

Transgressive niche across a salamander hybrid zone revealed by microhabitat analyses

Running title: transgressive niche in hybrid zones

Gentile Francesco Ficetola^{1,2}, Enrico Lunghi^{3,4,5,6}, Roberta Cimmaruta⁷, Raoul Manenti^{1*}

1 Department of Environmental Science and Policy, Università degli Studi di Milano, Milano, Italy

2 Laboratoire d'Ecologie Alpine (LECA), CNRS, Université Grenoble Alpes, Grenoble, France

3 Universität Trier Fachbereich VI Raum-und Umweltwissenschaften Biogeographie, Universitätsring 15, 54286 Trier, Germany

4 Museo di Storia Naturale dell'Università di Firenze, Sezione di Zoologia "La Specola", Via Romana 17, 50125 Firenze, Italy

5 Natural Oasis, Via di Galceti 141, 59100 Prato, Italy

6 Institute of Zoology, Chinese Academy of Sciences

7 Dipartimento di Scienze Ecologiche e Biologiche, Università della Tuscia, Viterbo, Italy

* Corresponding author

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Abstract

Aim: Hybrid populations can have intermediate, conserved or transgressive niches, compared to the parental species. Fine-scale analyses can improve our understanding of niche evolution, but information on microhabitat differences between parental species and hybrids is extremely scarce for animal populations. We assessed the pattern and role of niche variation along a hybrid zone, by investigating differences in microhabitat selection between terrestrial salamander species (*Hydromantes ambrosii* and *H. italicus*) and their hybrids. We tested whether introgressed populations show intermediate, conserved or transgressive niche, compared to parental species. Furthermore, we compared body condition index among individuals observed in different microhabitats (different areas of caves), to assess the potential advantages of exploiting specific microhabitats.

Location: We surveyed ~700 cave sectors in Italy, across the whole range of the two parental species and along their contact zone

Methods: We recorded salamander distribution and measured microhabitat features influencing salamander distribution: humidity, temperature and incident light. We tested niche differences between parental species and hybrids, and analysed spatial variation of body condition.

Results: We detected significant niche shifts between parental species and hybrids. Introgressed populations showed a transgressive niche and exploited sectors with warmer temperature, lower humidity and more light, compared to the non-introgressed ones. Introgressed salamanders were better able to exploit the microhabitat conditions that are frequently found nearby cave entrance. For males, body condition index was significantly higher in individuals found close to the cave entrance.

Main conclusions: The transgressive niche of hybrids between *H. ambrosii* and *H. italicus* indicates niche expansion toward harsh environmental conditions, and such transgressive segregation allows exploiting environments with higher food availability. The ability to exploit dry and warm sectors can provide important advantages to hybrid populations, that can better persist under stressful conditions. Transgressive niche can be important for long-term persistence and evolution within hybrid zones.

Keywords: Amphibians; microclimate; niche expansion; niche similarity; Transgressive niche; underground environment

1 INTRODUCTION

Since Mayr's definition of species as 'interbreeding natural populations that are reproductively isolated from other such groups' (Mayr, 1942), the existence of hybrid zones between different species has been regarded as a central topic of evolutionary biology, as hybrid zones can determine the collapse of reproductive isolation (Hewitt, 2001; Tanaka, 2007). Genomic evidence has increasingly revealed the importance of hybridization and interspecific gene flow during speciation events (e.g. Soubrier et al., 2016; Arnason et al., 2018; Palkopoulou et al., 2018), and hybridization is now considered a major constructive force of evolution, that can even promote adaptive radiations (Mallet, 2007; Ficetola & Stock, 2016; Bay & Rugg, 2017). Hybrid zones often occur between the ranges of closely related species, and can help to understand the evolutionary importance of hybridization events (Otis et al., 2017).

The long term fate of hybrid zones strongly depends on the fitness of hybrids, and on occurrence of ecological and reproductive barriers. On the one hand, hybrids can have limited fitness compared to parental species. This is expected to limit the extent of hybrid zones, and can even prompt evolution of pre-zygotic reproductive barriers (Seehausen, 2004). On the other hand, gene flow between related species can also be a source of potentially beneficial alleles. For example, the different stages of introgression recorded between Darwin's finch species having different beaks size can play a strong adaptive role during long droughts (Grant & Grant, 2016). The high mortality recorded during dry seasons produced a strong selective pressures on finches with large beak size, favoured hybridisation between species (Grant & Grant, 2016) and supported idea that contact zones are great opportunities to assess the adaptive role of genetic introgression (Bay & Rugg, 2017).

The genetic aspects involved in the occurrence and maintenance of hybrid zones are increasingly studied (Arntzen *et al.*, 2017), while less attention has been paid to the characterization of the ecological niche of hybrid populations. Differences in ecological niche between closely-related species may be impressive, but limited information exists on niche differences between hybrid populations and parental species, and how they may be related to introgression phenomena (Arntzen *et al.*, 2017). Hybrid populations can have conserved, intermediate, or transgressive niches, compared to the parental species. Conserved niches occur when the niches of hybrids are extremely similar to one of the parental species (Ficetola & Stock, 2016). However, hybrids are often assumed to have intermediate niche compared to their parental species (intermediate niche hypothesis) (Moore, 1977). Finally, the transgressive niche hypothesis proposes that hybrid populations can show niches that differ from those of both parental species (Rieseberg *et al.*, 1999; Ficetola & Stock, 2016). Assessing whether the niches of hybrids are conserved, intermediate or transgressive is extremely important to predict the fate of hybrid populations, and to understand long-term dynamics of species ranges. For instance, transgressive niches can allow hybrids to exploits environmental features that are unsuitable for both parental species. Transgressive niches can thus provide fitness advantages under some environmental conditions, potentially promoting hybrid speciation and expansion to new geographic areas.

The study of ecological niche in hybrid zones is challenging. In the last years, studies on niche evolution increasingly focused on the classical Grinnelian niche, as scenopoetic variables (e.g. temperature, humidity, terrain aspect) are key parameters determining species

distribution and fitness (Soberon & Nakamura, 2009; Slatyer *et al.*, 2013). The Grinnelian niche is often analysed on the basis of a macroecological broad-scale (Soberon & Nakamura, 2009). Macroecological analyses are powerful in that they allow patterns to be drawn over large taxonomic and geographic extents, but also have some limitations. First, they frequently have a coarse resolution (Beck *et al.*, 2012; Lira-Noriega *et al.*, 2013), but species often interact with their environment at finer (microhabitat) scale. Microhabitat conditions are not always captured by macroecological variables, and can provide a more accurate description of the effect of environmental variation on individuals (Scheffers *et al.*, 2014; Ficetola *et al.*, 2018a; Moore *et al.*, 2018). Second, hybrid zones often occur between the geographical ranges of parental species and have limited extensions (Garrick *et al.*, 2014). Macroecological data can be subject to strong spatial autocorrelation, thus making it difficult to disentangle biological patterns from patterns caused by the geographical proximity of localities (Warren *et al.*, 2014). Fine-scale analyses can improve our understanding of niche evolution, providing information on the microhabitat conditions selected by individuals (Ficetola *et al.*, 2018a; Moore *et al.*, 2018). Therefore, microhabitat variables can provide extremely useful data on niche divergences between hybrids and parental species. However, such fine-scale information remains limited, with most of studies performed on plants (Rieseberg *et al.*, 1999).

European terrestrial salamanders (genus *Hydromantes*; see (Wake, 2013) for taxonomical discussion) are active at the surface during cool and wet seasons (from autumn to spring), while exploit underground environments during dry and hot periods (late spring and summer) (Manenti, 2014; Lunghi *et al.*, 2015). These salamanders are at thermal equilibrium with the environment (Lunghi *et al.*, 2016) and, when underground, they select sectors with microclimatic conditions fitting their physiological requirements (e.g. temperature below the tolerance threshold, high humidity and lack of light (Ficetola *et al.*, 2012; Lunghi *et al.*, 2016). Microhabitat features at which these salamanders are observed during the stressful summer periods provide clear information on their operational temperature and other requirements (Ficetola *et al.*, 2018b), thus *Hydromantes* are an excellent study system to analyse niche evolution at the microhabitat level. Moreover caves and subterranean habitats can be very useful system to understand factors determining species distribution and to fill important knowledge gaps (Mammola & Leroy, 2018).

Here, we aim to understand the potential role of niche for fitness variation along hybrid zones, by investigating differences in microhabitat selection between terrestrial salamander species (*Hydromantes ambrosii* and *H. italicus*) and their hybrids. *Hydromantes ambrosii* and *H. italicus* show a narrow (<20 km), hybrid zone derived from a secondary contact event, possibly occurred after the last glacial maximum (Fig. 1; Ruggi *et al.*, 2005). Within this hybrid zone populations of both *H. ambrosii* and *H. italicus* are introgressed with foreign alleles, showing recombinant genotypes without parental specimens neither F1 hybrids (Nascetti *et al.*, 1996; Ruggi *et al.*, 2005). First, we tested whether introgressed populations show intermediate or conserved environmental requirements, compared to the parental species, or exploit different habitat conditions (transgressive niche hypothesis), in order to assess the importance of fine-scale niche variation for the differentiation between hybrids and parental species. Second, we compared body condition index (BCI) among individuals observed in different areas of caves, to assess the potential advantages of selecting

specific sectors. Sectors close to the cave entrance are generally more rich in prey (Ficetola *et al.*, 2018a; Lunghi *et al.*, 2018a) and we expect that they are associated with higher BCI. Our study highlights how selecting transgressive microhabitats can affect the fate of hybrid zones.

