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WOUTER BEUKEMA
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SUPERVISORS:
Dr. A. G. Toxopeus
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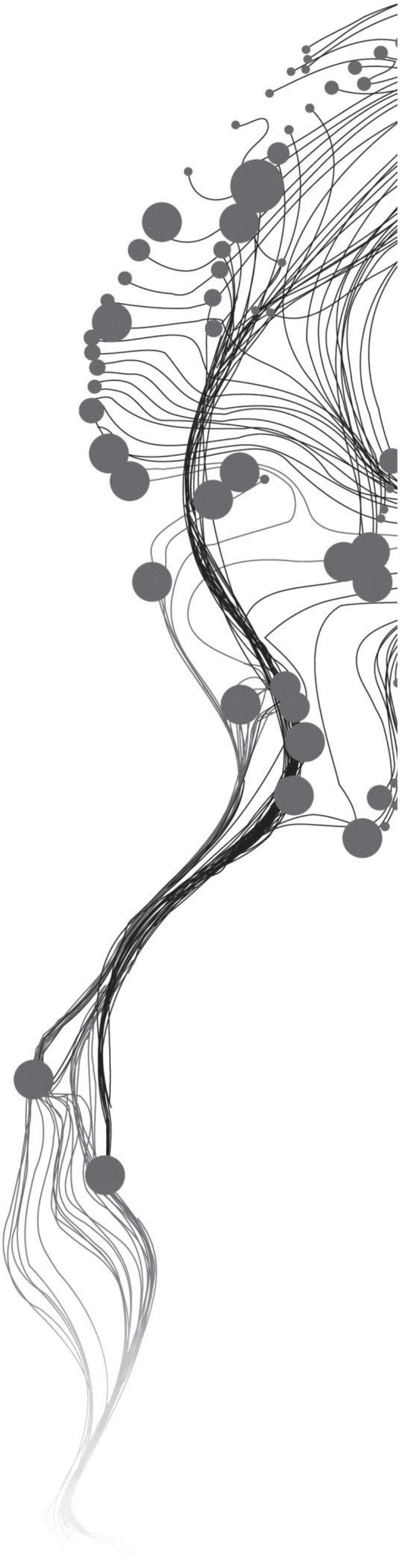

Homoplastic viviparity revisited: On the biogeography of salamanders in the Mediterranean Basin, with emphasis on different reproduction modes

WOUTER BEUKEMA
Enschede, the Netherlands, March 2012

Thesis submitted to the Faculty of Geo-Information Science and Earth Observation of the University of Twente in partial fulfilment of the requirements for the degree of Master of Science in Geo-information Science and Earth Observation.
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SUPERVISORS:
Dr. A. G. Toxopeus
Dr. T. A. Groen

THESIS ASSESSMENT BOARD:
Dr. Y. A. Hussin
Dr. J. F. Duivenvoorden (External Examiner, University of Amsterdam)

ABSTRACT

In general, it is assumed that related organisms display similar biological traits (also known as homology). As species relations where traditionally assessed based on morphological features, the advance of phylogenetic methods identified considerable amounts of non-homology (known as homoplasy) throughout the tree of life. Homoplasy is identified when non-related organisms show similar biological traits. Various mechanisms have been suggested to lie at the base of homoplasy, which are mostly influenced by genetic constraint and environmental factors. Despite the indispensability of phylogenetic analyses to identify homoplasy, comparative studies which adress the niche of organisms in relation to certain traits have remained a valuable component of evolutionary research. Indeed, the simple question whether similar traits have arisen in unrelated organisms as a response to similar environmental conditions underlies hypothesis testing in convergent traits, while the identification of a relation between the environment and a particular trait is the first step in the exploration of its cause. Niche modelling has been identified as a promising technique to test for such relations.

As a case study, the occurrence of homplastic viviparity amongst salamanders in the Mediterranean Basin was explored. Viviparity (i.e. reproduction by means of direct development) has evolved in several related and unrelated taxa and is endemic to salamanders of the study area. By means of testing for (1) intra- and intergeneric niche divergence, (2) niche overlap between and within reproductive groups, and (3) niche breadth of reproductive groups the general research question whether Grinnellian niche conditions differ between reproductive groups was tested. An uncorrelated (Pearsons $r_s < 0.7$) dataset of environmental predictors composed of topogeographical, climatic and vegetation derived data was combined with a total of 527 occurrence records for 36 taxa and identified, yet undescribed lineages within the genera *Lyciasalamandra*, *Salamandra* and *Speleomantes*. Two different ordination techniques were used to calibrate niches in multivariate environmental space; PCA-env and ENFA. Niche calibration and subsequent pairwise tests for niche similarity based on Schoener's *D* for 34 of the 36 study organisms were implemented within R by means of a recently presented framework. This resulted in a total of 561 models and overlap values per technique, besides 1122 similarity tests. Only significant similarity tests (n = 144 for PCA-env, n = 289 for ENFA) were used for further statistical analyses in which Independent T Tests were used to assess differences between intra- and intergeneric niche similarity, while One Way Anova Tests were used to assess differences between reproductive groups. Additionally, ten bootstrap replicate MaxEnt runs were performed of which the results were used in ENMTools 1.3 to assess differences in niche breadth between reproductive groups.

Results of PCA-env analyses show intrageneric niche similarity only to be significantly higher in the genus *Speleomantes*, while ENFA only shows higher intrageneric niche similarity for the genus *Salamandra*. One Way Anova Tests based on PCA-env similarity scores did not find significant differences between reproductive groups, while results based on ENFA scores show overlap values among non-viviparous taxa to be significantly higher. Niche breadth analyses based on MaxEnt output showed viviparous taxa to occupy significantly smaller ranges and narrower niches when compared to non-viviparous taxa. While not significantly higher, viviparous taxa do show a higher occurrence of overlap with each other than when compared to other reproductive groups, despite their relative narrow niches. It is therefore proposed that occurrence of reproductive behaviour does correlate with specific Grinnellian niche conditions. Future analyses of local scale conditions could shed more light on the exact causes for the reproductive transition.

Keywords: Viviparity, Niche Overlap, Niche Similarity, Intraspecific, Grinnellian, Mediterranean Basin, PCA, ENFA, MaxEnt, Salamandridae, Plethodontidae.

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The current thesis mainly focuses on the various types of reproductive behaviour among my favourite model organisms, which has been a fascinating topic of research from my very first publication (Beukema, 2006) up to the currently presented results. Therefore, I would like to dedicate this thesis to my parents for their continuous support.

Wouter Beukema
Enschede, the Netherlands
March, 2012

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GLOSSARY

Ancestral trait:	A biological trait shared by a group of organisms as a result of descent from a common ancestor.
Bionomic predictor:	Predictors describing biotic interactions between organisms.
Convergence:	The acquisition of similar biological traits in unrelated organisms.
Eltonian niche:	The niche constrained by biotic interactions.
Eltonian noise hypothesis:	Eltonian factors do not influence distribution at large scales/coarse resolution.
Fundamental niche:	The requirements of a species to persist, disregarding biotic interactions.
Grinnellian niche:	The set of scenopoetic factors among which an organisms can persist.
Homology:	Similar biological traits in related organisms.
Homoplasy:	Similar biological traits in non-related organisms.
Interspecific:	Between species.
Intraspecific:	Within species.
Niche:	The relational position of organisms in their ecosystem to each other.
Niche breadth:	The range along the environmental gradient within which a niche occurs.
Niche conservatism:	The tendency for related organisms to have similar niches; also, the tendency for the niche of an organism to be little changed over time.
Niche divergence:	Ecological divergence among related organisms or within a species.
Parallelism:	The acquisition of similar biological traits in related organisms descending from the same common ancestor.
Phylogenetic signal:	The tendency for more closely related organisms to have more similar characteristics.
Plasticity:	The ability of an organism to change its phenotype in response to changes in the environment.
Realized niche:	The part of the fundamental niche in which a species persists, limited by biological interactions.
Scenopoetic predictor:	Environmental predictors non-related to biotic interactions.
Viviparity:	Development of an embryo inside the body leading to live birth.

1. INTRODUCTION

1.1. On Homology and Homoplasy

Related organisms are generally expected to display similar phenotypical traits. In this aspect, the phenotype does not only represent morphology, but also (reproductive) behaviour, physiology and might even be extended to all the effects a gene has on the outside world that may influence its chances of being replicated (Dawkins 1982). This similarity is defined as homology; for example, an ancestral trait shared by two species is homologous between the two. Before the appearance of phylogenetic methodology, species relations where mostly constructed based on morphology. With the advance of phylogenetics, the first examples of non-homology were discovered based on incongruence between genetic and for example morphological datasets (Avise & Wollenberg, 1997). For instance, species which display similar morphological characters were discovered not to be related on genetic level. These patterns initially frustrated phylogenetists, while non-homology was presumed to be an uncommon phenomenon (Rieppel, 1989; Sanderson & Donoghue, 1989). Subsequently however, large-scale analyses recovered considerable presence of non-homology throughout the tree of life (e.g. Wake, 1991), initiating an interest for the causal factors leading to the observed situation. Homoplasy (Figure 1) was consequently coined to describe cases of unrelated organisms which display similar phenotypical traits. This phenomenon covers three classes; (1) reversal to an ancestral trait, (2) convergence; homoplasy as a result of different genetic mechanisms but similar selective pressures, and (3) parallelism; homoplasy as a result of the same genetic mechanisms (review in Losos *et al.*, 2011a; Wake *et al.*, 2011). These classes however do not have to be contradictory, while their discrimination can be troublesome. One of the most famous examples of homoplasy in general is the resemblance between the long-diverged placental mammals from Eurasia and the Americas compared to the marsupials on the Australian continent.

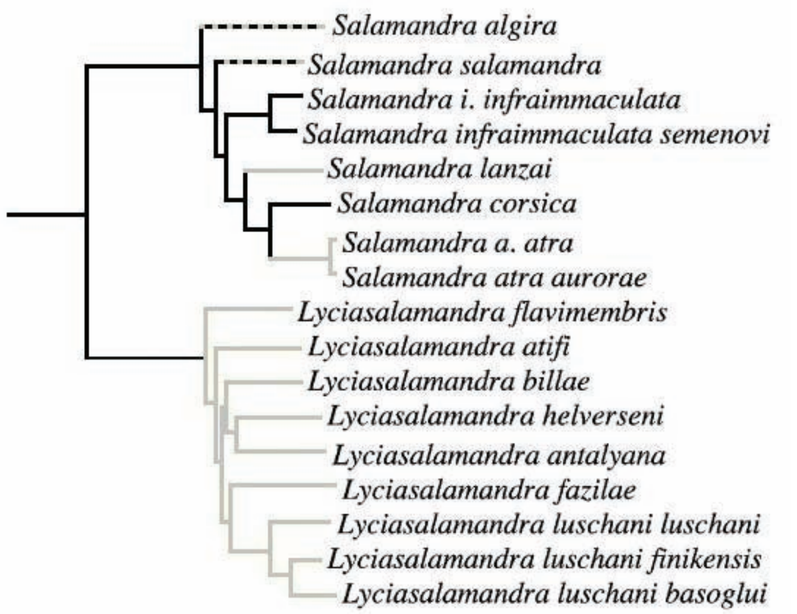

Figure 1: Molecular relationships between the amphibian genera *Salamandra* and *Lyciasalamandra*. Black and grey lines display two different types of reproductive behaviour, in which the grey is homoplastic within *Salamandra*. In the case of *S. algira* and *S. salamandra* both behaviours occur intraspecificly. Modified from Weisrock *et al.* (2006).

