Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Niche partitioning at microscale level and coexistence in two alpine vipers: Vipera berus walser and Vipera aspis aspis

Alessandro Prezzi
University of Turin
Lorenzo Laddaga
Società di Scienze Naturali del Verbano Cusio Ossola
Samuele Ghielmi
Museo delle Scienze
Mauro Fanelli
Marco Sassoè-Pognetto
University of Turin
Lorenzo Garizio
lorenzo.garizio@alumni.uniroma2.eu

University of Rome Tor Vergata

Research Article

Keywords: Ecological Niche Model (ENM), niche overlap, generalized additive model (GAM), microhabitat use, viper, syntopy

Posted Date: April 24th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-9417028/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: No competing interests reported.

Abstract

Niche differentiation between species displaying high ecological similarity often relies on fine-grained environmental partitioning that is easily overlooked at the macrohabitat scale. Identifying these mechanisms is essential to clarify the co-occurrence patterns and competitive dynamics of closely related taxa in shared locations. To this end, assessing the utilization of habitat features at a microscale level is pivotal for investigating how species sharing similar macrohabitats can coexist. The Walser viper (*Vipera berus walser*) is an endemic subspecies from Italian Western Alps with a very limited distribution. Sympatry with congeneric species occurs only with asp viper (*Vipera aspis aspis*) in a few localities. In contact zones, the two species predominantly inhabit mountain open areas. We assessed the utilization of microhabitat features and quantified the niche overlap between Walser and asp vipers in two syntopic locations. Moreover, we investigated the temporal activity pattern across the sampling season. Our results indicate that the two species primarily differ in their altitudinal ranges. We additionally detected a slight difference in microhabitat utilization, specifically in the use of shrubby patches. Although the niche overlap between asp and Walser vipers is high, the two species retain distinct ecological niches. Seasonal activity patterns highly overlap following similar bimodal trends. The study results indicate that the microhabitat structural complexity represents a key feature in promoting the two species' coexistence, highlighting the importance of preserving heterogeneous open areas in the alpine environment.

1. Introduction

The geographical range of animal populations results from the combination of multiple determinants. On a broad scale, an important role is played by the distribution of the closest parental lineages from which populations diversify (Sexton et al. 2009). On a finer scale, natural selection, random allele mutations, migrations, genetic drift, epigenetics, phenotypic plasticity and interspecific interactions mold the ecological niche and consequently the actual area of occupancy (Sexton et al. 2009; Rodríguez et al. 2015). Hutchinson defined the ecological niche as a hypervolume in an *n*-dimensional environmental space that allows a population to persist indefinitely (Hutchinson, 1957). He distinguished between fundamental and realized niches: the fundamental niche does not consider biotic interactions nor dispersal capability, whereas the realized niche is influenced by the relationships established between organisms, such as competition and predation (Hutchinson, 1957) and also their biogeography, meaning that suitable areas might have not been colonized due to geographical barriers and therefore be left outside of the realized niche. Phylogenetically related species may show a high degree of similarity in their niches, as a result of inheriting ecological traits from their common ancestor (Harvey and Pagel, 1991; Wiens and Graham, 2005). In case of allopatric speciation, closely related species displaying similar ecological niches that come into secondary contact can competitively exclude each other (Lucek et al. 2020). However, the association between phylogenetic distance and ecological niche is not always detectable (*e.g.* Rice et al. 2003; Losos et al. 2003; Knouft et al. 2006), suggesting that niche conservatism is not ubiquitous (Pearman et al. 2008; Losos, 2008; Peterson, 2011). Indeed, closely related species may respond differently to at least one environmental condition, which can lead to shifts

in the niches' environmental space, modifications of their shape, or changes in volume (*e.g.*, Jackson and Overpeck, 2000, Pigot et al. 2016). Niche conservatism should thus be empirically evaluated at context-specific level, taking into account both the environmental and biological factors affecting the ecological niche of the target organism within the study area.

Understanding the environmental features that predominantly shape organisms' niches and quantifying the degree of similarity between them are key tasks for conservation biology. In fact, a deep knowledge of species' ecological niches is necessary to evaluate, among other factors, the impact of alien species, the response of organisms to climate change effects, the species' risk of extinction, and the repercussions of altered communities on ecosystems (Carscadden et al. 2020). Moreover, a quantitative definition of ecological niches is pivotal to assess the degree of competition between sympatric species. When occurring in the same area, competition between species displaying similar ecological niches may arise, especially for trophic resources and habitat features (*i.e.* refuges, nesting areas), and lead to competitive exclusion (Hardin, 1960). Stable coexistence is thus promoted by mechanisms reducing the niche overlap (Schoener 1974; Pastore et al. 2021). Ecological niches are generally partitioned on the basis of three main components: (1) temporal, including diurnal and nocturnal activity or even different seasonal activity patterns (*e.g.*, Houadria et al. 2015); (2) spatial, meaning a different land and microhabitat use (*e.g.*, Wereszczuk and Zalewski, 2015); (3) trophic, in which competitors select different sources of food in order to not deplete the same resource (*e.g.*, Kingsbury et al. 2020). Niche partitioning in sympatric reptiles is primarily associated with differences in habitat use, whereas temporal segregation is rare (Toft, 1985). The degree of partitioning in trophic niches is often taxa-dependent. For example, food generally constitutes the principal resource partitioned between snakes, but in vipers the trophic niches are frequently similar and can completely overlap in contact zones (Luiselli, 2006; Martínez-Freiría et al. 2010). At microscale level, habitat selection by reptiles has been demonstrated to be correlated with the thermal properties of the habitat (Martín and Salvador, 1997; Harvey and Weatherhead, 2010; Gifford et al. 2012; Ferreira et al. 2017). As ectotherms, reptiles indeed display high thermal and hydric sensitivity, which makes them particularly susceptible to external alterations (Dezetter et al. 2021). To maintain suitable body temperature, reptiles need to spend prolonged periods in basking, exposing fully or partially their body to either direct sunlight or diffuse radiation. Optimal basking sites thus can become a source of both intraspecific and interspecific competition (Downes and Bauwens, 2002; Himes, 2003; Luiselli, 2006). Nevertheless, the structural complexity of microhabitats may facilitate the co-occurrence of species with different requirements and thermal optima, as environmental heterogeneity involves an increased availability of resources for different ecological needs (Ferreira et al. 2017). A more diverse habitat indeed implies a broader thermal landscape and thus promotes the coexistence of multiple species.

The Walser viper (*Vipera berus walser*) is a recently described taxon that was originally classified as a species (Ghielmi et al. 2016), but its taxonomic status has been under discussion due to discrepancies in genetic analysis. The mtDNA suggested that the closest lineages are Caucasian vipers (*Vipera kaznakovi*, *V. darevskii*, *V. dinniki*; Ghielmi et al. 2016; Doniol-Valcroze et al. 2021), but phylogenetic analysis of the nuclear genome highlighted its inclusion within the alpine clade of the adder *Vipera berus*

marasso (Schmidtler and Hansbauer, 2020; Doniol-Valcroze et al. 2021). Therefore, the taxon has been reclassified as a subspecies of *V. berus* (Dufresnes et al. 2024). The Walser viper displays a very restricted distribution, occurring only in a few areas of Italian Western Alps. Its range is disjunct, with a northern section of about 45 km^2, and a southern portion of about 225 km^2 (Ficetola et al. 2020). The altitudinal range is also limited, as populations of the Walser viper are found from approx. 1300 to 2300 m a.s.l. (for comparison, the elevation range of *V. berus marasso* in Central Alps is 550–2500 m a.s.l.; Bernini et al. 2004). The suitable habitat of *V. berus walser* is mainly composed of south-exposed open areas with high annual precipitation levels (Ficetola et al. 2020). Since its distribution and altitudinal ranges are very restricted, the Walser viper realized niche appears to be extremely narrow, potentially leading to high risk of extinction (Slatyer et al. 2013). Although quantitative information on population and habitat trends is still missing, and thus this subspecies has not been evaluated according to the IUCN criteria, the available data would classify *V. berus walser* as Endangered (Ficetola et al. 2020).