2 MATERIALS AND METHODS

2.1 Study sites and sampling

We surveyed 121 natural and artificial caves along the hybrid contact zone and across the whole range of the two parental species (*Hydromantes ambrosii* and *H. italicus*; Fig. 1a). Surveys were performed in early summer (June-July 2012-2016), when conditions outside the caves are unfavorable and the detection of salamanders active underground is highest (Lunghi *et al.*, 2015). We performed all the surveys during the central hours of dry and sunny days. We subdivided each cave in 3-m longitudinal intervals (hereafter: sectors), as this size approximately corresponds to the *Hydromantes* home ranges (Salvidio *et al.*, 1994; Lanza *et al.*, 2006). Underground environments were explored until the end or until the point where the progression was impossible without speleological equipment. In each sector, we used visual encounter surveys (Crump & Scott, 1994) to detect active salamanders and measured three abiotic variables known to influence their distribution: relative humidity (%), air temperature (°C) and maximum incident light (illuminance, measured in lux) (Lunghi *et al.*, 2015). Air temperature and humidity were recorded using a LAFAYETTE TDP-92 thermo-hygrometer (accuracy: 0.1°C and 0.1%). Illuminance was recorded using a EM882 light-meter (PCE Instruments; minimum detectable light: 0.01 lux), by performing >10 measures in the portions of the sector receiving more light. See (Ficetola *et al.*, 2012; Ficetola *et al.*, 2013) for additional details on sampling methods. Caves were similar in structure and the same microhabitats were available in all caves. To quantify salamanders microhabitat selection, measurements covered the whole cave if salamanders were found at the end of the cave, or were performed or until the first empty sector after the deepest salamander.

A subset of observed individuals was captured by hand, measured (total length; mm), weighed (accuracy: 0.1g) and sexed. Adult males were identified on the basis of sexual characters (mental gland and premaxillary teeth); individuals without male sexual characters but ≥ 73 mm were considered adult females, as 73 mm was the size of the smallest observed adult males (see results).

2.2 Statistical analyses

Definition of the genetic status of study populations

In order to assign sample sites into groups based on the degree of introgression, existing genetic data (allozymes from muscle tissue) were used (Ruggi *et al.*, 2005; Ruggi, 2007). These data are the most complete genetic sampling so far available and include dense sampling within the contact zone. Ruggi *et al.* (2005; 2007) analysed 24 allozyme loci at 646 individuals from 39 populations covering the whole range of both species; 18 of these populations (385 individuals) were within the hybrid zone (Fig. 1c, Supplementary Table S1, Fig. S1). Seven out of the 24 allozymes were discriminating between the two species; see

Appendix S1 for details on methods used for genetic analyses. Allozyme data have some limitations, since they have lower variability than more recent markers, some loci may be under selection, and they can be subject to gene expression in the tissue type that was used in the analysis (Schlötter, 2004). Even though the panel of available molecular tools is quickly broadening, the conclusions obtained using allozymes continue to be deemed reliable (Allendorf, 2017). Unfortunately, no microsatellites are currently available for European *Hydromantes*. Furthermore, salamanders have huge genomes with very high levels of duplication, which makes it challenging the application of approaches based on high-throughput sequencing (but see: Wielstra *et al.*, 2014; Newman & Austin, 2016; McCartney-Melstad *et al.*, 2018). For these reasons we relied on available allozyme data.

The mean allelic frequencies and the values of the Introgression Index I were used to assign the study populations to four genetic groups; pure populations of 1) *H. ambrosii* and 2) *H. italicus*, each with mean frequencies of introgressed alleles $<10\%$ and $I < 5\%$ (Gerchen *et al.*, 2018), 3) introgressed *H. ambrosii* populations, with mean allele frequencies of *H. italicus* genes $>10\%$ and $I_{italicus} > 5\%$, 4) introgressed *H. italicus* populations with $>10\%$ of *H. ambrosii* alleles and $I_{ambrosii} > 5\%$ (see Table S1). Several populations surveyed for ecological parameters were not the same studied for genetics, but all study populations were geographically close to the genotyped ones. Within the hybrid zone, the geographical distance between ecologically and genetically studied populations was always small (average \pm SD: 746 ± 590 m). Given the strong spatial pattern of introgression (Fig. 1c), we assumed that each study site had genetic features similar to the nearest population with available genetic data (Table S1).

Niche variation

First, we used generalized linear mixed models (GLMM; binomial error distribution) to assess the relationships between the distribution of salamanders of each group and the recorded microhabitat features. In GLMM, detection/non detection was the dependent variable, microhabitat features (temperature, humidity and light) were the independent variables, and cave identity was included as random effect. Non-detection of salamanders in a given sector may represent either a real absence or a failure in detection, and not taking into account potential misdetections can influence regression results (MacKenzie *et al.*, 2006). With our sampling protocol, detection probability is high but lower than one (approx. 0.75 per visit) (Ficetola *et al.*, 2012; Lunghi *et al.*, 2015). Therefore, in our models absences received a weight of 0.75 (following Gomez-Rodriguez *et al.*, 2012).

We built models with all the combinations of independent variables, and ranked them using the Akaike's Information Criterion (AIC) to identify the minimum adequate model for each species, i.e. the model explaining more variation with fewer variables (Burnham & Anderson, 2002). In caves there was a strong negative correlation between air temperature and humidity (Pearson's $r = -0.6$, $P < 0.0001$), and such collinearity can bias the results of regression analyses (Graham, 2003). Therefore, models including both these correlated variables were excluded from the list of candidate models. For each candidate model, we also calculated the AIC weight (w) (Lukacs *et al.*, 2007). Before analyses, light intensity was log-transformed, while humidity (%) was square-root-arcsine transformed to improve normality and reduce skewness. GLMM were fit using the lme4 package (Bates *et al.*, 2015).

We then used the PCA-env approach (Broennimann *et al.*, 2012; Petitpierre *et al.*, 2012; Di Cola *et al.*, 2017) to assess niche shifts between pure and introgressed salamander populations. First, we built univariate plots, to compare the frequency distribution of pure and introgressed populations under the range of available environmental conditions. Subsequently, we performed multivariate niche comparisons on the basis of Principal Component Analyses of environmental conditions (PCA-env), using the three microhabitat features measured in cave sectors (air temperature, relative humidity and maximum incident light). Niche overlap was calculated using the Schoener's *D* statistics (Schoener, 1970; Warren *et al.*, 2008b), which ranges between 0 (lack of overlap) and 1 (complete overlap). Schoener's *D* shows excellent performance compared to other overlap metrics (Warren *et al.*, 2008a; Rodder & Engler, 2011), and is among the most widespread metrics of niche overlap in ecological, evolutionary and biogeographical studies. The niche expansion of introgressed populations compared to the parental ones was the proportion of the occurrence density of introgressed populations that lay outside the conditions occupied in the native distribution (Di Cola *et al.*, 2017)

We used equivalency tests (Warren *et al.*, 2008a; Broennimann *et al.*, 2012) to assess the significance of niche differences between taxa (999 random permutations). Equivalency tests determine whether the niches of two taxa in two geographical ranges are equivalent, and a significant equivalency test indicates that the niche overlap between two entities is lower than expected by chance. Tests were performed using the `ecospat.niche.equivalency.test` function with the R software `ecospat` (Di Cola *et al.*, 2017).

Populations were assigned to the different groups (introgressed vs. pure) on the basis of subjective thresholds. In the main analyses, we then used the four genetic groups detailed above. In a second analysis, we considered populations with frequency of introgressed allozyme alleles > 20% as the introgressed ones (Endler, 1977), populations with frequency of introgressed alleles < 10% as the pure ones, and repeated analyses to assess the robustness of our results to this subjective threshold.

Distance from surface, microhabitat and body condition

First, we used linear mixed models (LMM) to assess how habitat features (temperature, humidity and light) are related to distance from cave entrance. In LMM, cave identity was used as a random factor.

