Eco-climatic indicators for three Culicoides *species of the Obsoletus complex in Italy*

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Summary
Bluetongue, Schmallenberg and African horse sickness viruses are transmitted by vectors belonging to the genus *Culicoides* (Diptera: Ceratopogonidae). Among this genus, species of the Obsoletus complex play a crucial role in Europe. In Italy the complex includes three species: *Culicoides obsoletus sensu strictu, Culicoides scoticus* and *Culicoides montanus*. These three sibling species were identified in 80 collection sites across Italy through a multiplex PCR test. Sixty-four sites were classified with a predominant species through a Bayesian approach. Environmental, topographic and climatic factors together with landscape metrics were investigated in each species group using the non-parametric Mann-Whitney test. *Culicoides obsoletus s.s.* resulted the most widely distributed species across the peninsula (51 sites). This species was collected in many eco-climatic conditions, at a wide range of temperature and altitudes, mainly in anthropogenic landscape. Conversely *C. scoticus* collection sites were dominated by natural vegetated areas or forest, at medium higher altitudes, preferably in a wilder and more pristine environment (predominant species in 8 sites). *Culicoides montanus* was a rarer species, statistically predominant in only 3 sites located in Southern Italy, characterized by temperatures higher than the other species. These results, together with other on-going researches on the vector competence of the species belonging to the Obsoletus complex, and with investigation of larval and breeding habitats, could greatly improve epidemiological knowledge of possible spreading *Culicoides*-borne viruses.

Indicatori eco-climatici per tre specie del Culicoides *Obsoletus complex in Italia*

Parole chiave
Analisi del paesaggio,
Bluetongue,
Culicoides montanus,
Culicoides obsoletus sensu strictu,
Culicoides scoticus,
Italia,
Obsoletus complex,
Telerilevamento.

Riassunto
I virus della Bluetongue, di Schmallenberg e della Peste equina sono trasmessi da artropodi vettori del genere *Culicoides* (Diptera: Ceratopogonidae) e, tra questi, l'Obsoletus complex ha in Europa un ruolo cruciale nella trasmissione di queste malattie. Tre specie dell'Obsoletus complex, ossia *Culicoides obsoletus sensu strictu, Culicoides scoticus* e *Culicoides montanus*, sono presenti in Italia e molto simili morfologicamente, distinguibili tramite tecnica PCR. Dai dati di sorveglianza entomologica per Bluetongue, sono stati selezionati 80 siti in cui l'Obsoletus complex è risultato il *Culicoides* prevalente. Applicando l'approccio Bayesiano, 64 di questi sono stati caratterizzati da una specie predominante (*C. obsoletus s.s., C. scoticus, C. montanus*). Le variabili considerate sono state di tipo ambientale (uso del suolo, indici di vegetazione), topografico (altitudine, pendenza, intensità di rilievo), climatico (temperatura diurna al suolo) insieme alle principali metriche di paesaggio (superficie, numero di poligoni, *Shape index*) e sono state utilizzate per confrontare tra loro le specie mediante il test non parametrico di Mann-Whitney. *Culicoides obsoletus s.s.* si è rivelata la specie più diffusa e più abbondante in Italia (predominante in 51 siti) mentre *C. scoticus* è risultata una specie diffusa ma poco abbondante. Infine *C. montanus* è risultata una specie poco diffusa e poco abbondante. *C. obsoletus s.s.* è risultata una specie più facilmente adattabile alle varie condizioni climatico-ambientali, essendo stata individuata in siti con un ampio range di temperature e di altitudini e con maggior impatto antropico. Invece *C. scoticus* è risultato più selettivo nelle

preferenze di habitat con minore capacità di adattamento mostrando preferenza per i siti con prevalenza di vegetazione naturale e boschiva, con vegetazione spontanea e minima antropizzazione (specie predominante in 8 siti). *C. montanus* è risultata una specie più rara e probabilisticamente rilevante in soli 3 siti, tutti nel Sud Italia e con temperature più alte rispetto alle altre specie. La ricaduta di questi risultati, unitamente ai dati di competenza vettoriale (attualmente in fase sperimentale) e allo studio dei siti larvali, potrebbero fornire utili informazioni per l'epidemiologia delle malattie trasmesse da *Culicoides*.

