Habitat and occurrence of ixodid ticks in Liguria region, northwest Italy

DOI:10.1007/s10493-014-9794-y

(Article begins on next page)

UNIVERSITÀ DEGLI STUDI DI TORINO

The final publication is available at Springer via http://dx.doi.org/DOI 10.1007/s10493-014-9794-y; [Erratum] DOI 10.1007/s10493-014-9818-7

Habitat and occurrence of ixodid ticks in Liguria region, northwest Italy.

Experimental and Applied Acarology, published on-line March 2014; DOI 10.1007/s10493-014-9794-y.
Erratum, published on-line May 2014; DOI 10.1007/s10493-014-9818-7.

Leonardo A. Ceballos[1], Maria D. Pintore[2], Laura Tomassone[1*], Alessandra Pautasso[2], Donal Bisanzio[3], Walter Mignone[2], Cristina Casalone[2] and Alessandro Mannelli[1].

[1] Department of Veterinary Sciencies, University of Turin, Italy.
[2] Experimental Zooprophylactic Institute of Piedmont, Liguria and Aosta Valley, Turin, Italy.
[3] Department of Environmental Studies, Emory University, Atlanta, Georgia, USA.
* Corresponding Author: Laura Tomassone. Dipartimento di Scienze Veterinarie Università degli Studi di Torino via L. da Vinci 44, 10095 Grugliasco (Torino), Italy. Tel. +39- 011-6709195, Fax +39-011-6709196, E-mail: laura.tomassone@unito.it

Keywords: Questing ticks, *Ixodes ricinus*, habitat, Liguria, Italy.

Running title: Ixodid ticks in northwest Italy

Abstract

Questing ticks were collected during monthly dragging sessions (March-August 2011) in three provinces of Liguria region, north-western Italy, to evaluate the species occurrence, spatial distribution and relative abundance. A total of 1464 specimens were collected in 94 dragging sites. *Ixodes ricinus* was the most abundant species (81.3% of collected ticks), followed by *Haemaphysalis punctata* (10.9%), *Dermacentor marginatus* (5.5%), *Ixodes frontalis* (1.3%), and *Rhipicephalus* spp. (0.9%). *I. frontalis* is reported for the first time in Liguria. An aggregation of *I. ricinus* positive sites was observed in inland areas characterized by dense forests dominated by deciduous trees (*Castanetum* and *Fagetum* phytoclimatic zones), especially in the west of the region where the differences in the Normalized DifferenceVegetation Index (NDVI) were higher between inland and coastal sites. Negative binomial regression for repeated measures was used to model the associations of NDVI and season with counts of host-seeking *I. ricinus* nymphs. The NDVI was a good predictor of *I. ricinus* nymphs abundance, and confirmed its utility in discriminating habitat suitability for this vector in north-western coastal Italy, where dry habitat conditions may limit the distribution of this species.

Introduction

Hard ticks (*Ixodidae*) are vectors and reservoir of zoonotic diseases worldwide. In the last few decades, an increased number of human cases of tick-borne zoonoses (TBZ) and a change in their geographical range were observed in Europe (Vorou et al. 2007). This was accompanied by a latitudinal and altitudinal shift in the distribution of ticks, particularly of *Ixodes ricinus*, the most important tick vector in Europe (Medlock et al. 2013).

TBZ infection risk is generally associated with open-air recreational and occupational activities, since ticks mainly inhabit sylvatic habitats (Randolph et al. 2010; Rizzoli et al. 2011). Different tick species are associated with particular ecological habitats, where they find appropriate environmental factors (i.e., microclimate, vegetation) and suitable vertebrate hosts (Gray et al. 2009; Jaenson et al. 2009). Habitat characteristics strongly influence the survival, distribution, abundance, and seasonal activity pattern of ticks (Estrada-Peña et al. 2004, 2013a; Jaenson et al. 2009). Most tick species are very susceptible to desiccation, and need humid microhabitats to periodically take up atmospheric water (Daniel and Dusbábek 1994). In particular, dry habitat conditions may limit the distribution of hygrophilic and exophilic species such as *I. ricinus* (Estrada-Peña et al. 2004; Tagliapietra et al. 2011). Lower densities of this species are observed in the Mediterranean zone, where it is too warm and dry except at medium-high altitude (Beugnet and Marié 2009). Accordingly, on the Mediterranean coast of Tuscany, north-western Italy, relatively moist, deciduous woods were more favourable for immature *I. ricinus* than dry pine woods (*Pinus pinaster*, *P. pinea*) (Mannelli et al. 1999). In a limited district of Genoa province (Liguria region, Italy), Mannelli et al. (2003) highlighted a greater probability of finding host-seeking *I. ricinus* larvae in inland than in coastal sites. Such difference might be explained by limited dry condition which can be found in coastal site habitats.

Lyme borreliosis is considered endemic in Liguria, where the first Italian case was reported (Crovato et al. 1985). Cimmino et al. (1992) calculated an incidence of 17 cases per 100,000 inhabitants per year in this region, and since 1983 (first record) to 1996, 230 cases were registered (Ciceroni and Ciarrocchi 1998). In Savona province Manfredi et al. (1999) estimated about 500 tick bites per 100,000 residents per year; biting ticks were mostly *I. ricinus*, followed by *Rhipicephalus sanguineus* and *Dermacentor marginatus*. Despite of this, the knowledge on tick distribution and ecology in the region is limited and fragmentary. The understanding of the environmental conditions allowing the maintenance and distribution of tick populations is important to evaluate the acarological risk for humans.

On the basis of the previous research in Genoa province, in this work we studied more in depth and on a wider geographic scale the ixodid tick fauna of Liguria. In particular, we analyzed the environmental factors that can limit the distribution and abundance of the free-living ticks in three provinces. The mountainous morphology, vegetation and climate, make this coastal region suitable to evaluate the effect of different habitats typologies on tick populations. A special reference is made to *I. ricinus*, to gain new insights into the population dynamics of this vector in southern Europe.

Materials and methods

Study area

The study was carried out in the provinces of Imperia, Genoa and La Spezia, Liguria region, north-western Italy (44.27° N, 8.48° E). Liguria has a mild Mediterranean climate all year-round. The rainfall quantities follow a growing trend progressing from the west to the east and from the coast to the inland areas, although the latter is less regular due to the action exerted by the complex orography on the rain-producing air masses (Pedemonte 2004). Due to the orographic effect, the humidity steadily increases in the inland, leading to temperate forest vegetation. The inland is characterized by the presence of small scattered villages which are embedded in a matrix of wooded areas with different degrees of conservation. At the sea coast, land morphology is characterized by a hilly territory where the habitat is relatively dry and vegetation is typically Mediterranean, with densely-populated settlements at the rivers' outlets to the sea.

Wild ungulates, which are important maintenance hosts for tick populations, are widespread in the region inland, especially wild boars (*Sus scrofa*) and roe deer (*Capreolus capreolus*). The roe deer, more abundant in the west, has expanded in eastern Liguria in the recent years thanks to conservational and restocking programs carried out in the east part of Genoa province. Moreover, the Maritime Alps in the Imperia province represent the south-west limit of the Alpine chamois (*Rupicapra rupicapra*) home range. Finally, the fallow deer (*Dama dama*) is present in Genoa, in isolated but consistent groups (Carnevali et al. 2009; Raganella-Pelliccioni et al. 2013).

Sampling strategy

In each province, tick collection sites were selected along a route starting close to the coast towards the inland, reaching the Northern Apennines (Genoa and La Spezia provinces) and the Maritime Alps (Imperia province). Distance of sites from the sea was comprised between 1 and 23 km, and altitude between 37 and 1100 m above sea level (a.s.l).

A convenience sampling was performed with the objective of covering an extensive area, so we chose easily accessible sites along the selected route. All sites were located on hills covered by deciduous woods, with the exception of a few sites with olive cultivations, or Mediterranean shrubs and pine wood in the proximity of sea.