To assess how microhabitat variation can affect the performance of individuals, we analysed the spatial variation of the body condition index (BCI) of all the captured adults. As BCI measure, we used the Residual Index, which is the difference between observed and the predicted body mass, and is considered among the most reliable body condition indexes (Bancila *et al.*, 2010; Labocha *et al.*, 2014). To calculate BCI, we regressed salamander weight against their total length, and for each individual we extracted the residuals of the regression (Bancila *et al.*, 2010; Labocha *et al.*, 2014). Length and weight were log-transformed to improve linearity. We then used LMM to test whether BCI changes among salamanders observed at different distances from the surface. Spatial distribution can be strongly different between sexes being strongly because of their breeding activity (Salvidio & Pastorino, 2002; Ficetola *et al.*, 2013; Lunghi *et al.*, 2018b), thus we analysed males and females separately. Juveniles were not considered, as their sex cannot be assessed on the basis

of external features. In LMM, genetic group was included as fixed factor, while cave identity and cave sector were included as random factors. In preliminary analyses we also considered the interactions between genetic group and distance from surface, but all the interactions were not-significant. Sample size was not homogeneous among random factors; therefore degrees of freedom were approximated and could be non-integer (Satterthwaite, 1946). Mixed models were built using lme4 and lmerTest in R 3.3.3 (R Development Core Team, 2017); the amount of variation explained by mixed models was assessed using marginal and conditional R^2 (R^2_M and R^2_C , respectively; Nakagawa & Schielzeth, 2013).

3 RESULTS

Overall, we monitored 121 caves across the whole range of both species (694 cave sectors) and observed 1190 salamanders in 278 sectors (Fig. 1; Table 1). The genetic analyses identified pure *H. ambrosii* populations that totally lack or had low frequency of introgressed *H. italicus* allozyme alleles ($\leq 8\%$) in the west, and *H. italicus* populations with limited frequency of introgressed allozyme alleles in the east of the study area (Fig. 1c, Table S1). Within the hybrid zone, there is a continuous decline of *H. ambrosii* alleles from west to east; the average frequency of introgressed alleles was 35% (Fig. 1c, Table S1). The frequency of introgressed alleles and the introgression index (Table S1) allowed assignment of the monitored populations to the four groups reported in Fig. 1.

3.1 *Hydromantes ambrosii*: introgressed vs. pure populations

For pure *H. ambrosii* populations, the best-AIC model suggested that salamanders were strongly associated to sectors with lack of light and cold temperature (Table 2). The relationships between microhabitat and salamander presence were analogous for introgressed *H. ambrosii*. For introgressed populations, the best-AIC model indicated a relationship with low temperature, but an alternative candidate model, with very similar AIC value, also suggested association with the darkest sector.

Despite both groups being related to similar microhabitat, the conditions of occupied sectors were not identical, as introgressed *H. ambrosii* were often observed in sectors with warmer temperature, lower humidity and more light, compared to the non-introgressed populations. For instance, 50% of cave sectors occupied by introgressed *H. ambrosii* showed temperature $>14.4^\circ\text{C}$, humidity below 91% and light above 0.13 lux (Fig. 2a-c). These conditions were also available in caves with pure *H. ambrosii* (Fig. 2), but were only rarely occupied. As a consequence, the average conditions of sectors with pure *H. ambrosii* were characterized by lower temperature, higher humidity and lower light (Fig. 2).

Overall, PCA-env showed strong and significant niche shift ($P = 0.001$) of introgressed populations, with expansion toward harsher conditions (warmer, drier and more illuminated sectors) compared to the non-introgressed populations (Fig. 3a).

3.2 *Hydromantes italicus*: introgressed vs. pure populations

In pure *H. italicus* populations, the best-AIC model suggested association with the darkest and most humid sectors. A very similar microhabitat association was also detected in introgressed populations (Table 2).

Despite both groups being related to similar microhabitats, the conditions of occupied sectors were not identical, as introgressed *H. italicus* were also observed in sectors with lower humidity and more light, compared to the pure populations. For instance, introgressed populations were more often observed in cave sectors with humidity below 92% and light above 0.5 lux, compared to pure populations (Fig. 2e-f).

Overall, introgressed *H. italicus* showed a significant niche shift ($P = 0.006$), with expansion toward harsher conditions (with lower humidity and more light; Table 3, Fig. 3b).

All the results remained identical if, as introgressed sites, we only considered populations with proportion of introgressed genes $\geq 20\%$ (niche similarity test: $I = 0.636$, $P = 0.022$). Equivalency tests did not detect significant differences between introgressed *H. ambrosii* and introgressed *H. italicus* (Table 3).

3.3 Distance from surface, microhabitat and body condition

Distance from cave entrance was a very strong determinant of microhabitat features. Light intensity quickly decreased in sectors far from the surface (LMM; $F_{1,674.4} = 480.3$, $P < 0.0001$). Furthermore, sectors far from the surface showed higher humidity ($F_{1,566.8} = 192.4$, $P < 0.0001$) and lower temperature ($F_{1,560.6} = 250.1$, $P < 0.0001$; Fig. S2).

Overall, we captured, measured and weighed 291 adult salamanders (132 males and 159 females). The smallest individual with male sexual characters showed total length = 73 mm, therefore individuals with this size or larger were assumed to be adults. Body size measures were comparable between sexes and between genetic groups (Table S2). The Body Condition Index (BCI) did not show correlation with salamanders' length ($r < 0.01$, $N = 291$, $P > 0.9$).

For males, BCI was significantly higher in individuals observed close to the cave entrance (LMM, $F_{1,109.7} = 6.8$, $P = 0.01$; Fig. 4a). After taking into account the distance from cave entrance, some BCI differences were observed between genetic groups ($F_{3,17.2} = 3.4$, $P = 0.04$), as introgressed *H. ambrosii* showed slightly higher BCI than the other groups (Fig. 4b).

For females, the relationship between BCI and distance from cave entrance was negative but not significant ($F_{1,61.0} = 0.33$, $P = 0.57$; Fig. 4c). After taking into account distance from cave entrance, some BCI differences were observed between genetic groups ($F_{3,22.9} = 3.3$, $P = 0.04$), as pure *H. italicus* showed slightly higher BCI than the other groups (Fig. 4d). For both males and females, mixed models explained a good amount of BCI variation (males: $R^2_M = 0.26$; $R^2_C = 0.52$; females: $R^2_M = 0.16$; $R^2_C = 0.57$).

4 DISCUSSION

Although species often interact with their environment at fine (microhabitat) scale, analyses of niche variation in hybrid populations frequently use coarse-scale habitat measures (Glennon *et al.*, 2014; Ficetola & Stock, 2016). Fine-scale analyses are essential to understand the impact of small scale processes on populations. Our analyses of salamander hybrid zones

provide unique insights on the occurrence of transgressive niches for hybrid populations. Furthermore, the better body conditions of individuals living nearby the surface provides insights on the potential advantages of exploiting a broader niche, shading light on the relationships between habitat selection and the fate of hybrid zones.

4.1 Transgressive niche of hybrid populations

The ability to exploit new (transgressive) niches has been proposed as one of the mechanisms that might enhance the fitness of hybrid populations, allowing them to live in environments different from the parental ones (Rieseberg *et al.*, 1999; Ficetola & Stock, 2016). Microhabitat selection is pivotal for the persistence of ectotherms in complex landscapes (Moore *et al.*, 2018; Suggitt *et al.*, 2018), yet there is still limited information regarding fine-scale niche differentiation along hybrid zones in animals. Our study showed transgressive segregation in hybrid populations of terrestrial salamanders, as they were able to live in harsher conditions (i.e., warmer, dryer and more luminous sectors) compared to the parental species, and this can have key consequences for the ecological interpretation of hybrid zones.

Our microhabitat analyses considered a very large number of caves across the distribution range of both *H. ambrosii* and *H. italicus*, and confirmed that microhabitat features (light, temperature and humidity) represent simple environmental gradients that strongly influence the distribution of salamanders inside caves (Table 2; (Cimmaruta *et al.*, 1999; Lunghi *et al.*, 2014; Ficetola *et al.*, 2018a). Plethodontid salamanders are lungless and mostly breathe through their humid skin, thus must remain in moist sectors to maintain water balance. Furthermore, these salamanders are mostly active under darkness, have a limited thermal tolerance and generally prefer temperatures below 20-24 °C (Brattstrom, 1963; Spotila, 1972). During summer, salamanders move underground where microclimatic conditions are constantly within their preferred range. Within caves, there is a continuous environmental gradient: sectors close to the surface have conditions similar to outside caves (light, warm temperature, low humidity), while the microhabitat becomes progressively wetter, colder and darker in deep sectors (Fig. S2; (Lunghi *et al.*, 2015). Therefore, the habitat selection of *Hydromantes* during summer is analogous to a habitat selection experiment along a gradient of light, humidity and temperature (Ficetola *et al.*, 2018a). Our findings confirm that the distribution of all study taxa along cave habitats is strongly determined by these environmental gradients (Table 2), but also highlights striking niche differences between parental species and hybrid populations.

Both *H. ambrosii* and *H. italicus* introgressed groups showed a significant niche shift compared to the non-introgressed groups (Table 3). Hybrids were found in drier, warmer and more luminous sectors compared to the parental species (Fig. 2), and this indicates niche expansion toward harsher environmental conditions. The ability to exploit dry and warm sectors can provide important advantages to hybrid populations, which can better persist under stressful conditions and in sub-optimal habitats. Transgressive segregation for ecological tolerance has been repeatedly observed in experimental studies on plants, which showed that hybrids can have increased tolerance to multiple stressors, such as cold, heat and drought (Rieseberg *et al.*, 1999). Tests of hybrid tolerance to abiotic stressors are less frequent in animals (Rieseberg *et al.*, 1999, but see also Matsukura *et al.*, 2016; Lockwood *et al.*, 2018

for recent examples), possibly because of the difficulty of measuring niche parameters in the wild. However, recent advances in ecological methods now allow a better assessment of the variation of ecological niche, and ecological analyses can be even combined with ecophysiological approaches to obtain a more complete picture of species tolerance in the wild.