Introduction

The genus *Culicoides* (Diptera: Ceratopogonidae) includes midges responsible for the transmission of some viruses such as Bluetongue virus (BTV), Schmallenberg virus (SBV) and African horse sickness virus (AHSV). These viruses may cause diseases that affect ruminants (BT, Schmallenberg) and equids (AHS).

In Europe, besides the historical vector *Culicoides imicola* Kieffer, 1913, other species belonging to the subgenera *Culicoides* (*Avaritia*) and *Culicoides* (*Culicoides*) are involved in BTV (Savini *et al.* 2005, Meiswinkel *et al.* 2007, Dijkstra *et al.* 2008, Romón *et al.* 2012, Goffredo *et al.* 2015) and SBV transmission (Elbers *et al.* 2013, Goffredo *et al.* 2013, Balenghien *et al.* 2014).

Within the subgenus *Culicoides* (*Avaritia*), the Obsoletus complex is a group of cryptic species, very similar in their morphology, which includes *Culicoides obsoletus* (Meigen), 1818, *Culicoides scoticus* Downes and Kettle, 1952, and *Culicoides montanus* Schakirzjanova, 1962 distinguishable through molecular approach (Meiswinkel *et al.* 2004, Gomulski *et al.* 2005).

Some studies investigated the association of the vectors at complex level with eco-climatic variables at European scale: Brugger and Rubel (Brugger and Rubel 2013b) estimated the Obsoletus complex to be most abundant into fully humid climates characterized by warm summers; Guis and colleagues (Guis *et al.* 2012) integrated climate observations (mean annual temperature, annual precipitation, their variation coefficients and a sun index) and model simulations within a mechanistic model of BT transmission risk, providing future scenarios on probability of presence of *Culicoides imicola*, Obsoletus complex and BT spread across Europe.

Other national researches, investigated possible factors successfully associated to the Obsoletus group: Brugger and Rubel (Brugger and Rubel 2013a) found that Austrian air temperature and precipitation well describe the fluctuating vector densities throughout the year; in Spain, Ducheyne and colleagues (Ducheyne *et al.* 2013) found that the occurrence of the *C. obsoletus* group was clearly determined by land surface temperature (LST), both in the day and during night; in mainland Portugal, Ribeiro and colleagues (Ribeiro *et al.* 2015) found that the most important factors were month, diurnal temperature range, and linear and quadratic terms for median monthly temperature; in the Netherlands, Scolamacchia and colleagues (Scolamacchia *et al.* 2014) found a broadly consistent association across species, with larger catches linked to temperature-related variables and lower wind speed.

In Italy, the Obsoletus complex is widespread and abundant (Goffredo *et al.* 2004, Goffredo *et al.* 2016), but due to the difficulty in separating the single species, the environmental context of vector life cycle has been studied, so far, for the whole complex (Conte *et al.* 2007, De Liberato *et al.* 2010). Minimum temperature, altitude, aridity index, terrain slope, normalised difference vegetation index and percentage of forest coverage, have been used to characterize the 100 largest collections, identifying for Obsoletus complex preferences for more shaded habitat, tolerating undulate topography and encountered regularly on montainous terrains of the peninsula (Conte *et al.* 2007).

However, part of the variability remains unexplained from previous results and detailed data on breeding preferences are scarce. Other approaches based on landscape analysis have been proved to be of help for environment species investigations (Guis *et al.* 2007, Ippoliti *et al.* 2013, Lambin *et al.* 2010). The landscape composition, configuration and connectivity affect the availability of the habitat and the spatio-temporal probability of contact between vectors and hosts, significant for understanding pathogen transmission and spread (Hartemink *et al.* 2014).

This study is an attempt to identify eco-climatic and landscape indicators that broadly determine the abundance of the three species (*C. obsoletus s.s.*, *C. scoticus* and *C. montanus*) of the Obsoletus complex found in Italy.