Vipera apsis is a widespread species occurring in Italy, France, Switzerland and Spain, with localized populations in Slovenia and Germany. The populations in the study areas are attributed to the nominal subspecies (*V. aspis aspis*; Fig. 1) and belong to the “atra-type” group (previously referred as *Vipera aspis atra*, Meisner, 1820). Despite the taxonomic reassignment (Ursenbacher et al. 2006; Golay et al. 2008), these vipers might retain an ecological identity, especially when compared to the other subspecies found in Italy (i.e. *francisciredi* and *hugyi*). Indeed, such populations can be found at higher elevations (over 2000 m a.s.l., with exceptional records at 2800 m; Sindaco et al. 2006) suggesting a higher tolerance to cold temperatures, although further research on their physiology are necessary to evaluate these potential adaptations. Asp vipers inhabit a wide range of environments, mainly ecotones such as drystone walls, shrubs, rocky riverbanks, and alpine pastures (Saint-Girons, 1952). Despite the geographical ranges of the two taxa fully overlap (Fig. 1), sympatry between Walser and asp vipers is seldom observed due to separation in the altitudinal ranges. Presumably, asp viper of the “atra-type” can colonize higher elevations only when in lack of competitors.

The aim of this study was to investigate the ecological factors influencing the co-occurrence of *V. berus walser* and *V. aspis aspis* in two syntopic locations (*sensu* Rivas; Rivas, 1964). Since Walser and asp vipers share the same macrohabitat within the study sites (mountain open areas) and are often found in close proximity, we focused on the microhabitat structure. At microscale level, we analyzed the utilization of habitat features and evaluated the degree of niche overlap between the two species. By leveraging occurrence data collected during field surveys, we fitted Generalized Additive Models (GAMs) to assess the main environmental factors affecting the presence probability of the two vipers. We adopted the PCA-env approach proposed by Broennimann et al. (2012) to quantify the overlap in ecological niches and to test the hypothesis of similarity. Finally, we evaluated the temporal activity patterns during the sampling season, in order to detect potential differences between the two species.

2. Materials and methods

2.1. Species occurrences and environmental data

Data were collected during field activity conducted in 2022 and 2023, from late May to early October (Tables SM1-3). We chose the study areas on the basis of occurrence data of both Walser and asp vipers gathered from previous surveys (Ghielmi et al. 2016; Ficetola et al. 2020), aiming at maximizing our sampling effort in syntopic locations. As a result, we selected 4 distinct patches of suitable habitat in Strona valley and 5 patches in Chiobbia Valley (Fig. 1). The surveys were performed approx. between 8:00 a.m. and 5:00 p.m., by adopting the Visual Encounter Survey method (VES), namely the searching for active animals within the study sites, during basking or moving outside their shelters. Encounters outside of the sites presented in Fig. 1 were discarded. Date, time, species and coordinates were recorded at each of the 93 encounter events. In 78 events vipers were captured, and we additionally collected information on sex, snout-vent-length (SVL), photos of head pholidosis (dorsal and lateral view) and dorsal pattern. These data, especially when considered together, allow to distinguish different individuals and thus to identify potential recaptures (Di Nicola et al. 2021b). For the remaining 15 encounter events, the vipers were not caught but we included only sightings of which we are sure of the identification, based on coloration, ventral pattern and head pattern. For these cases, we assumed that the individuals were not recaptures. Due to the small sample of these sightings, we believe that it doesn't affect the results of the work. By contrast, brief sightings were discarded due to the uncertainty on the identification.

To assess the differences in terms of habitat utilization between *Vipera aspis aspis* and *Vipera berus walser* at fine-grain level, we built high resolution maps of microhabitat features for each sampling site. Maps were generated by combining the geolocalization of microhabitat features (using a Garmin eTrex 20 GPS device) with high-resolution satellite imagery from Google Earth Pro (https://www.google.com/intl/it/earth/about/versions/). Specifically, field-collected GPS points provided the primary occurrence information, while the satellite imagery was used as a visual reference to guide the manual delineation of patch boundaries for those microhabitats that were large enough to be clearly identified remotely. For smaller features that are not clearly separable on the imagery, GPS locations were used to create representative polygons capturing their local extent. Digitization was performed in QGIS (v. 3.22.5 "Białowieża"). Maps were further elaborated in the R environment (v. 4.2.3) by merging similar microhabitat variables (Table SM4). As a result, we identified four types of microhabitat coverages available within the study sites, namely grass, shrubs, trees, and rocks. Additionally, since study sites were placed at different elevations and Walser and asp vipers display different altitudinal ranges, we downloaded the Digital Elevation Model (DEM) at a 10-m-resolution (Tarquini et al. 2012).

2.2. Microhabitat utilization

We investigated the microhabitat utilization for both species by estimating their probability of occurrence in response to environmental features. Occurrence probability was modeled for each species separately, through the use of GAMs. To assume independence in our observations, we randomly retained one occurrence for each individual found in the period 2022–2023. Additionally, 28 occurrence data (13 of *V. aspis* and 15 of *V. walser*) from previous research (Ficetola et al. 2020) or occasionally collected by the authors during past surveys were included in our analyses. The final dataset included a

total of 89 observations, 46 for *V. walser* and 43 for *V. aspis.* A set of 138 pseudo-absences was randomly generated over the study area and used to fit the two GAMs. Models' predictors were selected considering the main environmental factors related to vipers' ecological requirements. The elevation was included as an independent variable because it constitutes a main driver of reptile distribution, also representing a proxy for the thermal conditions (McCain 2010). Moreover, the two species display different altitudinal ranges, especially when in sympatry (as presumed by other cases of syntopy of asp viper with cold-adapted vipers such as *V. berus marasso* and *V. ursinii*, see Luiselli et al. 2007; Scali et al. 2011). To assess the effect of microhabitat features on vipers' occurrence, we extracted and computed the proportion of microhabitat categories within a 5-m-radius buffer around both occurrences (Table SM5) and pseudo-absences. We included in our models the proportions of rocks and shrubs, as they represent the principal and most abundant shelters within the study area. Since the two vipers mostly inhabit ecotones, we indeed expected that these two variables represent the microhabitat features mainly affecting the vipers' occurrence. Grass and tree proportions were not included among predictors after performing model comparison based on the results of Likelihood Ratio Tests (LRTs) and the Akaike information criterion (AIC; Table SM6). Due to the limited amount of data, we did not consider any differential effect between the Chiobbia and Strona valleys. Furthermore, the study sites placed in the two valleys are similar in terms of both altitudinal ranges and microhabitat features (Figures SM1-2). Therefore, we assumed that the occurrence of vipers is equally influenced by such environmental factors in both valleys. Prior to model fitting, we assessed pairwise Pearson correlations and excluded multicollinearity as a potential issue ($r < 0.35$ for each pairwise combination; Table SM7). We fitted the two GAMs with binomial error distribution and logit link function. For both models, the elevation was set as a linear predictor, whereas both rocks and shrubs proportions were included as smooth terms. Indeed, these variables are expected to have non-linear relation with the occurrence probability due to the ecotonal ecology of vipers. We set the number of knots to 3 for both smooth terms, to avoid ecologically unreasonable relationships with the vipers' occurrence probability. GAMs were performed in the R environment (v. 4.2.3) using the function *gam()* of the *mgcv* package (Wood and Wood, 2015).

2.3. Niche overlap and equivalency

We quantified the niche overlap between Walser and asp vipers by adopting the PCA-env approach proposed by Broennimann et al. (2012), using the *ecospat* R package (Broennimann et al. 2015). The framework involves performing Principal Component Analyses (PCA) of environmental variables and kernel density functions of occurrences in the multivariate PCA space (Broennimann et al. 2012). Elevation and proportions of microhabitat types (Figures SM1,2) were extracted from a 10x10 m grid covering the whole study area and used to represent the available environment as a bi-dimensional space by performing PCA. For both species, we computed the density of occurrences in the bivariate space defined by the first and second PCA axes by fitting a kernel density function. The degree of intersection between the two niches estimated with kernel densities was quantified using Schoener's D metric (Schoener, 1970). This metric varies between 0 (no overlap) and 1 (complete overlap). Schoener's D metric is one of the most applied metrics to evaluate overlap in ecological niches (Warren et al. 2008). On the basis of the Schoener's D metric, we also assessed niche similarity by performing the niche

equivalency test (Warren et al. 2008). The niche equivalency test evaluates whether the environmental conditions of the two niches are equal, by comparing the observed overlap with a null overlap distribution obtained by pooling and randomly reallocating the grid cells at occurrence locations between the two species. The null hypothesis of niche equivalency is rejected when the observed value of D falls outside the 95% density of the simulated values. We specifically tested for niche divergence between the two species, namely whether the observed niche overlap is significantly lower than random. We performed the equivalency test with 1000 simulations.