4.2 Advantages of transgressive niche

What might be the fitness advantages of tolerance to drought and light? Terrestrial salamanders feed on a large range of invertebrates. In cave environments, food resources are limited, and the richness of invertebrates is generally highest close to the surface. The possibility to exploit dry and luminous sectors can allow hybrid populations to exploit shallow cavities or to live into cave sectors close to the surface, where food availability is higher. In agreement with this hypothesis, individuals found close to the surface showed better Body Condition Index, compared to the ones found far from the surface (Fig. 4). A poor BCI was evident in males living far from the surface, while the relationship between distance from cave entrance and BCI was weaker in females. Breeding activity of males and females causes different patterns of cave exploitation that can influence these differences. Females lay eggs in the deepest sectors of caves, where microclimatic conditions are most suitable (constantly high humidity and low temperature) and remain stable through the year (Lunghi *et al.*, 2018b). Gravid females are heavier than females without eggs. Therefore, females with high BCI can be present both close to cave entrance (e.g. foraging individuals) and in deep sectors (e.g. females close to oviposition, searching for suitable breeding sites). Conversely, the better BCI of males close to the surface probably represents the better foraging opportunities of individuals that are able to exploit this environment.

The niche analyses were informed by existing genetic data, but there is a temporal discrepancy between the collection of the two datasets. Because hybrid zones can show temporal shifts, for instance in response to environmental changes (Taylor *et al.*, 2015; Leache *et al.*, 2017), there could be a mismatch between the microhabitat and genetic data. However, it is unlikely that the hybrid has shifted for our study species during this time frame (2005-2016). Firstly, previous studies on the hybrid zone showed it was roughly stable in location over a period of more than 20 years (Lanza *et al.*, 1995; Ruggi *et al.*, 2005; Ruggi, 2007). Secondly, cave salamanders have long generation time (age at maturity: 3-4 years; longevity: > 10 years) and very limited dispersal ability (Lanza *et al.*, 2006), consequently the mismatch between ecological and genetic data is small. Therefore, we expect that the groupings that were assigned based on the existing genetic data would be consistent over the time scale within which we worked.

4.3 Habitat selection and the fate of hybrid zones

In some hybrid zones, microhabitat differences between parental species can limit interspecific encounters. If hybrids are not able to exploit new habitats, these niche differences can strongly reduce the frequency of hybridization, thus microhabitat selection becomes one of the factors that promote mating isolation (Mebert *et al.*, 2015). Nevertheless,

hybrid zones often occur along ecological gradients, where transitional environments may allow the contact between species with different niches (Culumber *et al.*, 2012). Under these circumstances, the hybrid zone can be maintained if hybrids exploit intermediate niches along environmental gradients (Johannesson *et al.*, 2010; Arntzen *et al.*, 2017). However, the situation is completely different when hybrids occupy transgressive niches and are able to exploit habitat that are unsuitable for either parental species. The transgressive segregation for ecological tolerance can be one of the most important traits to facilitate niche divergence, and can foster adaptation and invasion by hybrids into novel niches (Rieseberg *et al.*, 1999; Kagawa & Takimoto, 2018). The situation can become particularly complex in view of ongoing global change. Rapid phenological and distribution shifts are facilitated by global change, thus putting species in contact that currently are isolated, and increasing the frequency of hybridization events (Krosby *et al.*, 2015; Canestrelli *et al.*, 2017; Grabenstein & Taylor, 2018). Given the rapid environmental changes taking place, we expect that species will either adapt to the new conditions, migrate into new sites, or become extinct. The possibility of hybrids to exploit new niches is an intriguing hypothesis that must be considered in global change analyses. For instance, species distribution modelling suggests that cave salamanders might lose suitable habitats because of increased length of hot and dry seasons (Araujo *et al.*, 2006; Ficetola *et al.*, 2016). The hybrid zone between *H. ambrosii* and *H. italicus* currently is narrow (~20 km; Fig. 1), suggesting that some unidentified factor limits the extent of introgression. A similar case has been reported for the *Zerynthia* cryptic butterflies (Zinetti *et al.*, 2013), where no sign of introgression in the narrow contact zone is reported and no information on the limiting factors are available. However, the enhanced tolerance of hybrids to stressful conditions might improve their fitness under the new environmental conditions. Relationships between global changes and hybridization are becoming a major research theme of evolutionary biology, and the ability of some hybrids to exploit transgressive niches can be an important mechanism shaping patterns of biodiversity in the future (Rieseberg *et al.*, 1999; Grabenstein & Taylor, 2018).

This study is one of the first to use fine-scale data to test the hypothesis of transgressive niche in hybrid populations. Such an approach allowed us a high-resolution assessment of the importance of niche breadth on fitness variation.

Tables

Table 1. Number of surveyed caves for the four salamanders groups.

Group	<i>N</i> caves	<i>N</i> cave sectors	<i>N</i> sectors with presence
Pure <i>H. ambrosii</i>	41	276	136
Introgressed <i>H. ambrosii</i>	10	37	16
Introgressed <i>H. italicus</i>	28	187	66
Pure <i>H. italicus</i>	42	194	60

Table 2. Relationships between the presence/absence of salamanders and microhabitat features. For each group, we show the best AIC model and, if any, candidate models within four AIC units from the best model (Richards, 2005). For each model, we list the predictors and, in parentheses, the regression coefficients. *K*: number of parameters in the model; *w*: AIC weight; R^2_M : marginal R^2 ; R^2_C : conditional R^2 (Nakagawa & Schielzeth, 2013). For introgressed *H. ambrosii* populations, two alternative models are reported as their AIC values were very similar.

Group	Model	<i>K</i>	AIC	<i>w</i>	R^2_M	R^2_C
a) <i>H. ambrosii</i>	Light (-0.35); temperature (-0.32)	4	285.9	0.97	0.34	0.39
b) <i>H. ambrosii</i> (introgressed)	temperature (-0.47)	3	39.8	0.49	0.41	0.50
	Light (-0.46); temperature (-0.40)	4	40.0	0.43	0.44	0.52
c) <i>H. italicus</i>	Light(-0.56); humidity (14.0)	4	162.5	0.99	0.63	0.63
d) <i>H. italicus</i> (introgressed)	Light(-0.54); humidity (5.7)	4	188.7	0.89	0.28	0.31

Table 3. Niche similarity (Shoener's D) and niche equivalency tests between pure and introgressed populations of salamanders, and comparisons between introgressed *H. ambrosii* and introgressed *H. italicus*, and between pure *H. ambrosii* and pure *H. italicus*.

	D	P	Niche expansion
<i>H. ambrosii</i> vs. introgressed <i>H. ambrosii</i>	0.624	0.001	0.26
<i>H. italicus</i> vs. introgressed <i>H. italicus</i>	0.654	0.006	0.22
Introgressed <i>H. ambrosii</i> vs. introgressed <i>H. italicus</i>	0.609	0.078	
pure <i>H. ambrosii</i> vs. pure <i>H. italicus</i>	0.757	0.001	

Figure legends.

Figure 1. Distribution of study localities within Southern Europe. a-b): localities sampled for microhabitat analyses. The blue and orange polygons are the range of *H. ambrosii* and *H. italicus*, respectively. c): localities with available scores for genetic analyses (see also Table S1 and Fig. S1). Diagrams show the relative frequency of *H. ambrosii* (blue) and *H. italicus* (orange) alleles across populations.

Figure 2. Density of occurrence of pure and introgressed salamander populations, in relation to environmental features (solid coloured areas). Grey areas represent the overlap between pure and introgressed populations; lines represent the environmental conditions available to each group of populations.

Figure 3. PCA-env performed on pure and introgressed salamander populations. Grey areas represent the overlap between pure and introgressed populations, while solid coloured areas are the environmental conditions only occupied by one group of populations. The solid and dashed contour lines represent, respectively, 100% and 75% of the available (background) environment. The figure also show the correlation circles of principal component analyses performed on the data, and the amount of variation explained by PCA axes.

Figure 4. Variation of body condition index among individuals observed at different distances from cave entrance, and from different genetic groups: partial regression plots. Left panel: males; right panels: females. Shaded areas are 95% confidence intervals.