Materials and methods

Entomological data

In the frame of the Italian Entomological Surveillance Plan for BT, permanent traps for the collection of *Culicoides* are widely distributed on the national territory. Data have been collected mostly on regular basis (weekly), according to standardised methods (Goffredo and Meiswinkel 2004) since 2000.

From this extensive entomological dataset, only the most abundant collections in terms of Obsoletus complex (i.e. collections where the number of Obsoletus complex specimens is greater than 80% of the total number of *Culicoides* specimens) were retained. The collections were representative of the locations of the trap (referred as sites) and solely georeferenced locations were considered.

Species belonging to Obsoletus complex are morphologically undistinguishable and then, from each collection, a number of at least 20 specimens were randomly selected and individually identified by a multiplex polymerase chain reaction (PCR) test (Monaco *et al.* 2010), to identify *C. obsoletus s.s.*, *C. scoticus* and *C. montanus*.

To classify each site as most abundant for a single species, a Bayesian approach based on *Beta* (α_1, α_2) distribution was used to define the 95% confidence interval of the species predominance:

$$Beta(\alpha_1,\alpha_2)=\frac{x^{\alpha_1-1}(1-x)^{\alpha_2-1}}{\int_0^1 t^{\alpha_1-1}(1-t)^{\alpha_2-1}dt}$$

where α_1 = midges belonging to species A + 1;

α_2 = midges tested – midges belonging to species A + 1.

The predominance is considered statistically different in the two species if the confidence intervals don't overlap (overlapping less than 0.05%). Table I reports the values considered for the characterization of the site.

Environmental data and landscape measures

Given the importance of having the correct coordinates of the selected sites, an accurate validation of latitude and longitude values was performed through Google Maps orthophotos.

A circular buffer of 1,000 meters radius was created around each site and the values of all predictors were referred to this geographical area. According to the type of variables, mean or median or majority values of the pixels in the buffers were calculated.

A geodatabase was designed to gather entomological, administrative (region, province and municipality boundaries) and eco-climatic layers.

The collected data were: MODIS Normalised Difference Vegetation Index (NDVI) and Enhanced Vegetation Index (EVI), MODIS Land Surface Temperature daytime (LSTd), landuse (photointerpretation of orthophoto images), orography data (elevation and derived measures).

All geographical data manipulation and analyses were performed with ArcGIS 10 (ESRI® Inc., Redlands, CA, USA) software, and raster data management was done through Idrisi Taiga software (Clark Labs, University, Worcester, MA, USA). The coordinate system used was the Universal Transverse Mercator WGS84 UTM zone 33 North.

MODIS NDVI, EVI and LSTd

Time series of MODIS/Terra images from the global 250 m 16 day Vegetation Index Product (MOD13Q1,

Table I. *95% Confidence intervals of* Beta (α_1, α_2) *distribution.* If the upper confidence limit is lower than 50% (light grey cells), the species A (e.g. *Culicoides obsoletus sensu strictu*) is not predominant, meaning that species B (e.g. *Culicoides scoticus*) is the predominant one. The site is associated to the species B. Vice-versa, when the lower confidence limit is higher than 50% (dark grey cells) the specie A is predominant. In all other cases, there is not a statistical predominant species.

Percentage of species A in the sample	Lower confidence level 95%	Upper confidence level 95%
0%	0.0%	13.3%
5%	1.2%	23.8%
10%	3.0%	30.4%
15%	5.4%	36.3%
20%	8.2%	41.9%
25%	11.3%	47.2%
30%	14.6%	52.2%
35%	18.1%	57.0%
40%	21.8%	61.6%
45%	25.7%	66.0%
50%	29.8%	70.2%
55%	34.0%	74.3%
60%	38.4%	78.2%
65%	43.0%	81.9%
70%	47.8%	85.4%
75%	52.8%	88.7%
80%	58.1%	91.8%
85%	63.7%	94.6%
90%	69.6%	97.0%
95%	76.2%	98.8%
100%	86.7%	100.0%

version 5, EVI and NDVI) and a time series on Land Surface Temperature daytime (MOD11A2, version 5, Land Surface Temperature/Emissivity 8-Day L3 Global 1 km) covering the entire Italy, were downloaded from Moderate Resolution Imaging Spectroradiometer MODIS database[1].