2.4. Seasonal activity patterns

We investigated the temporal activity pattern across the season, using only data from 2022–2023 surveys. Indeed, occurrence data from previous years were mostly occasionally collected and thus could represent a bias. The filtered dataset thus included 61 occurrences (29 *V. aspis*, 32 *V. walser*) in the whole study area. For each species, we fitted a kernel density function to the vipers' abundance per Julian day (*i.e.*, the number of individuals recorded in each survey date). The two densities curves represent the activity patterns over days of the year. The temporal overlap between the two species was calculated integrating the area of intersection of the two curves. The integration was then used to estimate the overlap coefficient (Δ), which varies from 0 (no overlap) to 1 (complete overlap).

3. Results

3.1. Microhabitat utilization

The results of GAMs (Table 1; Fig. 2) highlighted a slightly positive association between elevation and the probability of occurrence of Walser vipers in the study area (p-value: 0.055). Moreover, we detected a significant effect for both rock (p-value < 0.001) and shrub (p-value = 0.008) coverage. The two smooth functions reach a peak at approx. 0.5 to 0.7 coverage of the two types of refugia. Differently, the model for asp viper indicated a significantly negative relation between altitude and occurrence probability (p-value < 0.001). Rock proportion has a significant effect on species' occurrence probability (p-value < 0.001), displaying a trend with a peak at approx. 0.5 of coverage. By contrast, the shrub coverage does not affect the probability of occurrence of asp viper (p-value: 0.965).

Table 1
Model coefficients of the two Generalized Additive Models (GAMs) fitted to estimate the probability of occurrence of *V. berus walser* (Walser viper) and *V. aspis aspis* (asp viper). Estimated model values (*Estimate*), standard errors (*SE*), and p-values (*p*) for linear predictors (*Term*) are reported for each model. Effective degrees of freedom (*edf*), Chi-squared statistics (*Chisq*) and *p*-values (*p*) are shown to report the approximate significance of smooth terms.

Walser viper			
Term	*Estimate*	*SE*	*p*
Elevation	0.002	0.001	0.055
Approximate significance of smooth terms			
Smooth term	*edf*	*Chisq*	*p*
Rock coverage	1.718	21.00	< 0.001
Shrub coverage	1.809	11.32	0.008

Asp viper			
Term	*Estimate*	*SE*	*p*
Elevation	− 0.007	0.002	< 0.001
Approximate significance of smooth terms			
Smooth term	*edf*	*Chisq*	*p*
Rock coverage	1.832	15.69	< 0.001
Shrub coverage	1.000	0.002	0.964

3.2. Niche overlap and equivalency

The environmental variability within the study area was represented as a bidimensional space performing PCA (Fig. 3). The first two PCA axes explain 61.03% of the variation at sampling sites. Specifically, PC1 is positively associated with the proportion of grass and negatively related to the proportion of shrubs, whereas PC2 shows a positive relation with both tree and rock proportions and is negatively associated with the elevation (Figure SM3). The kernel densities fitted on the PCA cells corresponding to occurrence data were used to quantify the ecological niches of both Walser and asp vipers. V. *berus walser* density is higher in cells associated with shrubby and rocky areas at higher elevations (central-left portion of the PCA space), whereas *V. aspis aspis* density tends to occupy cells at lower elevations and habitats richer in rocks and grass (Fig. 3). The niche overlap calculated using the Schoener's D metric is high (*sensu* Rödder and Engler, 2011), amounting to 0.64. The results of the

equivalency test reject the null hypothesis (p-value = 0.03; Figure SM4), providing the evidence of divergence in the two ecological niches.

3.3. Seasonal activity patterns

The result obtained by integrating the area under the curve of the intersection of kernel densities suggests a high grade of overlap in seasonal activity patterns (overlap proportion of kernel densities = 0.84; Fig. 4). Both species highlighted a bimodal distribution, with two higher peaks: for *V. aspis* these were on the 16th of June (167th day of the year) and on the 15th of August (227th day of the year), whereas for *V. b. walser* were on the 25th of June (176th day of the year) and on the 6th of August (218th day of the year).

4. Discussion

Our study highlighted an altitudinal differentiation between Walser and asp vipers in contact zones. Although the two species overlap up to approx. 1750 m a.s.l. in the study area, *V. aspis aspis* primarily occurs at lower elevations, whereas *V. berus walser* predominantly inhabits higher altitudes. These results align with existing knowledge of the species' ecology (Ficetola et al. 2020; Di Nicola, 2021a) and corroborate previous research on other European vipers (Mebert et al. 2015; Luiselli et al. 2007; Scali et al. 2011). The elevational gradient is strongly related to temperature, and thus the observed separation might reflect differences in thermal requirements between the two species. Although distinct subspecies and populations can differ in terms of thermal tolerance, asp vipers generally occupy warmer areas compared to *V. berus* clades (*e.g.*, Scali et al. 2011), which inhabit colder environments and are distributed also at higher latitudes. The results of our models additionally indicate a slight difference in habitat use at a fine-grained level. On the one hand, we observed that rocky areas constitute a fundamental microhabitat type for both asp and Walser vipers. Indeed, rock coverage significantly affects the occurrence probability of the two species, with similar estimated trends and a broad overlap in their confidence intervals. On the other hand, shrub coverage does not influence the presence of asp vipers in the study area, whereas it represents a significant variable in explaining the occurrence of the Walser viper. This difference might also be related to the thermal requirements of the two species, as the thermal properties of the site affect the microhabitat selection in reptiles (Martín and Salvador, 1997; Harvey and Weatherhead, 2010; Gifford et al. 2012; Ferreira et al. 2017). Vegetation coverage is in fact negatively correlated with ground temperature, suggesting that bushes can provide a more suitable shelter for the Walser viper compared to the thermophilic asp viper, especially at high altitudes.

The ecological niche overlap in the study area estimated by applying the PCA-env approach is high (*sensu* Rödder and Engler, 2011), amounting to 0.64. This result aligns with our expectation, as we selected the sampling locations on the basis of previous evidence of syntopy between the two species. However, the results of the equivalency test indicate that asp and Walser vipers retain distinct ecological niches.

The seasonal activity patterns of the two species are very similar, with an overlap of 0.84. Both vipers show a bimodal trend, with the first peak occurring around mid-June and the second peak around mid-August. The observed patterns are consistent with the activity periods of most European vipers (*e.g.*, Brito, 2003), reflecting the species' behavior during the reproductive season. However, we refrain from considering our results fully representative of the whole annual activity period. Our sampling indeed does not cover the entire duration of vipers' activity between the hibernation phases. European vipers' activity generally ranges between early spring and middle fall, with marked geographical variations as it mainly depends on daily temperature and snowmelt timing. Nevertheless, our main aim was to compare the activity of the two species during the study period, in order to assess the degree of differentiation between asp and Walser vipers.