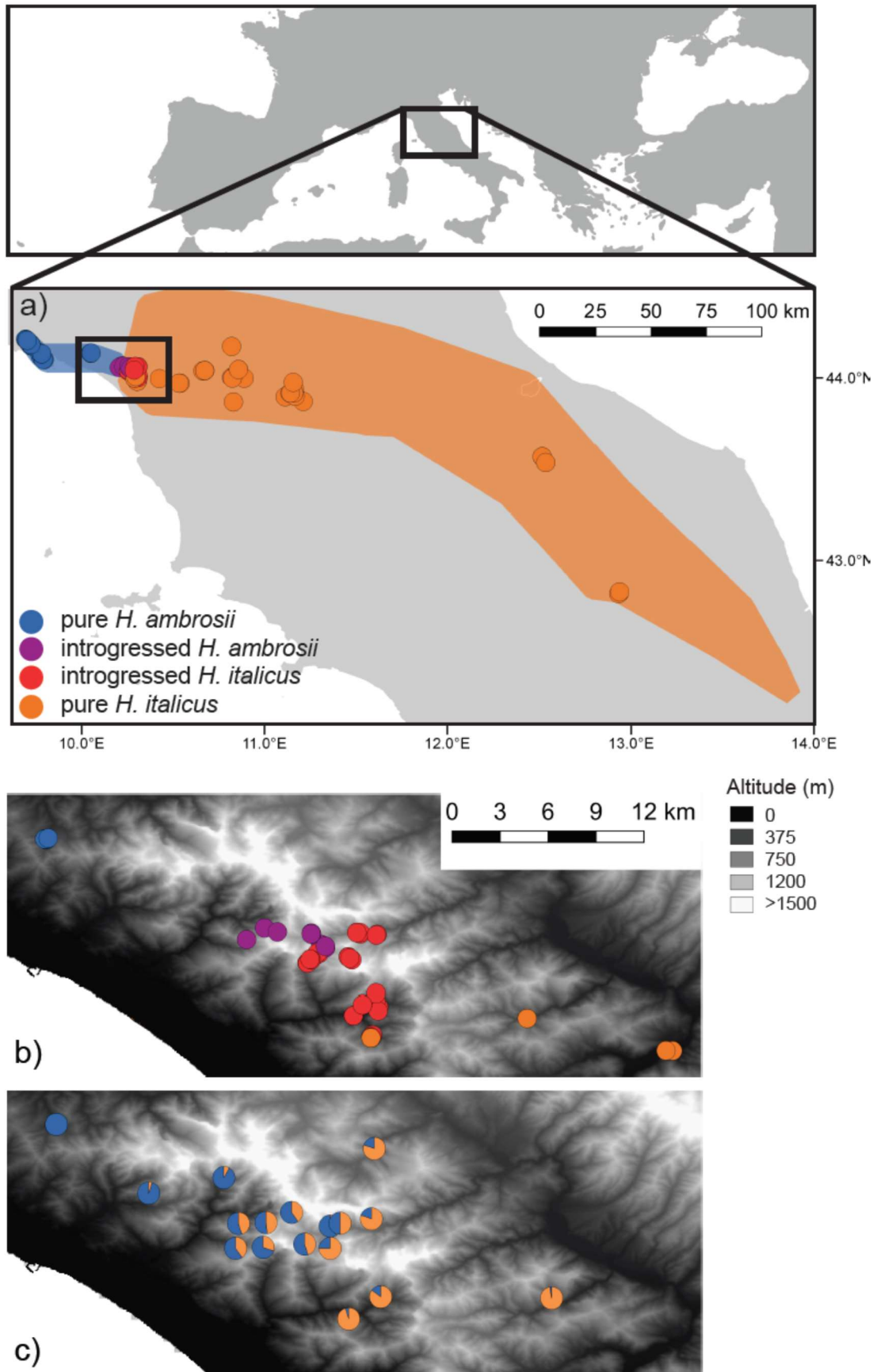


Figure 1

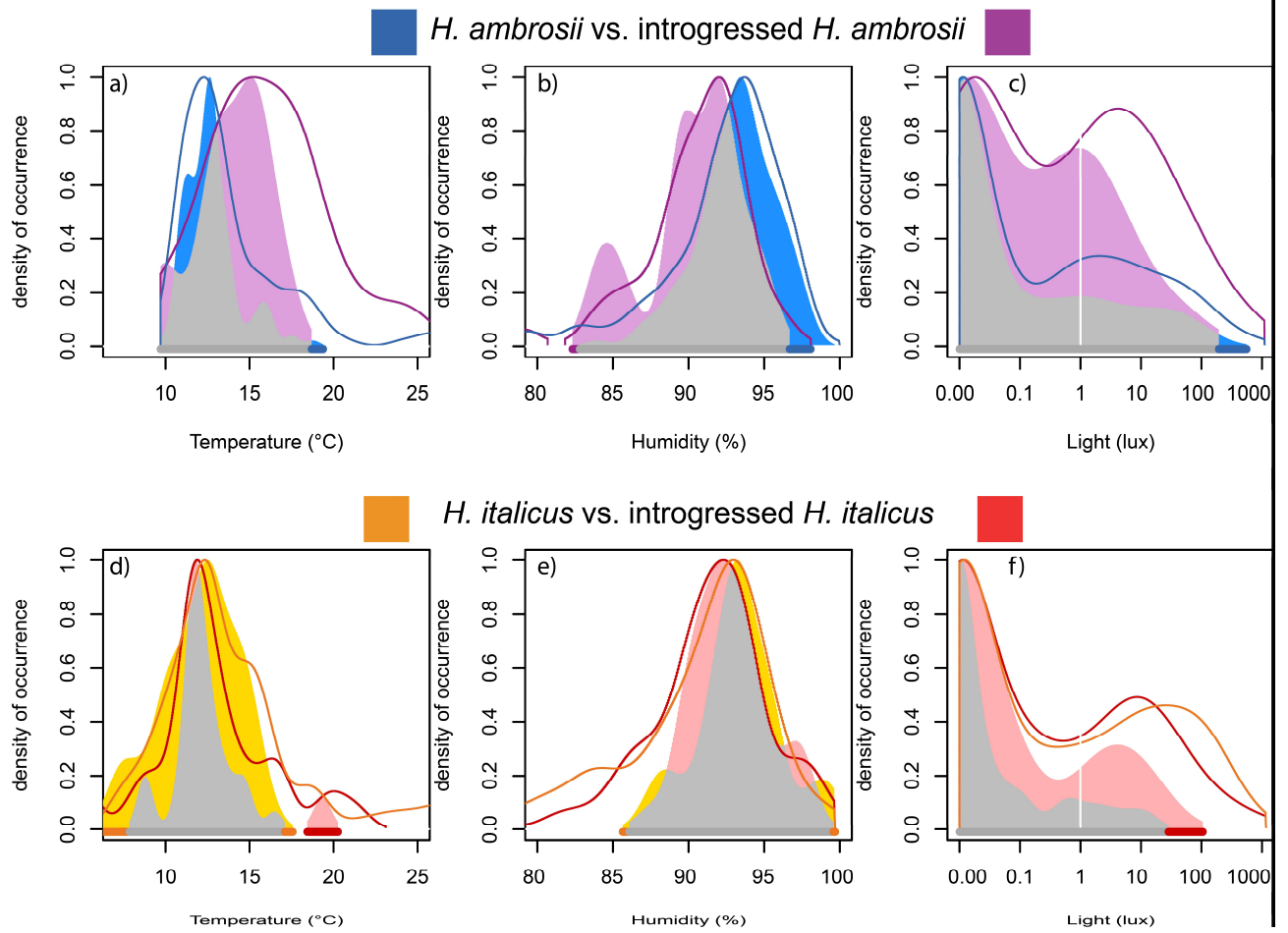


Figure 2

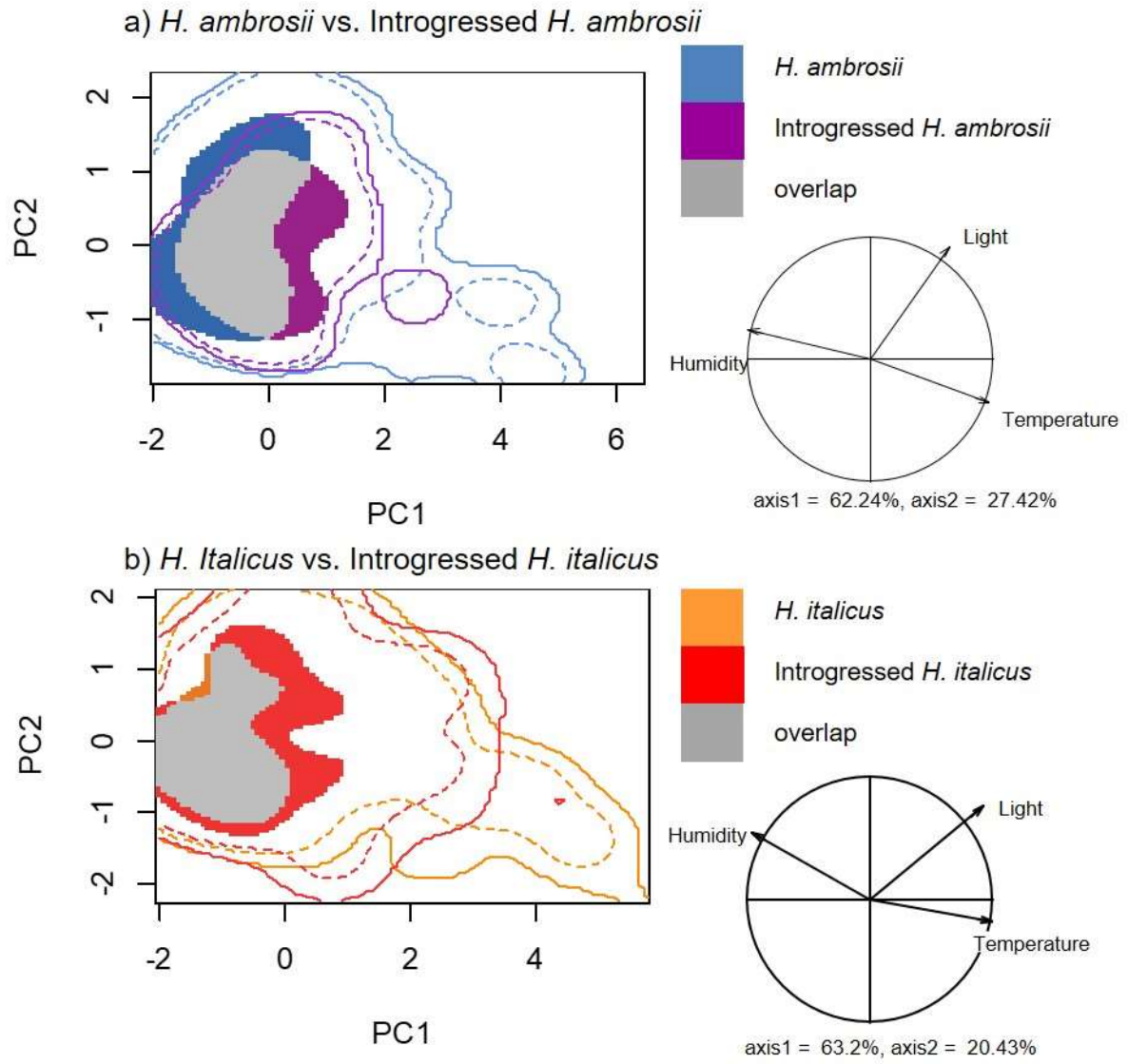


Figure 3

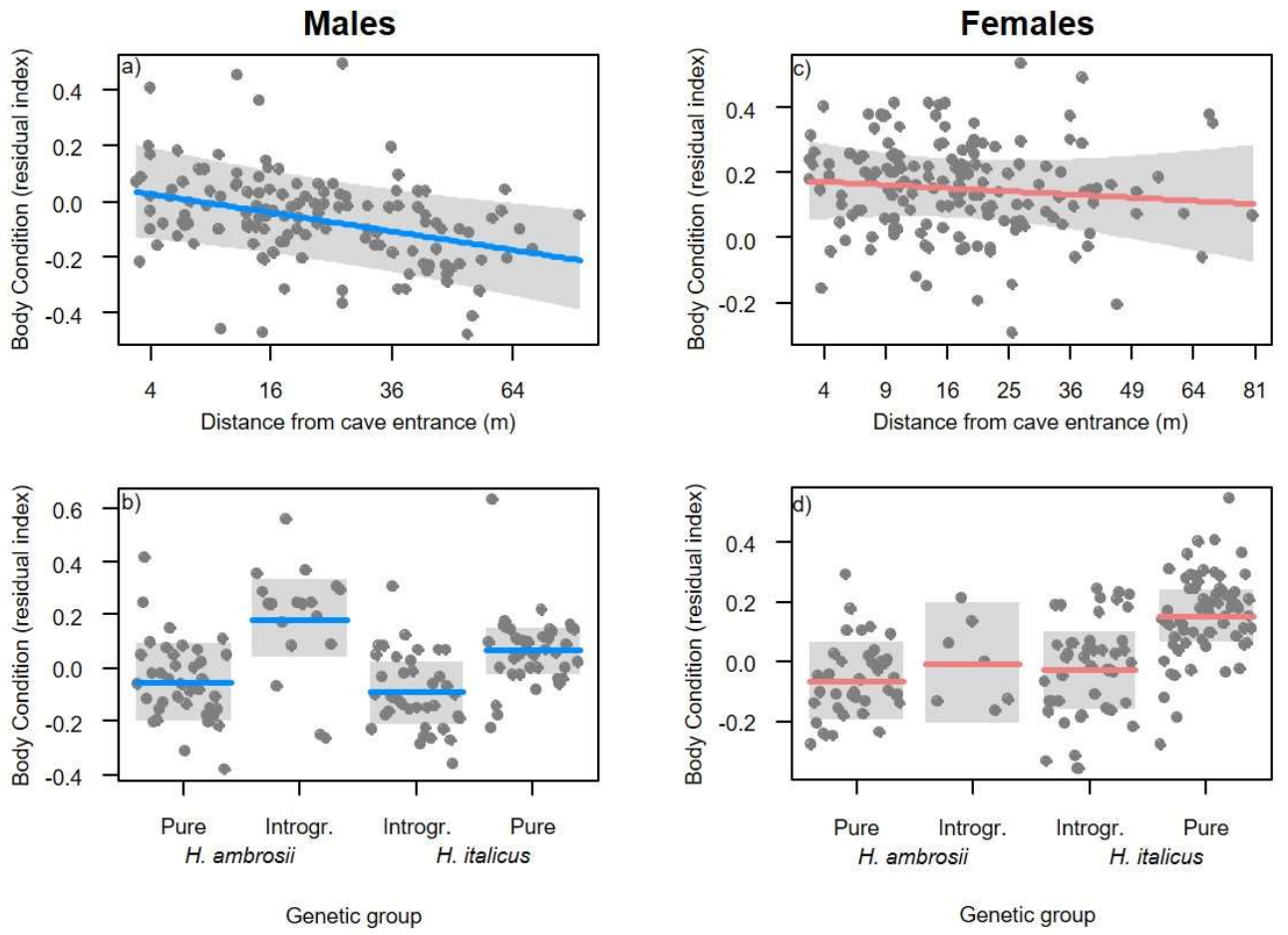


Figure 4

References

- Allendorf, F.W. (2017) Genetics and the conservation of natural populations: allozymes to genomes. *Molecular Ecology*, 26, 420-430.
- Araujo, M.B., Thuiller, W. & Pearson, R.G. (2006) Climate warming and the decline of amphibians and reptiles in Europe. *Journal of Biogeography*, 33, 1712-1728.
- Arnason, U., Lammers, F., Kumar, V., Nilsson, M.A. & Janke, A. (2018) Whole-genome sequencing of the blue whale and other rorquals finds signatures for introgressive gene flow. *Science Advances*, 4
- Arntzen, J.W., de Vries, W., Canestrelli, D. & Martinez-Solano, I. (2017) Hybrid zone formation and contrasting outcomes of secondary contact over transects in common toads. *Molecular Ecology*, 26, 5663-5675.
- Bancila, R.I., Hartel, T., Plaiasu, R., Smets, J. & Cogalniceanu, D. (2010) Comparing three body condition indices in amphibians: a case study of yellow-bellied toad *Bombina variegata*. *Amphibia-Reptilia*, 31, 558-562.
- Bates, D., Maechler, M., Bolker, B. & Walker, S. (2015) Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software*, 67, 1 - 48.
- Bay, R.A. & Ruegg, K. (2017) Genomic islands of divergence or opportunities for introgression? *Proceedings of the Royal Society B-Biological Sciences*, 284, 20162414.
- Beck, J., Ballesteros-Mejia, L., Buchmann, C.M., Dengler, J., Fritz, S.A., Gruber, B., Hof, C., Jansen, F., Knapp, S., Kreft, H., Schneider, A.K., Winter, M. & Dormann, C.F. (2012) What's on the horizon for macroecology? *Ecography*, 35, 673-683.
