sand fly species within each location, $P(x_i)$ represents the probability of finding sand fly species x_i within the given collection sites, and n is the total number of species present in that site.

2.7. Statistical analyses

To compare the presence/absence sites based on the characteristic climatic conditions and the sites where different sand fly species were collected, Principal Coordinate (PCoA) and Principal Component Analysis (PCA) were performed. The use of both analyses is justified by the fact that there are specific advantages to these tests. While PCA is often used for dimensionality reduction, visualization, feature extraction, and data compression, PCoA is particularly useful for visualizing and exploring relationships among samples in datasets where pairwise distances or dissimilarities between samples are meaningful (e.g., ecological data, genetic data; [42]). Hence, these two analyses complement each other effectively, offering supplementary insights that enhance the application of a singular test.

Bioclimatic variables are commonly used in ecological analysis and niche modelling [43]. The involved bioclimatic factors were as follows: wind speed in m s-1, bio1: annual mean temperature, bio2: mean diurnal range, bio3: isothermality, bio4: temperature seasonality, bio5: max temperature of warmest month, bio6: min temperature of coldest month, bio7: temperature annual range, bio8: mean temperature of the wettest quarter, bio9: mean temperature of the driest quarter, bio10: mean temperature of the warmest quarter, bio11: mean temperature of the coldest quarter, bio12: annual precipitation, bio13: precipitation of the wettest month, bio14: precipitation of the driest month, bio15: precipitation seasonality, bio16: precipitation of the wettest quarter, bio17: precipitation of the driest quarter, bio18: precipitation of the warmest quarter, bio19: precipitation of the coldest quarter.

In addition, a cluster heatmap was created to reveal the inner structure of the mean climatic values related to different sand fly species. Cluster heatmaps are useful to visualize and explore multidimensional data [44]. Furthermore, the Random Trees method was used to determine the feature importance of each climatic variable that can contribute to the occurrence of sand fly taxa. For this purpose, the data was prepared with Microsoft Excel, and consequent statistical analyses were performed in Scientific Python Environment (Spyder) v. 5.2.2.

2.8. Feature importance evaluation

The Random Trees method was employed to assess the relative importance of various climatic features associated with the species richness of sand fly collection sites in Kosovo. This method is efficient for environmental exploration [45]. The analysis incorporated a range of climatic variables, including wind speed (m/s) and bioclimatic variables (abbreviated as bio1–bio19) representing parameters such as temperature and precipitation. By utilizing the Random Trees algorithm, which is a machine learning technique capable of handling complex and high-dimensional datasets, we were able to evaluate the importance of each climatic variable in predicting the species richness of sand fly habitats [46]. This approach provides insights into the key environmental factors driving the distribution and abundance of sand fly populations in Kosovo. Understanding the relative importance of different climatic features enables researchers to prioritize conservation efforts and implement targeted management strategies to preserve habitats critical for sand fly species. Moreover, by identifying the most influential climatic variables, this analysis contributes to our broader understanding of the ecological dynamics governing sand fly populations and their responses to environmental changes. Random Trees analysis was performed in Scientific Python Environment (Spyder) v. 5.2.2.

2.9. Modelling of the climatic suitability patterns

For modelling the potential climatic suitability patterns, the minimum and maximum climatic values related to each species were separately determined. This method follows the logic of niche modelling [47]. The involved factors were the same as those used in the PCA and the Random Trees analyses. The modelling of the climatic suitability values was based on Climate Envelope Modelling, which is mathematically based on Boolean algebra [48] (Eq. (2)):

$$c_i = \begin{cases} 1 \ if \ l_i \leq x_i \leq u_i \\ 0 \ if \ u_i < x_i < l_i \end{cases} \tag{2}$$

Where $f_1, f_2, \ldots f_{20}$ represents the 20 climatic factors, l_i represents the lower limit for factor f_i, u_i represents the upper limit for factor f_i, x_i represents the value of factor f_i.

Based on the above-described equation, the climatic suitability value for the species can be calculated by summing up all the satisfied conditions (Eq. (3)):

$$Climatic\ suitability = \sum_{i=1}^{20} c_i \tag{3}$$

This equation represents the sum of all the Boolean conditions (c_i) satisfied by the climatic factors within their respective lower and upper limits. It gives you a numerical value indicating the overall suitability of the climate for the species based on the given factors.

In the case of the calculation of the number of species, the 95 % climatic suitability limit was applied. The above-described calculations were conducted, and the results were depicted using QGIS 3.34.3 with GRASS GIS 8.3.1.

3. Results

3.1. Spatial structure of positive sites

The Voronoi tessellation of positive sand fly collection sites in Kosovo shows that the density of occurrence is linked to the topographical patterns of Kosovo. The area of the polygons is the smallest in the Dukagjin Plain, and the tessellation is much less dense in the Kosovë Plain. While it can be expected in the case of the higher ranges of the Dinaric Alps Mountains such as the Accursed (Bjeshkët e Nëmuna), Sharri, and Kopaonik Mountains, surprisingly, the mid-elevation ranges like the Carralevë Range in Central Kosovo or the Zhegoc Mountains in Eastern Kosovo also determine the habitat patterns of sand flies (Fig. 2).

3.2. Graph analysis

The graph analysis results reveal that, based on its occurrence pattern, *P. perfiliewi* plays a central role in the sand fly fauna of Kosovo. Following closely, *P. neglectus* emerges as the second most influential node, with a pronounced correlation observed in the spatial structure connectivity between these two species. *Phlebotomus balcanicus*, *P. simici*, *P. tobbi*, and *Sergentomyia minuta* represent nodes of relatively lower importance. Additionally, the significance of *P. alexandri*, *P. mascittii*, and *P. papatasi* in the graph is deemed negligible. Notably, a moderate to strong interconnectedness is observed within the triad comprising *P. neglectus*, *P. tobbi*, and *P. simici*, as well as within the triangle formed by *P. balcanicus*, *P. neglectus*, and *P. simici*. Interestingly, while *P. perfiliewi* emerges as the most pivotal node in the graph, its prominence is partly facilitated by its strong connection with *P. neglectus*. In terms of connections with other species, *P. simici* and *P. neglectus* exhibit the highest connectivity, with eight and seven connections, respectively, whereas *P. mascittii* demonstrates the least, with only three connections (Fig. 3).