Data downloaded for the years 2001-2010, were pre-processed and submitted to a Fourier analysis, to extract the annual mean, the amplitude and the phase of the annual and bi-annual cycle for vegetation indices and temperatures.

The following pre-processing activities were carried out on the downloaded MODIS data using Python scripts in ESRI® ArcGIS environment: extraction of the layers of interest from the .hdf files and conversion in .img format, mosaicking of the 4 frames covering Italy, re-projection from the original Sinusoidal to WGS84 UTM zone 33 N coordinate system, subset to Italian area, conversion of original pixel values (temperature values were converted from Kelvin to degree Celsius; vegetation index values were scaled to the range -0.002 - 1).

To avoid incomplete images (noisy due to clouds or snow contamination) potentially leading to skewed results in the ecological indicators, all scenes were submitted to a Missing Data Interpolation (MDI) procedure, consisting of one or more of the following functions: linear temporal interpolation (missing pixel values are filled in interpolating the values from the previous and the following images); spatial interpolation (missing pixel values were filled in, taking the median value of first order neighbours pixels in the same image); climatology or temporal median (the missing pixel values were filled in with the median of all corresponding pixels in images dataset. For example, if the missing pixel was from the first week in January 2005, the new pixel value was the median of all pixels of the first week in January of the years 2001-2010).

The new pixel-complete series of data were analysed through the module STA (Seasonal Trend Analysis) in Idrisi Taiga software (Eastman *et al.* 2009). The temporal Fourier analysis describes the variation through time of satellite data as a series of sine curves with different frequencies, amplitudes and phases.

The mean, the first and the second harmonic of the Fourier decomposition of the series, were retained as they contain the major variation for yearly phenomena (Jakubauskas *et al.* 2001, Rogers *et al.* 1996).

Landuse

The landuse classification was done through photointerpretation of the images covering the buffer areas. The legend was designed according to the Corine Land Cover Legend - level 3 (CORINE Land Cover - EEA 2007), to guarantee a generic character of the classification scheme and the detailed level to derive landscape descriptors (Ippoliti *et al.* 2013).

A landscape analysis was conducted on the landuse map to characterize its spatial structure, and three main metrics were included in the analyses:

1. the percentage of each landcover type (class) in the 1 km radius buffer;
2. the number of patches (NumP) in each buffer, as it is an indicator of landscape fragmentation. An increase in NumP indicates increased fragmentation in the landscape with more patches of smaller size;
3. the Shape Index, a measures of the shape complexity of the patches, calculated as perimeter/[4*radq(area)] (McGarigal *et al.* 2012).

The CORINE level 3 classes were then re-aggregated into 6 land cover types, nearly corresponding to CORINE level 1: the aggregation was oriented by the interaction of human activities and vegetation (Vanhuysse *et al.* 2010).

Orography data

From Digital Elevation Model (DEM) 20 meters resolution[2], the following factors were derived: elevation (in meters), relief intensity (difference between the highest and lowest point, in meters), slope (in degrees).

Statistical analysis

The eco-climatic and topographic variables in the two groups of sites with *C. obsoletus s.s.* and *C. scoticus* as predominant species, were compared through the Mann-Whitney non-parametric test for independent samples (Siegel and Castellan Jr 1988).

Moreover, mean and 95% confidence intervals were calculated for each variable.

The low number (5 of 64, see Results section) of sites with *C. montanus* as predominant species, was only characterised through descriptive analysis.

The statistical analysis was performed using open-source statistical software R (R Development Core Team, 2009).

[1] http://e4ftl01.cr.usgs.gov/MOLT, last access on 5/12/2014.

[2] http://www.sinanet.isprambiente.it/it/sia-ispra/download-mais last access on 07/07/2015.

Results

Land-cover classification

Figure 1 shows an example of the photointerpretation in 1 km radius buffer around the site. A total of 29 CORINE level 3 classes were used to classify polygons in the buffers, and they were re-aggregated into 6 land-cover types, named urban, cultivated, semi-natural vegetation, forest, spontaneous vegetation, water.