Overall, the study results indicate that the syntopy between the two congeneric species is driven by ecological processes depending on environmental conditions at microscale level. First of all, asp and Walser vipers display a high niche overlap and similar activity patterns, suggesting the existence of competitive interactions. Furthermore, rocky areas are shared by the two species as both shelters and basking spots. Rocky microhabitats thus are likely to represent a source of competition between the two congenerics, especially where their altitudinal ranges overlap. This hypothesis is supported by evidence showing that close populations of *V. aspis aspis* in Western Alps can reach higher elevations (up to 2800 m a.s.l.) where *V. berus walser* is absent (Sindaco, 1993; Corti et al. 2011). Therefore, the competition with Walser vipers for microhabitats such as rocky areas may limit the distribution of asp vipers at elevated altitudes where their ranges overlap. However, coexistence between the two species in contact zones is allowed by a differentiation, even limited, in their ecological niches. Despite the high degree of overlap, asp and Walser vipers' niches are in fact not interchangeable. We detected the differential effects of two main environmental factors in explaining the observed partitioning between the two species within the study area. First, the altitudinal optimum, as Walser vipers occur at higher elevation compared to the asp viper range. Second, the microhabitat selection, with *V. berus walser* that inhabits shrubby ecotones whereas *V. aspis aspis* occurrence is not affected by bush coverage. This suggests that shrub availability may mitigate the competition between the two vipers, allowing their co-occurrence. Our results thus highlight the role of the habitat structural complexity at microscale level in promoting the two species' coexistence. In contact zones, competitive interactions are likely more intense where the habitat structure is simplified. In such environments, the presence of the species better adapted to local conditions (*i.e.*, altitude) may lead to the potential exclusion of the other, as previously reported in several cases for vipers (Saint-Girons, 1975; Sindaco, 1993; Scali et al. 2011). On the contrary, strongly diversified environments generally offer more types of shelters and basking spots, facilitating the coexistence between the two species.

Co-occurrence may also be promoted by partitioning in trophic niches. Although we did not investigate the species' diet preferences in our study, European vipers generally display a high degree of similarity in their trophic resources (Luiselli, 2006). Previous studies indicate that both *V. berus* and *V. aspis* predominantly prey on micromammals, with reptiles and amphibians representing a smaller portion of their diets (Luiselli and Anibaldi, 1991; Martínez-Freiría et al. 2010). This suggests that food resources

may constitute an additional source of competition between the two species, which would coexist mainly through the diversification in microhabitat selection. Nevertheless, vipers' diet displays variations depending on age, sex, and season (*e.g.*, Santos et al. 2007). Therefore, further studies on both diet preferences and prey availability are essential to fully understand the extent of trophic overlap between Walser and asp vipers in syntopic areas, and to clarify the interspecific interactions driving their co-occurrence.

From a conservation perspective, the potential competition deriving by the high similarities in asp and Walser vipers' ecological niches may pose a severe threat for the two species, especially for the *V. berus walser* which has a limited distribution. Climate change effects are indeed leading to alpine species shifting their elevation ranges at higher altitudes (Vitasse et al. 2021). This shift may cause an enlargement of the altitudinal overlap between the two species, since lowland species have a higher capacity to shift upward than high-elevation species. As a result, competition is intensified, potentially excluding the Walser viper in its lower-altitude distribution. Moreover, both global warming and the abandonment of traditional livestock farming induce the loss of open vegetation areas through the increase of alpine forest extension (Theurillat and Guisan, 2001; Falcucci et al. 2007). Mountain open areas offer heterogeneous habitats for alpine vipers, with high availability of rock and shrub formations that provide shelters and basking spots. The loss of such suitable habitat thus can lead to a decrease in both range extension and population abundance, as well as a decline in the number of locations where the two species can coexist.

Declarations

Author Contribution (CRediT)

A.P. Conceptualization, Investigation, Data curation, Writing – original draft and Writing – review and editing; L.L. and S.G. Conceptualization, Methodology, Investigation; M.F. Investigation; M.S-P. Investigation, Writing – review and editing; L.G. Conceptualization, Data curation, Formal analysis, Methodology, Writing – original draft and Writing – review and editing. All the authors read and accepted the manuscript before the submission.

Ethics statement

Field activities included standard viper manipulation procedures (capture, measurements and release) conducted under the following permits: MATTM reg. 0141665, 17.12.2021; MiTE reg. 0023649, 17/02/2023; ISPRA Prot. 31037, 11.6.2021.

Declaration of Competing Interest

The authors declare no conflict of interest.

Author Contribution

A.P. Conceptualization, Investigation, Data curation, Writing – original draft and Writing – review and editing; L.L. and S.G. Conceptualization, Methodology, Investigation; M.F. Investigation; M.S-P. Investigation, Writing – review and editing; L.G. Conceptualization, Data curation, Formal analysis, Methodology, Writing – original draft and Writing – review and editing. All the authors read and accepted the manuscript before the submission.

Acknowledgement

The authors would like to express their gratitude to Prof. Massimo Delfino (University of Turin) for his support and guidance, to Prof. Gentile Francesco Ficetola (University of Milan) for his contribution to project planning, and to Prof. Andrea Galimberti (University of Milan) for his assistance with permit acquisition.

Data Availability

The majority of the data generated or analyzed during this study are included within the manuscript and its supplementary information files. However, precise geographical coordinates of the sighting and capture locations are not publicly available due to conservation concerns regarding this rare endemic taxon. These data can be made available from the corresponding author upon reasonable request for legitimate scientific purposes.

References

1. Bernini, F., Bonini, L., Ferri, V., Gentilli, A., Razzetti, E., & Scali, S. (2004). *Atlante degli Anfibi e dei Rettili della Lombardia*. Provincia di Cremona.
2. Brito, J. (2003). Seasonal and daily activity patterns of *Vipera latastei* in northern Portugal. *Amphib-Reptil*, *24*(4), 497–508. http://dx.doi.org/10.1163/156853803322763954
3. Broennimann, O., Fitzpatrick, M. C., Pearman, P. B., Petitpierre, B., Pellissier, L., Yoccoz, N. G., Thuiller, W., Fortin, M. J., Randin, C., Zimmermann, N. E., Graham, C. H., & Guisan, A. (2012). Measuring ecological niche overlap from occurrence and spatial environmental data. *Global Ecology And Biogeography*, *21*, 481–497. http://dx.doi.org/10.1111/j.1466-8238.2011.00698.x
4. Broennimann, O., Petitpierre, B., Randin, C., Engler, R., Cola, V. D., Breiner, F., D'Amen, M., Pellissier, L., Pottier, J., Pio, D., Mateo, R. G., Hordijk, W., Dubuis, A., Scherrer, D., Salamin, N., & Guisan, A. (2015). ecospat: Spatial ecology miscellaneous methods. R package version 1.1. Available at: http://CRAN.R-project.org/package=ecospat
5. Carscadden, K. A., Emery, N. C., Arnillas, C. A., Cadotte, M. W., Afkhami, M. E., Gravel, D., Livingstone, S. W., & Wiens, J. J. (2020). Niche breadth: causes and consequences for ecology, evolution, and

conservation. *The Quarterly Review Of Biology*, *95*(3), 179–214. http://dx.doi.org/10.1086/710388