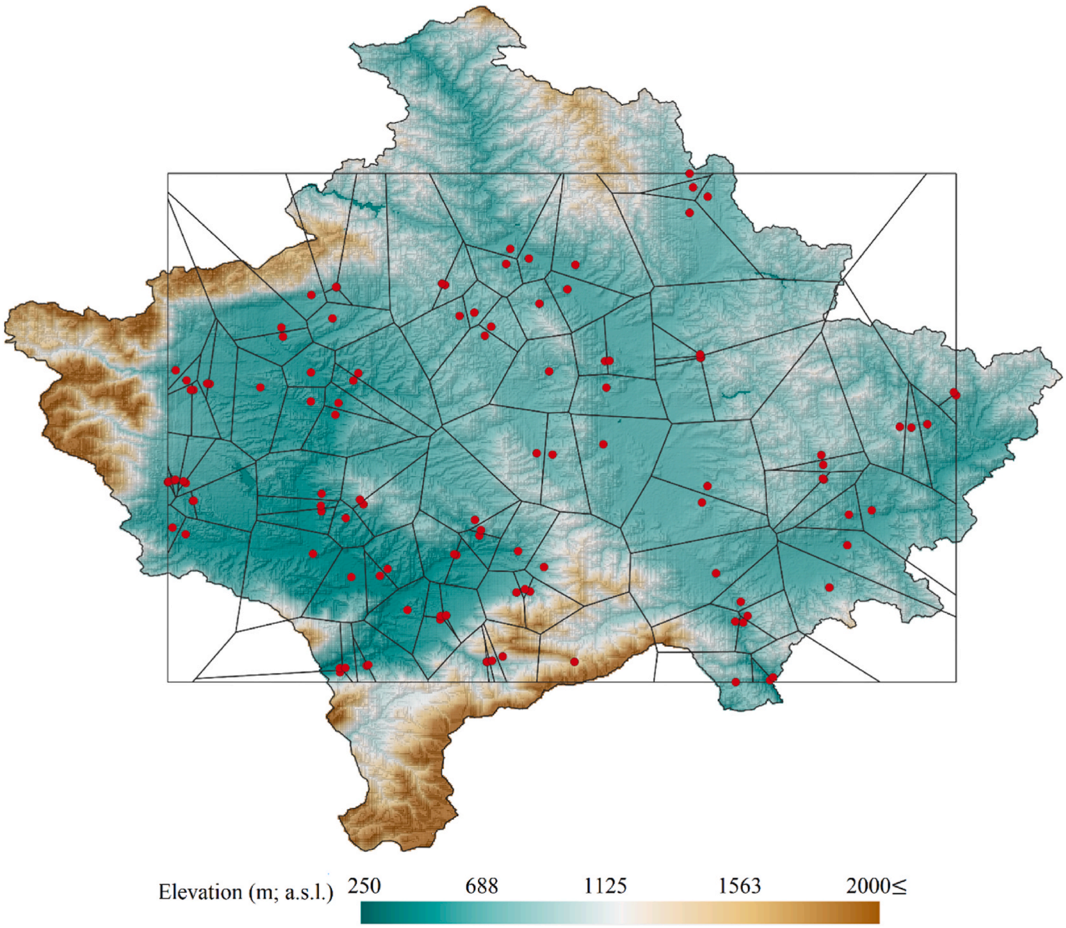

Fig. 2. The Voronoi tessellation plot of the sand fly occurrences in Kosovo is projected onto the topographical map of the country. The grid was determined by the most extreme delimiting coordinates of the positive trapping sites. Red points show the collecting sites that are positive for sand flies.

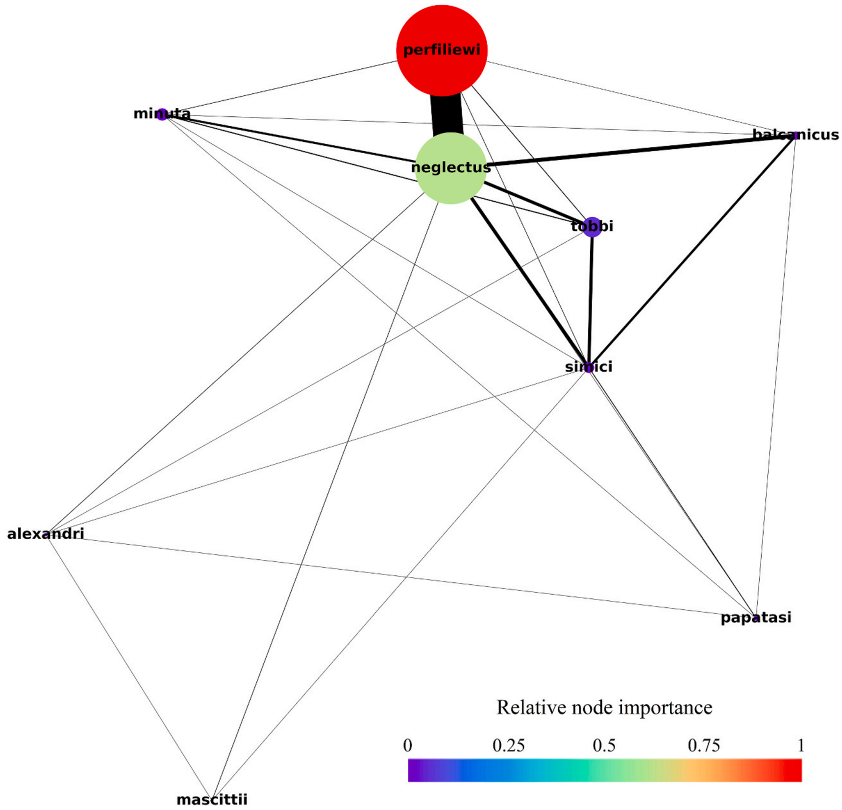

Fig. 3. The outcome of the graph analysis, which is derived from the occurrence matrix of sand fly species collected in Kosovo. Node sizes correspond to the relative importance of each node, while edge thickness indicates the level of nodal connectedness.