- Brattstrom, B.H. (1963) A preliminary review of the thermal requirements of Amphibians. *Ecology*, 44, 238-255.
- 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.
- Burnham, K.P. & Anderson, D.R. (2002) *Model selection and multimodel inference: a practical information-theoretic approach*. Springer Verlag, New York.
- Canestrelli, D., Biscconti, R., Chiochio, A., Maiorano, L., Zampiglia, M. & Nascetti, G. (2017) Climate change promotes hybridisation between deeply divergent species. *PeerJ*, 5
- Cimmaruta, R., Forti, G., Nascetti, G. & Bullini, L. (1999) Spatial distribution and competition in two parapatric sibling species of European plethodontid salamanders. *Ethology Ecology & Evolution*, 11, 383-398.
- Crump, M.L. & Scott, J., N. J. (1994) Visual Encounter Surveys. *Measuring and monitoring biological diversity: standard methods for Amphibians* (ed. by W.R. Heyer, M.A. Donnelly, R.W. McDiarmid, L.C. Hayek and M.S. Foster), pp. 84-92. Smithsonian Institution Press, Washington.
- Culumber, Z.W., Shepard, D.B., Coleman, S.W., Rosenthal, G.G. & Tobler, M. (2012) Physiological adaptation along environmental gradients and replicated hybrid zone structure in swordtails (Teleostei: Xiphophorus). *Journal of Evolutionary Biology*, 25, 1800-1814.
- Di Cola, V., Broennimann, O., Petitpierre, B., Breiner, F.T., D'Amen, M., Randin, C., Engler, R., Pottier, J., Pio, D., Dubuis, A., Pellissier, L., Mateo, R.G., Hordijk, W., Salamin, N. & Guisan, A. (2017) ecospat: an R package to support spatial analyses and modeling of species niches and distributions. *Ecography*, 40, 774-787.
- Endler, J.A. (1977) *Geographic variation, speciation and clines*. Princeton University Press. , Princeton.

