New methods for testing phylogenetic niche conservatism with an application to the squamates of Madagascar

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A thesis submitted in partial fulfilment of the
requirements for the degree of:

Doctor of Philosophy of
University College London
2017

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I, Laura Gil de Almeida Afonso Abranches Nunes, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Abstract

Phylogenetic niche conservatism (PNC), whereby closely-related species share similar ecological preferences, is often assumed to play a role in speciation processes. However, this trend is challenged by cases of phylogenetic niche divergence (PND), whereby closely-related species diverge in their ecological preferences. I examine the incidence of PNC and PND in the endemic scaled reptiles (Order, Squamata) of Madagascar. Firstly, I develop new tools to test for PNC and PND for a pair of species or populations. I introduce a novel measure of niche overlap and a null biogeographic test. I begin by comparing their performance with existing methods and case studies and I find the outputs from this new methodology to be consistent with evolutionary theory. I then conduct an assessment on the sensitivity of these tools to common sources of uncertainty identified in other niche-based methodologies. The methodology shows sensitivity to environmental spatial autocorrelation but not size of background region or sampling bias. I then applied these tools to sister taxa of squamates in Madagascar. I find cases of both PNC and PND and a tendency for niche conservatism in regions of high topographic complexity. In order to have an understanding of factors driving species divergence at a local scale, I also test for character displacement. I find character displacement to also be positively associated with topographic complexity. These findings suggest potential shifts in local scale niche traits and conservatism of broad scale niche traits. Overall in this thesis, I describe novel approaches to the study of PNC and PND. I also propose topographic complexity as an important factor in speciation processes in Madagascar. These findings provide hypotheses on the ecological processes involved in speciation on topographic complex regions while the tools presented in this thesis can be applied to many study systems.

Impact Statement

Our understanding of speciation is mostly based on the genetic processes that have lead to species divergence. One of the greatest challenges in current evolutionary research lies in understanding the ecological processes involved in evolutionary processes. There is an ongoing debate as to whether ecological differentiation between species occurs before or after speciation. While cases of niche conservatism, that is closely-related species sharing similar ecological niches, have been observed in some sister taxa, there are also cases of niche divergence among other closely-related taxa. There is not a clear distinction between the factors which lead to niche conservatism in some taxa and niche divergence in other taxa. Knowledge on the ecological processes which drive speciation patterns is of critical importance to enhance our understanding of the emergence of the biodiversity patterns observed today.

This thesis tackles this pressing issue by showcasing novel tools for testing for niche conservatism and divergence and new insight into evolutionary processes occurring in Madagascar, a well-recognised global biodiversity hotspot.The work presented in this thesis is the first to test for niche conservatism or divergence among a large number of taxa in this island.

This thesis also describes novel methodologies specifically designed to analyse niche-based questions at the landscape level and for data sets with few occurrence record, which are key limitations of existing methods. The methods have been presented to a wide audience, from specialised statistical conferences to broader ecological and evolution international conferences. The work in this thesis has also been published in a leading international peer reviewed journal. These tools have also been made freely available so that other scientists may apply them to answer the same set of questions in other study systems. Thus this thesis has an impact at an international level as well as across all major taxonomic *groups.*

The other outputs of this thesis will be brought about through publications in high-impact journals in both broad and specialist research areas in evolution and ecological modelling. Due to the unique expertise obtained from this thesis and the rising cross-disciplinary interest in how ecological niches may evolve through time, there is also great potential for collaborations with other academic institutions.

Acknowledgements

Firstly, I would like to thank my PhD supervisors Richard G. Pearson and Mark Wilkinson for their support over the past three years. Their guidance and advice were instrumental. I value their continued enthusiasm, motivation, and mostly their patience in turning my clumsy ramblings into scientific manuscripts. I would also like to thank Kate Jones for agreeing to be my thesis advisor, her input has been very helpful.

I have been fortunate enough to work alongside a fantastic group of people in a small corner of the world called the Centre for Biodiversity and Environment Research (CBER). Every single person there has made me feel very welcomed. It was a joy to arrive at the office every day. In particular, I would like to thank Alison Fairbrass, Tatsiana Barychka, Guilherme Ferreira, Sean Jordan, Céline Bellard, Roee Maor, Prabu Sivasubramaniam, Phillip Staniczenko, Henry Ferguson-Gow and Tim Lucas. A special mention to Chris Langridge for always being available to answer any query, large or small, and always having time for a chat over a cup of coffee (with a biscuit or two).