3.3. *Topographic roughness*

The topographic roughness of the collection sites by species is as follows: *P. alexandri*: 31.5 m km^{-1} (SD: ±19.1) (Fig. 4 A), *P. balcanicus*: 34.2 m km^{-1} (SD: ±27.5) (Fig. 4 B), *P. mascittii*: 76.0 m km^{-1} (SD: ±82.0) (Fig. 4C), *P. neglectus*: 53.4 m km^{-1} (SD: ±43.2) (Fig. 4 D), *P. papatasi*: 56.0 m km^{-1} (SD: ±36.8) (Fig. 4 E), *P. perfiliewi*: 46.0 m km^{-1} (SD: ±38.7) (Fig. 4 F), *P. simici*: 51.4 m km^{-1} (SD: ±29.8) (Fig. 4 G), *P. tobbi*: 64.8 (SD: ±40.3) (Fig. 4H), *S. minuta*: 45.4 m km^{-1} (SD: ±30.2) (Fig. 4 I), respectively. These values indicate that sand fly occurrences are generally linked to topographically relatively less varied environments, although some species, like *P. mascittii,* can be found in low-to-moderately rough reliefs.

3.4. *Diversity patterns*

The number of species in different sites and its IDW interpolated map exhibit regional differences between the eastern and western parts of Kosovo. West of the Carralevë Range in the Dukagjin Plain, the general number of collected sand fly species is higher than east of this mountain range in the Kosovë Plain. Sites with relatively rich sand fly fauna can also be seen in the southeastern part of Kosovo (Fig. 5 A).

The entropy values based on the species richness and the number of captured sand fly individuals by species in Kosovo show that, in general, the southwest part of Kosovo exhibits the highest entropy values, and a minor centre of higher entropy can also be seen in the southeastern part of the country, while the east-central region shows lower entropy (Fig. 5 B).

3.5. *Köppen-Geiger characterization*

Among the studied sites, 1 % belong to the BSk (arid, cold winters, steppe) type, 22 % to the Cfa (temperate, no dry season, hot summer) type, 1 % to the Dfa (continental, cold winters, no dry season, hot summer) type, and 76 % to the Dfb (continental, cold winters, no dry season, warm summer) type. Several sand fly species can be found both in temperate (Cfa) and continental (Dfa or Dfb) climatic regions in Kosovo (Fig. A–B, D, and F–I). *Phlebotomus mascittii* dwells only in Dfb (Fig. 6 C), and *P. papatasi* (Fig. 6 E) inhabits only Cfa regions. Some species (*P. neglectus*, *P. simici*, and *P. tobbi*) can be found at a cold arid (BSk) site in Hani i Elezit municipality, Ferizaj District (Fig. 6 D, G, H). Sand fly species with wide occurrences primarily inhabit humid continental regions, while in the case of other taxa, temperate climate regions generally have more notable participation in their occurrences.

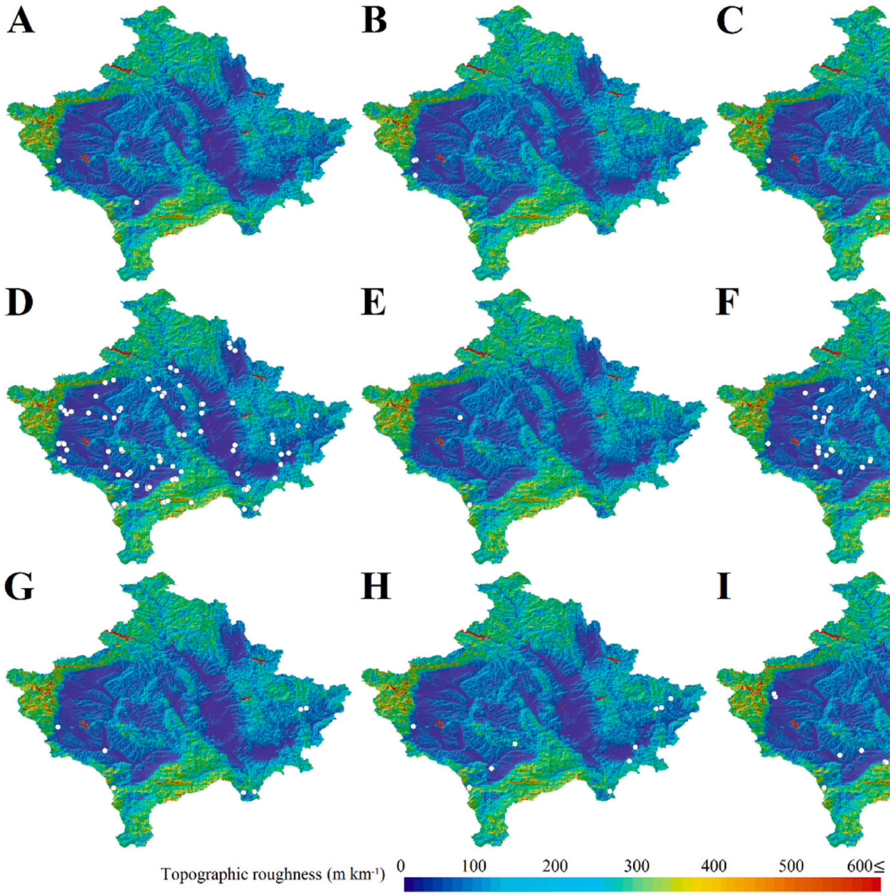

Fig. 4. The sand fly collection sites projected to the calculated topographic roughness pattern of Kosovo. White points show the occurrence sites of sand fly species. **A**: *Phlebotomus alexandri*, **B**: *Phlebotomus balcanicus*, **C**: *Phlebotomus mascittii*, **D**: *Phlebotomus neglectus*, **E**: *Phlebotomus papatasi*, **F**: *Phlebotomus perfiliewi*, **G**: *Phlebotomus simici*, **H**: *Phlebotomus tobbi*, **I**: *Sergentomyia minuta*.