- Ficetola, G.F. & Stock, M. (2016) Do hybrid-origin polyploid amphibians occupy transgressive or intermediate ecological niches compared to their diploid ancestors? *Journal of Biogeography*, 43, 703–715.
- Ficetola, G.F., Pennati, R. & Manenti, R. (2012) Do cave salamanders occur randomly in cavities? An analysis with *Hydromantes strinatii*. *Amphibia-Reptilia*, 33, 251-259.
- Ficetola, G.F., Pennati, R. & Manenti, R. (2013) Spatial segregation among age classes in cave salamanders: habitat selection or social interactions? *Population Ecology*, 55, 217–226.
- Ficetola, G.F., Colleoni, E., Renaud, J., Scali, S., Padoa-Schioppa, E. & Thuiller, W. (2016) Morphological variation in salamanders and their potential response to climate change. *Global Change Biology*, 22, 2013-2024.
- Ficetola, G.F., Lunghi, E., Canedoli, C., Padoa-Schioppa, E., Pennati, R. & Manenti, R. (2018a) Differences between microhabitat and broad-scale patterns of niche evolution in terrestrial salamanders. *Scientific Reports*, 8
- Ficetola, G.F., Lunghi, E., Canedoli, C., Padoa-Schioppa, E., Pennati, R. & Manenti, R. (2018b) Differences between microhabitat and broad-scale patterns of niche evolution in terrestrial salamanders. *Scientific Reports*, 8, 10575.
- Garrick, R.C., Benavides, E., Russello, M.A., Hyseni, C., Edwards, D.L., Gibbs, J.P., Tapia, W., Ciofi, C. & Caccone, A. (2014) Lineage fusion in Galapagos giant tortoises. *Molecular Ecology*, 23, 5276-5290.
- Gerchen, J.F., Dufresnes, C. & Stöck, M. (2018) Introgression across hybrid zones is not mediated by Large X-effects in green toads with undifferentiated sex chromosomes. *The American Naturalist* 192, E178–E188.
- Glennon, K.L., Ritchie, M.E. & Segraves, K.A. (2014) Evidence for shared broad-scale climatic niches of diploid and polyploid plants. *Ecology Letters*, 17, 574-582.
- Gomez-Rodriguez, C., Bustamante, J., Diaz-Paniagua, C. & Guisan, A. (2012) Integrating detection probabilities in species distribution models of amphibians breeding in Mediterranean temporary ponds. *Diversity and Distributions*, 18, 260-272.
- Grabenstein, K.C. & Taylor, S.A. (2018) Breaking Barriers: Causes, Consequences, and Experimental Utility of Human-Mediated Hybridization. *Trends in Ecology & Evolution*, 33, 198-212.
- Graham, M.H. (2003) Confronting multicollinearity in ecological multiple regression. *Ecology*, 84, 2809-2815.
- Grant, P.R. & Grant, R. (2016) Introgressive hybridization and natural selection in Darwin's finches. *Biological Journal of the Linnean Society*, 117, 812-822.
- Hewitt, G.M. (2001) Speciation, hybrid zones and phylogeography — or seeing genes in space and time. *Molecular Ecology*, 537-549.
- Johannesson, K., Panova, M., Kempainen, P., Andre, C., Rolan-Alvarez, E. & Butlin, R.K. (2010) Repeated evolution of reproductive isolation in a marine snail: unveiling mechanisms of speciation. *Philosophical Transactions of the Royal Society B-Biological Sciences*, 365, 1735-1747.
- Kagawa, K. & Takimoto, G. (2018) Hybridization can promote adaptive radiation by means of transgressive segregation. *Ecology Letters*, 21, 264-274.
- Krosby, M., Wilsey, C.B., McGuire, J.L., Duggan, J.M., Nogeire, T.M., Heinrichs, J.A., Tewksbury, J.J. & Lawler, J.J. (2015) Climate-induced range over-lap among closely related species. *Nature Climate Change*, 5, 883– 886.
- Labocha, M.K., Schutz, H. & Hayes, J.P. (2014) Which body condition index is best? *Oikos*, 123, 111-119.

- Lanza, B., Caputo, V., Nascetti, G. & Bullini, L. (1995) *Morphologic and genetic studies of the European plethodontid salamanders: taxonomic inferences (genus Hydromantes)*. Museo Regionale di Scienze Naturali,, Torino.
- Lanza, B., Pastorelli, C., Laghi, P. & Cimmaruta, R. (2006) A review of systematics, taxonomy, genetics, biogeography and natural history of the genus *Speleomantes* Dubois, 1984 (Amphibia Caudata Plethodontidae). *Atti del Museo Civico di Storia Naturale di Trieste*, 52
- Leache, A.D., Grummer, J.A., Harris, R.B. & Breckheimer, I.K. (2017) Evidence for concerted movement of nuclear and mitochondrial clines in a lizard hybrid zone. *Molecular Ecology*, 26, 2306-2316.
- Lira-Noriega, A.L., Sobéron, J. & Miller, C.P. (2013) Process-based and correlative modeling of desert mistletoe distribution: a multiscalar approach. *Ecosphere*, 4, 1–23
- Lockwood, B.L., Gupta, T. & Scavotto, R. (2018) Disparate patterns of thermal adaptation between life stages in temperate vs. tropical *Drosophila melanogaster*. *Journal of Evolutionary Biology*, 31, 323-331.
- Lukacs, P.M., Thompson, W.L., Kendall W.L., Gould, W.R., Doherty, P.F., Burnham, K.P. & Anderson, D.R. (2007) Concerns regarding a call for pluralism of information theory and hypothesis testing. *Journal of Applied Ecology*, 44, 456-460.
- Lunghi, E., Manenti, R. & Ficetola, G.F. (2014) Do cave features affect underground habitat exploitation by non-troglobite species? *Acta Oecologica*, 55, 29 - 35.
- Lunghi, E., Manenti, R. & Ficetola, G.F. (2015) Seasonal variation in microhabitat of salamanders: environmental variation or shift of habitat selection? *Peerj*, 3
- Lunghi, E., Manenti, R., Canciani, G., Scari, G., Pennati, R. & Ficetola, G.F. (2016) Thermal equilibrium and temperature differences among body regions in European plethodontid salamanders. *Journal of Thermal Biology*, 60, 79-85.
- Lunghi, E., Cianferoni, F., Ceccolini, F., Veith, M., Manenti, R., Mancinelli, G., Corti, C. & Ficetola, G.F. (2018a) What shapes the trophic niche of European plethodontid salamanders? *Plos One*, 13
- Lunghi, E., Corti, C., Manenti, R., Barzaghi, B., Buschetti, S., Canedoli, C., Cogoni, R., De Falco, G., Fais, F., Manca, A., Mirimin, V., Mulargia, M., Mulas, C., Muraro, M., Murgia, R., Veith, M. & Ficetola, G.F. (2018b) Comparative reproductive biology of European cave salamanders (genus *Hydromantes*): nesting selection and multiple annual breeding. *Salamandra*, 54, 101-108.
- MacKenzie, D.I., Nichols, J.D., Royle, J.A., Pollock, K.H., Bailey, L.A. & Hines, J.E. (2006) *Occupancy estimation and modeling: inferring patterns and dynamics of species occurrence*. Academic Press, Burlington, MA.
- Mallet, J. (2007) Hybrid speciation. *Nature*, 446, 279-283.
- Mammola, S. & Leroy, B. (2018) Applying species distribution models to caves and other subterranean habitats. *Ecography*, 41, 1194-1208.
- Manenti, R. (2014) Dry stone walls favour biodiversity: a case study from the Appennines. *Biodiversity and Conservation*, 23, 1879-1893.
- Matsukura, K., Izumi, Y., Yoshida, K. & Wada, T. (2016) Cold tolerance of invasive freshwater snails, *Pomacea canaliculata*, *P. maculata*, and their hybrids helps explain their different distributions. *Freshwater Biology*, 61, 80-87.
- Mayr, E. (1942) *Systematics and the origin of species*. Columbia Univ. Press, New York.
- McCartney-Melstad, E., Vu, J.K. & Shaffer, H.B. (2018) Genomic data recover previously undetectable fragmentation effects in an endangered amphibian. *Molecular Ecology*, 27, 4430-4443.