Entomological distribution of species

Among the Italian collections, the Obsoletus complex was found to be the predominant group in 80 georeferenced sites.

According to the PCR technique and the *Beta* distribution approach (Table I), 51 sites were classified as *C. obsoletus s.s.* predominant, 8 as *C. scoticus* predominant and 3 as *C. montanus* predominant (Figure 2). No unique predominant species were found in the remanent sites (n = 18) .

Figure 2 shows that sites where *C. obsoletus s.s.* is the predominant species were widely distributed across the peninsula, while *C. scoticus* was predominant in few sites, mainly located in Central-Southern Italy. The three sites in which *C. montanus* was the predominant species were located in Southern Italy.

In two sites, namely Alghero in Sassari province and Policoro in Matera province, *C. montanus*, although not satisfying the statistical requirements established, represented the 24% and 27% of the specimens analysed. Due to the scarceness of *C. montanus*, these two sites were assigned to this species and used for the descriptive analysis.

Figure 1. *Example of orthophoto map interpretation based on CORINE Land Cover.* The figure shows a trap site (red triangle in the center) and the landuse classification in 1 km radius buffer around it (e.g.: 111: Continuous Urban Fabric; 211: Agricultural areas, non-irrigated arable land; 312: Forests).

C. obsoletus s.s. and *C. scoticus* sites characteristics

Table II shows the mean, the 95% confidence intervals, the Mann-Whitney U value and the p-value for the eco-climatic and landscape factors. Six variables (LSTd amplitude 1 and amplitude 2 and phase 1, annual mean of NDVI, annual mean of EVI, percentage of urban in the buffer) showed significant statistical differences between *C. obsoletus s.s.* and *C. scoticus* sites.

Culicoides obsoletus s.s. predominant sites had higher values of annual (LSTd - A1) and bi-annual (LSTd - A2) amplitudes (12.55°C and 1.94°C respectively) than *C. scoticus* (10.93°C and 1.50°C) ($p < 0.01$), while the mean temperature throughout the year (variable amplitude 0, not statistically significant) was similar (18.34°C mean value for *C. obsoletus s.s.* and 18.25°C for *C. scoticus*).

The mean values of vegetation indices (amplitude 0), revealed a significant higher greenness vegetation cover in *C. scoticus* sites (NDVI = 0.67, EVI = 0.40) in

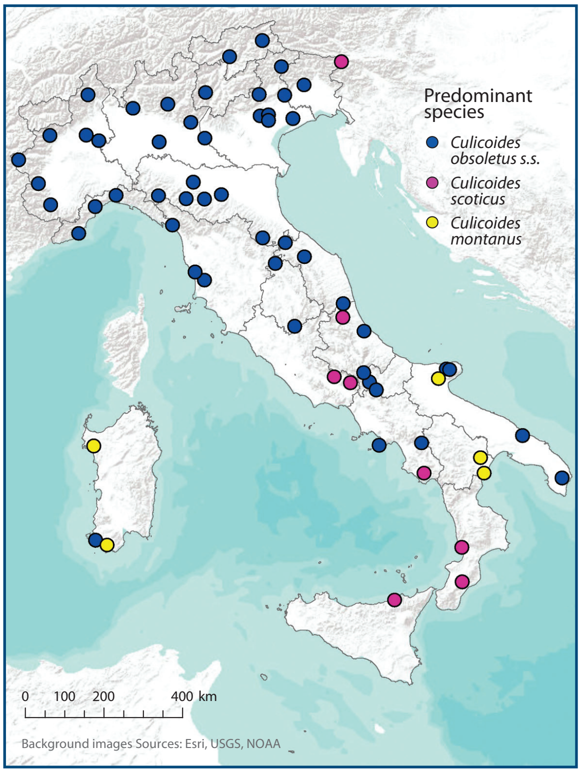

Figure 2. *Geographical distribution of 64 trap sites in Italy, characterised by the predominant species:* Culicoides obsoletus sensu strictu *(n = 51, blue points),* Culicoides scoticus *(n = 8, pink points) and* Culicoides montanus *(n = 5, yellow points).*

Table II. *Mean, relative 95% confidence interval and Mann-Whitney test for each variable in* C. obsoletus s.s. *and* C. scoticus *sites.* Six variables showed significant statistical differences between *C. obsoletus s.s.* and *C. scoticus* sites (p-value < 0.05 are highlighted with asterisks in the table).