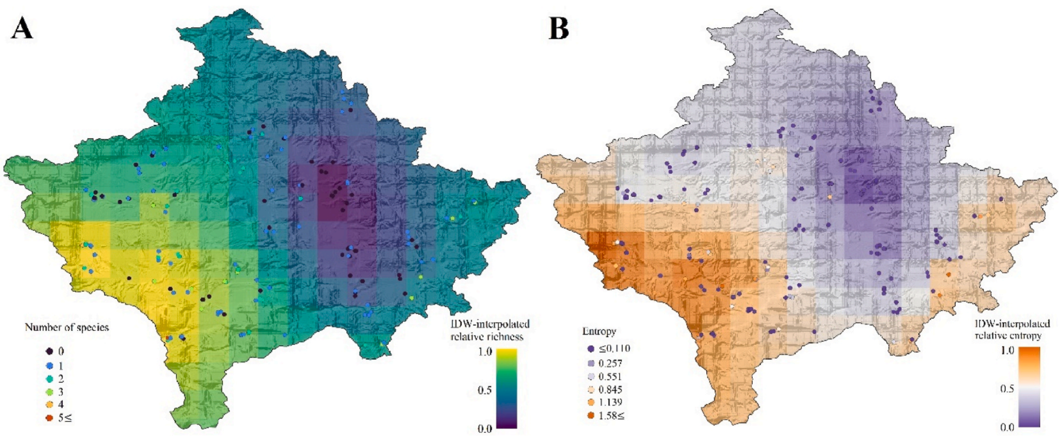

Fig. 5. **A**: The number of sand fly species in Kosovo at the analysed collection sites with the IDW interpolated values. **B**: The collected sand fly species-individual number-based entropy values with the IDW interpolated values in Kosovo. Points show the collecting sites that are positive for sand fly species.

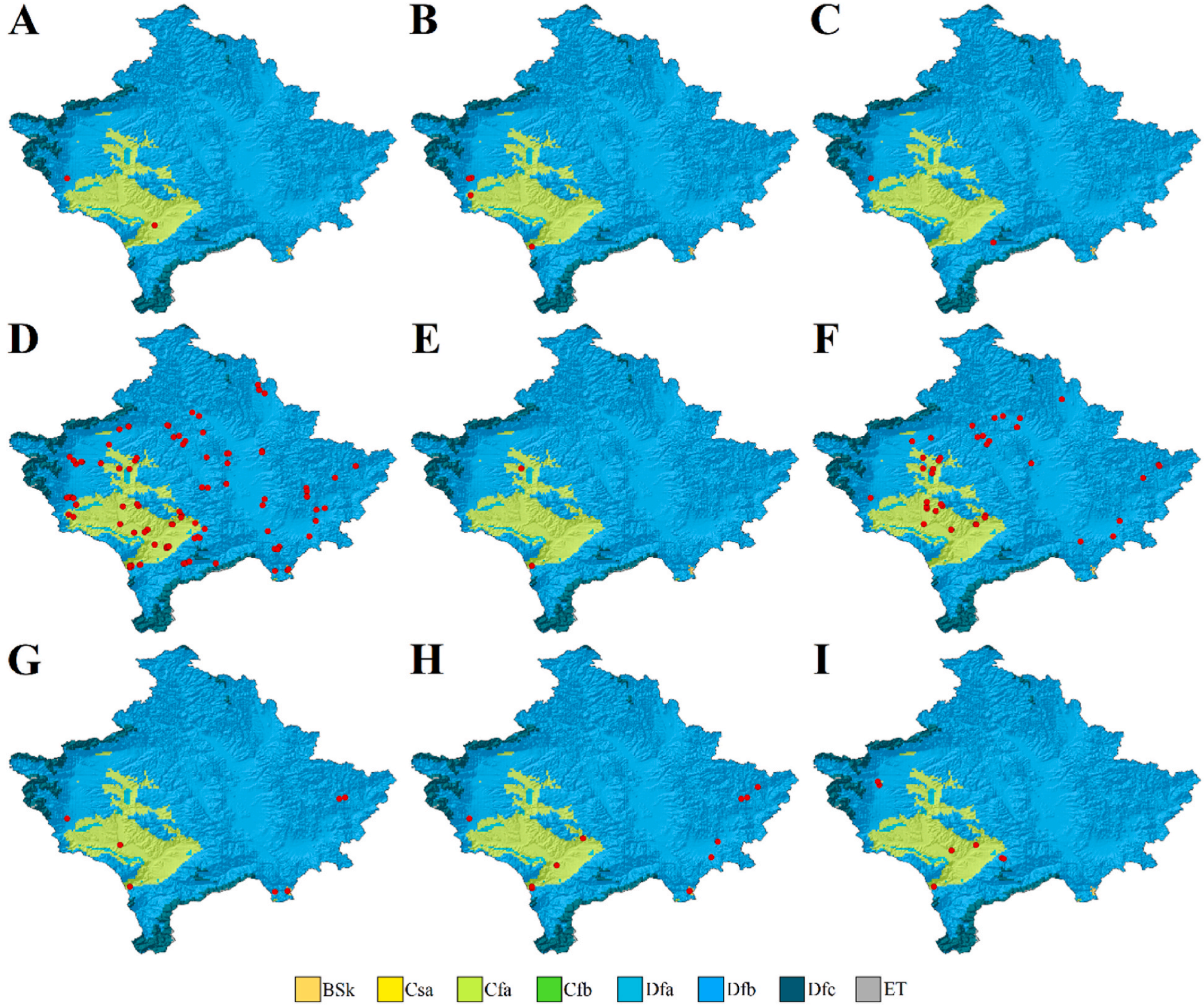

Fig. 6. The projection of sand fly collection sites to the Köppen-Geiger climate map of Kosovo by species. **A**: *Phlebotomus alexandri*, **B**: *Phlebotomus balcanicus*, **C**: *Phlebotomus mascittii*, **D**: *Phlebotomus neglectus*, **E**: *Phlebotomus papatasi*, **F**: *Phlebotomus perfiliewi*, **G**: *Phlebotomus simici*, **H**: *Phlebotomus tobbi*, **I**: *Sergentomyia minuta*. BSk: cold semi-arid (steppe) climate, Csa: hot-summer Mediterranean climate, Cfa: humid subtropical climate, Cfb: temperate oceanic climate, Dfa: hot-summer humid continental climate, Dfb: warm-summer humid continental climate, Dfc: subarctic climate, ET: tundra. Red points show the collecting sites that are positive for the species.

3.6. Principal Component and coordinate analyses

The PCoA of the presence and absence sites does not exhibit a clear separation since these points largely overlap with each other. The convex hulls indicate that the field of the points belonging to the absence sites is embedded into the field of the points of the presence sites. It indicates that there is no notable difference between presence and absence sites in the climatic sense (Fig. 7 A).