While homoplasy was initially perceived as a problem in evolutionary biology and taxonomy (e.g. Rieppel, 1989; Sanderson & Donoghue, 1989), this opinion changed during the early '90 (e.g. Wake, 1991).

Currently, homoplasy is considered a relatively common phenomenon and considerable research has been done to uncover causal mechanisms or factors (review in Wake *et al.*, 2011). Mainly, such research has focused on (similar) genetic mechanisms, thus often exploring patterns of parallelism. It has however been acknowledged that similar environmental pressures can lead to similar features by means of coincidence, adaptation or correlated response due to genetic constraint (Losos, 2011a).

1.1.1. Homoplasy and Environmental Factors

Especially in the context of convergence, environmental factors can act as drivers which may result in similar features (see Losos, 2011a for a comprehensive overview of this topic). The possibility of organisms to develop for instance new morphological features is limited by genetic constraint, thus similar features do not necessarily appear by means of similar environmental conditions only, especially among related species (Wake, 1991; Wake *et al.*, 2011). However, when non-related organisms show similar features (such as wings in bats and birds), the environment has often been suggested to act as driving factor. While exploring causes for homoplasy, it is important to emphasize that often there is not a single factor which drives the phenomenon; multifarious selection describes the combination of factors which may lead to a similar outcome (Wade & Kalisz, 1990). In general, relations between factors and certain features are tested within environmental space by means of correlative analyses. However, long term experiments are indispensable (but generally strenuous) to confirm these observations and test whether change within particular variable(s) does indeed lead to an evolutionary response (Wade & Kalisz, 1990).

Recently, trait evolution and (ecological) speciation have been explored by means of correlating time-calibrated phylogenies of multiple species with environmental predictors (e.g. Graham *et al.*, 2004). In the case of trait evolution, ancestral niches can be reconstructed through time by means of potential causal environmental drivers (e.g. Kamilar & Muldoon, 2010; Wiens *et al.*, 2011). Such analyses can provide an interesting insight in terms of selection and potentially driving factors among related species, leading to speciation and/or adaptation displayed by a particular trait. However, these analyses are based on a multitude of assumptions with regard to the niche of a species (see also paragraph 1.2), while ancestral reconstructions of a trait in a phylogenetic context are not straightforward and methodologies are continuously developing (Wiens *et al.*, 2011). Recent critique on such methods (e.g. Grandcolas *et al.*, 2011) should therefore be taken into account. Despite the indispensability of phylogenetic analyses to identify homoplasy, comparative studies addressing the niche of organisms in relation to certain traits remain a valuable component of evolutionary research (e.g. Losos, 2011b). Indeed, the simple question whether similar traits have arisen in unrelated organisms as a response to similar environmental conditions underlies hypothesis testing in convergent traits (Losos, 2011a), therefore the identification of a relation between the environment and a particular trait is the first step in the exploration of its cause (Wade & Kalisz, 1990). Niche modelling (review in Elith & Leathwick, 2009) is a promising technique to test for such relations (e.g. Harmon *et al.*, 2005).

1.2. Niche Modeling

Despite the fact that niche-related studies have been topic of research since the early twentieth century, interest in the niche concept has been revitalised and increased in recent years (Soberón & Nakamura, 2009). Many definitions to explain the term ‘niche’ have been proposed during the last century (e.g. Grinnell, 1917; Elton, 1927; Hutchinson, 1957; Soberón, 2007). Most commonly, a distinction is made between the fundamental niche (the range of conditions, biotic and abiotic, in which an organism can persist) and the realized niche (the part of the fundamental niche that is actually occupied as a result of e.g. competition with other organisms). When these concepts are transferred to mechanistic calibration of niches based on environmental predictors, it is important to make a distinction between conditions and resources (Soberón, 2007). Herein, condition-related predictors are scenopoetic, while resource-related predictors are bionomic (Hutchinson, 1978). Respectively, this corresponds to the definition of the Grinnellian niche (Grinnell, 1917) and the Eltonian niche (Elton, 1927). The Grinnellian niche has been the main foundation for recent niche-related studies, as scenopoetic spatial data regarding temperature, precipitation or topography is readily available on a global scale (e.g. Hijmans *et al.*, 2005). Additionally, such environmental conditions can explain large-scale biogeographic, ecological and distributional patterns which have been major research topics (e.g., but certainly not limited to Losos *et al.*, 2003; Graham *et al.*, 2004; Broennimann *et al.*, 2007; Fitzpatrick *et al.*, 2007; Hof *et al.*, 2010; Kamilar & Muldoon, 2010). In contrast, biotic interactions and resource–consumer dynamics represented by the Eltonian niche in bionomic predictors are best applied at local scale (Soberón, 2007). Data on such processes is far more challenging to measure, remains specific for each organism, and can in general not explain large scale patterns (but see e.g. Wiens, 2011).

Two main methodological approaches (Broennimann *et al.*, 2012) have been used during recent years to model niches in environmental space, which are both commonly applied in the context of a Geographical Information System (GIS). Both methods use a combination of species occurrence records with a set of environmental predictors to characterize niches. Species Distribution Models (SDMs) apply calibration of statistical or machine-learning functions, occasionally in combination with jack knife tests to assess predictor importance in fitting the niche of an organism (Elith & Leathwick, 2009). In contrast, ordination techniques select orthogonal and linear combinations of predictors in environmental space which maximize environmental variance from the entire dataset, thus offering a more 'direct' means for niche characterization (Broennimann *et al.*, 2012). Ordination techniques have received less attention (but see e.g. Graham *et al.*, 2004; Hof *et al.*, 2010), even though these can provide highly accurate results in terms of niche calibration (Broennimann *et al.*, 2012). Additionally, these techniques closely resemble the original description of the fundamental niche by Hutchinson (1957), defined as multidimensional space of environmental predictors in which an organism can persist. Besides studies related to niche modelling, ordination techniques but especially SDMs have been applied for other purposes among which biogeography (including hindcasting of models), conservation and climate change effects (Elith & Leathwick, 2009).

1.2.1. Niche Evolution

The integration of GIS and evolutionary biology is a relatively recent but promising development (Kozak *et al.*, 2008). When niches of several organisms have been calibrated, these can be compared by means of niche overlap metrics to assess the degree of niche evolution (review in Rödder & Engler, 2011). Niches are expected to remain conserved, which means that related organisms should occupy related niches. Niche divergence is identified when related organisms occupy different niches. The question whether niches are conserved or diverge over space and time is a long debated subject in ecology and evolutionary biology, which has attracted an increasing amount of interest during the last decade (review in Pearman *et al.*, 2008). In general, there seems to be a tendency for niche conservatism among taxa (i.e. preservation of niche attributes, which are shared with closely related taxa) over short to moderate time spans (Peterson, 2011). Niche divergence seems to become more pronounced over longer time spans. However, exceptions in the form of niche shifts have been suggested to occur, among others revealed by contrasting phylogenetic signal (e.g. Losos *et al.*, 2003; Graham *et al.*, 2004; Kamilar & Muldoon, 2010) and comparison between native and invasive species' niches (e.g. Broennimann *et al.*, 2007; Fitzpatrick *et al.*, 2007). Knowledge on the factors that can cause these niche shifts is hitherto largely lacking (Wiens, 2004).

There has been considerable debate on what the "best" approach to calibrate and especially compare (overlap) of niches is (e.g. Pearman *et al.*, 2008; Peterson, 2011). Recent studies have begun to shed light on the question, and compared niches either in a phylogenetic framework or independently (Warren *et al.*, 2010; Struwe *et al.*, 2011; Broennimann *et al.*, 2012).

1.3. Case study

A well-known homoplastic trait is formed by the evolutionary transition from ovi(vi)parous reproduction (encompassing an aquatic stage) to viviparity (i.e. giving birth to terrestrial, fully metamorphosed individuals). Viviparity has evolved in both invertebrates and vertebrates (e.g. Tyndale-Biscoe & Renfree, 1987; Blackburn, 2000; Kohler *et al.*, 2004). Environmental factors, particularly cold climates, have often been suggested to act as selective agents (Shine, 1989). Within the Class Amphibia, viviparity is an uncommon trait, especially among salamanders in which the phenomenon only occurs in the Mediterranean Basin (Vitt & Caldwell, 2009). Within this area, a high number of salamander species has diverged, mainly during the past five million years as a result of biogeographical causes (Weisrock *et al.*, 2006; Zhang *et al.*, 2008). These salamanders are characterized by their largely or fully terrestrial lifestyle, as opposed to their counterparts the newts, which are largely aquatic (Vitt & Caldwell, 2009). Reproduction of salamanders takes place on land, after which larvae are deposited in aquatic biotopes in which they remain until metamorphosis. However, several salamander taxa in Europe, North Africa and the Near East have evolved to viviparous reproduction. Viviparous reproduction is known to occur in the sister genera *Salamandra* and *Lyciasalamandra* of the family Salamandridae (Vitt & Caldwell, 2009), both on species and intraspecific level (Figure 1). Reversal to an ancestral trait has been suggested as cause for the transition to viviparity (García-París *et al.*, 2003), while an important role has been attributed to the influence of environmental factors in this process (Gasser & Joly, 1972; García-París *et al.*, 2003; Velo-Antón *et al.*, 2007; Beukema *et al.*, 2010). However, the phenomenon has also been reported to occur in *Speleomantes sarrabusensis*, a member of the Plethodontidae family which occurs on the Italian island of Sardinia (Lanza & Leo, 2000).

Figure 2: Examples of Mediterranean salamander species. From left to right: *Speleomantes ambrosii*, La Spezia, Italy; *Salamandra salamandra gigliolii*, Calabria, Italy; *Lyciasalamandra billae*, Antalya, Turkey. All photos 2007-2010 © Author.

1.4. Problem statement

The recent report of viviparity in a Mediterranean member of the Plethodontidae (Lanza & Leo, 2000) is remarkable considering the fact that this family comprises more than 400 members, of which not a single species has been described to display viviparous reproduction (Vitt & Caldwell, 2009). As the ancestors of the Plethodontidae and Salamandridae split during the Late Jurassic (Zhang & Wake, 2009) reversal to an ancestral trait is an unlikely reason for the occurrence of viviparity in both families. Environmental factors, as suggested before, may have influence on the transition to viviparity, although comparative analyses encompassing multiple species are hitherto lacking. Several factors such as coldness, dryness or permeability of soils have been suggested to cause a change in reproductive behavior. In this aspect, viviparity can be seen as an adaptation, as well as specialization. Therefore it can be suspected that niches of viviparous taxa are more similar, narrower and are composed of comparable environmental conditions when compared to non-viviparous taxa. The purpose of the current study is to explore these assumptions regarding the presence of homoplastic viviparity in Mediterranean salamanders by means of Grinnellian niche characterization and overlap.

1.4.1. Specific objectives

1. To measure pairwise niche similarity between all salamander taxa.

2. To compare the degree of niche divergence within and between genera.

3. To compare the degree of niche divergence among reproductive groups.

4. To assess the relation between niche breadth and range size between reproductive groups.

1.5. Research Questions

1. Are niche attributes conserved among genera?

2. Are significant niche similarity indices of intra-reproductive group comparisons (vivi vs. vivi, non-vivi vs. non-vivi) higher than those of inter-reproductive group comparisons (non-vivi vs. vivi, vivi vs. non-vivi)?

3. Are niche breadth and range of viviparous taxa smaller compared to non-viviparous taxa?

1.6. Research Hypotheses

H_0 Intrageneric niche overlap is higher than intergeneric niche overlap.
H_1 Intrageneric niche overlap is not higher than intergeneric niche overlap.