Variables	*C. obsoletus s.s.* (n=51)			*C. scoticus* (n=8)			Mann-Whitney test	
	Mean	Lower confidence interval (95%)	Upper confidence interval (95%)	Mean	Lower confidence interval (95%)	Upper confidence interval (95%)	U	p-value
Elevation	366.87	277.851	455.886	462.75	253.892	671.611	152	0.260
Relief intensity	220.86	167.840	273.886	336.75	234.958	438.542	117.5	0.057
Slope	8.89	6.790	10.996	12.99	10.144	15.844	134	0.126
LSTd mean value A0	18.34	17.467	19.209	18.25	15.153	21.343	195	0.854
LSTd amplitude 1	**12.55**	**12.170**	**12.930**	**10.93**	**9.653**	**12.210**	**332**	**0.003****
LSTd amplitude 2	**1.94**	**1.789**	**2.087**	**1.50**	**1.312**	**1.682**	**322**	**0.007****
LSTd phase 1	**260.39**	**259.121**	**261.669**	**254.45**	**250.858**	**258.036**	**343**	**0.001****
LSTd phase 2	254.76	239.065	270.457	235.48	190.868	280.083	272	0.137
NDVI mean value A0	**0.58**	**0.556**	**0.596**	**0.67**	**0.627**	**0.709**	**62**	**0.001****
NDVI amplitude 1	0.15	0.127	0.165	0.11	0.066	0.151	280	0.095
NDVI amplitude 2	0.07	0.059	0.073	0.05	0.043	0.065	259	0.232
NDVI phase 1	221.23	205.270	237.185	203.99	136.900	271.085	221	0.720
NDVI phase 2	179.77	168.222	191.312	178.31	157.431	199.182	242	0.414
EVI mean value A0	**0.34**	**0.330**	**0.356**	**0.40**	**0.364**	**0.439**	**74**	**0.003****
EVI amplitude 1	0.13	0.120	0.148	0.11	0.087	0.138	262	0.207
EVI amplitude 2	0.05	0.044	0.055	0.05	0.043	0.058	171	0.479
EVI phase 1	240.55	224.804	256.297	228.88	169.934	287.832	181	0.625
EVI phase 2	167.52	155.267	179.770	154.74	122.815	186.661	249	0.331
Urban	**0.15**	**0.103**	**0.202**	**0.04**	**0.011**	**0.075**	**299**	**0.036***
Cultivated	0.50	0.406	0.585	0.40	0.166	0.631	241	0.418
Semi-natural vegetation	0.10	0.063	0.128	0.16	0.078	0.239	118	0.057
Forest	0.22	0.156	0.291	0.31	0.112	0.512	146	0.202
Spontaneous vegetation	0.02	0.000	0.043	0.07	0.000	0.228	180	0.484
Water	0.01	0.007	0.020	0.02	0.002	0.034	164.5	0.357
Num_poly	17.78	16.116	19.452	20.00	11.551	28.449	198	0.946
Shape_Index	1.54	1.499	1.572	1.58	1.472	1.684	193	0.820

*p < 0.05; **p < 0.01.

comparison to *C. obsoletus s.s.* sites (mean NDVI = 0.57, mean EVI = 0.34).

The sixth significant variable was urban class of landuse classification: anthropic areas represented the 15% of the *C. obsoletus s.s.* sites, while it was the 4% of the *C. scoticus* sites (p < 0.05).

C. montanus sites characteristics

Table III shows the descriptive statistics of the eco-climatic factors in 1-km radius buffers of *C. montanus* sites (n = 5): minimum, maximum, mean values, the 95% confidence intervals, the median and the coefficient of variation.

The sites are located in Southern Italy (Sardinia island, Basilicata and Puglia regions): the latitude ranges between 39° and 41°40' North.