At each site, host-seeking ticks were collected by one operator by dragging a 1 m^2 cotton cloth over the ground vegetation on 100 m transects. Collected ticks at each site were pooled together and maintained alive in humidified vials (nymphs and adults) and then frozen at -20°C, or preserved in 70% ethanol (larvae), for later molecular studies on tick-borne pathogens. Ticks were subsequently identified in our laboratories by using keys from Manilla (1998) and Cringoli et al. (2005). Environmental data (weather conditions, temperature and humidity) were registered. Each sampling site was characterized according to the habitat (forest, meadows, scrublands, cropland), the percentage of vegetation cover of the principal plant species (according to Braun-Blanquet 1979), the phenology of dominant and minor plant species and the presence of leafs on the ground. Tick collection was monthly performed from March to August 2011, excluding July due to adverse weather conditions.

Statistical analysis

Since almost all dragging sites were wooded areas, we compared the habitat typologies by grouping sites in phytoclimatic zones (climatic areas of botanical importance), according to the classification proposed by Pavari (1916). These categories represent the geographical distribution of a representative plant association composed by homogenous (and spontaneous) species. Each plant association is characterized by peculiar climatic requirements that occur consistently in that specific area.

To classify the sampling sites into the three phytoclimatic categories present in the study area (*Lauretum*, *Castanetum* and *Fagetum*), we considered the dominant tree species (Online Resource 1), the distance to the sea and altitude.

Numbers of ticks per 100 m^2 and percentage of positive sites were calculated per province, dragging session, and phytoclimatic zones.

In order to perform a spatial analysis of tick distribution, the Cartesian coordinates (UTM, Zone 32T, datum WGS84) were taken with a GPS device for each dragging location. Given the arbitrary distribution of collection sites, we performed an explorative global spatial analysis to determine the presence of spatial clustering of positive sites. The analysis was carried out for each tick species, including the three provinces and the five collection sessions. We used a binary join-count statistics (Cliff and Ord 1981) by comparing the observed data with Monte Carlo envelopes from multiple simulations of the null model. We tested whether positive sites joins occurred more frequently than would be expected if the sites were labeled in a spatially random way; values of $p < 0.05$ were considered significant. The analyses were performed using the *spdep* package (Bivand et al. 2013) in the software R (R Core Team 2013).

A negative binomial regression with robust standard errors was used to obtain estimates of mean numbers of host-seeking immature *I. ricinus* per transect, with 95% confidence intervals (95%CI). Negative binomial error was used in order to take into account the potential overdispersion of the distribution of host-seeking ticks among dragging sites. We applied intercept-only, generalized log-linear models using the GENMOD procedure in the SAS® system (Exp option in the ESTIMATE statement, SAS version 9.2) (Littell et al. 2002).

A particular attention was paid to *I. ricinus* nymphs, since this is the most likely stage for the transmission of tick-borne pathogens to people. We used the Normalized Difference Vegetation Index (NDVI) as a predictor of *I. ricinus* nymphs. This parameter was extensively used as indicator of the habitat suitability for hygrophilic ticks because it is a measure of the photosynthetic activity of the vegetation, and therefore of the environmental humidity (e.g., Kitron 1998, Bisanzio et al. 2008, Estrada-Peña 1999, 2006, 2013b). The NDVI was calculated on 16-day averages from MODIS satellite survey (250 m pixel size; NASA MODIS Website: http://modis.gsfc.nasa.gov/). For our analyses, we used images that were taken during the second week of July and August 2011, which corresponded to the peak of vegetation cover in summer, when differences in moisture between habitats were most likely to be detected by NDVI. Elevation data were obtained from the Shuttle Radar Topography Mission dataset (90 m pixel size; NASA SRTM Website: http://www2.jpl.nasa.gov/srtm/). All geographic data were merged in a Geographic Information System based on QGIS system and projected from geographic to planar units (UTM zone 32T, datum WGS84).

To compare the NDVI of different phytoclimatic zones and of positive sites for different tick species, we applied a non-parametric Kruskal-Wallis test followed by multiple comparison test using the *pgirmess* package (Giraudoux 2013) in the software R; values of $p < 0.05$ were considered significant.

The adjusted effects of NDVI and season on the odds of collecting at least one *I. ricinus* nymph on a 100 m transect was estimated by means of a random-effect logistic regression. A binary outcome, taking value 1 if one or more nymphs were collected, 0 otherwise, was used in the GLIMMIX procedure (likelihood approximation by Gauss-Hermite quadrature) in the SAS® system. Such an approach allowed accounting for correlation arising from repeatedly collecting ticks at the same sites across the study period (Molenberghs and Verbeke 2005). The seasonal pattern of nymphs was modeled through a sinusoidal fluctuation, with amplitude of 1 and period of 1 yr; the peak was chosen based upon exploratory analysis and the models residuals (Mannelli et al. 2003). To check for linearity, in the logit scale, of the effect of NDVI×10 on the outcome, observations were divided into four groups by using quartiles of NDVI×10 as break points. The effect of such grouping on the outcome was estimated in a logistic regression model, by using the first quartile as the reference group. Model checking was accomplished by goodness-of-fit statistics (Pearson's χ^2) and by plotting Pearson's residuals against sampling sessions (Littell et al. 2002).

Results

Tick collection

Dragging was performed in 94 sites, with a total capture effort of 447 transects. The 76% of dragging sites was positive for at least one questing tick (n= 71; 23/31 in La Spezia, 16/20 in Genoa and 32/43 in Imperia)

We collected 1464 host-seeking ticks: 1232 larvae, 192 nymphs, and 40 adults. Most of the arthropods were collected in La Spezia province (n= 774, mean no. of ticks per 100 m^2= 5.1, SD= 15.1), followed by Genoa (n= 413, 4.4 ticks/100 m^2, SD= 14.8) and Imperia (n= 277, 1.4 ticks/100 m^2, SD= 7.4). The vast majority were *I. ricinus* (81% of collected ticks, n= 1190), followed by *Haemaphysalis punctata* (n= 161), *Dermacentor marginatus* (n= 80), *Ixodes frontalis* (n= 19), and *Rhipicephalus* spp. (n= 14). *I. ricinus* were 96% of the collected ticks in Genoa and 93% in La Spezia. In Imperia, *I. ricinus* were less collected than *H. punctata* (27% vs. 56%). *Rhipicephalus* spp. were only collected in Imperia (25.6% of sites), the unique province where species of all five genera were found (Table 1, Online Resource 2).

Table 1 Questing ticks collected by dragging, from March to August 2011, in the provinces of La Spezia, Genoa and Imperia (Liguria)

Province (total no. transects[a])	La Spezia (153)			Genoa (94)			Imperia (200)		
	Ticks	Positive sites		Ticks	Positive sites		Ticks	Positive sites	
Species	collected	%	95%CI	collected	%	95%CI	collected	%	95%CI
I. ricinus	719	64.5	45.4-80.8	395	75	50.9-91.3	76	23.3	11.8-38.6
I. frontalis	7	9.7	2.0-25.8	3	15	3.2-37.9	9	14	5.3-27.9
D. marginatus	43	32.3	16.7-51.4	15	25	8.7-49.1	22	18.6	8.4-33.4
H. punctata	5	12.9	3.6-29.8	0	0	0-16.8	156	20.9	10.0-36.0
Rhipicephalus spp	0	0	0-11.2	0	0	0-16.8	14	25.6	13.5-41.2
Total	774	74.2	55.4-88.1	413	80	56.3-94.3	277	74.4	58.8-86.5

[a]: some sites were not evaluated in each field trip, due to bad weather or logistical impediments.