H_0 Significant niche similarity indices of intra-reproductive group comparisons are not higher than those of inter-reproductive group comparisons.
H_1 Significant niche similarity indices of intra-reproductive group comparisons are higher than those of inter-reproductive group comparisons.

H_0 Niche breadth and range of viviparous taxa are not significantly smaller than that of non-viviparous taxa.
H_1 Niche breadth and range of viviparous taxa are significantly smaller than that of non-viviparous taxa.

2. MATERIALS AND METHODS

2.1. Study Area

Global biodiversity hotspots, delineated based on biogeographical commonalities, have been defined as conservation priorities (Myers *et al.*, 2000; Lamoreux *et al.,* 2006). The current study focuses on one such hotspot, namely the Mediterranean Basin (which comprises the limits of Europe, Asia and Africa, Figure 3). The Mediterranean Basin is characterized by a high degree of endemism, especially regarding its flora (Myers *et al.*, 2000). The extent of primary vegetation has however declined to less than 4.7%, severely threatening the persistence of local biota. Despite this major deterioration, high species richness and high intraspecific genetic variability are distinctive for the Mediterranean Basin. This pattern has mainly been shaped by climate fluctuations and plate tectonics during the Miocene and Pliocene, and glaciations during the Pleistocene, which caused major range contractions, isolation and subsequent recolonization among species. This has led to extensive (sub)speciation in biota, especially in the three main glacial refugia of Iberia, Italy and the Balkans (Taberlet *et al.*, 1998).

The extent of the Mediterranean Biodiversity Hotspot was downloaded from www.biodiversityhotspots.org. The shapefile was subsequently clipped to exclude the Canary Islands, Azores and Madeira archipelago (Figure 3).

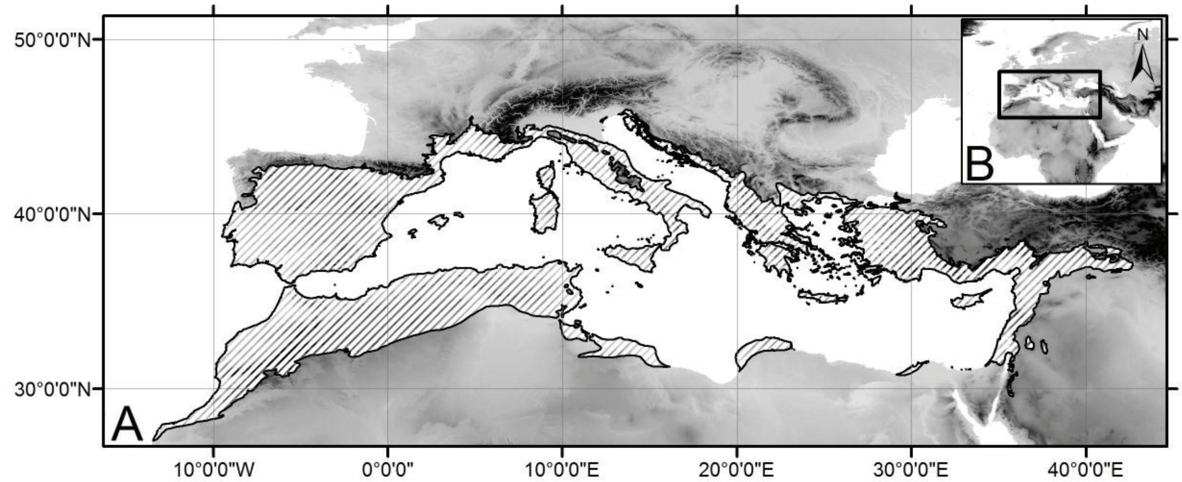

Figure 3: A; The Mediterranean Biodiversity Hotspot (striped) within the Mediterranean Basin. B; location of the Mediterranean Basin in the western Palearctic.

2.2. Materials

2.2.1. Occurrence records

As a model, 36 salamander taxa were used for analyses (Appendix 1). When a species is composed of several evolutionary lineages, in most cases represented by subspecies, it has been shown that niche calibration is more accurate when applied on lineage level compared to the species as whole (Pearman *et al.*, 2010; Thompson *et al.*, 2011). Even recently diverged lineages can occupy different niches (e.g. Beukema *et al.*, 2010; Pearman *et al.*, 2010). The current 36 model taxa are distributed amongst approximately 21 species of which several are well known regarding their ability for fast local genetic adaptation to environmental conditions (Weitere *et al.*, 2004; Goldberg *et al.*, 2009). Hence, the decision was made to calibrate niches of taxa rather than species, including several identified, yet not formally described evolutionary lineages (see Lanza *et al.*, 2005; Lanza *et al.*, 2006; Beukema *et al.*, 2010). These constitute all members of the genera *Lyciasalamandra, Salamandra* and *Speleomantes* which occur in the defined study area, apart from *Salamandra algira spelaea* which is only known from a single locality. The taxonomy proposed by Frost *et al.* (2011) was largely followed, but occasionally modified as proposed by Speybroeck *et al.* (2010). The recently described *Lyciasalamandra irfani* (Göçmen *et al.*, 2011) was regarded as part of *L. billae* due to doubtful taxonomic validity of the former. Intraspecific systematics of *Salamandra algira* are based on Bogaerts *et al.* (in prep). Intraspecific systematics and geographical boundaries of *Salamandra salamandra* in Italy were based on Lanza *et al.* (2005).

A total of 527 records were gathered. These distribution data consist of personal records (n= 41), records from colleagues (n= 328; see Acknowledgements) and records derived from literature (n= 158), at a maximum resolution of 1x1 km. Literature records were extracted from Cimmaruta *et al.* (1999), Veith *et al.* (2001), Johannesen *et al.* (2006), Lanza *et al.* (2006), Veith *et al.* (2008) and Akman *et al.* (2011). All records were georeferenced and stored as .kml files in Google Earth using the WGS84 coordinate system, and subsequently imported into ArcGIS 10.0.

2.2.2. Environmental predictors

A combination of climatic, topographical and vegetation-derived predictors embodies the environmental dataset (Appendix 2).

Climatic predictors were represented by the entire Bioclim 1.4 dataset (including temperature and precipitation data; Hijmans *et al.*, 2005), and the Bioclim derived Global Potential Evapo-Transpiration (Global-PET) and Global Aridity Index from the CGIAR-CSI database. These climatic predictors were generated through interpolation of average monthly climate data from weather stations on a 2.5' resolution grid (approximately 5 km^2 resolution).

The topographical predictor altitude was also downloaded from the Bioclim dataset, but is derived from SRTM data gathered in 2000. The predictors slope and aspect were derived from the altitude layer using Spatial Analyst Tools in ArcGIS 10.0.

A Normalized Difference Vegetation Index (NDVI) was generated based on SPOT imagery that was collected between 1999 and 2009 with a ten-day interval. The total amount of 396 images was stacked in ERDAS IMAGINE 2011, after which time profiles of the stack were smoothed in ENVI 4.8. Subsequently, monthly median NDVI values were computed in ERDAS.

Initially, a predictor describing superficial geology in the study area was compiled from USGS data for Europe, the Arabian Peninsula and Africa (Open File Report 97-470I, Open-File Report 97-470B and Open-File Report 97–470A respectively, http://pubs.er.usgs.gov/). This categorical predictor was composed of 64 classes. Categorical data, especially when composed of a large amount of classes, can both hamper niche calibration and subsequent interpretation. Additionally, geological or soil characters can vary on much smaller scales than the resolution of GIS data in general (John *et al.*, 2007). Therefore, it was decided not to include this predictor in the succeeding analyses.

2.3. Methods

A general overview of the methodology can be found in Figure 4.

2.3.1. Multicollinearity analysis

Multicollinearity among environmental predictors can have serious impact on model performance, both in regards to the prediction (i.e. overfitting), as well as the contribution of each predictor in a model (Guisan *et al.*, 2002; Heikkinen *et al.*, 2006; Peterson, 2011). Especially SDM accuracy can become negatively influenced by large, correlated datasets (Heikkinen *et al.*, 2006; Broennimann *et al.*, 2012). This problem is less severe in regards to ordination techniques, as these select limited orthogonal and linear combinations of predictors which maximize environmental variance from the entire dataset (Broennimann *et al.*, 2012). Several algorithms have been built to overcome the issue of multicollinearity (such as MAXENT, Elith *et al.*, 2011). However, the resulting combination of predictors might not be representative for the studied organism from an ecological viewpoint when significant explaining predictors have been removed due to correlation with other, less-explaining ones. Variance Inflation Factor (VIF) analysis (Belsley *et al.,* 1989) has often been used to derive a set of uncorrelated predictors from the original dataset by means of stepwise omission (e.g. Guisan & Thuiller, 2005; Heikkinen *et al.*, 2006; Beukema *et al.*, 2010). Collinearity is assessed by comparison of each predictor with the entire dataset. While this method does result in a limited dataset with the highest explanatory variability, no pairwise comparisons are made due to which preference for each predictor from a correlated pair cannot be taken into account. A solution to this problem has been proposed in the form of *a priori* selection, in which both the amount and type of predictors are included based on the ecology of the organism (Austin, 2007; Hengl *et al.*, 2009; Elith *et al.*, 2011). However, this approach does not solve earlier mentioned issues regarding multicollinearity.

To reduce the current dataset of environmental predictors, a hybrid approach was used based on Pearsons' correlation coefficient (r_s). This analysis was performed in ENMTools 1.3 based on the spatial files of the environmental predictors. The result is a table of pairwise predictor comparisons, after which predictor omission can be performed based on ecological knowledge. Predictors were considered to be intercorrelated if the resulting r_s was > 0.7. To allow comparison between different niche building techniques, the resulting dataset was used for both SDM and ordination calibration (see below), regardless of the ability of the latter to deal with multicollinearity. To assess independence of the final dataset using another approach, stepwise linear regression was performed in SPSS16 during which a collinearity diagnosis was made based on the VIF. Multicolinearity is identified when the VIF > 10 (Belsley *et al.,* 1989).

2.3.2. Niche modelling

Although the calibration of niches has received an increasing amount of attention during the past decade (Soberón & Nakamura, 2009), methods to measure and quantify niche overlap have received limited interest. A relatively large amount of studies have compared proposed techniques for niche calibration, (e.g. Elith *et al.*, 2006; Hernandez *et al.*, 2006; Jiménez-Valverde *et al.*, 2008). Results of these studies showed large differences with regard to the performance of various algorithms, which were furthermore often based on questionable yet ubiquitous validation metrics (e.g. Area Under the Curve, Lobo *et al.*, 2008). Only recently, several authors have summarized niche metrics and proposed techniques to measure niche overlap (e.g. Warren *et al.*, 2010; Rödder & Engler, 2011; Struwe *et al.*, 2011; Broennimann *et al.*, 2012).