- Mebert, K., Jagar, T., Grzelj, R., Cafuta, V., Luiselli, L., 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, 364-376.
- Moore, D., Stow, A. & Kearney, M.R. (2018) Under the weather?-The direct effects of climate warming on a threatened desert lizard are mediated by their activity phase and burrow system. *Journal of Animal Ecology*, 87, 660-671.
- Moore, W.S. (1977) An evaluation of narrow hybrid zones in vertebrates. *The Quarterly Review of Biology*, 52, 263-277.
- Nakagawa, S. & Schielzeth, H. (2013) A general and simple method for obtaining R^2 from generalized linear mixed-effects models. *Methods in Ecology and Evolution*, 4, 133-142.
- Nascetti, G., Cimmaruta, R., Lanza, B. & Bullini, L. (1996) Genetic and geographic variation in the European plethodontid salamanders (genus *Hydromantes*). *Journal of Herpetology*, 30, 161-183.
- Newman, C.E. & Austin, C.C. (2016) Sequence capture and next-generation sequencing of ultraconserved elements in a large-genome salamander. *Molecular Ecology*, 25, 6162-6174.
- Otis, J., Thornton, D., Rutledge & Murray, D.L. (2017) Ecological niche differentiation across a wolf-coyote hybrid zone in eastern North America. *Diversity and Distribution*, 23 529-539.
- Palkopoulou, E., Lipson, M., Mallick, S., Nielsen, S., Rohland, N., Baleka, S., Karpinski, E., Ivancevici, A.M., To, T.H., Kortschak, D., Raison, J.M., Qu, Z.P., Chin, T.J., Alt, K.W., Claesson, S., Dalen, L., MacPhee, R.D.E., Meller, H., Rocar, A.L., Ryder, O.A., Heiman, D., Young, S., Breen, M., Williams, C., Aken, B.L., Ruffier, M., Karlsson, E., Johnson, J., Di Palma, F., Alfoldi, J., Adelsoni, D.L., Mailund, T., Munch, K., Lindblad-Toh, K., Hofreiter, M., Poinar, H. & Reich, D. (2018) A comprehensive genomic history of extinct and living elephants. *Proceedings of the National Academy of Sciences of the United States of America*, 115, E2566-E2574.
- Petitpierre, B., Kueffer, C., Broennimann, O., Randin, C., Daehler, C. & Guisan, A. (2012) Climatic Niche Shifts Are Rare Among Terrestrial Plant Invaders. *Science*, 335, 1344-1348.
- R Development Core Team (2017) *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna.
- Richards, S.A. (2005) Testing ecological theory using the information-theoretic approach: examples and cautionary results. *Ecology*, 86, 2805-2814.
- Rieseberg, L.H., Archer, M.A. & Wayne, R.K. (1999) Transgressive segregation, adaptation and speciation. *Heredity*, 83, 363-372.
- Rodder, D. & Engler, J.O. (2011) Quantitative metrics of overlaps in Grinnellian niches: advances and possible drawbacks. *Global Ecology and Biogeography*, 20, 915-927.
- Ruggi, A. (2007) *Descrizione di una zona di contatto e ibridazione tra *Speleomantes italicus* e *Speleomantes ambrosii bianchii* (Amphibia - Plethodontidae) sulle Alpi Apuane mediante marcatori nucleari e mitocondriali*. Università degli Studi della Tuscia, Viterbo.
- Ruggi, A., Cimmaruta, R., Forti, G. & Nascetti, G. (2005) Preliminary study of a hybrid zone between *Speleomantes italicus* and *S. ambrosii* on the Apuane Alps, using RFLP analysis. *Annali Museo Civico di Storia naturale Giacomo Doria di Genova*, 97, 135 - 144.
- Salvidio, S. & Pastorino, M.V. (2002) Spatial segregation in the European plethodontid *Speleomantes strinatii* in relation to age, and sex. *Amphibia-Reptilia*, 23, 505-510.
- Salvidio, S., Lattes, A., Tavano, M., Melodia, F. & Pastorino, M.V. (1994) Ecology of a *Speleomantes ambrosii* population inhabiting an artificial tunnel. *Amphibia-Reptilia*, 15, 35-45.
- Satterthwaite, F.E. (1946) An approximate distribution of estimates of variance components. *Biometrics*, 2, 110-114.

- Scheffers, B.R., Edwards, D.P., Diesmos, A., Williams, S.E. & Evans, T.A. (2014) Microhabitats reduce animal's exposure to climate extremes. *Global Change Biology*, 20, 495-503.
- Schoener, T.W. (1970) Nonsynchronous spatial overlap of lizards in patchy habitats. *Ecology*, 51, 408-418.
- Seehausen, O. (2004) Hybridization and adaptive radiation. *Trends in Ecology and Evolution*, 19, 198 - 207.
- Slatyer, R.A., Hirst, M. & Sexton, J.P. (2013) Niche breadth predicts geographical range size: a general ecological pattern. *Ecology Letters*, 16, 1104-1114.
- Soberon, J. & Nakamura, M. (2009) Niches and distributional areas: Concepts, methods, and assumptions. *Proceedings of the National Academy of Sciences of the United States of America*, 106, 19644-19650.
- Soubrier, J., Gower, G., Chen, K., Richards, S.M., Llamas, B., Mitchell, K.J., Ho, S.Y.W., Kosintsev, P., Lee, M.S.Y., Baryshnikov, G., Bollongino, R., Bover, P., Burger, J., Chivall, D., Cregut-Bonnoure, E., Decker, J.E., Doronichev, V.B., Douka, K., Fordham, D.A., Fontana, F., Fritz, C., Glimmerveen, J., Golovanova, L.V., Groves, C., Guerreschi, A., Haak, W., Higham, T., Hofman-Kaminska, E., Immel, A., Julien, M.A., Krause, J., Krotova, O., Langbein, F., Larson, G., Rohrlach, A., Scheu, A., Schnabel, R.D., Taylor, J.F., Tokarska, M., Tosello, G., van der Plicht, J., van Loenen, A., Vigne, J.D., Wooley, O., Orlando, L., Kowalczyk, R., Shapiro, B. & Cooper, A. (2016) Early cave art and ancient DNA record the origin of European bison. *Nature Communications*, 7
- Spotila, J.R. (1972) Role of temperature and water in the ecology of lungless salamanders. *Ecological Monographs*, 42, 95 - 125.
- Suggitt, A.J., Wilson, R.J., Isaac, N.J.B., Beale, C.M., Auffret, A.G., August, T., Bennie, J.J., Crick, H.Q.P., Duffield, S., Fox, R., Hopkins, J.J., Macgregor, N.A., Morecroft, M.D., Walker, K.J. & Maclean, I.M.D. (2018) Extinction risk from climate change is reduced by microclimatic buffering. *Nature Climate Change*, 8, 713.
- Tanaka, Y. (2007) Introgressive hybridization as the breakdown of postzygotic isolation: A theoretical perspective. *Ecological Research*, 22, 929 - 939.
- Taylor, S.A., Larson, E.L. & Harrison, R.G. (2015) Hybrid zones: windows on climate change. *Trends in Ecology & Evolution*, 30, 398-406.
- Wake, D.B. (2013) The enigmatic history of the European, Asian and American plethodontid salamanders. *Amphibia-Reptilia*, 34, 323-336.
- Warren, D.L., Glor, R.E. & Turelli, M. (2008a) Environmental niche equivalency versus conservatism: quantitative approaches to niche evolution. *Evolution*, 62, 2868-2883.
- Warren, D.L., Glor, R.E. & Turelli, M. (2008b) Environmental niche equivalency versus conservatism: Quantitative approaches to niche evolution. *Evolution*, 62, 2868-2883.
- Warren, D.L., Cardillo, M., Rosauer, D.F. & Bolnick, D.I. (2014) Mistaking geography for biology: inferring processes from species distributions. *Trends in Ecology and Evolution*, 29, 572 - 580.
- Wielstra, B., E, D., P, L., Lammers, Y., Meilink, W.R.M., Ziermann, J.M. & Arntzen, J.W. (2014) Parallel tagged amplicon sequencing of transcriptome-based genetic markers for *Triturus* newts with the Ion Torrent next-generation sequencing platform. *Molecular Ecology Resources*, 14, 1080-1089.
- Zinetti, F., Dapporto, L., Vovlas, A., Chelazzi, G., Bonelli, S., Balletto, E. & Ciofi, C. (2013) When the rule becomes the exception. No evidence of gene flow between two *Zerynthia* cryptic butterflies suggests the emergence of a new model group. *Plos One*, 8, e65746.

Biosketches

G. Francesco Ficetola is a researcher combining multiple approaches (landscape ecology, macroecology, evolutionary ecology and environmental DNA) to improve knowledge of the factors determining biodiversity. Raoul Manenti has a special research interest in the biology of amphibians and of subterranean environments. GFF and RM conceived the study; GFF, EL, RC and RM collected the data; GFF and EL performed the analyses; RC performed genetic analyses; GFF, EL, RC and RM wrote the paper.