The PCA of sand fly collection sites in Kosovo, based on 22 environmental factors, reveals interesting insights into the habitat preferences and environmental tolerances of different sand fly species. While the overall habitat preferences of sand flies show some similarities, certain species exhibit broader environmental tolerances compared to others. For instance, comparing the convex hull areas of *P. neglectus* and *S. minuta* highlights this difference. It is observed that *S. minuta* tends to inhabit milder and more wind-protected environments, whereas *P. neglectus* demonstrates a higher tolerance for temperature seasonality, temperature annual range, and more humid conditions compared to *S. minuta*. Additionally, the analysis suggests that *P. simici* and *P. tobbi* occupy similar environmental niches within Kosovo. Conversely, *P. perfiliewi* prefers higher elevations and more windy regions than the former two species. These findings provide valuable insights into the ecological preferences and adaptability of different sand fly species in Kosovo, which can inform further studies on sand fly ecology and disease transmission dynamics (Fig. 7B).

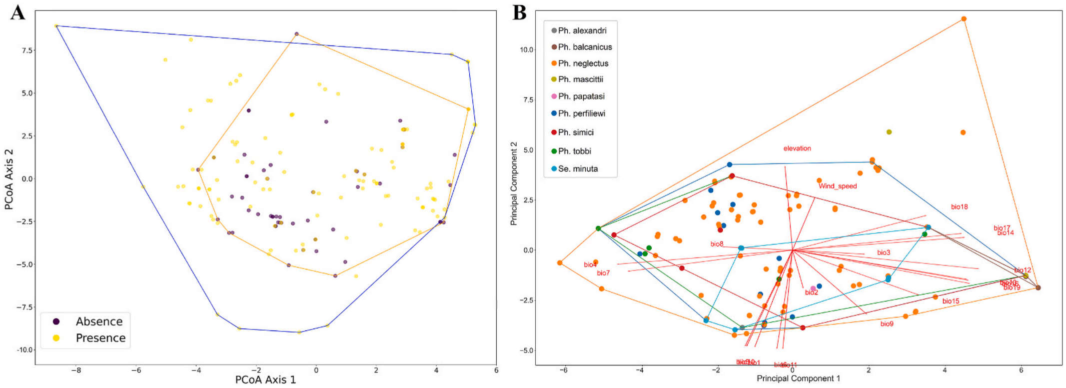

Fig. 7. A: The PCoA of presence and absence sites based on climatic values with convex hulls. **B**: PCA result depicting the relationships among studied environmental variables and sand fly collection sites, with accompanying biplots illustrating the correlation between environmental factors and sand fly species distribution. Explanation of the biplot labels: elevation: elevation in *m*, a.s.l, Wind_speed: wind speed in m s^{-1}, bio1: annual mean temperature, bio2: mean diurnal range, bio3: isothermality, bio4: temperature seasonality, bio5: max temperature of the warmest month, bio6: min temperature of the coldest month, bio7: temperature annual range, bio8: mean temperature of the wettest quarter, bio9: mean temperature of the driest quarter, bio10: mean temperature of the warmest quarter, bio11: mean temperature of the coldest quarter, bio12: annual precipitation, bio13: precipitation of the wettest month, bio14: precipitation of the driest month, bio15: precipitation seasonality, bio16: precipitation of the wettest quarter, bio17: precipitation of the driest quarter, bio18: precipitation of the warmest quarter, bio19: precipitation of the-coldest quarter.

3.7. Cluster heatmap

As the cluster heatmap of sand fly species based on their mean climatic values in Kosovo suggests, *P. neclectus* and *P. perfiliewi* have very similar mean environmental values, and the same can be noted in the comparison of *P. simici* and *P. tobbi*. In Kosovo, *P. alexandri* and *P. mascittii* have the most distinct niches compared to the other sand fly species. A strong correlation can be seen in the case of the occurrence of *P. alexandri* and the related mean bio6 and bio11 values, as well as the occurrence of *P. mascittii* and the related mean bio2 values. In contrast, strong negative correlations can be seen for the occurrence of *P. mascittii* and the mean bio4, bio7, and bio8 values. Also, a relatively strong negative correlation can be found between the occurrence of *S. minuta* and the mean wind speed values (Supplementary Fig. 2).

3.8. Feature importance of climatic factors

Based on Random Trees, wind speed and temperature seasonality are the most influential factors regarding the number of collected sand fly taxa in Kosovo. The relative importance of the factors based on Random Trees is, in decreasing order, as follows: wind speed: 0.323, bio4: 0.123, bio19: 0.054, bio15: 0.043, bio9: 0.042, bio16: 0.042, bio12: 0.040, bio6: 0.037, bio8: 0.037, bio14: 0.035, bio13: 0.034, bio1: 0.034, bio11: 0.031, bio10: 0.027, bio17: 0.025, bio5: 0.023, bio18: 0.022, bio7: 0.022, bio2: 0.005, bio3: 0.001 (Suppl. Fig. 3).

3.9. Climatic suitability patterns

The modelled climatic suitability values of the nine collected sand fly species in Kosovo follow well observed patterns but also indicate further territories where the investigated species can occur. Not surprisingly, *P. neglectus*, the most widespread sand fly species in the country, is potentially absent only in the higher-elevation regions. The potential range of *P. perfiliewi* (Fig. 8 F) is very similar to that of *P. neglectus* (Fig. 8 D), but it is limited to a somewhat narrower area. Although the number of collection sites for *P. simici* (Fig. 8 G) and *P. tobbi* (Fig. 8H) is lower than the positive collection sites for *P. perfiliewi* (Fig. 8 F), these species exhibit a similarly wide potential range in Kosovo. The potential ranges of *P. alexandri* (Fig. 8 A) and *S. minuta* (Fig. 8 I) are limited to the western part of Kosovo. *Phlebotomus balcanicus* (Fig. 8 B), *P. mascittii* (Fig. 8C), and *P. papatasi* (Fig. 8 E) are potentially restricted to relatively small western regions of the country.