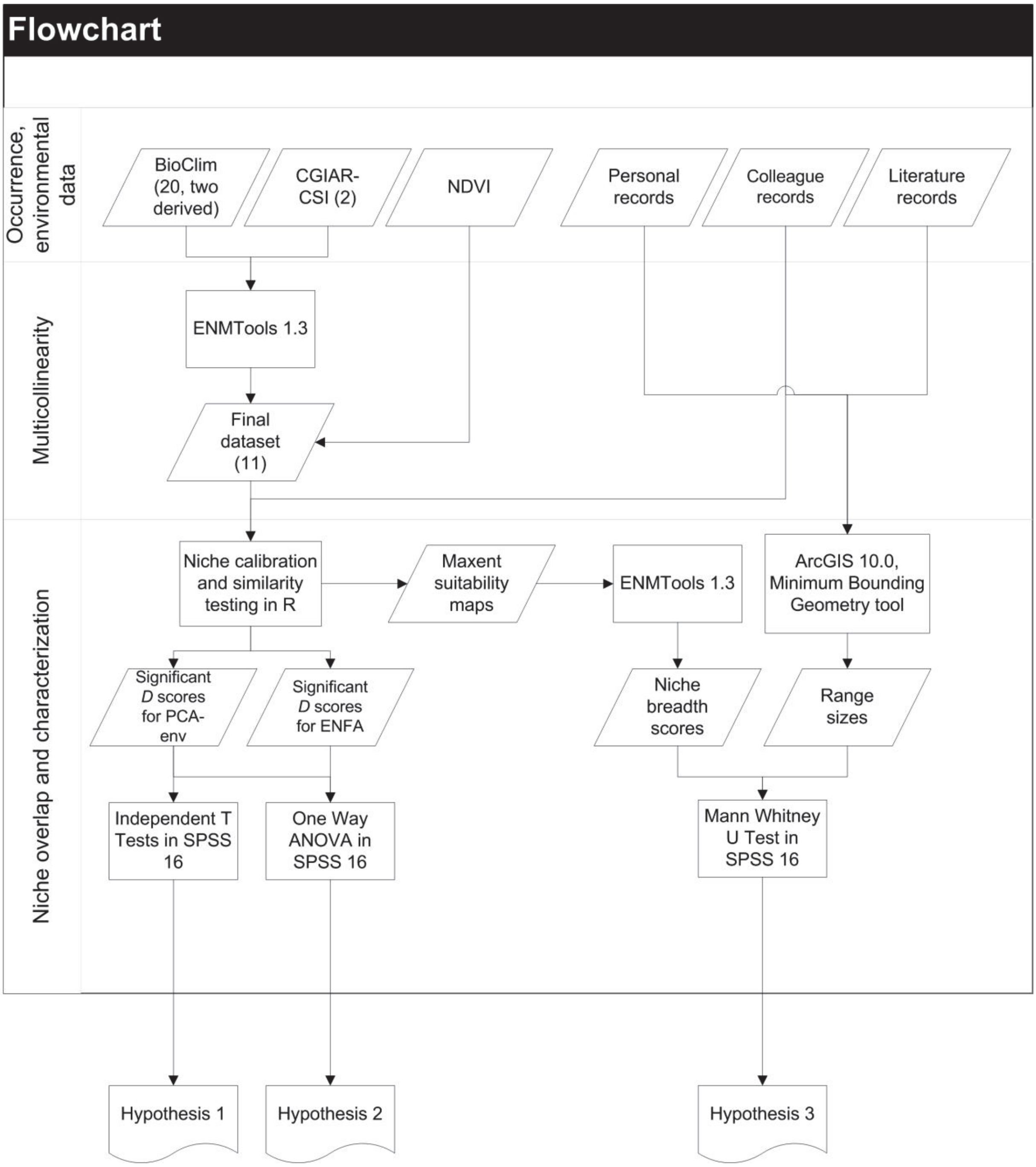

Figure 4: Flowchart displaying the main methodological steps.

However, these methods were never robustly tested, while the earlier mentioned differences in niche calibration performance should at least be taken into account. Broennimann *et al.* (2012) recently presented a framework which was designed to include both ordination and SDM techniques, and can be divided into three parts: (1) calibration of niches along environmental axes of a multivariate analysis based on presence only data; (2) measurements of niche overlap; and (3) statistical tests of niche overlap as presented by Warren *et al.* (2008). The strength of this framework lies in the fact that it was tested using simulated, virtual entities ('study organisms') with predefined environmental boundaries by which performance of niche calibration techniques can be tested. Subsequently, the most appropriate technique to calibrate niches for a particular case study can be chosen. Additionally, the framework dealt with several methodological artefacts common to niche calibration. Firstly, the amount of occurrence records (i.e. number of grid cells) of a study taxa represented by a given combination of environmental conditions is divided by the total number (grid cells) of such environmental conditions in the study area. Without this correction for the relative occurrence of environments, niche overlap is underestimated (Broennimann *et al.*, 2012). Secondly, occurrence- and environmental datasets used to calibrate niches might differ in temporal and spatial resolution, as a result of e.g. different origin (atlas data, personally collected data) or time of collection. To measure niche overlap in multivariate environmental space, it is therefore necessary to standardize the occurrence density of an organism. This is performed by application of a kernel smoother which is independent of grid size. A similar approach has also been used by Hengl *et al.* (2009) in combination with Ecological Niche Factor Analysis (Hirzel *et al.*, 2002). As preparation for the current analyses, a Gaussian kernel with standard bandwidth corresponding to 0.9 times the minimum of the standard deviation and the inter-quartile range of the data divided by 1.34 times the sample size to the negative one-fifth power was used for this purpose. This is a standard bandwidth proposed by Silverman (1986).

For the current study, niches were calibrated by means of two ordination techniques (Figure 4) which in general outperform any other SDM method. Both approaches closely resembles the original description of the fundamental niche by Hutchinson (1957), defined as multidimensional space of environmental predictors in which an organism can persist.

Firstly, Principal Component Analyses – environment (hereafter PCA-env) was applied. This technique to calibrate niches has been shown to outperform others based on evaluation by means of simulated study organisms with predetermined niches (Broennimann *et al.*, 2012). A PCA analysis in the current context selects limited, uncorrelated orthogonal and linear combinations of environmental predictors. The first principal component accounts for as much variability in the study area as possible, followed by the second component. Niches are calibrated upon the first two components, where changes in position display environmental differences. In this aspect, PCA-env is calibrated on the entire environmental space of the study area rather than using only climatic values corresponding to occurrence records. The latter technique has been commonly used before (e.g. Nakazato *et al.*, 2010), but generally underestimates niche overlap (Broennimann *et al.*, 2012). The output of each pairwise comparison between study taxa consists of (1) a PCA which shows contribution of the environmental predictors to, and the percentage of intertia explained by the two axes (Figure 7); (2) the niches of both taxa along the PCA axes (Figure 8); and (3) two similarity tests (see paragraph 2.2.3, Figure 5). PCA axes are the same for all pairwise comparisons as these are fitted on the same geographical extent for each taxon.

Secondly, Ecological Niche Factor Analysis (Hirzel *et al.*, 2002, hereafter ENFA) was applied. ENFA works in a similar way as the PCA analyses described in the previous paragraph as it compares niches in environmental space along two axes. However, instead of explaining as much variability as possible, the first component explains 100% of the 'marginality factor': this is defined as the difference between the average conditions in the kernel density of occurrences (distribution) of an organism to the conditions in the centroid of environmental space. In other words, marginality indicates to what degree a niche is dissimilar from the average conditions in the study area, with higher values indicating higher marginality. The next components consist of 'specialization factors': axes which maximize the ratio of variance between the kernel density of occurrences and the study area (Hirzel *et al.*, 2002; Basille *et al.*, 2008). Marginality can also be seen as an indication for specialization; higher marginality means higher specialization. A varying part of the specialization can therefore already be explained within the marginality factor, with the remainder being described in the specialization factors. However, as specialization values

can range from 1 to infinity, 'tolerance' has been used as an alternative. Tolerance is calculated as the inverse of specialization (1/S) which ranges from 0 (specialist species) to 1 (generalist species).
The output of each pairwise comparison between study taxa consists of (1) a PCA which shows contribution of the environmental predictors to the axes, in which the horizontal axis represents marginality, and the vertical axes tolerance; (2) the niches of both taxa along these PCA axes; and (3) two similarity tests (see paragraph 2.2.3, Figure 5).

Additionally, MAXENT was applied as SDM technique to calibrate niches in order to analyse niche breadth of species (see paragraph 2.3.4). MAXENT produces high quality predictions that are often more successful than those produced with other SDM methods (Elith *et al.*, 2006; Hernandez *et al.*, 2006; Jiménez-Valverde *et al.*, 2008). Also, MAXENT has a successful prediction power even when using low sample sizes (Pearson *et al.*, 2006; Wisz *et al.*, 2008). Maximum entropy is achieved by the constraint that the expected value of each environmental predictor must equal the mean value at the occurrence record (the empirical average) (Phillips *et al.*, 2006). As several study taxa are represented by a relative low amount of occurrence records, MAXENT was set to produce 10 bootstrap replicates, producing an average prediction (also known as 'Ensemble Forecasting', Araújo & New, 2007) of habitat suitability.

2.3.3. Calculating niche similarity

To test comparative hypothesis of niche overlap, various tests have been developed which are dependent of the scenario to be tested (Graham *et al.*, 2004; Warren *et al.*, 2008; Glor & Warren, 2011). Two main tests have been proposed; the Identity or Equivalency test can be used to measure whether niches of two organisms are identical. This is mainly useful for overlapping (e.g. sympatric or parapatric) distributions. The Background or Similarity tests addresses the question whether the calibrated niche of organism A predicts the niche of organism B better than expected by chance, taking into account the local environmental heterogeneity of the study area (full explanation below). This is especially useful when comparing non-overlapping (allopatric) organisms. For the current case only the similarity test was applied, as nearly all study taxa have non-overlapping ranges.

The following explanation is based on the information provided by Warren *et al.* (2008), Warren *et al.* (2010) and Broennimann *et al.* (2012). The similarity test is conducted by comparing calibrated niches, i.e. the area in environmental space where organism A and B are predicted to occur. First, the actual (also known as observed or true) similarity between two niches as defined by the metric Schoener's D (see below) is calculated. This actual overlap is compared to a number of randomizations, to tests whether the two niches are more similar to each other than expected in regard to the environmental conditions in the study area. The randomizations are made by placing the kernel density of occurrences at random within the predicted niche of organism A, upon which a null model is built which is compared to the predicted niche of organism B. This process is usually repeated 100 times in both directions (A vs. B and B vs. A) to construct a null distribution of D values which can be compared to the actual D value (example in Figure 5). When the actual overlap value is significantly ($p < 0.05$) higher or lower than expected from the null distribution based on a two-tailed test, the null hypothesis that the two organisms are not more similar to each other can be rejected.

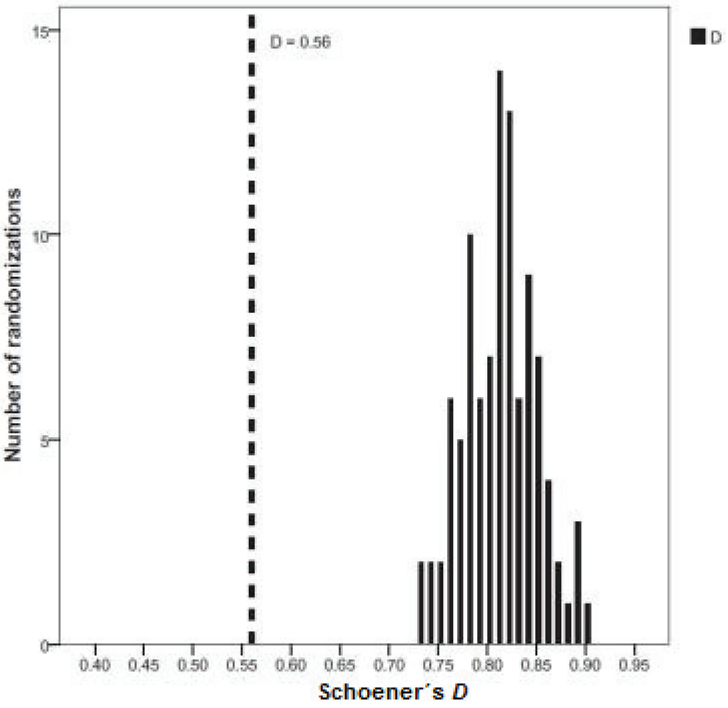

Figure 5: An example of niche similarity testing based on Schoener's *D*; the observed value of niche overlap (dotted line) is significantly lower than expected when compared to 100 randomizations in environmental space (histogram). Consequently, these organisms do not occupy similar niches. Modified from Beukema *et al.* (2010).