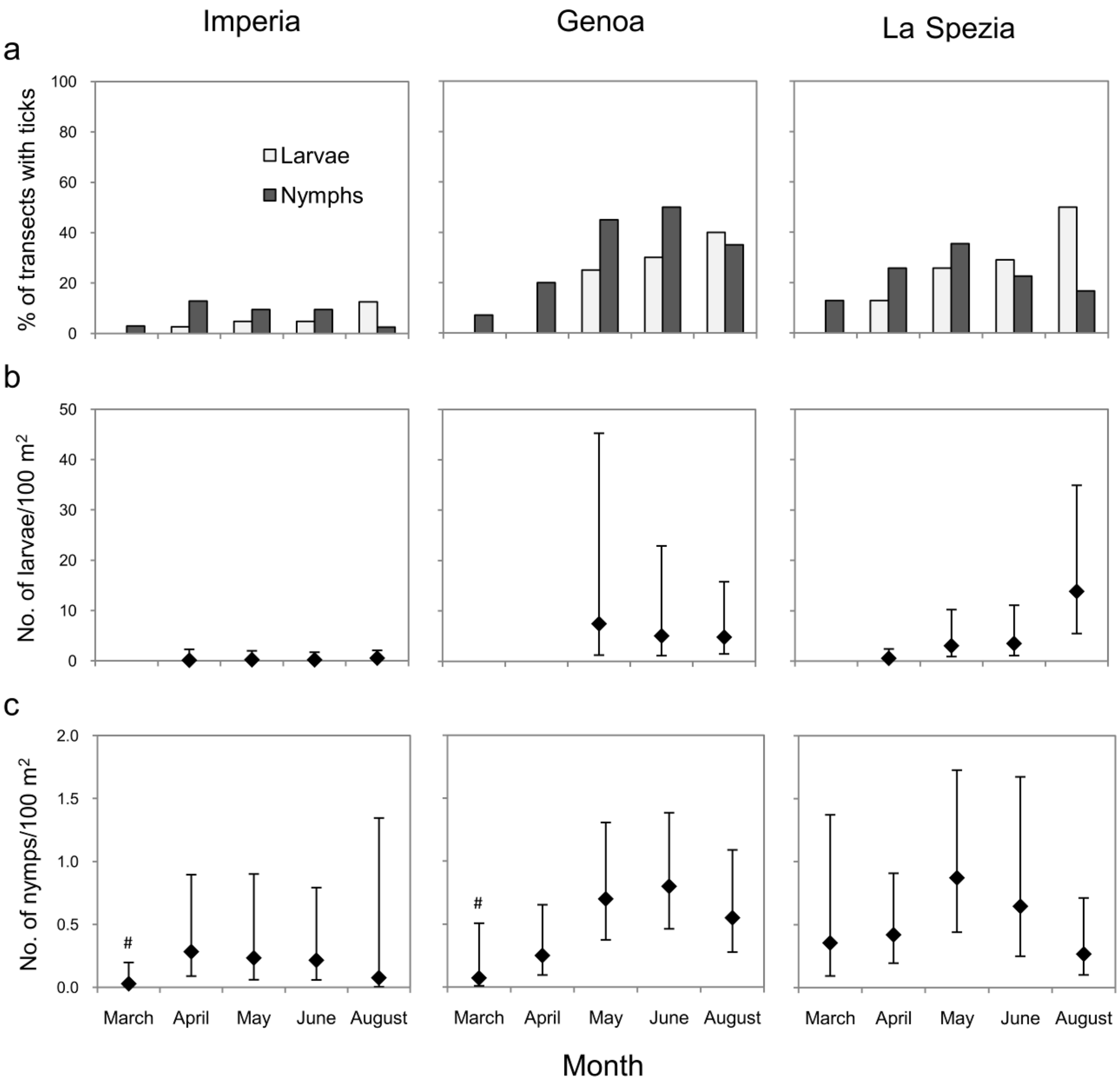
As regards the tick developmental stage, the majority of collected *I. ricinus, D. marginatus* and *H. punctata* were larvae (85.9, 81.3 and 88.8%, respectively), while 84.2% (16/19) of *I. frontalis* were nymphs. Only adults of *Rhipicephalus* spp. were collected.
Considering the number of sites which were found positive in at least one dragging session, we observed a difference between provinces (Table 1). In general, 75% of visited sites were positive at least once for one of the tick species. Overall, the prevalence of positive sites for *I. ricinus* was significantly different amongst provinces, being higher in La Spezia and Genoa compared to Imperia (respectively: 64.5, 75.0 and 23.3%; Fisher Exact test, $p< 0.001$). On the contrary, no significant difference among provinces was registered for *D. marginatus* (respectively: 32.3, 25.0 and 18.6%; $p= 0.388$), *I. frontalis* (9.7, 15.0 and 14.0%; $p= 0.851$), and between La Spezia and Imperia for *H. punctata* (12.9 and 20.9%; $p= 0.538$). In Imperia the percentage of positive sites did not differ significantly for the species of the five tick genera ($p= 0.727$), while in the other provinces *I. ricinus* positive sites were more frequent than positive sites of other species (Table 1).
Spatial clustering of *I. ricinus* positive sites, as evaluated by join-counts, was statistically significant.
As regards the tick distribution according to the distance from the sea, we collected *I. ricinus* throughout the studied area in Genoa, where the captures were dispersed from the sites nearest to farthest from the sea (3.6-19.9 km). Instead, in the other two study areas, *I. ricinus* were mostly constrained to inland sites, with more than 98% of the total collection at a distance greater than 10 km from the sea, up to 22.9 Km. Likewise, a significantly spatial clustering of *H. punctata* and *Rhipicephalus* spp. positive sites was detected. *H. punctata* was mostly gathered in the inland (95.3% of collected ticks at a distance greater than 14 km from the sea); only 8 specimens from Imperia were found at less than 6 km from the coast. Thirteen of 14 collected *Rhipicephalus* spp. were found within 6 km of the sea. In contrast, *D. marginatus* was sparse in all three provinces (2.3-19 km), and the few specimens of *I. frontalis* were collected throughout the studied areas. These latter species showed no spatial clustering (Online Resource 2).
As far as the tick altitudinal distribution is concerned, we mostly observed *I. ricinus* in sites above 600 m a.s.l. in La Spezia and Imperia (respectively 61.1% and 97.9% of collected specimens, relative to the capture effort for each altitudinal range of 300m). On the contrary, 84.3% of *I. ricinus* collected in Genoa were found below 600 m. This tick species was found up to 1070 m a.s.l. *D. marginatus* was generally collected below 600 m (84.1%), and never above 810 m. *H. punctata* was mostly found above 900 m in Imperia (84.7% of collected ticks); the five specimens from La Spezia were found in sites between 570 and 870 m. *Rhipicephalus* spp. were only present below 600 m in Imperia. *I. frontalis* was fairly homogenously distributed, from 50 to 1000 m a.s.l.

Relative abundance and seasonality of immature I. ricinus

The seasonal distribution was analyzed for immature *I. ricinus* only, since the numbers of collected ticks were too small for the adults (3 males and 3 females in La Spezia, and 2 males in Genoa) and for the other species. The mean numbers of all collected *I. ricinus* larvae were similar for La Spezia (4.11 larvae/100 m^2, 95%CI= 2.32-7.30) and Genoa (3.73 larvae/100 m^2, 95%CI= 1.82-7.64), but more than eighteen times smaller in Imperia (0.20 nymphs/100 m^2, 95%CI= 0.08-0.55). *I. ricinus* larvae were collected starting from April in La Spezia and Imperia, and from May in Genoa; the percentage of positive dragging sites gradually increased to reach its maximum in August in the three provinces (Fig. 1a). In La Spezia, larvae showed a peak of activity in August (13.83 larvae/100 m^2, 95%CI= 5.48-34.9) when 50% of the sites were infested. In Genoa the mean number of larvae was more stable over time (range: 4.80-7.45 larvae/100 m^2), but with greater variability between sites. In Imperia both the proportion of larvae-positive sites (max.: 13%) and the mean number of larvae (range: 0.10-0.53 larvae/100 m^2) were lower than in the other two provinces (Fig. 1a,b). The mean numbers of all collected *I. ricinus* nymphs were similar for La Spezia (0.50 nymphs/100 m^2, 95%CI= 0.28-0.90) and Genoa (0.51 nymphs/100 m^2, 95%CI= 0.31-0.84), but more than four times smaller in Imperia (0.16 nymphs/100 m^2, 95%CI= 0.04-0.60). Nymphs were captured during all dragging sessions in the three provinces and a peak in the proportion of nymphs-positive sites was observed in mid-spring and early summer (May and June) in La Spezia and Genoa, and in April in Imperia (Fig. 1a). The mean numbers of nymphs were slightly variable, but once again lower values were observed in Imperia (maximum mean number of nymphs/100 m^2 [95%CI] per province: La Spezia= 0.87 [0.44-1.73], Genoa= 0.80 [0.46-1.38], Imperia= 0.28 [0.09-0.89]) (Fig. 1c). *I. ricinus* adults were collected only sporadically, in 7 out of 94 dragging sites. Overall, the prevalence of positive sites was significantly different among provinces, both for *I. ricinus* larvae (Fisher Exact tests, $p< 0.001$) and nymphs ($p= 0.003$). In both cases the prevalence was higher in La Spezia and Genoa compared to Imperia (respectively: 51.6, 60.0 and 14.0% for larvae, and 45.2, 60.0 and 18.6 % for nymphs).