The sites are homogeneous from the climatic point of view, considering that the mean daily temperature (LSTd), for the period 2001-2010 varied between 23.6°C and 25.3°C. The temperatures were apparently higher compared to *C. obsoletus s.s.* (18.3°C; 95% c.i. 17.4-19.1°C) and to *C. scoticus* (18.2°C; 95% c.i. 15.1-21.3°C) predominant sites.

Concerning the vegetation indices, the *C. montanus* sites recorded a value of 0.52 for NDVI and 0.32 for EVI.

Discussion

Among the *Culicoides* species associated to livestock in Italy, *C. obsoletus s.s.* and *C. scoticus* are both widespread across the whole Italian peninsula, although the former is relatively more abundant (Goffredo *et al.* 2013, Goffredo *et al.* 2016). The two species share the same breeding habitats, mainly

Table III. *Summary statistics and 95% confidence interval of the mean, for each variable in* C. montanus *sites (5 trap sites): minimum, maximum, mean values, the 95% confidence intervals of the mean, the median and the coefficient of variation of the variables, calculated in 1-km radius buffer.*

Variable	Mean	C.I. (95%)	Minimum	Median	Maximum	CV (standard deviation/ mean)
Elevation	110.88	0 - 298.99	9.93	50.02	376.39	136.6%
Relief intensity	124.80	0 - 259.81	18.00	102.00	280.00	87.1%
Slope	4.21	0 - 8.80	0	1.13	17.35	175.2%
LSTd mean value A0	24.10	23.24 - 24.99	23.57	23.88	25.28	2.9%
LSTd amplitude 1	13.86	12.52 - 15.19	12.43	13.76	15.19	7.8%
LSTd amplitude 2	1.61	1.22 - 1.99	1.22	1.74	1.92	19.3%
LSTd phase 1	253.76	250.48 - 257.05	250.49	254.53	256.33	1.0%
LSTd phase 2	145.63	68.13 - 223.13	82.87	132.36	243.99	42.9%
NDVI mean value A0	0.52	0.45 - 0.58	0.46	0.51	0.60	10.0%
NDVI amplitude 1	0.13	0.07 - 0.18	0.06	0.14	0.17	37.0%
NDVI amplitude 2	0.05	0.03 - 0.07	0.03	0.05	0.06	25.8%
NDVI phase 1	83.79	28.73 - 138.72	47.12	59.77	146.04	52.9%
NDVI phase 2	205.76	177.42 - 234.10	186.84	192.76	240.84	11.1%
EVI mean value A0	0.32	0.28 - 0.37	0.28	0.33	0.37	10.7%
EVI amplitude 1	0.09	0.05 - 0.13	0.05	0.10	0.13	33.7%
EVI amplitude 2	0.04	0.03 - 0.05	0.03	0.04	0.05	24.3%
EVI phase 1	118.27	10.51 - 226.04	44.43	72.91	213.86	73.4%
EVI phase 2	215.75	180.5 - 250.99	185.19	214.35	251.55	13.2%
Urban	0.071	0 - 0.17	0.01	0.03	0.19	108.0%
Cultivated	0.48	0.24 - 0.73	0.27	0.52	0.68	40.8%
Semi-natural vegetation	0.16	0 - 0.38	0	0.09	0.47	116.4%
Forest	0.18	0 - 0.43	0	0.11	0.44	107.8%
Spontaneous vegetation	0.07	0 - 0.19	0	0.03	0.24	139.1%
Water	0.04	0 - 0.10	0	0	0.10	138.8%
Num_poly	15.4	7.37 - 23.43	6	17	22	42.0%
Shape_Index	1.52	1.30 - 1.73	1.28	1.48	1.71	11.4%

linked to vegetation and leaf litter (Harrup *et al.* 2013, Ramilo *et al.* 2012).

To identify eco-climatic indicators which can be associated to the 'biological success' of each species of the Obsoletus complex, we selected collection sites where a single species resulted statistically predominant.