In this aspect, it is possible that significant niche similarity is recovered for organism A vs. B, but not the other way around. A possible explanation for this phenomenon is a more heterogeneous (or potentially wider) niche in environmental space of A when compared to B (Nakazato *et al.*, 2010). Organism A partially occurs in environmental conditions of the niche of B, while organism B does not occur in the majority of environmental conditions of A. Therefore, A vs. B will result in significant similarity, while B vs. A will lead to rejection of the hypothesis.

The metric *D* as defined by Schoener (1968, hereafter Schoener's *D*), was used to measure niche similarity following Warren *et al.* (2008) based on the formula in Figure 6. In this formula, p_A and p_B are the suitability scores according to the calibrated niches of organisms A and B in cell *i* of the study area. The metric varies between 0 (no overlap) and 1 (complete overlap).

$$D = 1 - \frac{1}{2}\left(\sum_{i} \left| p_{A,i} - p_{B,i} \right|\right)$$

Figure 6: Equation for Schoener's *D*.

Secondly, only the niche indices which were more similar than expected by chance were divided into six groups to assess intra- and intergeneric niche overlap; (1) within genus *Lyciasalamandra*; (2) between *Lyciasalamandra* and members from another genus; (3) within genus *Salamandra*; (4) between *Salamandra* and members from another genus; (5) within genus *Speleomantes*; (6) between *Speleomantes* and members from another genus. Comparisons were tested by means of independent t-tests including Levene's tests for homogeneity of variances in SPSS16, as all group wise data had a normal distribution based on Shapiro Wilk tests ($P > 0.05$).

Finally, significant *D* overlap scores were divided into four new groups which also had a normal distribution; (1) viviparous vs. viviparous taxa; (2) viviparous vs. non-viviparous taxa; (3) non-viviparous vs. viviparous taxa; and (4) non-viviparous taxa vs. non-viviparous taxa. A one way ANOVA test in combination with a Homogeneity of Variances- and post-hoc Tukey test were used to assess differences, as well as to produce descriptive statistics within each group.

2.3.4. Assessment of niche breadth and range

The physical environment and biotic interactions influence the breadth of a niche over evolutionary time spans (e.g. Feinsinger *et al.*, 1981 and references therein). It has hence been proposed that organisms with a broad niche should be geographically widespread and abundant throughout their range (Brown, 1984), as a result of access to a wide range of different habitat types and resources. On the other hand, organisms with a narrow niche have been argued to occupy small ranges due to adaptation and subsequent specialization to local conditions (Kickpatrick & Barton, 1997). A comparison was consequently made between niche breadth, range and reproductive type of all study taxa.

- Niche breadth was calculated by means of the inverse concentration as presented by Levins (1968) in ENMTools 1.3 based on the continuous average habitat suitability output files from MAXENT.
- Range was defined as the geographical area within which an organism can be found. Therefore, Minimum Convex Polygons (MCP) were created in ArcGIS 10.0 using the Minimum Bounding Geometry tool, of which the surface in km^2 was subsequently calculated.

As both niche breadth and range were not normally distributed (Shapiro Wilk test $P < 0.00$) and did not have a linear relationship even when outliers were removed, a Spearman's Correlation was performed in SPSS16 after the variables were transformed into ranks. Subsequently, a Mann-Whitney U test was performed to assess differences between reproductive groups.

3. RESULTS

3.1. Multicollinearity analyses

Multicollinearity analysis found 37 pairwise predictor combinations with an $r_s > 0.7$. Consequently, based on ecological knowledge, fifteen predictors were removed from the dataset. The final dataset including r_s values is displayed in Table 1a. For more information on the predictors, see Appendix 2.

Predictor	Slope	Aspect	Annual Mean Temp	Isothermality	Temperature Seasonality	Mean Temp Wettest Quarter	Annual Precip	Precip Seasonality	Precip Coldest Quarter	PET
Slope	0	0	-0.32	-0.14	0.05	-0.33	0.30	0.03	0.30	-0.21
Aspect	0	0	0.03	0.09	-0.08	-0.02	0.02	0.08	0.05	0.02
Annual Mean Temp	0	0	0	0.34	-0.15	0.56	-0.42	0.57	-0.11	0.67
Isothermality	0	0	0	0	-0.37	0.18	-0.43	0.37	-0.21	0.62
Temperature Seasonality	0	0	0	0	0	-0.32	-0.10	-0.15	-0.08	0.29
Mean Temp Wettest Quarter	0	0	0	0	0	0	-0.42	-0.10	-0.48	0.23
Annual Precip	0	0	0	0	0	0	0	-0.08	0.84	-0.62
Precip Seasonality	0	0	0	0	0	0	0	0	0.41	0.44
Precip Coldest Quarter	0	0	0	0	0	0	0	0	0	-0.29
PET	0	0	0	0	0	0	0	0	0	0

Table 1a: Dataset of environmental predictors (pairwise $r_s < 0.7$) after multicollinearity analysis.

Predictor	VIF
Slope	1.295
Aspect	1.018
Annual Mean Temp	11.456
Isothermality	11.578
Temperature Seasonality	9.893
Mean Temp Wettest Quarter	3.305
Annual Precip	15.490
Precip Seasonality	5.956
Precip Coldest Quarter	16.940
PET	23.070

Table 1b: Dataset of environmental predictors after multicollinearity analysis based on the VIF.

3.2. Niche similarity

Speleomantes genei B and *Lyciasalamandra luschani luschani* were omitted from analyses, as their range (see also Table 3a) and number of occurrence records was too limited. Conclusively, a total of 561 pairwise combinations were made between the study taxa by means of PCA-env and ENFA. This resulted in 561 *D* scores per modeling technique, as well as 1122 significance values for all similarity tests. Only significant *D* scores of higher-than-expected overlaps were taken into account (n = 144 for PCA-env, n = 289 for ENFA). No lower-than-expected significant overlaps were found.

3.2.1. PCA-env

Equal variance was met for all inter- and intrageneric comparisons based on Levene's test ($P = 0.437$, $P = 0.144$ and $P = 0.111$ for the genera *Lyciasalamandra*, *Salamandra* and *Speleomantes* respectively). Based on the independent t tests, no statistically significant differences were found regarding inter- and intrageneric niche similarity for the genera *Lyciasalamandra* and *Salamandra* ($t(61) = -0.709$, $P = 0.481$; $t(56) = 1.529$, $P = 0.132$ respectively). Thus, *D* values were not significantly higher within the genera when compared to members of other genera. Intrageneric niche similarity was higher in the genus *Speleomantes* when compared to intergeneric similarity ($t(21) = 3.562$, $P = 0.002$), 0.433 ± 0.131 versus 0.201 ± 0.181.

Subsequently, significant *D* scores were divided into four groups; (1) viviparous vs. viviparous taxa (n = 41); (2) viviparous vs. non-viviparous taxa (n = 24); (3) non-viviparous taxa vs. viviparous taxa (n = 25); and (4) non-viviparous taxa vs. non-viviparous taxa (n = 54). For descriptive statistics, see Table 2a. A one way ANOVA test in combination with a Homogeneity of Variances and post-hoc Tukey test were used to assess differences, as well as to produce descriptive statistics within each group. Levene's test of Homogeneity of Variance was non-significant ($P = 0.238$), meaning the assumption of homogeneity of variances was met. Results of the ANOVA analysis showed no statistically significant difference between group means (ANOVA ($F = 1.229$, $P = 0.302$). A subsequent post-hoc test was therefore not implemented.

Group	N	Mean	SD	SE	95% CI for Mean		Min	Max
					Lower bound	Upper bound		
Vivi vs. vivi	41	0.285	0.184	0.029	0.227	0.343	0.020	0.665
Vivi vs. non-vivi	24	0.332	0.210	0.043	0.243	0.421	0.004	0.702
Non-vivi vs. vivi	25	0.282	0.204	0.041	0.197	0.366	0.000	0.702
Non-vivi vs. non-vivi	54	0.347	0.163	0.022	0.302	0.391	0.000	0.638
Total	144	0.316	0.185	0.015	0.285	0.346	0.000	0.702

Table 2: Descriptive statistics of Schoener's *D* scores from PCA-env within compared groups.

As five of the non-viviparous taxa (*S. a. tingitana* B, *S. algira* "Rif + Atlas", *S. i. infraimmaculata*, *S. s. gallaica* and *S. s. longirostris*) show considerable overlap with all viviparous taxa (excluding *S. sarrabusensis*) concluding from Figure 8, all significant *D* values between these and the other study taxa were omitted for re-analyses. This resulted in a total of 82 significant *D* values, which were distributed among two groups; (1) viviparous vs. viviparous taxa (n = 41) and (2) non-viviparous taxa vs. non-viviparous taxa taxa (n = 40). Equal variance was met based on Levene's test ($P = 0.060$). No statistically significant difference was recovered based on an independent t test ($t(79) = -1.545$, $P = 0.126$).

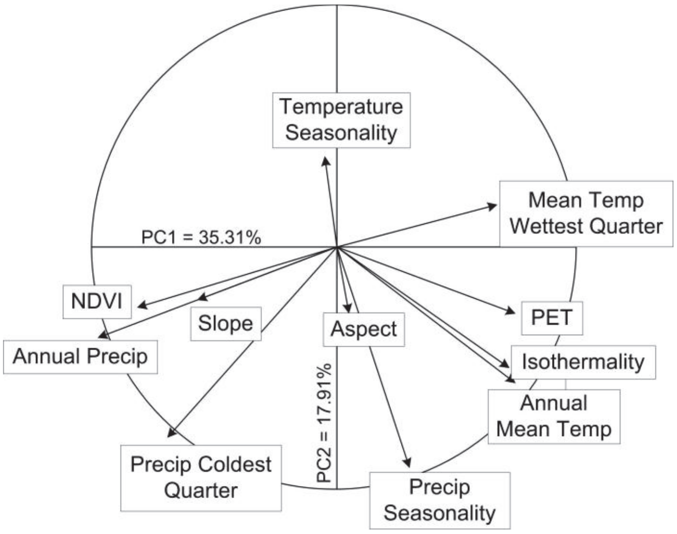

Figure 7: The contribution of environmental predictors on the two PCA axes of the PCA-env, and the percentage of inertia explained by the axes.