We compared our results to the findings in Genoa province in 1998-9 (high Ligurian Sturla Valley, Municipalities of Borzonasca and Chiavari; Mannelli et al. 2003), considering the same period of the year (March-August) and only sites within 10 km from the old study area (n= 11). The mean number of questing *I. ricinus* nymphs showed a decrease (0.4 [CI= 0.2-0.8] *vs.* 1.2 [CI= 0.7-2.0] nymphs/100m^2). On the contrary, the mean number of larvae increased almost fivefold (3.8 [CI= 1.7-8.7] *vs.* 0.8 [CI= 0.4-1.5] larvae/100m^2).

Fig. 1 Percentages of capture sites with at least one questing immature *I. ricinus* (a) and mean numbers and 95%CIs of *I. ricinus* larvae (b) and nymphs (c) collected by dragging, from March to August 2011, in the provinces of Imperia , Genoa and La Spezia (Liguria). # : mean and 95%CI were estimated with a Poisson distribution, since the regression model did not fit with a negative binomial distribution.



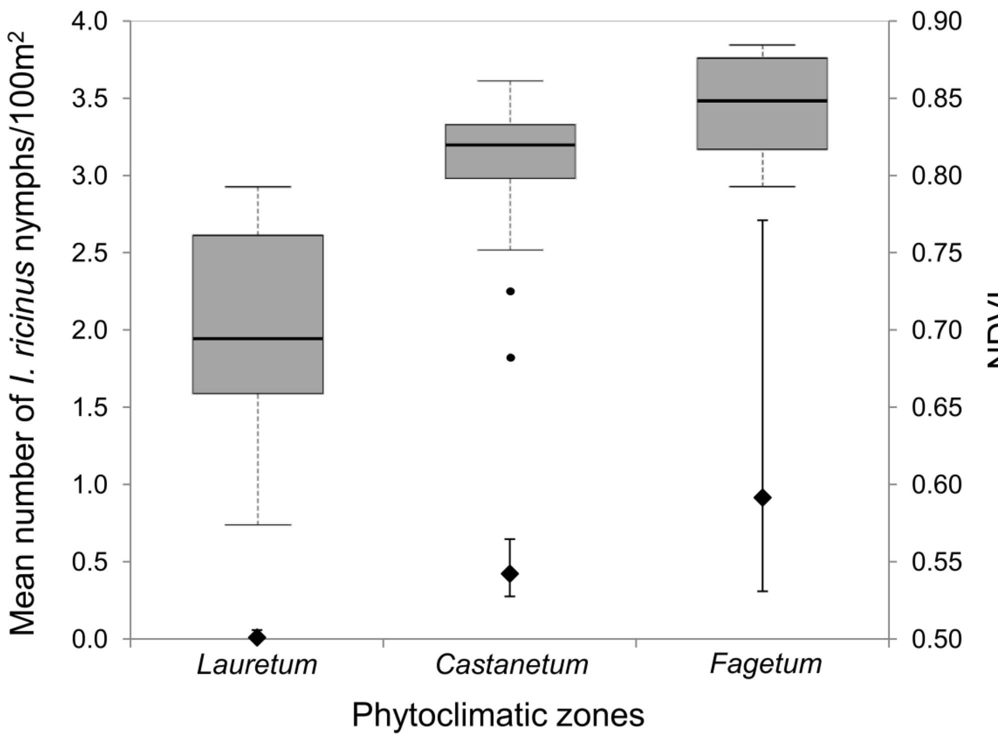
Relationship between immature I. ricinus *abundance and vegetation*

In our study areas, the dominant species of trees varied continuously and gradually with the overlapping of typical species of each phytoclimatic zone at their boundaries.

In general, a xerophilic vegetation was more present in sites close to the sea, especially at the southern hill side, with typical Mediterranean plants (mainly tree heath, *Erica arborea*, and holly oak, *Quercus ilex*), olive cultivations (*Olea europaea*), and maritime pine (*Pinus pinaster*), typical of *Lauretum*. While moving away from the sea, the vegetation was gradually more represented by the mesophilic species of deciduous trees, especially at the northern hill side. The dominant species in this zone, *Castanetum*, were the chestnuts (*Castanea sativa*), oaks (mainly *Quercus pubescens* in the west, and *Quercus cerris* in the east), hop hornbeam (*Ostrya carpinifolia*), and the exotic black

locust (*Robinia pseudoacacia*). In the inland (i.e. >10 km of the sea), the forests were dominated by the deciduous trees listed above, with a greater presence of other mesophilic species as the manna ash (*Fraxinus ornus*), and the hazelnut (*Corylus avellana*), which increased their presence with altitude. Finally, above 800 m a.s.l., we found the beech (*Fagus sylvatica*), a species characterizing the *Fagetum* (Online Resource 1).
As regards the classification of sites by phytoclimatic zones, the mean numbers of larvae were greater in the sites classified as *Castanetum*, followed by *Fagetum* and *Lauretum* (respectively, 3.18 [95%CI= 1.95-5.18], 2.51[95%CI= 1.02-6.22], and 0.01 larvae/100m^2 [95%CI= 0.00-0.06]). The mean number of *I. ricinus* nymphs was greater in the *Fagetum*, followed by *Castanetum* and *Lauretum* (respectively, 0.91 [95%CI= 0.31-2.71], 0.42[95%CI= 0.28-0.65], and 0.01 nymphs /100m^2 [95%CI= 0.00-0.06]) (Fig. 2).

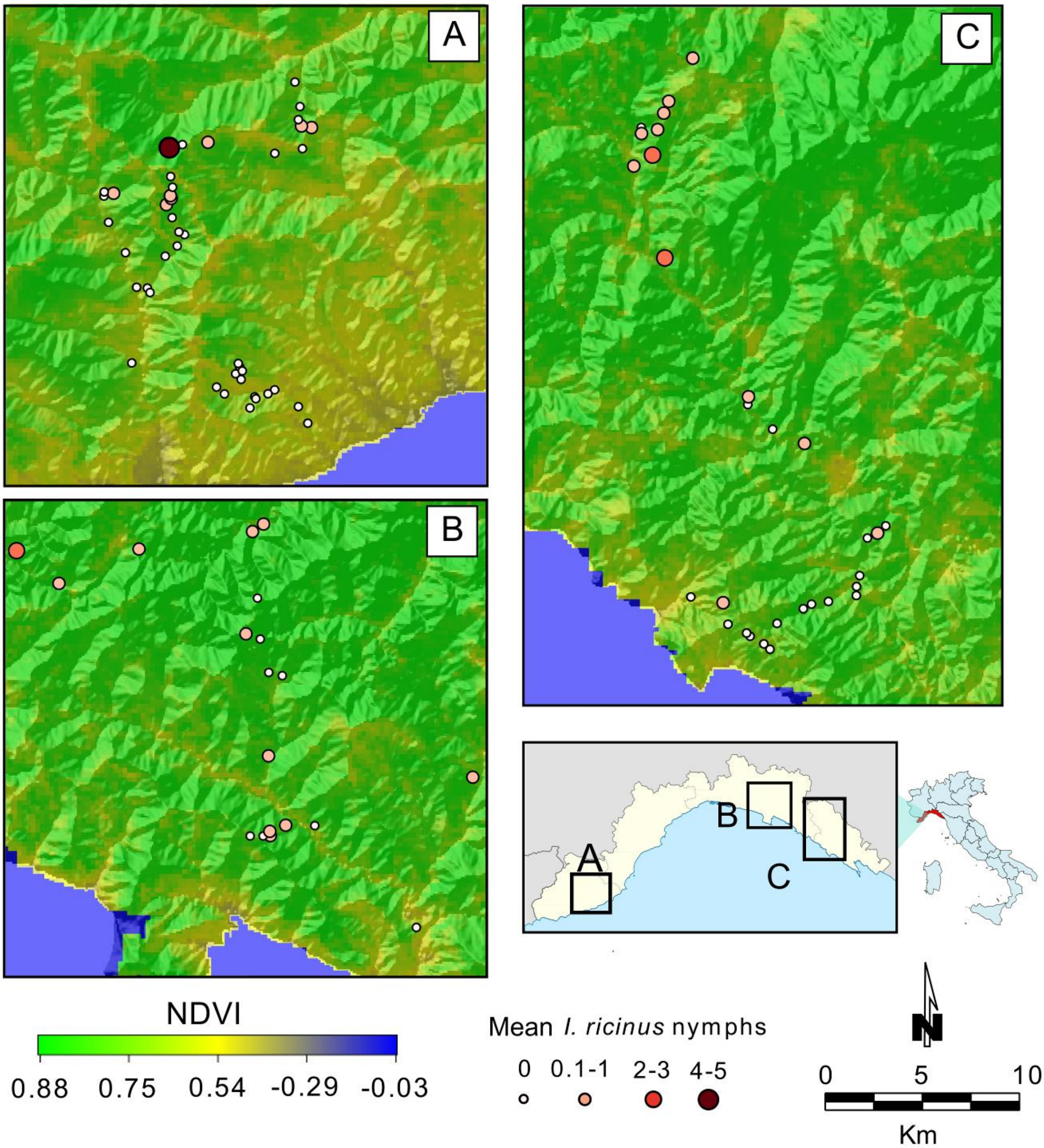
Fig. 2 Mean numbers (diamond-shaped points) and 95%CIs of questing immature *I. ricinus* collected by dragging from March to August 2011 and boxplots of NDVI values in the *Lauretum* (n= 26), *Castanetum* (n= 60) and *Fagetum* (n= 8) dragging sites, in La Spezia, Genoa and Imperia provinces (Liguria).