The modelled mean climatic suitability values are the lowest in the westernmost part of the country and in the eastern ranges of Alpet Shqiptare. Low values can also be seen in the southwestern part of the country in the Mali i Sharrit ranges and in the northern region along the Kopaonik ranges. The highest values are characteristic of the western lowland area, the Dukagjin Plain, and the western hill land area, west to the Carralevë range. Mid-values can be seen in the eastern valleys and lowlands, including, e.g., the Kosovë Plain (Fig. 9 A). The potential number of species based on at least 95 % satisfied climatic limits exhibits similar spatial patterns. In this map, the highest number of potential sand fly species can be seen in the northern part of the Dukagjin Plain (Fig. 9 B).

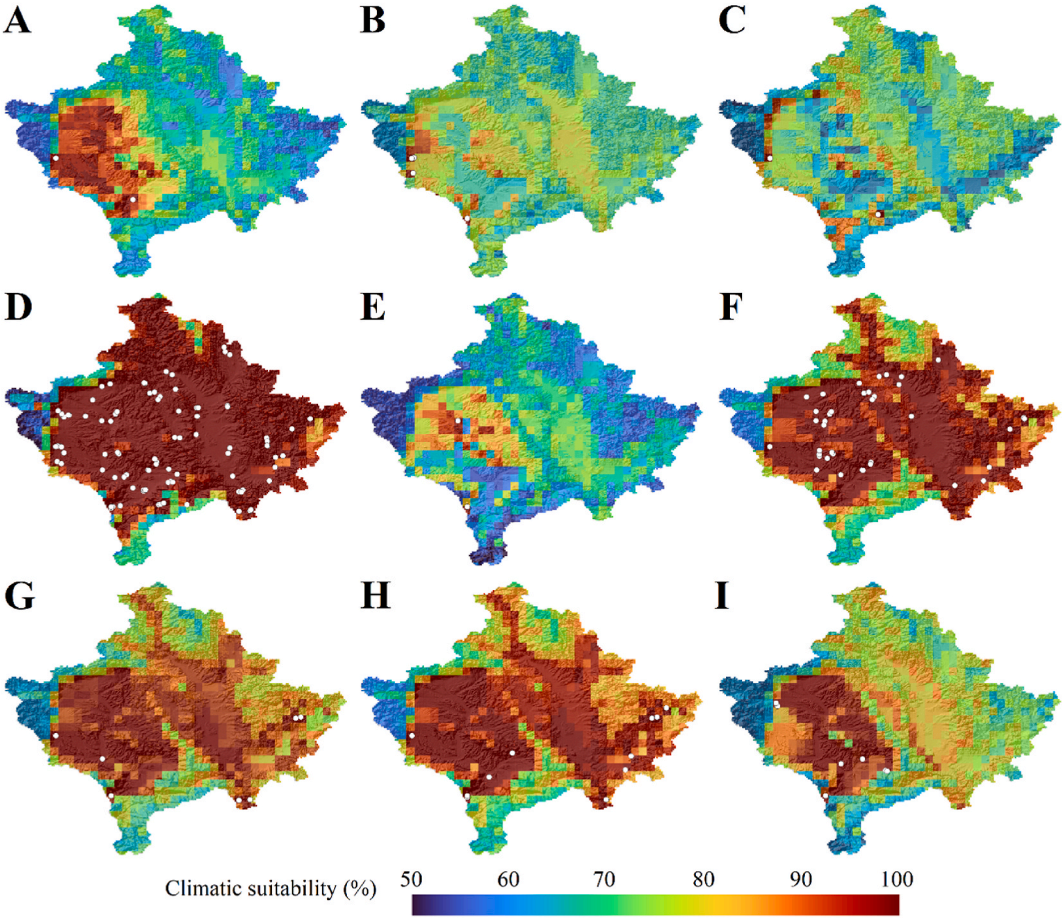

Fig. 8. The modelled suitability values of different sand fly species in Kosovo. White points show the collecting sites that are positive for the species. **A**: *Phlebotomus alexandri*, **B**: *Phlebotomus balcanicus*, **C**: *Phlebotomus mascittii*, **D**: *Phlebotomus neglectus*, **E**: *Phlebotomus papatasi*, **F**: *Phlebotomus perfiliewi*, **G**: *Phlebotomus simici*, **H**: *Phlebotomus tobbi*, **I**: *Sergentomyia minuta*.

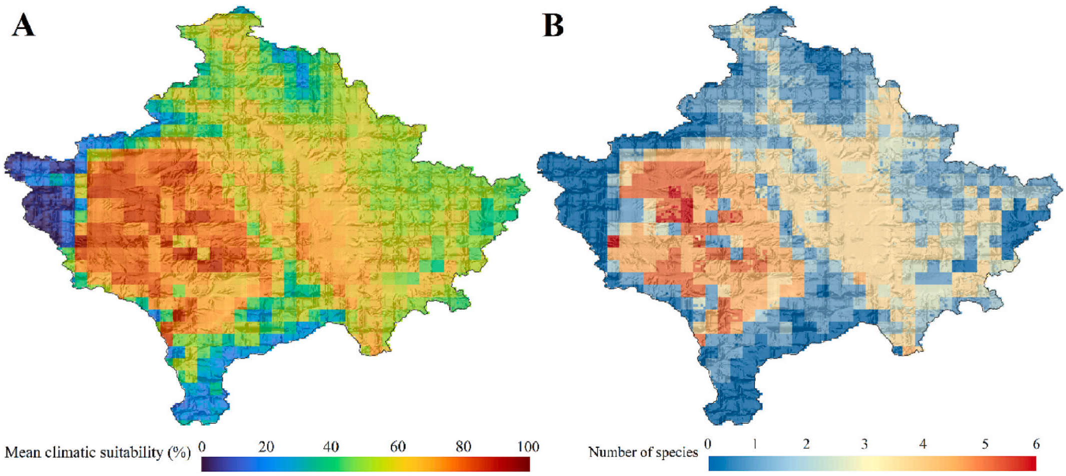

Fig. 9. The potential mean climatic suitability values (A) and the number of sand fly species based on the at least 95 % satisfied climatic limits (B) in Kosovo.