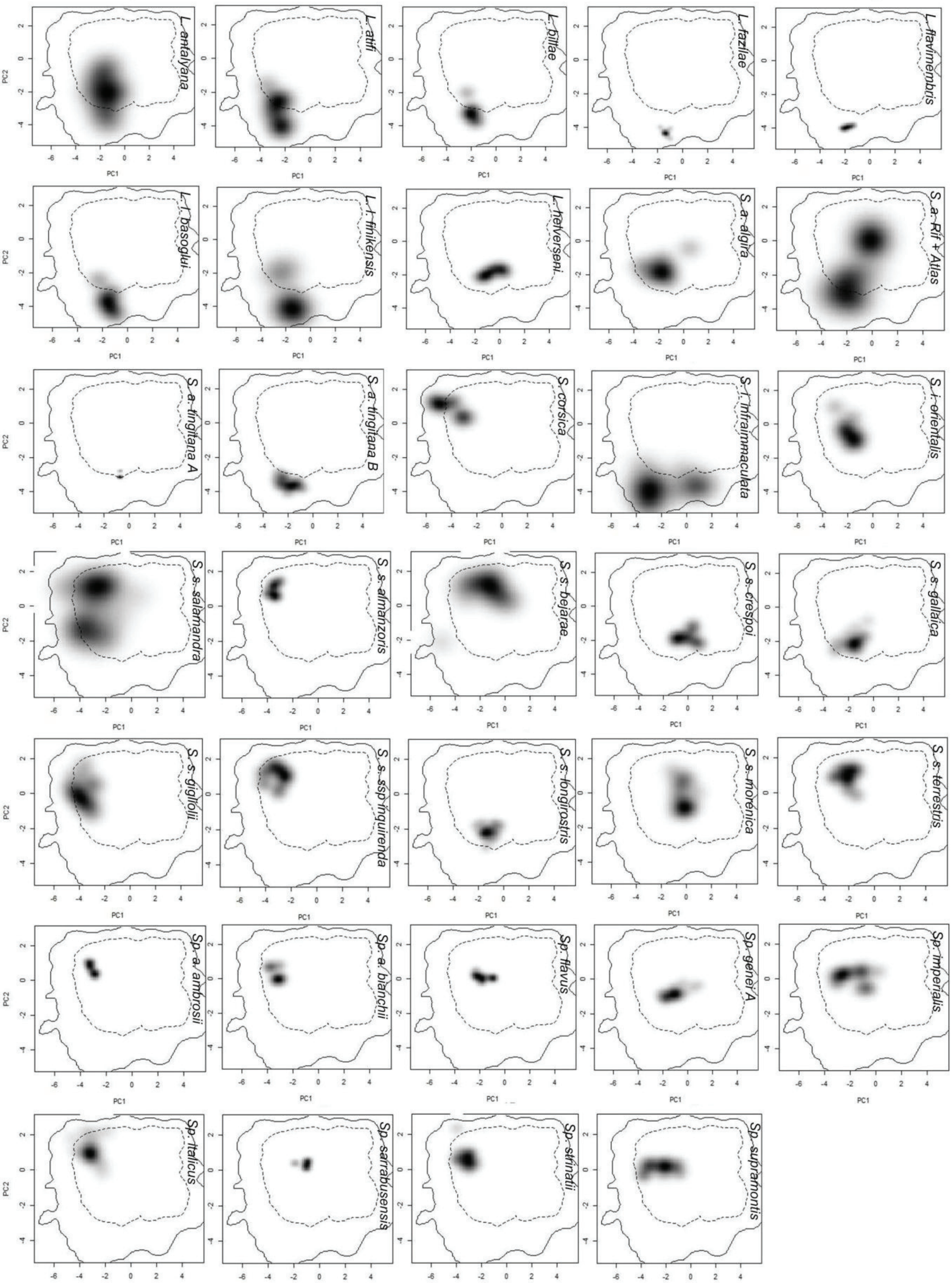

Figure 8: Niches bounded by PCA-env axes. Black shading represents the density of occurrences of each taxon. Solid and dashes contour lines show respectively 100 and 50% of the background environment.

As the bounding PCA-env axes and conclusively the environmental background are the same for all calibrated niches, these can be compared by means of the vectors represented by environmental predictors in the PCA illustrated in Figure 7. Due to the fact that multicollinearity analysis was performed prior to PCA-env analysis, the percentage inertia for both axes is relatively low, as only two are taken into consideration to calculate niche overlap. PC1 is mainly correlated with NDVI, Annual Precipitation, Mean Temperature of Wettest Quarter, PET, Annual Mean Temperature and Isothermality, while PC2 is correlated with Precipitation of Coldest Quarter and Precipitation Seasonality.

3.2.2. ENFA

Based on ENFA results, equal variance was not met for inter- and intrageneric comparisons regarding *Lyciasalamandra* (Levene's test $P = 0.001$) while it was met for the genera *Salamandra* and *Speleomantes* ($P = 0.075$ and $P = 0.241$ respectively). Based on the independent t tests, no statistically significant differences were found regarding inter- and intrageneric niche similarity for the genera *Lyciasalamandra* and *Speleomantes* ($t(76.508) = 1.166$, $P = 0.247$; $t(76) = -0.497$, $P = 0.621$ respectively). Thus, D values were not significantly higher within the genera when compared to members of other genera. Intrageneric niche similarity was higher in the genus *Salamandra* when compared to intergeneric similarity ($t(131 = 3.832$, $P < 0.000$), 0.464 ± 0.194 versus 0.340 ± 0.177.

For descriptive statistics of the one way ANOVA based on ENFA similarity scores, see Table 3a. Levene's test of Homogeneity of Variance was significant ($P = 0.001$), meaning that the assumption of homogeneity of variances was not met. Therefore, a Welch test was applied to test for significant differences between groups. This test was significant ($P < 0.000$) leading to the conclusion that there are significant differences.

Group	N	Mean	SD	SE	95% CI for Mean		Min	Max
					Lower bound	Upper bound		
Vivi vs. vivi	52	0.317	0.211	0.029	0.259	0.376	0.022	0.681
Vivi vs. non-vivi	40	0.308	0.140	0.022	0.264	0.353	0.055	0.739
Non-vivi vs. vivi	42	0.294	0.176	0.027	0.239	0.348	0.000	0.739
Non-vivi vs. non-vivi	155	0.415	0.187	0.015	0.385	0.445	0.033	0.791
Total	289	0.365	0.191	0.011	0.343	0.387	0.000	0.791

Table 3a: Descriptive statistics of Schoener's D scores from ENFA within compared groups.

Group		Mean difference	SE	Sig.	Lower bound	Upper Bound
Vivi vs. vivi	Vivi vs. non-vivi	0.009	0.039	0.996	-0.091	0.109
	Non-vivi vs. vivi	0.024	0.038	0.925	-0.075	0.123
	Non-vivi vs. non-vivi	-0.098	0.030	**0.006**	-0.174	-0.021
Vivi vs. non-vivi	Vivi vs. vivi	-0.009	0.039	0.996	-0.109	0.091
	Non-vivi vs. vivi	0.015	0.041	0.984	-0.091	0.120
	Non-vivi vs. non-vivi	-0.107	0.033	**0.007**	-0.191	-0.022
Non-vivi vs. vivi	Vivi vs. vivi	-0.024	0.038	0.925	-0.123	0.075
	Vivi vs. non-vivi	-0.015	0.041	0.984	-0.120	0.091
	Non-vivi vs. non-vivi	-0.121	0.032	**0.001**	-0.204	-0.039
Non-vivi vs. non-vivi	Vivi vs. vivi	0.098	0.030	**0.006**	0.021	0.174
	Vivi vs. non-vivi	0.107	0.033	**0.007**	0.022	0.191
	Non-vivi vs. vivi	0.121	0.032	**0.001**	0.039	0.204

Table 3b: Results of the post-hoc Tukey test based on Schoener's D scores from ENFA.

The post-hoc Tukey test (Table 3b) showed that in all cases, Schoener's *D* values from the non-viviparous taxa vs. non-viviparous taxa were significantly higher than compared to the other groups (Table 3b).

3.3. Niche breadth

MAXENT models calibrated in order to permit niche breadth analyses were characterized by very high AUC values (0.954 – 1.000) as shown in Table 4a. Spearman's correlation between the ranked parameters Niche Breadth and Range was weak and non-significant (r_s = 0.310, *P* = 0.066). The subsequent Mann-Whitney test reported a significant difference between reproductive groups regarding both Niche Breadth and Range; in both cases the viviparous group was shown to possess narrower niches and smaller ranges, as can be concluded from the Mean Rank in Table 4b.

Taxon	**Reproductive type**	**Niche breadth**	**Range (km²)**	**AUC**
Lyciasalamandra antalyana	Viviparous	0.1991	208.13	0.982
Lyciasalamandra atifi	Viviparous	0.0701	1636.21	0.994
Lyciasalamandra billae	Viviparous	0.0240	32.53	0.999
Lyciasalamandra fazilae	Viviparous	0.0044	532.20	0.999
Lyciasalamandra flavimembris	Viviparous	0.0030	174.18	1.000
Lyciasalamandra helverseni	Viviparous	0.0022	488.27	1.000
Lyciasalamandra luschani luschani	Viviparous	0.0397	2.38	0.999
Lyciasalamandra luschani basoglui	Viviparous	0.0674	404.53	0.997
Lyciasalamandra luschani finikensis	Viviparous	0.0186	229.47	0.997
Salamandra algira algira	Non-viviparous	0.3395	26140.53	0.962
Salamandra algira "Rif + Atlas"	Non-viviparous	0.0631	6500.06	0.997
Salamandra algira tingitana A	Viviparous	0.0353	526.48	0.998
Salamandra algira tingitana B	Non-viviparous	0.0203	1078.02	0.999
Salamandra corsica	Non-viviparous	0.0093	1913.16	0.999
Salamandra infraimmaculata infraimmaculata	Non-viviparous	0.0316	18635.14	0.997
Salamandra infraimmaculata orientalis	Non-viviparous	0.1641	7222.33	0.974
Salamandra salamandra salamandra	Non-viviparous	0.2232	230013.38	0.965
Salamandra salamandra almanzoris	Non-viviparous	0.0334	707.74	0.998
Salamandra salamandra bejarae	Non-viviparous	0.1243	61393.59	0.991
Salamandra salamandra crespoi	Non-viviparous	0.0891	8333.30	0.991
Salamandra salamandra gallaica	Non-viviparous	0.0394	32705.22	0.994
Salamandra salamandra gigliolii	Non-viviparous	0.0202	21039.32	0.997
Salamandra salamandra ssp. '*inquirenda*'	Non-viviparous	0.0479	43103.99	0.995
Salamandra "salamandra" longirostris	Non-viviparous	0.0670	3347.44	0.997
Salamandra salamandra morenica	Non-viviparous	0.2086	19390.85	0.972
Salamandra salamandra terrestris	Non-viviparous	0.1135	46742.58	0.985
Speleomantes ambrosii ambrosii	Non-viviparous	0.0481	220.91	0.998
Speleomantes ambrosii bianchii	Non-viviparous	0.0769	31.89	0.998
Speleomantes flavus	Non-viviparous	0.0459	74.14	0.997
Speleomantes genei A	Non-viviparous	0.0485	1517.77	0.999
Speleomantes genei B	Non-viviparous	0.3738	7.17	0.954
Speleomantes imperialis	Non-viviparous	0.0515	2673.52	0.995
Speleomantes italicus	Non-viviparous	0.0631	23844.60	0.994
Speleomantes sarrabusensis	Viviparous	0.0201	47.70	1.000
Speleomantes strinatii	Non-viviparous	0.0288	7704.65	0.998
Speleomantes supramontis	Non-viviparous	0.0383	638.85	0.997

Table 4a: Reproductive type, niche breadth and range of all study taxa.

Parameter	Reproductive type	N	Mean Rank	Mann-Whitney *U*	*P*
Niche Breadth	Viviparous	11	12.55	72	0.024
	Non-viviparous	25	21.12		
Range	Viviparous	11	9.45	38	0.001
	Non-vivipaorus	25	22.48		

Table 4b: Results of the Mann-Whitney Test.