Spatial clustering of *I. ricinus* nymphs, as evaluated by join-counts, was statistically significant. The distribution of captures by province according to the distance from the sea had a similar pattern when we took into account nymphs only or all developmental stages together (Fig. 3 and Online Resource 2).
I. ricinus nymphs were collected in sites with different dominant tree species. Considering the positive sites for at least 3 dragging sessions, and with 6 or more collected nymphs (i.e., the most constantly and densely infested sites), they were characterized by dense forests dominated by deciduous trees, with abundant herbs and accumulation of leaves on the ground. Six of these seven sites were located in a *Castanetum* area and the other one in the *Fagetum* (1000 m a.s.l.).The dominant tree species in these sites were the chestnuts, the black locust and, above 800 m a.s.l., the

beech. Other well represented coexisting tree species were the hop hornbeam, the manna ash, the hazelnut, the Turkey oak *(Q. cerris)* and the maritime pine. On the other hand, the sites that were consistently negative had, as dominant tree species, the olives (alone, or with pubescent oaks), maritime pines (generally associated with tree heaths), hop hornbeams, or holly oaks. Only two out of 26 sites classified as *Lauretum* were positive for *I. ricinus*. Only one larva and one nymph were collected in the whole study period in these two sites (0.2 % of the *I. ricinus* total capture), where chestnuts was a well-represented tree species.

Fig. 3 Spatial distribution and mean capture (from March to August 2011) of *I. ricinus* nymphs and NDVI in the provinces of Imperia (A), Genoa (B) and La Spezia (C) (Liguria).



The median NDVI was not significantly different between *Fagetum* (median= 0.86) and *Castanetum* (0.82), but both medians were significantly higher than in *Lauretum* (0.71) (Kruskal-Wallis chi-square= 234.04, df= 2, p< 0.001, Multiple comparison test: p< 0.05) (Fig. 2 and Online Resource 3).
Results of the random-effect logistic regression showed that the odds of collecting at least one *I. ricinus* nymph on a 100 m transect were significantly associated with NDVI (p< 0.0001, Table 2). The rarity of *I. ricinus* nymphs at sites with relatively low NDVI (Fig. 3 and Online Resource 3) may explain such an association, which was strong and characterized by a high degree of uncertainty (odds ratio= 50.4, 95% CI= 8.4-302.8). Furthermore, the check for linearity yielded acceptable results. Based on goodness-of-fit statistics, the model was considered as appropriate for the analysis (Pearson's χ^2= 0.41). Plots of Pearson residuals, however, demonstrated the presence of an outlier (Pearson residual= 6.3) corresponding to a site in the province of La Spezia where one nymph was found in March and not in the subsequent dragging sessions. This site had a relatively low NDVI (0.75) compared to the median of the province (0.81, Q_1-Q_3= 0.79-0.83).

Other tick species
Regarding the habitat of the other tick species, *D. marginatus* was mainly collected in chestnut woods (76.3% of specimens), while consistently negative sites were dominated by hazelnuts or beech at highland (>800 m a.s.l.), and by olive groves under 500 m a.s.l.
Twelve out of the 14 *Rhipicephalus* spp. were collected in a *Lauretum* habitat characterized by pubescent oak. Pubescent oaks also characterized the sites where most *H. punctata* were collected (78.9%); specimens were also found in hazel groves, and occasionally in forests of chestnuts, Turkey oak, manna ash or olive cultivations. Chestnut forest was the habitat where *I. frontalis* was mainly captured (12 out of 19 specimens). Regarding the NDVI of the positive sites for each species, only the median of NDVI for *Rhipicephalus* spp. (0.68) was significantly different compared to the medians of positive sites of the other four species (medians range= 0.81-0.83; Kruskal-Wallis chi-square= 31.60, df= 4, p< 0.0001; Multiple comparison test: p< 0.05).

Discussion

In our work, we studied the ixodid tick fauna in three broad areas of Ligurian region comprising different types of wooded habitats. Differences in density (according to dragging captures) and spatial distribution of the species were found. This differential distribution of ticks may be the product of different microhabitat conditions. Dominant vegetation indirectly influences the occurrence, development and activity of ticks (e.g. questing activity), since it modulates the microclimate (Randolph and Storey 1999) and affects the abundance and richness of hosts because it provides food and/or shelter. Therefore, specific types of vegetation can be useful indicators of tick occurrence and of the ecosystems supporting tick-borne diseases (Daniel and Dusbábek 1994; Bisanzio et al. 2008).
I. ricinus was the most abundant species collected in our study areas. In particular, it was prevalent in the eastern provinces and in the inland. *I. ricinus* being a hygrophilic species, this result is consistent with the climatic and environmental differences characterizing the Ligurian territory (as described in M&M). Indeed, the optimal habitat for *I. ricinus* is the deciduous forest, where the immature stages find a convenient microhabitat in which litter keeps an appropriate relative humidity enabling the completion of their developmental cycle. *I. ricinus* has been recorded in most Italian regions (Cringoli et al. 2005), especially in temperate woods and shrubby habitats. In our

area, we mostly collected it in inland valleys, up to the highest study sites (1070 m), characterized by hardwood forests and higher relative humidity, dominated by chestnuts, black locust, or beech; these tree species are widely and abundantly distributed in Liguria region and in large part of northern Italy. It is noteworthy that both the peak and the mean capture numbers of *I. ricinus* nymphs were higher in the *Fagetum* zone (Fig. 2 and Online Resource 3). This supports the statement of altitudinal expansion of this species as seen in other parts of Europe (Medlock et al. 2013) and Italy (i.e., Tuscan Apennines: Martello E. in preparation; Ragagli C. in preparation). This interesting result is worth further studies.

This tick-habitat relation was reflected by the positive association between the abundance of *I. ricinus* nymphs and NDVI. Accordingly, in an area of Tuscany (Le Cerbaie Hills, province of Pisa, less than 40 km from Mediterranean sea and ca. 100 km from La Spezia sites), host-seeking *I. ricinus* were more abundant in relatively moist bottomland habitat covered by deciduous trees, than in dry upland habitat covered by Mediterranean vegetation (Bisanzio et al. 2008). In this last study, carried out within a small enclosed geographic area, a significant association between *I. ricinus* counts and NDVI had also been observed. NDVI thus appears to be a useful tool to predict *I. ricinus* distribution and density, and our results confirm its utility in discriminating habitat suitability for this vector in the northwestern Mediterranean areas of Italy, at different geographic scales.