Outside the office, I am grateful for the friends that have supported me through the years - with special mention to Laura Johnson, Aimie Robinson, Daniel Ingram, Kate Bruton, Tim Coker and Mic Richichi. I'm thankful for all the fantastic people I've met in London and who have made this city such a wonderful and fun place to live. In particular, I would like to thank Simone da Silva, Marie Meunier, Helen Moorfoot, Danielle Lawrence and Natalie King who were always around when I needed 'downtime' in the form of a chat, a drink, or karaoke. I would also like to thank my favourite starting eleven, each of whom I had the honour of playing football with on a muddy pitch in Hackney, East London every Sunday: Sarah Sackett, Birgul Oz, Charlotte Young, Cara Stacey, Emily Farley, Becky Grevitt, Kate Beer, Ekin Oklap, Lucy Key, Fran Higgins and Diede Fennema.

I would also like to say a heartfelt thank you to my housemates and life-long friends, Carolina Ricardo and Laura Coquereau. It would have been much harder to complete this thesis without seeing your faces everyday.

A special thank you to my family: To my mother Margarida and my father Artur for supporting me in every way they could and making this whole journey possible. Finally, to my sister Sara and my niece Alice, your company made the writing up process much more enjoyable and I cannot imagine doing it any other way.

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Chapter 1

Introduction

1.1 Biogeography and the study of Speciation

Speciation is the splitting of an ancestral species into two or more new species due to genetic isolation (Losos and Glor, 2003). In recent years there has been a better understanding of the phylogenetic relationships between species. Phylogenetic relationships are commonly described by a tree, where the emergence of new lineages is represented by the splitting of branches. Species with shared evolutionary history are grouped together, while distant relatives are further apart in the tree. The development of robust phylogenetic trees helps scientists to understand speciation at fine phylogenetic scales (Barraclough and Nee, 2001). Despite providing a comprehensive illustration of the Tree of Life, phylogenetic trees fail to explain the non-genetic processes that are responsible for the branching of the tree (Losos and Glor, 2003; Schluter, 2009; Nosil et al., 2012; Anacker and Strauss, 2014).

Two species may become genetically isolated due to geographic isolation, a process commonly referred to as vicariance or allopatric speciation (Jordan, 1905; Coyne and Orr, 2004; Pyron and Burbrink, 2010). In this scenario, the geographical disruption to an ancestral range leads to the loss of gene flow between the now disjunct populations, thus leading to a pair of genetically distinct species (i.e., sister species). In recent years there has been an accumulation of literature supporting geographic isolation as the dominant driver of speciation (Barraclough and Vogler, 2000; Turelli et al., 2001).

The occurrence of sister species with partial overlapping distributions (parapatric sister pairs) challenges the universality of speciation due to geographic isolation. One possible explanation for the presence of non-overlapping distributions between sister pairs is the re-connection of species ranges post-speciation (i.e., secondary contact) (Mayr, 1963) but cases of sister pairs with complete overlapping distributions (sympatric pairs) have further motivated scientists in proposing non-geographic speciation processes (Coyne and Orr, 2004; Bolnick and Fitzpatrick, 2007).

Understanding the factors that limit species distributions is a critical step for determining whether sister pairs have diverged because of geographic or non-geographic factors. The discipline of biogeography attempts to explain why species are found where they are and outline what limits such distributions (Violle et al., 2014). Species distributions may be limited by geographic barriers to dispersal or by unfavourable environmental conditions (Angert, 2009). While allopatric sister pairs may owe their genetic isolation to geographic disruptions, the speciation of parapatric and sympatric pairs may be explained by abiotic (e.g., climatic conditions) or biotic factors (e.g., interspecific competition).

The niche is one of the most central concepts in ecology and also one of the most challenging to address, given the different interpretations and attempts to measure and predict a species' niche (Godsoe, 2010). The most common description of a niche is by Hutchinson (1957), which described it as the set of conditions that allow a species to persist. Nowadays, a niche is best explained with the BAM diagram (Peterson et al., 2011) (Figure 1.1). This Venn diagram describes the different components which make up the niche of a species. The A component refers to the abiotic factors, such as environmental conditions. This set is essentially describing the fundamental niche of a species. The B element represents the biotic factors, such as competition and other species interactions, thus the overlap of the two sets represent the realised niche (Soberón and Peterson, 2005). Finally M represents the set of geographic grid cells that are accessible to a species given its intrinsic dispersal capacities (Godsoe, 2010). The intersection of all these sets would describe the current distribution of the species (Soberón and Peterson, 2005).