6. Corti, C., Capula, M., Luiselli, L., Razzetti, E., & Sindaco, R. (2011). *Fauna d'Italia, Reptilia*. Vol. XLV (Calderini.
7. Dezetter, M., Le Galliard, J. F., Guiller, G., Guillon, M., Leroux-Coyau, M., Meylan, S., Brischoux, F., Angelier, F., & Lourdais, O. (2021). Water deprivation compromises maternal physiology and reproductive success in a cold and wet adapted snake *Vipera berus*. *Conserv Physiol*, *9*(1), coab071. https://doi.org/10.1093/conphys/coab071
8. Di Nicola, M. R., Cavigioli, L., Luiselli, L., & Andreone, F. (2021a). *Anfibi & Rettili d'Italia*. Edizioni Belvedere.
9. Di Nicola, M. R., Pontara, A., Kass, G. E. N., Kramer, N. I., Avella, I., Pampena, R., Mercuri, S. R., Dorne, J. L. C. M., & Paolino, G. (2021b). Vipers of major clinical relevance in Europe: taxonomy, venom composition, toxicology and clinical management of human bites. *Toxicology*, *453*, 152724. https://doi.org/10.1016/j.tox.2021.152724
10. Downes, S., & Bauwens, D. (2002). An experimental demonstration of direct behavioural interference in two Mediterranean lacertid lizard species. *Animal Behaviour*, *63*(6), 1037–1046. https://doi.org/10.1006/anbe.2002.3022
11. Doniol-Valcroze, P., Ursenbacher, S., Mebert, K., Ghielmi, S., Laddaga, L., Sourrouille, P., Kariş, M., & Crochet, P. (2021). Conflicting relationships of *Vipera walser* inferred from nuclear genes sequences and mitochondrial DNA. *Jou Of Zoological Systemetics And Evoloutionary Res*, *59*(8), 2307–2320. https://doi.org/10.1111/jzs.12543
12. Dufresnes, C., Ghielmi, S., Halpern, B., Martínez-Freiría, F., Mebert, K., Jelić, D., Crnobrnja-Isailović, J., Gippner, S., Jablonski, D., Joger, U., Laddaga, L., Petrovan, S., Tomović, L., Vörös, J., İğci, N., Kariş, M., Zinenko, O., & Ursenbacher, S. (2024). Phylogenomic insights into the diversity and evolution of Palearctic vipers. *Molecular Phylogenetics And Evolution*, *197*. https://doi.org/10.1016/j.ympev.2024.108095
13. Falcucci, A., Maiorano, L., & Boitani, L. (2007). Changes in land-use/land-cover patterns in Italy and their implications for biodiversity conservation. *Landsc Ecol*, *22*, 617–631. http://dx.doi.org/10.1007/s10980-006-9056-4
14. Ferreira, D., Žagar, A., & Santos, X. (2017). Uncovering the rules of (reptile) species coexistence in transition zones between bioregions. *Salamandra* 53(2).
15. Ficetola, G. F., Fanelli, M., Garizio, L., Falaschi, M., Tenan, S., Ghielmi, S., Laddaga, L., Menegon, M., & Delfino, M. (2020). Estimating abundance and habitat suitability in a micro-endemic snake: the Walser viper. *Acta Herpetol*, *15*(2), 73–85. https://doi.org/10.13128/A_H-7771
16. Ghielmi, S., Menegon, M., Marsden, S. J., Laddaga, L., & Ursenbacher, S. (2016). A new vertebrate for Europe: the discovery of a range-restricted relict viper in the western Italian Alps. *Jou Of Zoological Systemetics And Evoloutionary Res*, *54*(3), 161–173. https://doi.org/10.1111/jzs.12138
17. Gifford, M. E., Clay, T. A., & Powell, R. (2012). Habitat use and activity influence thermoregulation in a tropical lizard, *Ameiva exsul*. *Journal Of Thermal Biology*, *37*(7), 496–501.

https://doi.org/10.1016/j.jtherbio.2012.05.003

18. Golay, P., Monney, J-C., Conelli, A., Durand, T., Thiery, G., Zuffi, M. A. L., & Ursenbacher, S. (2008). Systematics of the Swiss asp vipers: some implications for the European *Vipera aspis* (Linnaeus, 1758) complex (Serpentes: Viperidae) – A tribute to Eugen Kramer. *Amphib-Reptil*, *29*(1), 71–83. https://doi.org/10.1163/156853808783431460
19. Hardin, G. (1960). The competitive exclusion principle. *Science*, *131*(3409), 1292–1297. http://dx.doi.org/10.1126/science.131.3409.1292
20. Harvey, P. H., & Pagel, M. D. (1991). The comparative method in evolutionary biology. Oxford university press. http://dx.doi.org/10.1093/oso/9780198546412.001.0001
21. Harvey, D. S., & Weatherhead, P. J. (2010). Habitat selection as the mechanism for thermoregulation in a northern population of massasauga rattlesnakes (*Sistrurus catenatus*). *Écoscience*, *17*(4), 411–419. http://dx.doi.org/10.2980/17-4-3363
22. Himes, J. G. (2003). Intra- and interspecific competition among the water snakes *Nerodia sipedon* and *Nerodia rhombifer*. *J Herpetol*, *37*(1), 126–131. http://dx.doi.org/10.1670/0022-1511037%5B0126:IAICAT%5D2.0.CO;2.
23. Houadria, M., Salas-Lopez, A., Orivel, J., Blüthgen, N., & Menzel, F. (2015). Dietary and temporal niche differentiation in tropical ants—can they explain local ant coexistence? *Biotropica*, *47*(2), 208–217. https://doi.org/10.1111/btp.12184
24. Hutchinson, G. E. (1957). Concluding remarks. *Cold Spring Harbor Symposia On Quantitative Biology*, *22*, 415–427. https://doi.org/10.1101/SQB.1957.022.01.039
25. Jackson, S. T., & Overpeck, J. T. (2000). Responses of plant populations and communities to environmental changes of the late Quaternary. *Paleobiology*, *26*(S4), 194–220. http://dx.doi.org/10.1017/S0094837300026932
26. Kingsbury, K. M., Gillanders, B. M., Booth, D. J., & Nagelkerken, I. (2020). Trophic niche segregation allows range-extending coral reef fishes to co-exist with temperate species under climate change. *Glob Chang Biol*, *26*(2), 721–733. https://doi.org/10.1111/gcb.14898
27. Knouft, J. H., Losos, J. B., Glor, R. E., & Kolbe, J. J. (2006). Phylogenetic analysis of the evolution of the niche in lizards of the *Anolis sagrei* group. *Ecology*, *87*(sp7). https://doi.org/10.1890/0012-9658(2006)87[29:PAOTEO]2.0.CO;2S29-S38.
28. Losos, J. B., Leal, M., Glor, R. E., de Queiroz, K., Hertz, P. E., Rodríguez Schettino, L., Lara, A. C., Jackman, T. R., & Larson, A. (2003). Niche lability in the evolution of a Caribbean lizard community. *Nature*, *423*, 542–545. http://dx.doi.org/10.1038/nature01814
29. Losos, J. B. (2008). Phylogenetic niche conservatism, phylogenetic signal and the relationship between phylogenetic relatedness and ecological similarity among species. *Ecology Letters*, *11*(10), 995–1003. http://dx.doi.org/10.1111/j.1461-0248.2008.01229.x
30. Lucek, K., Butlin, R. K., & Patsiou, T. (2020). Secondary contact zones of closely-related *Erebia* butterflies overlap with narrow phenotypic and parasitic clines. *Journal Of Evolutionary Biology*, *33*(9), 1152–1163. http://dx.doi.org/10.1111/jeb.13669

31. Luiselli, L. M., & Anibaldi, C. (1991). The diet of the adder (*Vipera berus*) in two alpine environments. *Amphib-Reptil*, *12*(2), 214–217. http://dx.doi.org/10.1163/156853891X00194
32. Luiselli, L. M. (2006). Resource partitioning and interspecific competition in snakes: the search for general geographical and guild patterns. *Oikos*, *114*(2), 193–211. https://doi.org/10.1111/j.2006.0030- 1299.14064.x
33. Luiselli, L., Filippi, E., & Di Lena, E. (2007). Ecological relationships between sympatric *Vipera aspis* and *Vipera ursinii* in high-altitude habitats of central Italy. J Herpetol 41(3):378–384. https://doi.org/10.1670/0022-1511(2007)41[378:ERBSVA]2.0.CO;2.
34. Martín, J., & Salvador, A. (1997). Microhabitat selection by the Iberian rock lizard *Lacerta monticola*: effects on density and spatial distribution of individuals. *Biological Conservation*, *79*(2), 303–307. https://doi.org/10.1016/0006-3207(95)00110-7
35. Martínez-Freiría, F., Lizana, M., do Amaral, J. P., & Brito, J. C. (2010). Spatial and temporal segregation allows coexistence in a hybrid zone among two Mediterranean vipers (*Vipera aspis* and *V. latastei*). Amphib-Reptil 31(2):95–212. https://doi.org/10.1163/156853810791069001
36. McCain, C. M. (2010). Global analysis of reptile elevational diversity. *Global Ecology And Biogeography*, *19*(4), 541–553. http://dx.doi.org/10.1111/j.1466-8238.2010.00528.x
37. Mebert, K., Jagar, T., Grželj, R., Cafuta, V., Luiselli, L. M., Ostanek, E., Golay, P., Dubey, S., Golay, J., & Ursenbacher, S. (2015). The dynamics of coexistence: habitat sharing versus segregation patterns among three sympatric montane vipers. *Biological Journal Of The Linnean Society*, *116*(2), 364–376. https://doi.org/10.1111/bij.12582
38. Pastore, A. I., Barabás, G., Bimler, M. D., Mayfield, M. M., & Miller, T. E. (2021). The evolution of niche overlap and competitive differences. *Nat Ecol Evol*, *5*(3), 330–337. http://dx.doi.org/10.1038/s41559-020-01383-y
39. Pearman, P. B., Guisan, A., Broennimann, O., & Randin, C. F. (2008). Niche dynamics in space and time. *Trends In Ecology & Evolution*, *23*(3), 149–158. http://dx.doi.org/10.1016/j.tree.2007.11.005
40. Peterson, A. T. (2011). Ecological niche conservatism: A time-structured review of evidence. *Journal Of Biogeography*, *38*(5), 817–827. http://dx.doi.org/10.1111/j.1365-2699.2010.02456.x
41. Pigot, A. L., Trisos, C. H., & Tobias, J. A. (2016). Functional traits reveal the expansion and packing of ecological niche space underlying an elevational diversity gradient in passerine birds. *Proc R Soc B*, *283*(1822), 20152013. http://dx.doi.org/10.1098/rspb.2015.2013
42. Rice, N. H., Martínez-Meyer, E., & Peterson, A. T. (2003). Ecological niche differentiation in the *Aphelocoma* jays: a phylogenetic perspective. *Biological Journal Of The Linnean Society*, *80*(3), 369–383. http://dx.doi.org/10.1046/j.1095-8312.2003.00242.x
43. Rivas, L. R. (1964). A Reinterpretation of the concepts sympatric and allopatric with proposal of the additional terms syntopic and allotopic. *Systematic Zoology*, *13*(1), 42–43. http://dx.doi.org/10.2307/2411436
44. Rödder, D., & Engler, J. O. (2011). Quantitative metrics of overlaps in Grinnellian niches: advances and possible drawbacks. *Global Ecology And Biogeography*, *20*(6), 915–927.