In Genoa, *I. ricinus* was present also closer to the sea, and below 300m. This could be explained by the absence, in this area, of study sites characterized by the thermoxerophilous vegetation of the '*Lauretum*'. It is precisely in this zone (i.e., the northern part of the 'Riviera di Levante' and nearby inland) that the highest annual rainfall of the entire Ligurian coastal area is registered (Pedemonte 2004). Accordingly, the NDVI calculated in the positive collection sites was rather high (data not shown).

I. ricinus was the most abundant tick recorded also in other study areas in Italy (e.g., Mannelli et al. 1999; Bertolotti et al. 2006; Dantas-Torres and Otranto 2013), including another Liguria province, Savona. Here, Manfredi et al. (1999) reported *I. ricinus* as the main species infesting domestic animals and wildlife and biting humans, especially in areas with woodlands with high deer densities.

However, maximum mean numbers of questing *I. ricinus* nymphs/100 m^2 dragging in our study (La Spezia= 0.9) were lower than those reported from Le Cerbaie Hills (15.0; Bisanzio et al. 2008) or from Trento province, northeastern Italy (37.3; Rizzoli et al. 2002). Maximum numbers were also lower than those found in Genoa province in 1998 (1.9 nymphs/100 m^2; Mannelli et al. 2003). Nevertheless, by comparing our results in the same province and months of this previous study, we observed a decrease in nymphs and an increase of larvae numbers. Such findings are difficult to discuss, however they could be due to the limited study periods.

Although the numbers of the other collected tick species were not high, we could detect differences in their distribution. In contrast with *I. ricinus*, *H. punctata* was the most abundant tick collected in Imperia, with a relative capture ten times more than La Spezia. This species was mostly collected in the inland (95% to > 14 km from the sea). *H. punctata*, a generalist tick which transmit human pathogens such as rickettsiae and *Francisella tularensis*, is widespread in Italy, and preferentially infests calcareous dry sites with coppice and shrubs (Cringoli et al. 2005). Indeed, we mostly collected this tick species in sites characterized by a vegetation dominated by pubescent oaks. This oak species is characteristic of calcareous substrata, mainly at the south mountainsides.

Imperia was the province with the lowest capture numbers, despite being the only province where all five tick species were collected. The few *Rhipicephalus* specimens were mainly collected in the sites closest to the sea. Almost all these sites were characterized by pubescent oaks as dominant tree species and showed a low relative NDVI. Due to the difficulties in discriminating between *R. turanicus* and *R. sanguineus,* which are both present in Mediterranean habitats (Manilla 1998; Gray et al. 2013), and to the fact that the two species easily interbreed (Zahler et al. 1997), we cautiously maintained the denomination of *Rhipicephalus* spp. in our study. Previous studies (Durio et al. 1983) reported both *Rhipicephalus* species infesting dogs in Imperia province; in Savona, *R. sanguineus* was reported to infest humans, cats, domestic ruminants, roe deer and wild boars apart from dogs (Manfredi et al. 1999). This tick genus can transmit different pathogens such as rickettsiales and *Babesia* spp. (Cringoli et al., 2005).

D. marginatus and *I. frontalis* were more scattered in our study area. *D. marginatus* is a thermophilic tick frequently found in the Mediterranean region (Parola et al. 2009). It showed a wide distribution, and altitude appeared as the unique limiting factor, since it was not collected above 807m a.s.l. We collected it in typical Mediterranean sites close to the sea, but also in inland sites characterized by *Castanetum*. Indeed, the range of NDVI values in positive sites was wider and with a lower median value (data not shown) compared to *I. ricinus* positive sites, although such difference was not significant. *D. marginatus* is mainly associated with wild boars, which are abundant in the three provinces. In Italy, this tick species was previously reported in oak woods, shrubs, and prairies and steppes up to 1500 m a.s.l., including the northern Apennines (Mannelli et al. 1997). On the host, *D. marginatus* were collected from humans, sheep and wild boars in Savona province (Manfredi et al. 1999). As reported for *I. ricinus*, an extension and an increase in abundance of *Dermacentor* spp. is being observed in Europe, probably due to climate changes such as the shortening of the winter period and increasing annual minimum temperatures (Kriewra and Czulowska 2013; Beugnet and Marié 2009; Dautel et al. 2006). This can have an effect on the incidence of the tick-borne lymphadenopathy (TIBOLA), an emerging zoonotic rickettsiosis transmitted by *D. marginatus* and *D. reticulatus* (Parola et al. 2005) which was reported in Tuscany (Selmi et al. 2009).

Finally, *I. frontalis* was for the first time collected in Liguria, while it was reported in other Italian regions (Cringoli et al. 2005). This species infests avian hosts in large parts of Europe, and likes abundant cover and leafy undergrowth (Schorn et al. 2011). The finding of actively questing larvae, nymphs and one adult *I. frontalis* in 12 dragging sites from the three provinces is interesting, since it shows that its life cycle is established in Liguria where it could have been introduced thanks to migratory birds. The zoonotic role of *I. frontalis* is not defined; however ‘Candidatus *Neoehrlichia mikurensis*’ was recently found in this species (Movila et al. 2013).

In conclusion, our survey contributes to the knowledge on tick fauna in Liguria and highlights some aspects of *I. ricinus* phenology in southern Europe. The positive relationship between the abundance of *I. ricinus* nymphs and NDVI supports the hypothesis that, at least in Liguria, dry habitat conditions may limit the distribution of this species. However, only the spring-summer season of one year was studied, and no conclusive data on tick seasonality and distribution can be drawn. Further studies, comparing human health data and tick field collections, are needed, to evaluate the areas and activities at risk for tick bites and infection by tick borne pathogens.

Acknowledgments

The authors thank all personnel of Experimental Zooprophylactic Institute of Piedmont, Liguria and Aosta Valley, and University of Turin, directly or indirectly involved in this work, and especially those who helped us in the fieldwork: Debora Corbellini, Saverio Bessone and Elisa Martello. This study was supported by the Istituto Zooprofilattico Sperimentale di Piemonte, Liguria e Valle d'Aosta depending from the Ministry of Health, Italy (IZSPLV 02/09 RC).

References

Bertolotti L, Tomassone L, Tramuta C, Grego E, Amore G, Ambrogi C, Nebbia P, Mannelli A (2006) *Borrelia lusitaniae* and spotted fever group rickettsiae in *Ixodes ricinus* (Acari: Ixodidae) in Tuscany, central Italy. J Med Entomol 43:159-165

Beugnet F, Marié JL (2009) Emerging arthropod-borne diseases of companion animals in Europe. Vet Parasitol 163:298-305

Bisanzio D, Amore G, Ragagli C, Tomassone L, Bertolotti L, Mannelli A (2008) Temporal variations in the usefulness of normalized difference vegetation index as a predictor for *Ixodes ricinus* (Acari: Ixodidae) in a *Borrelia lusitaniae* focus in Tuscany, central Italy. J Med Entomol 45:547-555

Bivand R et al. (2013) spdep: Spatial dependence: weighting schemes, statistics and models. R package version 0.5-56. http://CRAN.R-project.org/package=spdep

Braun-Blanquet J (1979) Fitosociología. Bases para el estudio de las comunidades vegetales. Blume Ediciones, Madrid, pp 820

Carnevali L, Pedrotti L, Riga F, Toso S (2009) Banca Dati Ungulati: Status, distribuzione, consistenza, gestione e prelievo venatorio delle popolazioni di Ungulati in Italia. Rapporto 2001-2005. Biol Cons Fauna 117:1-168 [Italian-English text]

Ciceroni L and Ciarrocchi S (1998) Lyme disease in Italy, 1983-1996. New Microbiol 21:407-418

Cimmino MA, Fumarola D, Sambri V, Accardo S (1992) The epidemiology of Lyme borreliosis in Italy. Microbiologica 15:419-424

Cliff AD and Ord JK (1981) Spatial Processes: Models and Applications. Pion, London, pp 266

Cringoli G, Iori A, Rinaldi L, Veneziano V, Genchi C (2005) Zecche. Mappe Parassitologiche. Rolando, Napoli