4. DISCUSSION

4.1. Methodological remarks

As the main analytical part of the current research was based on a recently presented framework there are only few examples by which the current analyses and results can be compared. Calibration of niches based on 'simulated entities' (i.e. virtual organisms with known environmental restrictions) showed ordination techniques to outperform SDM techniques in general (Broennimann *et al.*, 2012). Applied analyses based on existing organisms by means of this framework are therefore currently only available regarding PCA calibration (Bassin *et al.*, 2011; Ballard *et al.*, in press) which was identified by Broennimann *et al.* (2012) as the most accurate technique to measure niche overlap. Rödder & Engler (2011) argued that comparison of niches in multivariate environmental space by means of e.g. PCA and ENFA analyses will provide an unbiased estimation of niche overlap, as each combination of environmental attributes along the axes can only be made once. This approach is highly similar to the concept of the fundamental niche by Hutchinson (1957). In contrast, multiple combinations can be found in geographical space, when represented by e.g. grid cells which might lead to overestimation, especially in case of significant overprediction. However, the same authors also noted the usefulness of SDM methods, as these incorporate the relative importance of multiple combinations within grid cells of the entire study area as weighting factor, resulting in potential suitability. The application of both PCA-env and ENFA can be seen as an appropriate combination, as these techniques take environmental variation within the entire study area into account, as opposed to conditions limited to exact occurrence sites of organisms in e.g. a general PCA. It has to be noted however that although both ordination techniques are calibrated by means of the entire study area, methodologies differ. In the case of PCA-env, niches are plotted along the two most explaining axes representing environmental variation in the entire study area. As a result, the background environment remains stable for each pairwise comparison between taxa (Figure 8). In contrast, calibration of niches by means of ENFA requires the use of pseudo-absences, which are separately created for each pairwise comparison leading to different background values. Therefore, resulting Schoener's *D* values can be compared, but the composition of marginality and tolerance axes in the PCA might differ per pairwise combination.

One of the main strengths of ENFA, especially amongst other ordination techniques, is its explanatory power with regard to the environmental conditions which encompass an organism's niche (Basille *et al.*, 2008). These can be analysed by means of the marginality and various specialization factors, thus gaining an overview of which environmental predictors most accurately explain the distribution of niches (e.g. Santos *et al.*, 2006; Soares & Brito, 2007). When applied to calculate niche overlap, most of this information gets lost. This is mainly due to the fact that niches are only compared along marginality and tolerance axes, rather than along multiple axes representing environmental conditions in the study area.

The application of kernel densities might correct for biased output as a result of outliers, which would be given an equal weight of importance in a general PCA analyses. Sampling bias overall was low, although several cases can be identified in which kernel densities in niche plots (Figure 8) show subdivisions while the true distribution is largely continuous. This is mainly due to incomplete distribution data regarding *S. corsica* (central Corsica), *S. i. infraimmaculata* (Libanon in general) and *S. s. salamandra* (eastern Adriatic coast). In other cases, such patterns are actual representations of isolated subpopulations (*S. algira* "Rif + Atlas", *S. imperialis*). Conclusively, the suggestion made by Broennimann *et al.* (2012) regarding the fact that a kernel density would corrected for biased sampling efforts should be mitigated.

Average AUC values from the bootstrap replicate runs in MAXENT turned out to be very high (0.954 – 1.000). There has been considerable criticism on the AUC as means for validation during recent years (e.g. Lobo *et al.*, 2008). Very high AUC values are not uncommon (e.g. Engler & Rödder, 2011; Rödder *et al.*, 2011) and might be influenced by heterogeneity of environmental predictors, extent of the study area, number and spatial distribution of occurrence points (Lobo *et al.*, 2008; Anderson & Raza, 2010). In the current case, the number of occurrence points does not seem to have a direct influence on the AUC as both taxa with very few occurrence records, and those represented by a considerable number all display high AUC values (Table 3a). This result is somewhat surprising, as calibration upon a large study area (i.e. large geographical extent in regard to the occurrence of a study species) usually results in lower AUC values when compared to calibration upon a limited area (Anderson & Raza, 2010). The amount of overprediction (i.e. identification of suitable habitat outside of the study species range which cannot be occupied due to biogeographical barriers) was minimal (MAXENT results not shown), likely as a result of the large study area (Anderson & Raza, 2010).

Pearson's *r* has been used on several occasions to reduce the amount of environmental predictors in a dataset, while retaining as much variance as possible (e.g. Kamilar & Muldoon, 2010). However, when compared to results based on the VIF, considerable multicollinearity was still present after pairwise correlations had been removed (Table 1b). The incongruence between these analyses should be taken into account for future analyses, especially in regard to SDM methods. However, the effect on the current study is likely minimal as both applied ordination techniques confine environmental space to two PCA axes.

4.2. Niche characteristics

As noted before, many presumptions have to be made to permit the calibration and measuring of niche divergence in multivariate environmental space. Niche conservatism lies at the base of these assumptions, as it is necessary to assume that an organism is in a pseudo-equilibrium with the environment to be able to predict a niche across space and time (Guisan & Thuiller, 2005; Pearman *et al.*, 2008; Elith & Leathwick, 2009). However, especially in the case of wide-ranging organisms, different environmental constraints can influence local distribution patterns which might lead to model over- or underprediction in general (Pearman *et al.*, 2010) which can be worsened due to e.g. sampling bias. Moreover, this phenomenon might be especially true for the Mediterranean Basin, where glacial refugia and successive recolonization events have had a profound impact on intra- and interspecific divergence (Taberlet *et al.*, 1998). The recent development of calibration of niches below species level might assist to overcome this bias (Pearman *et al.*, 2010). Studies which implement such analyses have, up to date, without exception obtained more accurate results than when compared to a model of the species as whole (e.g. Pearman *et al.*, 2010; Thompson *et al.*, 2011). The results of the current analyses support this approach, as even recently diverged lineages (i.e. subspecific *Salamandra* taxa) were shown to occupy significantly diverged niches in environmental space.

4.2.1. Inter- and intergeneric niche evolution

Much interest has been given to individual cases of niche divergence in the recent past (e.g. Losos *et al.*, 2003; Graham *et al.*, 2004; Broennimann *et al.*, 2007; Fitzpatrick *et al.*, 2007; Kamilar & Muldoon, 2010) although a tendency for species to display niche conservatism in general remains ubiquitous (Peterson, 2011). The reasons for different rates of overall niche divergence among genera or clades of species are however not well understood. In a study on North- and South American Plethodontidae salamanders, Kozak & Wiens (2010a) found that phylogenetic diversification rates of salamander clades were significantly correlated with the rate of (climatic) niche evolution, irrespective of clade age. While the size of the geographical area in which clades occur does not seem to be a limiting factor, clade overlap (i.e. competition) might negatively affect rates of niche evolution and diversification. Contrasting patterns in which competition does not seem to be a limiting factor have however been identified, even within the Plethodontidae (Kozak & Wiens 2010b).

As current analyses show, only the genus *Speleomantes* displays significantly higher intrageneric niche similarity based on results of PCA-env calibration, while only the genus *Salamandra* displays significantly higher intrageneric niche similarity based on results of ENFA. Both these cases can be interpreted as niche conservatism, but have to be carefully analyzed. Recently, Rödder *et al.* (2011) recovered a high degree of intrageneric niche conservatism among members of the genus *Lyciasalamandra* as a possible result of relative low genetic variability (Veith *et al.*, 2008), which in the current results does not show a significant difference between intra- and intergeneric overlap. When visually analyzed by means of niche plots in environmental space regarding PCA-env (Figure 8) it becomes clear that this is mainly due to the niches of the Moroccan *Salamandra* ssp. and *S. i. infraimmaculata*, which consequently overlap with members of the genus *Lyciasalamandra*. Indeed, apart from *L. helverseni* most niches of *Lyciasalamandra* are highly similar to each other, which is in agreement with the results of Rödder *et al.* (2011). While ENFA results do display a significant higher intrageneric niche overlap for the genus *Salamandra* it has to be noted that this technique is known to regard niches as more similar than they actually are in comparison with e.g. PCA-env (Broennimann *et al.*, 2012). As the niche breadth of *Salamandra* taxa is in general moderate to high when compared to other genera, these will also have a larger chance to overlap on an intraspecific level, as well as with taxa of *Lyciasalamandra* and *Speleomantes* which might lead to high interspecific overlap scores. Subsequently, intrageneric overlap scores of the genera *Lyciasalamandra* and *Speleomantes* which generally possess narrower niches with lesser chance of overlap are not significantly higher.

Although the current analyses were not combined with time-calibrated phylogenetics, some conclusions can be drawn based upon earlier published research (e.g. Steinfartz *et al.*, 2000; Carranza *et al.*, 2008). Diversification within the genera *Salamandra* and *Speleomantes* most likely occurred soon after the Messinian Salinity Crisis (5.3 million years ago), while intraspecific divergence mostly occurred as a result of the Pleistocene glaciations. Intraspecific, recent mitochondrial divergence is especially high in *S. salamandra* which also displays the highest rate niche divergence among its subspecies (Figure 8). Fast local genetic adaptation to environmental conditions has been repeatedly described as cause for the currently observed diversity (Weitere *et al.*, 2004; Goldberg *et al.*, 2009). This might support the theory of high phylogenetic diversification rates as a result of higher Grinnellian niche divergence rates (Kozak & Wiens, 2010a). While (currently known) intraspecific mitochondrial divergence in the genus *Speleomantes* is comparatively low, most reported cases originate from continental taxa rather than those found on Sardinia, while the former are sympatric with *S. salamandra* which would rule out competition as a limiting factor. In fact, these populations are often sympatric with yet another salamander genus, *Salamandrina*, which was not incorporated in the current analyses. These observations would support the relative unimportance of competition in relation to salamandrid diversity, comparable to what has been described for the North-American Appalachians (Kozak & Wiens 2010b).

4.2.2. Niche evolution in respect to reproductive behaviour

Results showed that niche similarity between the defined reproductive groups is not significantly higher or lower according to the one way ANOVA tests, apart from scores among non-viviparous taxa based on ENFA results (Table 3b). However, significant niche similarity scores within reproductive groups as opposed to scores between reproductive groups have a higher occurrence based on both PCA-env and

ENFA calibration. The omission of five study taxa from the PCA-env results caused complete disappearance of significant niche overlap between reproductive groups, while *D* values within the two remaining reproductive groups again did not show statistically significant differences. These results can be interpreted in several ways, in which the significant results between range, niche breadth and reproductive type (Table 4b) also have to be taken into account.

Conform with the assumption that widespread organisms should occupy a broader niche than organisms with a limited distribution as a result of availability of resources and adaptation/specialization (Brown, 1984; Kickpatrick & Barton, 1997), viviparous taxa did prove to have significantly smaller ranges and niche breadth when compared to non-viviparous taxa. Conversely, this could also be an indication of the fact that a smaller range will on average encompass a smaller subset of environmental variation than a large area. The former hypothesis is however more likely, as niches of viviparous taxa have proven to be significantly similar to each other in nearly all cases (Table 2, 3a; Figure 8). Indeed, as niche breadth of viviparous taxa is smaller, which can also be concluded from the plots in environmental niche space (Figure 8), it is noteworthy to mention that members of this reproductive group do regularly show overlap, both by means of PCA-env and ENFA calibration. This can again be interpreted as high Grinnellian niche similarity of viviparous taxa as these have less chance to overlap due to their narrow niches, while it has to be taken into account that several non-viviparous taxa do display similar environmental. Members of the non-viviparous group tend to occupy broader niches, which did result in a significantly higher overlap when compared to other groups based on ENFA calibration.