4. Discussion

The results show marked differences in potential diversity patterns of endemic sand fly species, particularly between the western and eastern parts of Kosovo, indicating that those regions are not equally vulnerable to the circulation of sand fly-borne diseases. We found that the plains of eastern Kosovo exhibit a less diverse sand fly fauna compared to the lowlands and valleys of the western region. These findings are corroborated by interpolated entropy patterns and the Voronoi tessellation which show that positive sites exhibit lower densities of sand fly habitats in the eastern part of the country. We determined that *P. neglectus* and *P. perfiliewi* stand out as the predominant sand fly species in Kosovo, which predominantly has continental climatic conditions. This conclusion is supported by the fact that while *P. neglectus* exhibits a broader distribution than other species, the absolute number of *P. perfiliewi* captured surpasses that of other sand fly taxa. These two species occur in regions of Europe that lie outside the typical range of habitats favoured by species like *P. perniciosus* or *P. sergenti*, which prefer mild winter areas.

The presence of *P. neglectus* and *P. perfiliewi* has been documented in northern distribution border areas such as South Hungary [49] and South Romania [50]. These regions in Kosovo and the above-mentioned countries typically exhibit a humid continental climate (Dfb), which aligns well with the general cold tolerance of *Phlebotomus* species.

Kosovo serves as a model for sand fly distribution due to its topographical and geographical characteristics. The country exhibits a decreasing southwestern to northeastern winter temperature trend, which is associated with the distance from the Adriatic Sea. Based on that, a rapid decrease in sand fly diversity within a few hundred kilometres in a territory without a natural geographic barrier can be observed. The unique combination of the topography and geographical setting of Kosovo, situated between maritime and continental climatic regimes, provides an advantageous opportunity to observe this phenomenon within a condensed spatial frame. Notably, *Phlebotomus* species are conspicuously absent in mountainous, boreal (Köppen Dfc) climate regions, a trend observed in Europe generally. It's crucial to note that while two species dominate the sand fly fauna in Kosovo based on the absolute number of individuals collected and their occurrence across various locations, numerous other species also inhabit the country alongside them, which is also observed in other countries such as Romania [50]. Conclusively, the border area's distribution pattern does not imply a low diversity of potential vectors, but rather suggests that one species may dominate, as revealed by entropy calculations and graph analysis results.

Based on our data, the main reason for this observation is the prevalent climatic conditions. The plains of the southwestern regions have a dominantly humid temperate climate, with milder winters, and the meteorological conditions are also humid in both halves of the year. In contrast, the eastern part of the country has harsher winters, which also accounts for the higher elevation regions, where sand flies are absent. The influence of temperature and humidity on sand fly distribution and activity has been extensively discussed in the literature. Particularly in temperate and Mediterranean regions, higher temperatures are the main factor in longer seasonal activity and the number of generations per season [19,51]. However, the influence of humidity has been discussed controversially. While Kniha et al. [19] observed a peak of abundance at 80 % relative humidity for *P. mascittii* in Austria, Prudhomme et al. [52] and Cazan et al. [53] observed a constant decline of activity with increasing humidity in France and Romania, respectively. In general, a humid climate is conducive to the presence of sand flies, while rainy and cool conditions can adversely affect larval development and sand fly activity, thus impacting species occurrence [48]. However, it is not necessarily the case that precipitation conditions in Kosovo restrict the occurrence of sand fly species overall. Several Mediterranean sand fly populations exhibit robust tolerance to dry atmospheric conditions and can thrive in arid environments, such as the Aegean Island of Santorini [54]. Species like *P. neglectus*, *P. papatasi*, *P. perfiliewi*, *P. simici*, *P. similis*, *P. tobbi*, *Sergentomyia dentata*, and *S. minuta* demonstrate successful adaptation to extremely low precipitation levels during the warmest quarter of the year, colonizing even the barren volcanic landscapes of such regions [20]. The only species that may be restricted by hot and dry Mediterranean summers is *P. mascittii*, which shows the highest abundance in humid temperate climates in Central Europe [55].

Interestingly, we observed that wind plays an important regional role in the presence of sand flies on a country-wide geographical scale. This is one of the profound results of the study because the possible role of winds on sand fly occurrence has rarely been investigated [56]. However, it is known that sand flies respond quite sensitively to wind speed [57], which may be related to their relatively poor flying ability and the consequently high risk of drifting due to even medium-strong winds. But it can also be hypothesized that wind can cause desiccation of the potential breeding sites, which can be detrimental for both larvae and adults, especially when strong winds are combined with low humidity and high ambient temperatures [58]. It is worth noting that sand fly taxa typically exhibit low-altitude flight patterns [59], which can mitigate the impact of wind drift. Furthermore, there is evidence for a significant negative correlation between wind speed and sand fly population density [60], with strong winds capable of diminishing or entirely halting sand fly activity [61]. At a local scale, heightened sand fly densities are often observed near wind-sheltered surfaces such as walls [62], underscoring the influence of wind dynamics on sand fly population dynamics. In summary, the findings of this study suggest that wind strength likely plays an indirect role in the regional prevalence of leishmaniasis by modulating the distribution patterns of its vectors. Additionally, it should be noted that the belt of continental climate coincides with the zone of permanent, relatively strong westerly winds that are also sensitive to climatic changes, as historical studies proved (e.g., Hu et al. [63]). This underscores the importance of studying the role of winds in sand fly distribution more deeply in future research endeavours.

Our findings related to topographic roughness and sand fly occurrence highlight the significance of topographic features in shaping the distribution patterns of sand fly species within the study area. The observed association between sand fly occurrence and topographically less varied environments corroborates the preference of certain species for habitats characterized by lower levels of terrain ruggedness. This relationship suggests that sand flies exhibit a propensity towards environments with relatively uniform topographic characteristics. This matches the common perception of the weak flight ability of sand flies, which would require short distances with low terrain ruggedness for natural dispersal by flying. Noteworthy, the presence of species such as *Phlebotomus mascittii* in low to moderately rough reliefs indicates nuanced responses of individual species to topographic heterogeneity. Despite the general trend towards less varied terrain, the occurrence of *P. mascittii* in such environments suggests a degree of adaptability or ecological niche differentiation, which is also supported by its observed patchy distribution in Central European countries [64–66]. These findings contribute to our understanding of the ecological preferences and habitat associations of sand fly species, highlighting the importance of considering topographic features in conservation and management strategies to mitigate vector-borne disease risks. Furthermore, identifying species-specific responses to topographic variability underscores the complexity of sand fly ecology and underlines the need for targeted research to elucidate the mechanisms driving species distributions in heterogeneous landscapes.