Crovato F, Nazzari G, Fumarola D, Rovetta G, Cimmino MA, Bianchi G (1985) Lyme disease in Italy: first reported case. Ann Rheum Dis 44:570-571

Daniel M and Dusbábek F (1994) Micrometeorological and microhabitat factors affecting maintenance and dissemination of tick-borne disease in the environment. In: Sonenshine DE and Mather TN (eds) Ecological dynamics of tick-borne zoonoses, 1st edn. Oxford University Press, New York , pp. 91-138

Dantas-Torres F and Otranto D (2013) Species diversity and abundance of ticks in three habitats in southern Italy. Ticks Tick Borne Dis 4:251-255

Dautel H, Dippel C, Oehme R, Hartelt K, Schettler E (2006) Evidence for an increased geographical distribution of *Dermacentor reticulatus* in Germany and detection of *Rickettsia* sp. RpA4. Int J Med Microbiol 296(Suppl. 40):149-156

Durio P, Durante G, Sobrero L (1983) Contributo alla conoscenza della fauna ixodologica italiana. Indagini sulla distribuzione della zecche del Piemonte e della Liguria. Riv Parassitol 43:345-352

Estrada-Peña A (1999) Geostatistics as predictive tools to estimate *Ixodes ricinus* (Acari: Ixodidae) habitat suitability in the Western Palearctic from AVHRR satellite imagery. Exp App Acarol 23:337-349

Estrada-Peña A, Bouattour A, Camicas JL, Walker AR (2004) Ticks of Domestic Animals in the Mediterranean Region. A Guide to Identification of Species, 1st edn. University of ZaragozaPress, Zaragoza, pp 131

Estrada-Peña A, Venzal JM, Sánchez Acedo C (2006) The tick *Ixodes ricinus*: distribution and climate preferences in the western Palaearctic. Med Vet Entomol 20:189-197

Estrada-Peña A, Gray JS, Kahl O, Lane RS, Nijhof AM (2013a) Research on the ecology of ticks and tick-borne pathogens-methodological principles and caveats. Front Cell Infect Microbiol 3:29

Estrada-Peña A, Farkas R, Jaenson TG, Koenen F, Madder M, Pascucci I, Salman M, Tarrés-Call J, Jongejan F (2013b) Association of environmental traits with the geographic ranges of ticks (Acari: Ixodidae) of medical and veterinary importance in the western Palearctic. A digital data set. Exp Appl Acarol 59:351-366

Giraudoux P (2013) pgirmess: Data analysis in ecology. R package version 1.5.7. http://CRAN.R-project.org/package=pgirmess

Gray J, Dantas-Torres F, Estrada-Peña A, Levin M (2013) Systematics and ecology of the brown dog tick, *Rhipicephalus sanguineus*. Ticks Tick Borne Dis 4:171-180

Gray JS, Dautel H, Estrada- Peña A, Kahl O, Lindgren E (2009) Effects of climate change on ticks and tick-borne diseases in Europe. Interdiscip Perspect Infect Dis ID 593232.

Jaenson TG, Eisen L, Comstedt P, Mejlon HA, Lindgren E, Bergström S, Olsen B (2009) Risk indicators for the tick *Ixodes ricinus* and *Borrelia burgdorferi* sensu lato in Sweden. Med Vet Entomol 23:226-237

Kiewra D and Czulowska A (2013) Evidence for an increased distribution range of *Dermacentor reticulatus* in south-west Poland. Exp Appl Acarol 59:501-506

Kitron U (1998) Landscape ecology and epidemiology of vector-borne diseases: tools for spatial analysis. J Med Entomol 35:435-445

Littel RC, Stroup WW, Freund RJ (2002) SAS® for linear models, 4th ed. SAS Institute Inc., Cary, North Carolina ,pp 466

Manfredi MT, Dini V, Piacenza S, Genchi C (1999) Tick species parasitizing people in an area endemic for tick-borne diseases in north-western Italy. Parassitologia 41:555-560

Manilla, G (1998) Acari, Ixodida (Fauna d'Italia 36). Edizioni Calderoni, Bologna, Italy, pp 280

Mannelli A, Tolari F, Pedri P, Stefanelli S (1997) Spatial distribution and seasonality of ticks (Acarina: Ixodidae) in a protected area in the northern Apennines. Parassitologia 39:41-45

Mannelli A, Cerri D, Buffrini L, Rossi S, Rosati S, Arata T, Innocenti M, Grignolo MC, Bianchi G, Iori A, Tolari F (1999) Low risk of Lyme borreliosis in a protected area on the Tyrrhenian coast, in central Italy. Eur J Epidemiol 15:371-377

Mannelli A, Boggiatto G, Grego E, Cinco M, Murgia R, Stefanelli S, De Meneghi D, Rosati S (2003) Acarological risk of exposure to agents of tick-borne zoonoses in the first recognized Italian focus of Lyme borreliosis. Epidemiol Infect 131:1139-1147

Medlock JM, Hansford KM, Bormane A, et al. (2013) Driving forces for changes in geographical distribution of *Ixodes ricinus* ticks in Europe. Parasit Vectors 6:1

Molenberghs G and Verbeke G (2005) Models for discrete longitudinal data. Springer, New York, pp 683

Movila A, Alekseev AN, Dubinina HV, Toderas I (2013) Detection of tick-borne pathogens in ticks from migratory birds in the Baltic region of Russia. Med Vet Entomol 27:113-117

Parola P, Paddock CD, Raoult D (2005) Tick-borne rickettsioses around the world: emerging diseases challenging old concepts. Clin Microbiol Rev 18:719-756

Parola P, Rovery C, Rolain JM, Brouqui P, Davoust B, Raoult D (2009) *Rickettsia slovaca* and *R. raoultii* in tick-bornerickettsioses. Emerg Infect Dis 15:1105-1108

Pavari A (1916) Parte generale. In: Ricci M1916 (ed.) Studio preliminare sulla coltura di specie forestali esotiche in Italia. Annali del Regio Istituto Superiore Nazionale Forestale, Firenze, vol. I (1914-15), pp 221

Pedemonte R (2004) Contributo alla classificazione dei climi della Liguria. 6- Distribuzione geografica delle precipitazioni annue I parte. Rivista Ligure di Meteorologia 11. http://www.nimbus.it/liguria/rlm11/clima_liguria.htm). Accessed October 2013

R Core Team (2013) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org

Raganella-Pelliccioni E, Riga F, Toso S (2013) Linee guida per la gestione degli Ungulati. Cervidi e Bovidi. ISPRA (Istituto Superiore per la Protezione e la Ricerca Ambientale). http://www.isprambiente.gov.it/it/pubblicazioni/manuali-e-linee-guida/linee-guida-per-la-gestione-degli-ungulati.-cervidi-e-bovidi. Accessed October 2013

Randolph SE, Anda P, Avsic-Zupanc T, et al. (2010). Human activities predominate in determining changing incidence of tick-borne encephalitis in Europe. Euro Surveill 15(27):24-31

Rizzoli A, Merler S, Furlanello C, Genchi C (2002) Geographical information systems and bootstrap aggregation (bagging) of tree-based classifiers for Lyme disease risk prediction in Trentino, Italian Alps. J Med Entomol 39:485-492

Rizzoli A, Hauffe H, Carpi G, Vourc H G, Neteler M, Rosà R (2011) Lyme borreliosis in Europe. Euro Surveill 16(27), pii:19906

Schorn S, Schöl H, Pfister K, Silaghi C (2011) First record of *Ixodes frontalis* collected by flagging in Germany. Ticks Tick Borne Dis 2:228-230

Selmi M, Martello E, Bertolotti L, Bisanzio D, Tomassone L (2009) *Rickettsia slovaca* and *Rickettsia raoultii* in *Dermacentor marginatus* ticks collected on wild boars in Tuscany, Italy. J Med Entomol 46:1490-1493