The omission of several study taxa from PCA-env calibration (*S. a. tingitana* B, *S. algira* “Rif + Atlas”, *S. i. infraimmaculata*, *S. s. gallaica* and *S. s. longirostris*) resulted in a complete disappearance of niche overlap between reproductive groups. For four of these (*S. a. tingitana* B, *S. algira* “Rif + Atlas”, *S. s. gallaica* and *S. s. longirostris*) the recovered pattern is likely a result of the analyses in Grinnellian niche space. All these taxa occur generally in well-forested humid areas which at the resolution of the current study might not be fully accounted for despite the inclusion of a NDVI predictor. Conversely, *S. i. infraimmaculata* does tend to occupy considerably xeric habitats (e.g. Goldberg *et al.*, 2009).

Finally, *Speleomantes sarrabusensis* deserves special mentioning regarding its assumed viviparity (Lanza & Leo, 2000) as only member of the family Plethodontidae. According to the similarity tests and niche space plots resulting from PCA-env analyses, *S. sarrabusensis* does not inhabit similar conditions compared to other viviparous taxa. Indeed, the position of *S. sarrabusensis* in environmental niche space is unique in the sense that this taxon does not show significant overlap with any other study taxon (Figure 8, unpublished results). ENFA analyses did show several cases of significant similarity with a number of viviparous taxa. However, as noted before, this is likely a result of niche characteristics (i.e. based on niche breadth (‘tolerance’) and deviation of the mean conditions of the study area) rather than niche attributes (true environmental similarity), as information on the latter gets largely lost during calculations of niche overlap in ENFA. This leads to the conclusion that the range of *S. sarrabusensis* encompasses a combination of environmental conditions not found for any other Mediterranean salamander, but does possess a small range and narrow niche corresponding to other viviparous taxa (see also Table 4a).

4.3. Dimensions and scale

The purpose of the current study was to assess Grinnellian niche evolution among Mediterranean salamanders with different reproductive behaviour. As the Grinnellian niche is composed of (usually coarse-scale) scenopoetic predictors (Grinnell, 1917; Soberón, 2007) it is most appropriately used to infer large-scale biogeographic patterns, rather than local scale conditions. Indeed, biotic interactions as covered by the Eltonian niche might become less evident on such resolutions, as proposed in the Eltonian noise hypothesis (Soberón & Nakamura, 2009). However, cases are known in which Eltonian factors such as competition might influence rates of niche evolution (Kozak & Wiens, 2010). As the study taxa of the current research shown little geographical overlap, while viviparous taxa are all allopatric, at least salamandrid competition does most likely not have an influence on the occurrence of viviparity. Additionally, the currently used Schoener's *D* statistic is highly independent of grid size (Engler & Rödder, 2011), making it a useful metric for the current analyses.

However, as only broad biogeographical patterns are assessed with the current approach, data on local-scale processes is not incorporated. Likely, these processes are decisive in regards to an evolutionary transition to different reproductive behaviour. Beukema *et al.* (2010) showed recently diverged, intraspecific lineages of Moroccan *S. algira* characterized by different reproduction modes to occupy significantly different environmental conditions. Although these analyses were similarly not applied at a microhabitat scale (albeit at higher resolution than the current analyses), the contribution of different geology- and vegetation communities, and climatic influences caused by the Atlantic ocean and Mediterranean sea were likely of great influence for separation of populations and existence of multiple reproduction modes. Another example consists of even more recently diverged (< 10.000 years) insular populations of *S. salamandra* in north-western Spain which have separately evolved to viviparity as a result of local scale conditions such as low availability of water bodies (Velo-Antón *et al.*, 2007; Velo-Antón *et al.*, in press). As opposed to the earlier mentioned 'cold climate hypothesis', Velo-Antón *et al.* (in press) therefore proposed a 'dry climate hypothesis' related to the transition to viviparity, at least regarding Mediterranean amphibians.

4.3.1. Multivariateness

Niche overlap measured along a single environmental predictor is highly prone to overestimation. Conversely, when measuring niche overlap by means of a large number of environmental predictors within multivariate space it might be possible to underestimate overlap due to model complexity (Peterson, 2011). When comparing allopatric organisms it is especially difficult to identify similar (i.e. convergent) conditions which might lead to a similar outcome (Samuels & Drake, 1997; Ernst *et al.*, in press). The *a priori* selection of environmental predictors has been proposed as an alternative means for accurate calibration of a species niche (Austin, 2007; Hengl *et al.*, 2009; Elith *et al.*, 2011). While such an approach might produce accurate results in regard to habitat suitability maps, the suitability for application in regard to measuring niche overlap can be argued. For the current analyses, a combination of multicollinearity analysis and *a priori* selection was used to construct a comprehensible dataset. In general, this technique performed well, as niches could be calibrated with high accuracy (e.g. Table 4a). However, only the results from PCA-env can give an indication of which environmental conditions encompass the distribution or niche of the different study taxa. From the PCA (Figure 7) and corresponding niche plots (Figure 8) it can be concluded that viviparous species (apart from *S. sarrabusensis*) are highly correlated with the predictor 'Precipitation during the coldest quarter', while 'Slope', 'Annual Precipitation' and 'NDVI' show correlation to a lesser extent. At first sight, this would seem to contrast the earlier mentioned 'dry climate hypothesis'. However, from an ecological viewpoint this can be interpreted as high dependency on winter rains, as this is the only period during which surface activity is permitted for viviparous taxa. As mentioned by e.g. Broennimann *et al.* (2012), the application of SDM methods such as MaxEnt which produce jack-knife tests of predictors importance and response curves for habitat suitability for each environmental predictor in relation to the modelled organism, is therefore highly useful to infer which environmental conditions characterize the niche or distribution.

5. CONCLUSIONS

Do the herein presented results reject the general hypothesis of environmental correlates within viviparous taxa as opposed to non-viviparous taxa? The general answer is no, although it is by no means straightforward. As shown by the analyses of niche breadth and partially by the tolerance of ENFA, niches of viviparous taxa are narrower which likely represents specialization. Despite the fact that niche similarity within reproductive groups was not significantly higher than between groups, overlap occurred much more often within reproductive groups, even despite the narrow niches of viviparous taxa. Results of PCA-env showed niches of viviparous taxa to cluster in conditions which from an ecological viewpoint agree with their need for survival (e.g. winter rains which permit activity). Non viviparous taxa in contrast generally occupy broader, and different conditions. Analyses by means of MAXENT jack-knife testing could increase the understanding of environmental conditions, both in negative and positive regard, which encompass these distribution patterns. However, several non-viviparous taxa challenge this pattern and occupy similar Grinnellian conditions as the viviparous taxa, without showing geographical overlap with these. Moreover, despite the display of significant similarity based on ENFA analysis, *S. sarrabusensis* does not show any niche overlap with other viviparous taxa according to PCA-env analyses. This observed pattern does however not necessarily contradict viviparous reproduction in this species, as local scale conditions could be of equal importance in driving the transition.

At the scale of scenopoetic variables, or the Grinnellian niche, viviparous taxa occupy narrower niches in environmental space when compared to non-viviparous taxa. While environmental conditions might well influence the existence of viviparity, local-scale conditions are most likely decisive, as has been suggested in recently published studies. While the current study therefore does identify environmental correlates among viviparous taxa, future local scale or landscape-genetic studies should provide more detailed information regarding the causes for transition to viviparous reproduction.

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APPENDIX 1

Taxon	**Occurrence (current study area)**	**Nr. records**
Lyciasalamandra antalyana	Turkey	7
Lyciasalamandra atifi	Turkey	11
Lyciasalamandra billae (incl. *L. "irfani"*)	Turkey	7
Lyciasalamandra fazilae	Turkey	10
Lyciasalamandra flavimembris	Turkey	5
Lyciasalamandra helverseni	Greece	6
Lyciasalamandra luschani luschani	Turkey	3
Lyciasalamandra luschani basoglui	Turkey/Greece	8
Lyciasalamandra luschani finikensis	Turkey	7
Salamandra algira algira	Algeria/Morocco	10
Salamandra algira "Rif + Atlas"	Morocco	36
Salamandra algira tingitana A	Morocco/ Spain	16
Salamandra algira tingitana B	Morocco	15
Salamandra corsica	France	17
Salamandra infraimmaculata infraimmaculata	Turkey/Syria/Lebanon/Israel	16
Salamandra infraimmaculata orientalis	Turkey	11
Salamandra salamandra salamandra	Italy/Balkan	29
Salamandra salamandra almanzoris	Spain	6
Salamandra salamandra bejarae	Spain	21
Salamandra salamandra crespoi	Portugal	9
Salamandra salamandra gallaica	Portugal/Spain	21
Salamandra salamandra gigliolii	Italy	22
Salamandra salamandra ssp. '*inquirenda*'	Italy	20
Salamandra "salamandra" longirostris	Spain	17
Salamandra salamandra morenica	Spain	16
Salamandra salamandra terrestris	France/Spain	12
Speleomantes ambrosii ambrosii	Italy	12
Speleomantes ambrosii bianchii	Italy	7
Speleomantes flavus	Italy	19
Speleomantes genei A	Italy	20
Speleomantes genei B	Italy	5
Speleomantes imperialis	Italy	37
Speleomantes italicus	Italy	23
Speleomantes sarrabusensis	Italy	5
Speleomantes strinatii	Italy/France	18
Speleomantes supramontis	Italy	23
		527

APPENDIX 2

Environmental predictors used for the current study. Predictors indicated in **bold** were retained after multicollinearity analysis.

Predictor	**Type**	**Period**	**Database**
Annual Mean Temperature	Climatic	1950-2000	WorldClim
Mean Diurnal Range: Mean of monthly (max temp - min temp)	Climatic	1950-2000	WorldClim
Isothermality: (P2/P7)* 100	Climatic	1950-2000	WorldClim
Temperature Seasonality (standard deviation *100)	Climatic	1950-2000	WorldClim
Max Temperature of Warmest Month	Climatic	1950-2000	WorldClim
Min Temperature of Coldest Month	Climatic	1950-2000	WorldClim
Temperature Annual Range (P5-P6)	Climatic	1950-2000	WorldClim
Mean Temperature of Wettest Quarter	Climatic	1950-2000	WorldClim
Mean Temperature of Driest Quarter	Climatic	1950-2000	WorldClim
Mean Temperature of Warmest Quarter	Climatic	1950-2000	WorldClim
Mean Temperature of Coldest Quarter	Climatic	1950-2000	WorldClim
Annual Precipitation	Climatic	1950-2000	WorldClim
Precipitation of Wettest Month	Climatic	1950-2000	WorldClim
Precipitation of Driest Month	Climatic	1950-2000	WorldClim
Precipitation Seasonality (Coefficient of Variation)	Climatic	1950-2000	WorldClim
Precipitation of Wettest Quarter	Climatic	1950-2000	WorldClim
Precipitation of Driest Quarter	Climatic	1950-2000	WorldClim
Precipitation of Warmest Quarter	Climatic	1950-2000	WorldClim
Precipitation of Coldest Quarter	Climatic	1950-2000	WorldClim
Global Potential Evapo-Transpiration	Climatic	1950-2000	CGIAR-CSI
Global Aridity Index	Climatic	1950-2000	CGIAR-CSI
Altitude	Topographical	2000	SRTM
Aspect	Topographical	2000	Derived SRTM
Slope	Topographical	2000	Derived SRTM