In recent years there has been a rising interest in the use of ecological niches to distinguish between geographic and non-geographic speciation processes. If sister pairs became isolated due to geographic disruptions, they are not expected to have significantly different ecological niches. Sympatric pairs would also have similar ecological niches given that they occupy the same geographic space. Parapatric pairs would also be expected to have similar niches, because their contact zones are assumed to be caused by secondary contact after diverging in geographic isolation. The expectation of niche similarity between sister pairs is associated with the notion that evolution of niche-related traits is slow (Wiens et al., 2010), thus preventing adaptations to novel environmental conditions and limiting species distributions (Wiens and Graham, 2005). The retention of ancestral niche conditions is referred to as phylogenetic niche conservatism (PNC) (Wiens, 2004). A species' intrinsic inability to diverge from

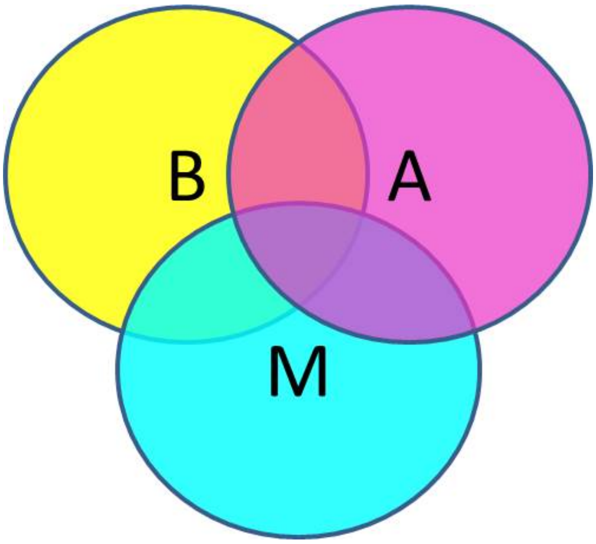

Figure 1.1 The BAM diagram. 'B' represents the biotic conditions, 'A' represents the abiotic conditions which translate into the fundamental niche of a species, and 'M' represents the accessible habitat for a species given its dispersal ability. The intersection of 'B' and 'A' is the realised ecological niche of a species while the intersection of 'B', 'A' and 'M' define the current distribution of a species (Soberón and Peterson, 2005).

its ancestral ecological niche could also explain cases of allopatric speciation where a clear geographical barrier is absent (Wiens, 2004; Pyron et al., 2014). As a result, an alternative form of allopatric speciation has been proposed. While 'hard' allopatry refers to the classic scenario of a geographical barrier splitting a population into two isolated distributions, 'soft' allopatry refers to an environmental disruption dividing the range of an ancestral population (Pyron and Burbrink, 2010). As dictated by PNC, the species are unable to diverge in their ancestral niche and persist in these new environmental conditions, therefore there is a disruption to gene flow and the populations become genetically isolated (Wiens, 2004). Phylogenetic niche conservatism is therefore associated with allopatric speciation.

The importance of PNC and allopatric speciation in propelling species diversification (Peterson et al., 1999) has been challenged by instances of ecological differentiation and speciation with gene flow among sister pairs (Butlin et al., 2008; Nosil, 2008). Ecological differentiation has emerged in recent years as another mode of speciation, where selection for different ecological conditions leads to disruptions in gene flow without the interference of a physical barrier (Coyne and Orr, 2004; Rundle and Nosil, 2005). The discovery of niche divergence between sister pairs, herein referred to as phylogenetic niche divergence (PND), suggest that speciation may also be mediated by ecology (Schluter, 2001; Schluter and Conte, 2009). PND supports a speciation process driven by adaptations to environmental gradients (Losos et al., 2003; Florio et al., 2012; Pyron et al., 2014; Anacker and Strauss, 2014). This speciation process could be expected in parapatric pairs, in cases where an ancestral species is distributed along an environmental gradient which results in adaptations and reproductive isolation between co-occurring populations (Florio et al., 2012).

Despite speciation via geographic isolation being regarded as the most common mode of species divergence, there has been an emergence of cases of speciation caused by species adaptations across environmental gradients (PND) or due to conservation of ancestral climatic niche preferences (PNC). Understanding the interplay between geographic isolation and environmental-mediated speciation, and the prevalence of niche conservatism in a phylogeny, may provide great insight into the causes of diversication patterns and trends of niche evolution (Wiens and Donoghue, 2004).

1.1.1 Existing methods for measuring a species ecological niche

The most common approach to infer an ecological niche relies on species distribution models (SDMs), also referred to as ecological niche models (ENMs). ENMs are statistical tools that attempt to correlate environmental conditions with species distributions (Buckley et al., 2010). These models are therefore inferring a species' ecological niche based on its distribution. This description of an ecological niche is referred to as the 'occupied niche' of a species (Peterson et al., 2011).

Correlative models are limited by the challenge of incorporating ecological theory, such as species response curves to environmental conditions (Austin, 2002), and choice regarding the number and type of environmental parameters used to calibrate the model (Rodda et al., 2011). A correlative approach will only define the niche according to the data available, which may be biased or limited (Godsoe, 2010). Given Hutchinson's definition of a niche describing conditions for long term persistence of a species, there is also an argument for removing sink populations when inferring ecological niches from distribution data (Godsoe, 2010). Correlative models also do not take into account regions which may be suitable for the species despite lack of known occurrences, possibly due to dispersal ability or time between recent extinction and recolonization events (Godsoe, 2010). Therefore, ENMs assume that species are at equilibrium with the environmental conditions where they are found and which are available to them (Pagel and Schurr, 2012). These methods are thus limited when inferring a species fundamental niche.