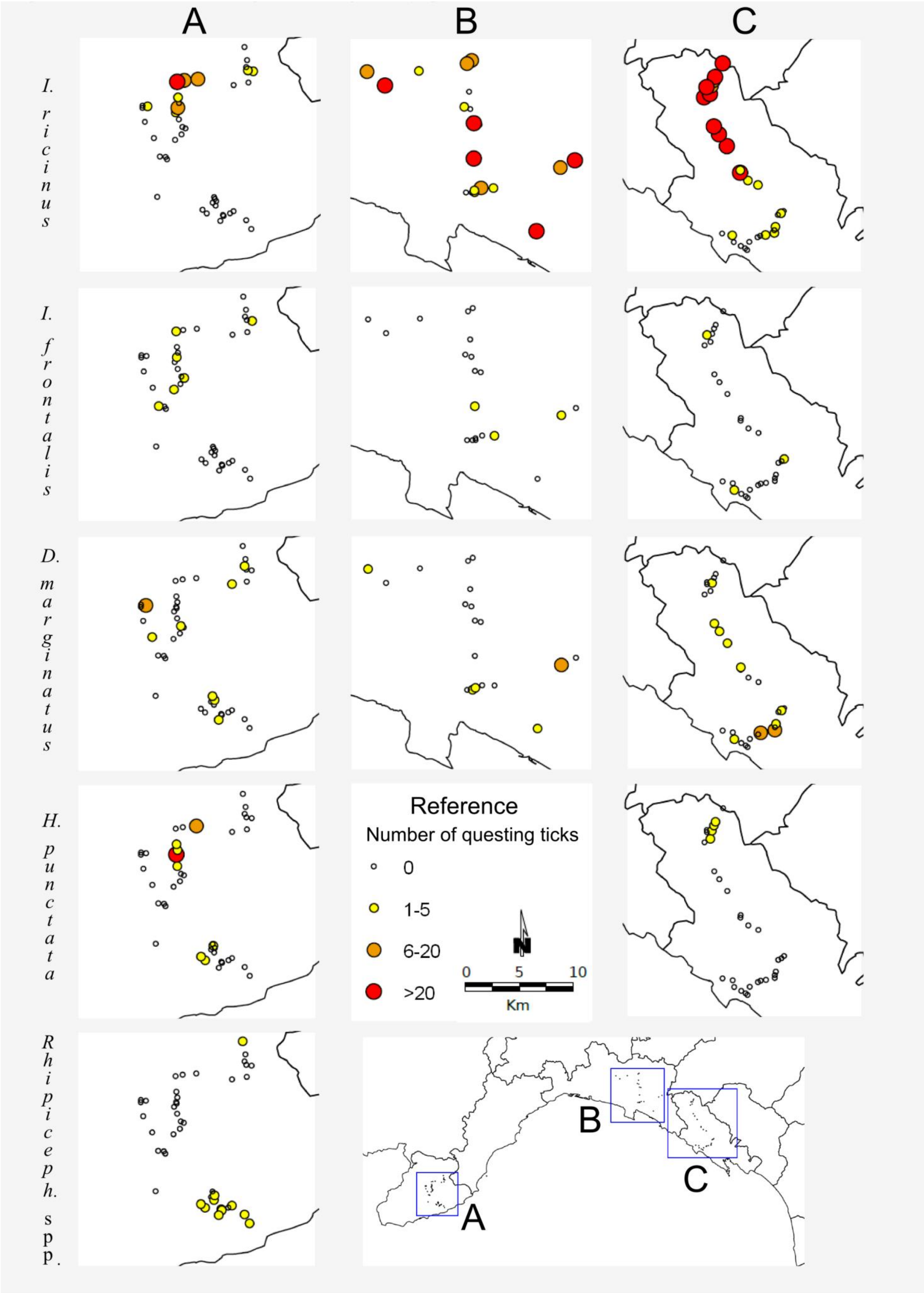
Tagliapietra V, Rosà R, Arnoldi D, Cagnacci F, Capelli G, Montarsi F, Hauffe HC, Rizzoli A (2011) Saturation deficit and deer density affect questing activity and local abundance of *Ixodes ricinus* (Acari, Ixodidae) in Italy. Vet Parasitol 183:114-124

Vorou RM, Papavassiliou VG, Tsiodras S (2007) Emerging zoonoses and vector-borne infections affecting humans in Europe. Epidemiol Infect 135:1231-1247

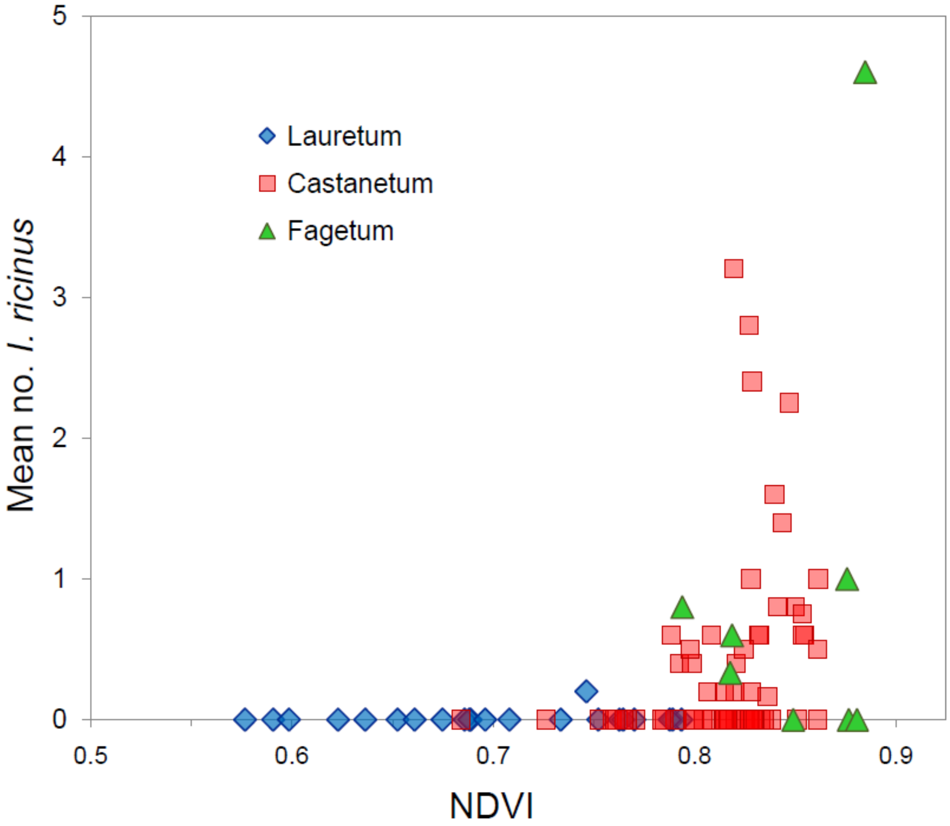
Zahler M, Filippova NA, Morel PC, Gothe R, Rinder H (1997) Relationships between species of the *Rhipicephalus sanguineus* group: a molecular approach. J Parasitol 83:302-306

Online Resource 1 Examples of tick collection sites in Liguria. Main types of vegetation associations: <u>Lauretum</u>: (a) olive cultivations, (b) shrubs and pubescent oak forest, (c) shrubland of tree heath with maritime pines, (d) holly oak forest; <u>Castanetum</u>: (e) pubescent oak forest, (f) chestnut forest, (g) ferns in chestnut forest; <u>Fagetum</u>: (h) meadow and beech forest, (i) beech forest.

Online Resource 2 Spatial distribution and total questing ticks collected (from March to August 2011) in the provinces of Imperia(A), Genoa (B) and La Spezia (C) (Liguria), by species.



Online Resource 3 Mean number of collected *I. ricinus* nymphs *vs.* NDVI in the *Lauretum*, *Castanetum* and *Fagetum* dragging sites, province of La Spezia, Genoa and Imperia (Liguria).



Erratum to: Habitat and occurrence of ixodid ticks in the Liguria region, northwest Italy

Erratum to: Exp Appl Acarol DOI 10.1007/s10493-014-9794-y

Due to an unfortunate turn of events, an error occurred in the abstract of the original publication. Two statistical techniques were mixed up and therefore the sentence ‘‘Negative binomial regression for repeated measures was used to model the associations of NDVI and season with counts of host-seeking *I. ricinus* nymphs.’’ is incorrect.

The correct sentence in the abstract reads ‘‘Random-effect logistic regression was used to model the associations of NDVI and season with the probability of finding host-seeking *I. ricinus* nymphs.’’