Culicoides obsoletus s.s. was the most successful species in many eco-climatic conditions, in rural-agricultural environments, apparently tolerating anthropogenic interference, at a wide range of temperature and altitudes. *Culicoides scoticus* was found to be significantly predominant in few sites, characterized by medium higher altitudes, more natural and greener landscape with less human influence. It is important to stress that the low number of predominant *C. scoticus* sites and the difference in the cardinality of the two groups (n = 51 of *C. obsoletus s.s.* compared to n = 8 of *C. scoticus*) might have influenced the significance of the comparisons.

Only six of the 26 variables investigated resulted statistically different between the two species; however, they allowed to broadly depict the macrohabitat of the two groups. Even if not statistically different, the rest of the variables concurred and encouraged to depict more greener and spontaneous vegetation for *C. scoticus* sites compared to *C. obsoletus s.s.* ones, at higher altitudes and steeper terrains.

According to its wider distribution across many eco-climatic zones, *C. obsoletus s.s.* seems to tolerate greater variations in temperatures during the year: the annual amplitude measures the annual excursion of temperature, while bi-annual amplitudes is a measure of the strength of the first spring or autumn warm days. Both variables registered higher values in *C. obsoletus s.s.* sites.

The phase of the first harmonic measures the offset of a sinusoidal peak from the origin, i.e. it indicates the day of the year in which temperatures re-start the annual cycle. The annual cycle starts earlier for

C. scoticus, according to its general higher altitudes (factor not statistically significant).

Concerning vegetation indices, both NDVI and EVI resulted statistically significant in amplitude 0, i.e. mean value of the whole series: *C. scoticus* predominant sites resulted with higher greenness vegetation cover along the year in comparison to *C. obsoletus s.s.*. Greener areas along the year are related to the presence of green vegetation and dense canopy (Moody and Johnson 2001).

The photointerpretation of high resolution orthophotos around the sites of midge collection, allowed to produce a very detailed landcover-landuse map in their surroundings. Although most of the landuse classes in the buffers resulted not statistically significant between the two species, their percentage distribution contributed to identify a wilder and more pristine environment for *C. scoticus*.

Among the landscape metrics on landuse/landcover, only the urban proportion of continuous or discontinuous urbanised areas in the buffers resulted statistically significant: anthropic areas represent the 15% of the *C. obsoletus s.s.* sites, while it resulted to cover the 4% of the *C. scoticus* sites. It is important to notice that 'urban' class refers mainly to discontinuous urban fabric in the buffer (data not shown). Discontinuous areas are defined when most of the land is covered by structures (buildings, houses, barns, stables, roads and artificially surfaced areas) associated with vegetated areas and bare soils[3]. This landcover class well represents the Italian landscape around farms, where patchy areas are composed of house of the owner, animal stables, pastures, grazing areas.

Although related to disease spread and not to vector presence, Faes and colleagues (Faes *et al.* 2013) found that, along with wind and precipitations, land cover is another important factor affecting the incidence of BTV8 in Northern Europe; high-risk areas for infection being characterized by a fragmentation of the land cover, especially the combination of forests and urban areas.

Orography variables although resulted not statistically significant, agreed in the general result of *C. scoticus* to be found at higher altitudes, higher average relief intensity, i.e. more abrupt terrains, compared to *C. obsoletus s.s.* species.

As concerning *C. montanus* species, it is rare in Italian territory and little is known about its range ecology worldwide ['Scientific Opinion of the Panel on Animal Health and Welfare on a request from the European Commission (DG SANCO) on Bluetongue', 2008]. The species was captured as predominant species in only 3 sites (out of 13 sites in which it was present). The common factor among the sites was an high land surface temperature, higher than the values registered in the *C. obsoletus s.s.* and *C. scoticus* sites.

These findings may serve as a springboard for further studies on the microhabitat drivers that underpin the 'biological success' of *C. obsoletus s.s.* and *C. scoticus* within forested habitats, i.e. further studies on breeding and larval sites are required to fully understand the niche preferences of the two species.

An effort should than follow to translate the gained ground-based knowledge into indices derived from remote sensed databases, either including other landscape indices and/or other satellite sensors, comprising the newly available European ESA Earth Observation Missions products.

[3] CORINE nomenclature at http://www.eea.europa.eu/publications/technical_report_2007_17.

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