http://dx.doi.org/10.1111/j.1466-8238.2011.00659.x

45. Rodríguez, J. P., Keith, D. A., Rodríguez-Clark, K. M., Murray, N. J., Nicholson, E., Regan, T. J., Miller, R. M., Barrow, E. G., Bland, L. M., Boe, K., Brooks, T. M., Oliveira-Miranda, M. A., Spalding, M., & Wit, P. (2015). A practical guide to the application of the IUCN Red List of Ecosystems criteria. *Phil Trans R Soc B*, *370*(1662), 20140003. https://doi.org/10.1098/rstb.2014.0003
46. Saint-Girons, H. (1952). Écologie et éthologie des Vipères de France. *Annales Des Sciences Naturelles - Zoologie Et Biologie Animale*, *14*, 263–343.
47. Saint-Girons, H. (1975). Coexistence de *Vipera aspis* et de *Vipera berus* en Loire-Atlantique: un problème de compétition interspécifique. *Rev Ecol (Terre Vie)*, *4*, 590–613. http://dx.doi.org/10.3406/revec.1975.4904
48. Santos, X., Llorente, G., Pleguezuelos, J., Brito, J., Fahd, S., & Parellada, X. (2007). Variation in the diet of the Lataste's viper *Vipera latastei* in the Iberian Peninsula: seasonal, sexual and size-related effects. *Animal Behaviour*, *57*(1), 49–61. http://dx.doi.org/10.1163/157075607780001998
49. Scali, S., Mangiacotti, M., Sacchi, R., & Gentilli, A. (2011). A tribute to Hubert Saint Girons: niche separation between *Vipera aspis* and *V. berus* on the basis of distribution models. *Amphib-Reptil*, *32*(2), 223–233. https://doi.org/10.1163/017353711X562171
50. Schmidtler, J. F., & Hansbauer, G. (2020). Die alpenkreuzotter (*Vipera berus marasso*), eine neue unterart in den Bayerischen Alpen. *Zeitschrift Für Feldherpetologie*, *27*, 136–148.
51. Schoener, T. W. (1974). Resource partitioning in ecological communities. *Science*, *185*(4145), 27–39. http://dx.doi.org/10.1126/science.185.4145.27
52. Sexton, J. P., McIntyre, P. J., Angert, A. L., & Rice, K. J. (2009). Evolution and ecology of species range limits. *Annual Review Of Ecology Evolution And Systematics*, *40*(1), 415–436. https://doi.org/10.1146/annurev.ecolsys.110308.120317
53. Sindaco, R. (1993). Anfibi e rettili della Valle d'Aosta: sintesi bibliografica e dati inediti. *Revue Valdôtaine d'Histoire Naturelle*, *47*, 141–153.
54. Sindaco, R., Doria, G., Razzetti, E., & Bernini, F. (2006). *Atlas of Italian amphibians and reptiles*. Edizioni Polistampa, Societas Herpetologica Italica.
55. Slatyer, R. A., Hirst, M., & Sexton, J. P. (2013). Niche breadth predicts geographical range size: a general ecological pattern. *Ecology Letters*, *16*(8), 1104–1114. http://dx.doi.org/10.1111/ele.12140
56. Tarquini, S., Vinci, S., Favalli, M., Doumaz, F., Fornaciai, A., & Nannipieri, L. (2012). Release of a 10-mresolution DEM for the Italian territory: comparison with global-coverage DEMs and anaglyphmode exploration via the web. *Computers & Geosciences*, *38*(1), 168–170. https://doi.org/10.1016/j.cageo.2011.04.018
57. Theurillat, J. P., & Guisan, A. (2001). Potential impact of climate change on vegetation in the European Alps: a review. *Climate Change*, *50*(1), 77–109. 10.1023/A:1010632015572
58. Toft, C. A. (1985). Resource partitioning in amphibians and reptiles. Copeia, 1985(1):1–21. https://doi.org/10.2307/1444785

59. Ursenbacher, S., Conelli, A., Golay, P., Monney, J-C., Zuffi, M. A. L., Thiery, G., Durand, T., & Fumagalli, L. (2006). Phylogeography of the asp viper (*Vipera aspis*) inferred from mitochondrial DNA sequence data: evidence for multiple Mediterranean refugial areas. *Molecular Phylogenetics And Evolution*, *38*(2), 546–552. https://doi.org/10.1016/j.ympev.2005.08.004
60. Vitasse, Y., Ursenbacher, S., Klein, G., Bohnenstengel, T., Chittaro, Y., Delestrade, A., Monnerat, C., Rebetez, M., Rixen, C., Strebel, N., Schmidt, B. R., Wipf, S., Wohlgemuth, T., Yoccoz, N. G., & Lenoir, J. (2021). Phenological and elevational shifts of plants, animals and fungi under climate change in the European Alps. *Biological Reviews*, *96*(5), 1816–1835. https://doi.org/10.1111/brv.12727
61. Warren, D. L., Glor, R. E., & Turelli, M. (2008). Environmental niche equivalency versus conservatism: quantitative approaches to niche evolution. *Evolution*, *62*(11), 2868–2883. https://doi.org/10.1111/j.1558-5646.2008.00482.x
62. Wereszczuk, A., & Zalewski, A. (2015). Spatial niche segregation of sympatric stone marten and pine marten – avoidance of competition or selection of optimal habitat? *PLoS One*, *10*(10), e0139852. https://doi.org/10.1371/journal.pone.0139852
63. Wiens, J. J., & Graham, C. H. (2005). Niche conservatism: integrating evolution, ecology, and conservation biology. *Annual Review Of Ecology Evolution And Systematics*, *36*(1), 519–539. http://dx.doi.org/10.1146/annurev.ecolsys.36.102803.095431
64. Wood, S., & Wood, M. S. (2015). Package 'mgcv'. *R package version*, *1*(29), 729.