Noteworthy, neither PCA nor HCA showed a sharp separation of sand fly taxa into clear ecological groups, which might be due to the relatively limited extension of the country or the sand fly species' environmental preference in Kosovo being similar; their resiliency to more extreme climatic conditions, however, is different. Also, sand fly trapping in European countries is usually somewhat biased, as mostly animal farms or locations close to human dwellings are surveyed. This was also the case for the sand fly data analysed in this study, which originates from three surveys focusing on the aforementioned trapping sites [14,16,28]. Based on the literature, most trapping locations exhibit similar prerequisites regarding habitat structure and host animals.

Generally, while there are similarities in the habitat preferences of sand flies, our analyses reveal notable differences in environmental tolerances between species. For example, *P. neglectus* demonstrates a great tolerance for temperature seasonality, temperature annual range, and high humidity levels, which might be reflected by its northern occurrence as far as Northern Italy [67] or the southern part of Hungary [49]. Furthermore, our analyses indicated similarities in environmental niches between species with similar geographic distributions, such as *P. simici* and *P. tobbi*. These findings contribute to our understanding of the ecological dynamics of sand fly populations in Kosovo and provide insights into the factors shaping species distributions and abundance patterns. By elucidating the ecological preferences and adaptability of different sand fly species, this analysis lays the groundwork for further research on sand fly ecology and disease transmission dynamics.

Our study identified *P. perfiliewi* and *P. neglectus* as the most important sand fly species of Kosovo. While *P. perfiliewi* emerges as the most pivotal node in our graph analysis, it is partly facilitated by its strong node connection with *P. neglectus*. This suggests that ecological roles and interactions of these two species may be closely linked, further emphasizing the importance of understanding their habitat preferences and ecological dynamics for effective sand fly management and disease control. Both species are confirmed vector species for *Leishmania infantum* as well as for phleboviruses and Xhekaj et al. [16] recently detected *L. infantum* DNA in specimens of both species and Vaselek et al. [14] found *L. tropica* DNA in a single *P. neglectus* specimen in Kosovo. Moreover, blood meal analysis revealed a multi-host feeding nature of both species, involving mainly cattle but also dogs and humans, among others, which could facilitate the spillover of *L. infantum* from dogs to humans. Indeed, the European distribution of these species is similar, occupying relatively northern habitats in Southeast Europe, and our climate modelling analysis confirmed similarly large suitability patterns for Kosovo.

Very importantly, the overall modelled climatic suitability for sand flies in Kosovo, which was highest in the western plain of the country, is in line with the spatial appearance of sand fly-borne pathogens. Firstly, the aforementioned sand flies found positive for *L. infantum* and *L. tropica* DNA were all trapped within this area of high climatic suitability. Secondly, *Leishmania* seroprevalences of as high as 21.6 % in dogs living in this region were documented [68]. Thirdly, Ayhan et al. [69] detected high rates of antibodies to the Toscana virus and Sicilian virus in cattle in this region. Additionally, the southwestern region of Kosovo is bordered, without any geographic boundary, by northern Albania, which has been identified as one of the hotspots of visceral leishmaniasis, with *P. neglectus* as the principal vector [70]. In 2015 and 2016, a novel phlebovirus, namely the Drin virus, was detected in *P. neglectus* in the same region [71], which further highlights the importance of this cross-border region for the emergence of sand fly-borne pathogens. Noteworthy, we also modelled a comparably lower but substantial climatic suitability for *P. neglectus*, *P. perfiliewi*, and *P. tobbi* in the eastern Kosovo plain, which is geographically connected to North Macedonia, potentially serving as a corridor for natural sand fly dispersal. All three species are highly abundant in the country, and despite the limited available data, canine and human leishmaniasis are endemic [12,72]. The isolation of a novel phlebovirus, the Bregalaka virus, from pools of *P. perfiliewi* further corroborates the circulation of phleboviruses in the country [73].

5. Conclusions

This study highlights differences in patterns of sand fly distribution across Kosovo and the potential impact on disease vulnerability and adaptation strategies. Lower diversity in eastern plains, contrasting with diverse western lowlands, implies varied transmission risks, supported by analysed entropy patterns and Voronoi tessellation. Prevailing climatic conditions are the major drivers of these disparities, and southwestern regions with milder winters and humidity favour sand fly abundance, while the harsher eastern climate limits suitable habitats. Wind speed was also shown to play a notable role in site selection, impacting sand fly distribution and breeding success. Overall, these findings clearly show the interplay of climate, habitat, and ecology in shaping sand fly distribution and disease risks in Kosovo. Understanding these patterns is crucial to understanding sand fly-borne disease transmission and applying targeted adaptation and control measures tailored to affected regions in Kosovo. In humid continental regions like Kosovo, we conclude that sand fly diversity displays significant geographical heterogeneities, and sand fly occurrences are highly sensitive to locally and regionally changing parameters such as wind speed, seasonal thermal variations, and rainfall conditions. Additionally, while a few sand fly taxa may become dominant under humid continental conditions, several other species can persist. This observation is of high importance for understanding the dynamics of transmittable sand fly-borne diseases within a region.

Data availability statement

All data are included in the article and Supplementary Material.

CRediT authorship contribution statement

Attila J. Trájer: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Ina Hoxha:** Writing – review & editing, Investigation, Formal analysis, Data curation. **Betim Xhekaj:** Writing – review & editing, Investigation, Formal analysis, Data curation. **Katharina Platzgummer:** Writing – review & editing, Investigation, Formal analysis, Data curation. **Vit Dvořák:** Writing – review & editing, Validation, Formal analysis, Data curation. **Adelheid G. Obwaller:** Writing – review & editing, Supervision, Project administration, Funding acquisition. **Jovana Stefanovska:** Writing – review & editing, Supervision, Project administration. **Aleksandar Cvetkovikj:** Writing – review & editing, Supervision, Project administration. **Julia Walochnik:** Writing – review & editing, Supervision, Resources, Investigation, Funding acquisition. **Kurtesh Sherifi:** Writing – review & editing, Supervision, Project administration. **Edwin Kniha:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Investigation, Funding acquisition, Formal analysis, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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