Figures

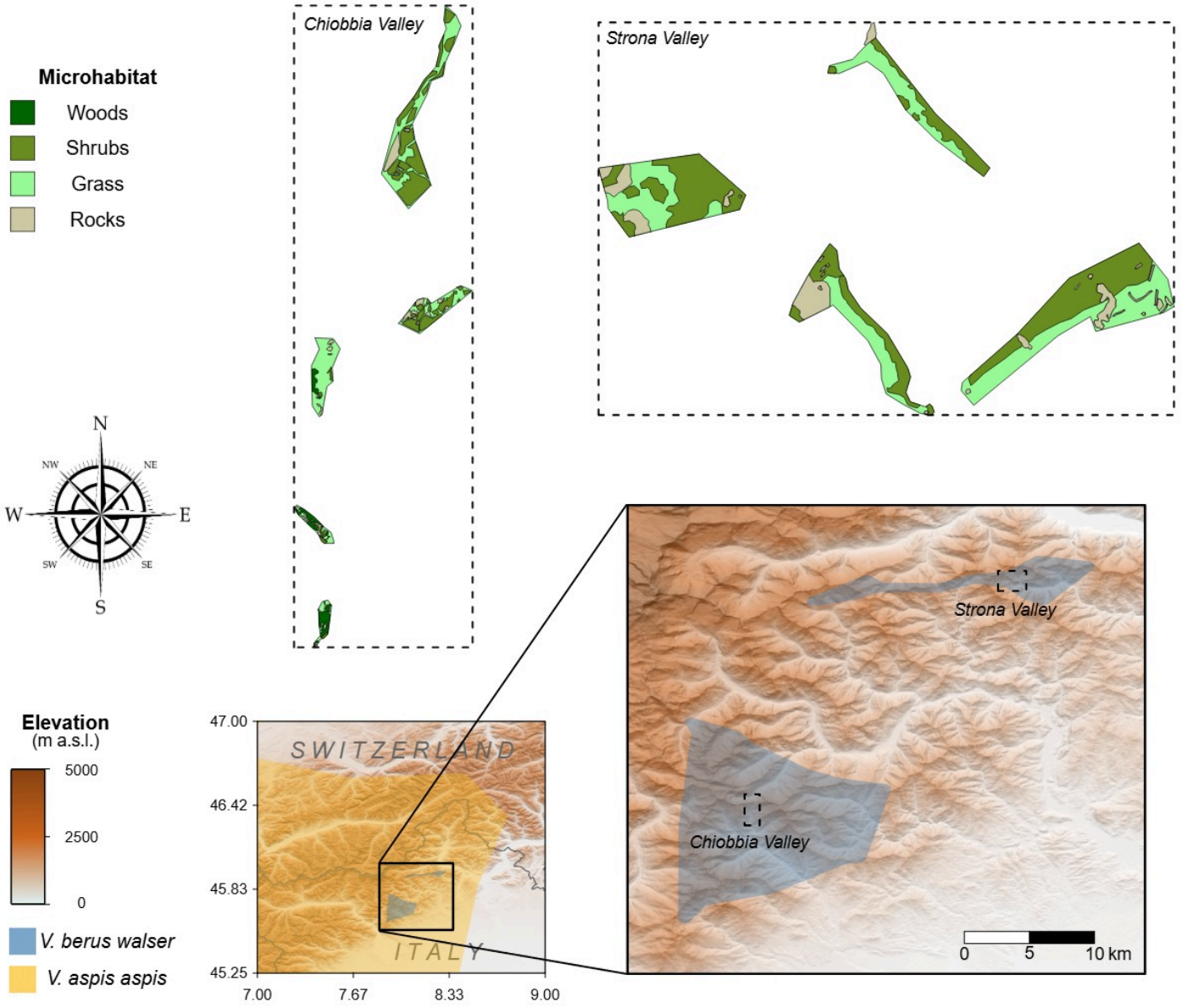


Figure 1

Hillshade map of the study area. Polygons report the distributions of *V. berus walser* (in light blue, see Ficetola et al. 2020) and *V. aspis aspis* (in orange, see Ursenbacher et al. 2006) in the Western Alps. The position of the two sampling locations (Strona Valley - 4 sampling sites - and Chiobbia Valley - 5 sampling sites) is indicated by the dashed rectangles. The exact coordinates of the sampling sites are not reported for conservation purposes. The microhabitat types identified within the sampling sites are visualized by different colors (woods: dark green; shrubs: green; grass: light green; rocks: grey).

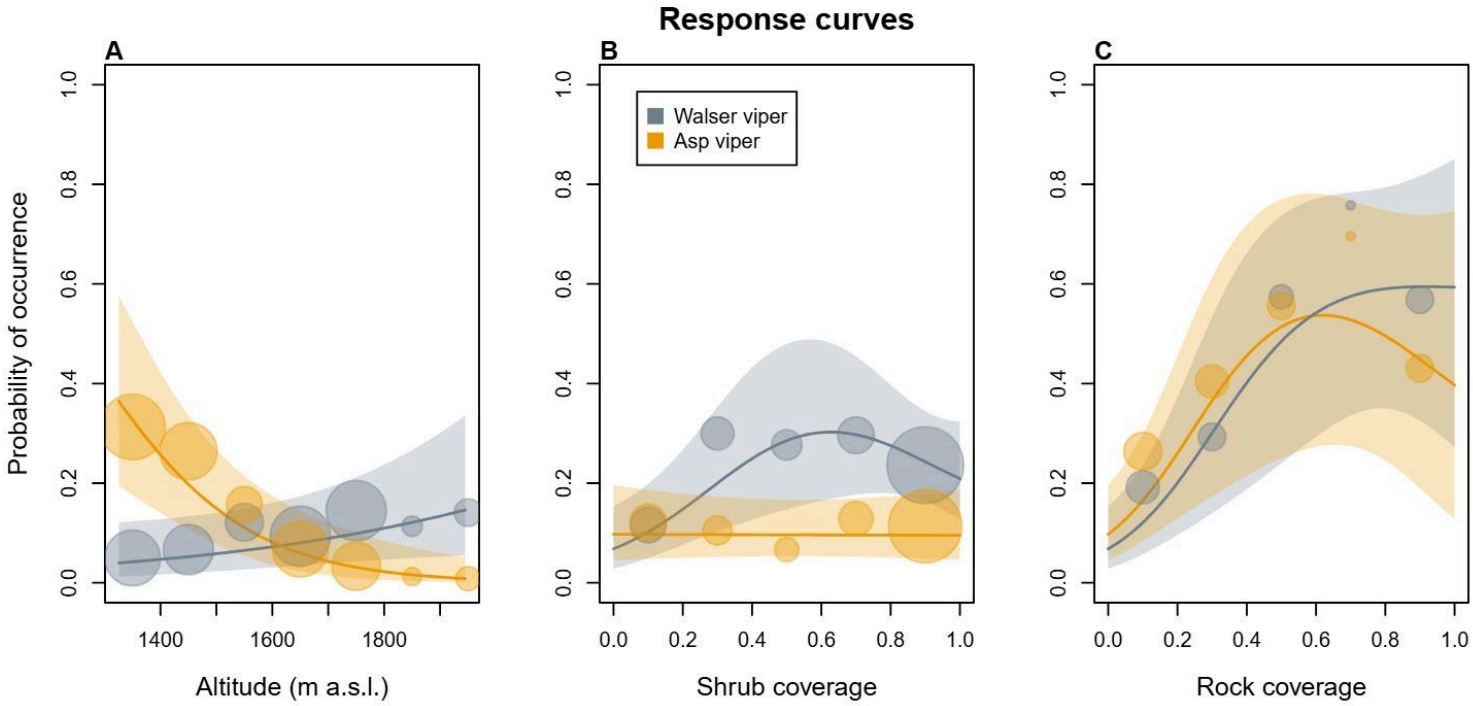


Figure 2

Occurrence probability of *Vipera berus walser* (light blue) and *V. aspis aspis* (orange) in response to the environmental variables estimated by fitting Generalized Additive Models (GAMs). The altitude (A) was set as a linear predictor, whereas the proportions of shrubs (B) and rocks (C) within a 5-m-radius buffer were included as smooth terms. Points represent the partial residuals estimated by GAMs, with sizes proportional to the number of vipers detected under each corresponding condition. Shaded areas indicate the 95% confidence intervals.

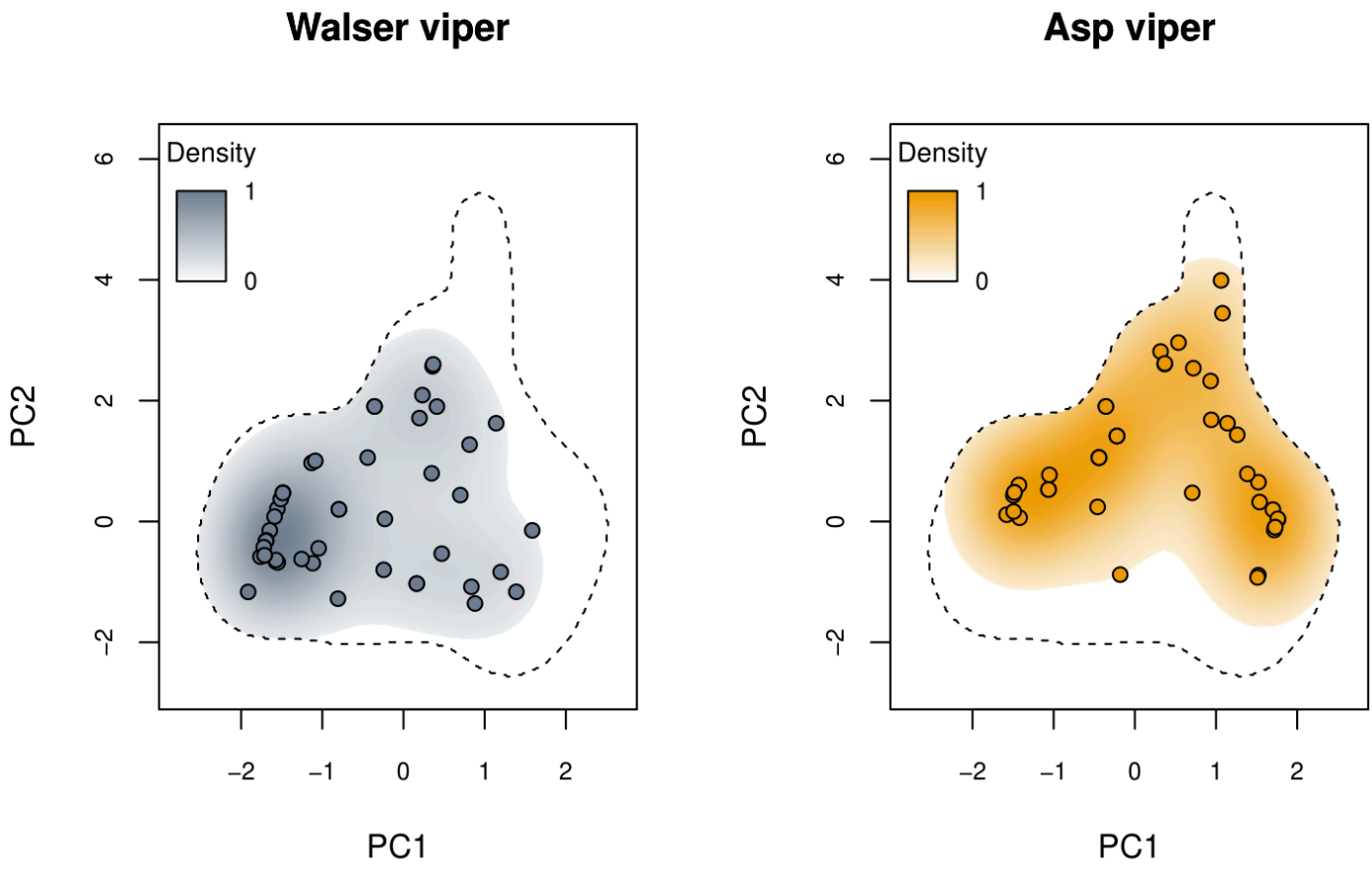


Figure 3

Realized niches of *Vipera berus walser* (light blue) and *V. aspis aspis* (orange) in the study area. Points report the occurrences of the two species in the PCA bidimensional space. Shaded areas indicate the density of occurrences by cell, with darker shading representing higher density values. Dashed contour lines illustrate the whole available environment.

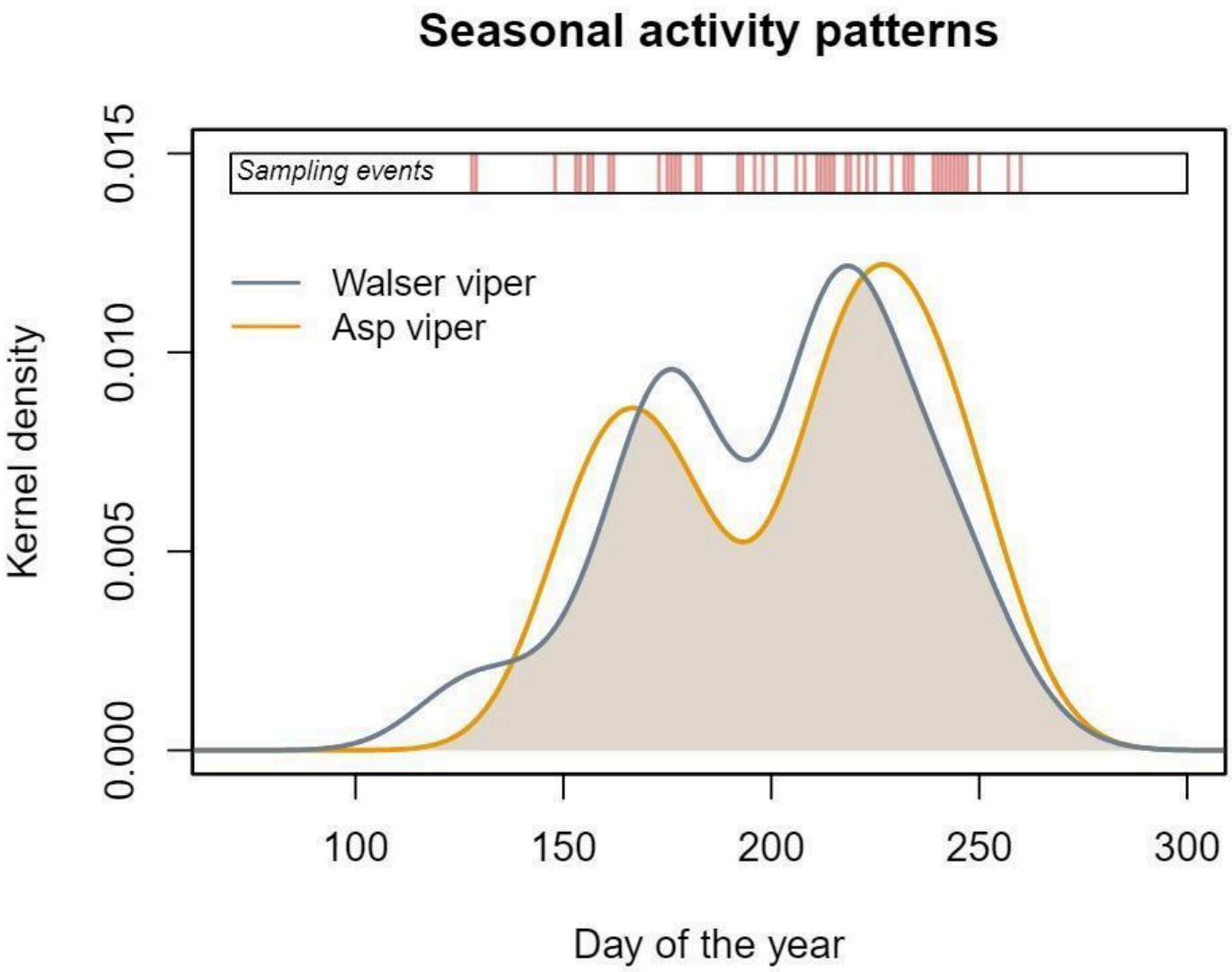


Figure 4

Seasonal activity patterns of *Vipera berus walser* (light blue) and *V. aspis aspis* (orange) during the sampling period. Lines report the densities of encounter events for both species. Gray area highlights the overlap between the two densities. Red lines in the upper bar report the sampling events conducted during the season.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- PrezzietalSupplementaryMaterials.docx