To measure a fundamental niche requires detailed knowledge of the tolerance of species to environmental stressors, which requires a larger investment in data collection and analysis compared to using ENMs. A mechanistic (or process-based) approach is a more suitable alternative to correlative models when defining the fundamental niche. These methods address some of the limitations of ENMs by regarding organisms as a set of behavioural, morphological and/or physiological traits (Kearney and Porter, 2009). These approaches may capture population growth rate and mortality and thus may be better at distinguishing between source and sink populations (Kearney et al., 2010). Mechanistic models have been used to describe many types of niches such as nutritional niches, biophysical niches and individual based models describing dynamic energy budgets (Kearney et al., 2010). These models may be more appropriate when estimating changes in the distributions and abundances due to environmental change (Kearney and Porter, 2009).

Considerable deterrents to the use of mechanistic models include: i) the current rarity of available data on functional processes that may limit species distribution (Elith et al., 2010); ii) the higher financial and computational efforts to collect such data (Anderson, 2013) and iii) the potential ethical and practical issues with data collection through experimental manipulations (Panzacchi et al., 2014). Indeed, perhaps one of the most comprehensive attempts at defining the fundamental niche of a group of species involved a large amount of field collections and laboratory work (Araújo et al., 2013). This study explored the differences between fundamental and realised niches using three parapatric species in the lizard genus *Liolaemus* (Araújo et al., 2013). Species distributions were recorded in the field and specimens were taken *ex situ* and tested for tolerances to temperature in the laboratory. The study found divergent realised niches, as expected for parapatric species, while analysis of their fundamental niches suggested divergence (i.e., niche evolution) in cold tolerances but conservatism in heat tolerances (Araújo et al., 2013). Two points can be inferred from this analysis. First, strictly speaking, this study is not quantifying the whole fundamental niche, as it is restricted to only one dimension. Second, given the amount of effort required to discern that single niche dimension, it highlights the difficulty in developing and applying accurate mechanistic approaches to niche theory and to large sample sizes. As a result, mechanistic models have only been applied to a few cases while correlative models generally dominate the research on distribution and niche modelling (Warren et al., 2008; Elith et al., 2010; Broennimann et al., 2012; Dormann et al., 2012; Blonder et al., 2014). This overwhelming preference for correlative models is also motivated by their simplicity, adaptability and the availability of user friendly computer applications such as MAXENT (Phillips et al., 2006) and BIOMOD (Thuiller et al., 2009).

1.2 Current knowledge of PNC/PND in extant lineages

1.2.1 Existing methods for measuring PNC in a sister pair

In recent years there have been significant advances in the study of niche conservatism with the emergence of geo-referenced environmental layers and statistical modelling tools (McCormack et al., 2010). Niche conservatism is best measured in terms of niche overlap, which is the proportion of the niche that is shared between two species (Colwell and Futuyma, 1971). High niche overlap is potentially explained by niche conservatism, and low niche overlap by niche divergence. A pioneering paper in this area is Peterson et al. (1999) work on allopatric speciation in the Isthmus of Tehuantepec, Southern Mexico. Using an articial intelligence algorithm (GARP) and a chi-square significance test, the authors found that often a taxon's ecological niche was able to predict the distribution of its sister taxon (and vice versa), thus suggesting niche conservatism within this allopatric set of species (Peterson et al., 1999). These findings were not ubiquitous among other systems (Graham et al., 2004), but Warren et al. (2008) attributed the disagreement to the studies using different approaches and in effect also looking at different questions: niche equivalency (indistinguishable niches) in Graham et al. (2004) and niche similarity in Peterson et al. (1999).

In order to resolve this problem, a new methodology was introduced by Warren et al. (2008) which placed niche equivalency and niche similarity at opposite ends of a spectrum thus testing both hypotheses at the same time. Two test statistics were introduced, one based on Schoener's statistic of niche overlap and another adapted from Hellinger's distance statistic (D and I respectively). Both work by performing a cell-to-cell comparison between the gridded potential distributions (i.e., ENMs) of two species derived from a distribution model (e.g., MAXENT). This approach is suitable for both continuous or binary probabilities of occurrence and the value of niche overlap ranges from 1 (niche equivalency) to 0 (niche divergence), with various degrees of niche similarity in between (Warren et al., 2008). In one of the novel significance tests introduced by Warren et al. (2008), which I refer to as the background test, the simulated D or I values are calculated by comparing the ENM of one taxon to an ENM derived from random points sampled within the range (background) of the other taxon (and vice versa). This process is repeated a number of times to create a collection of simulated niche overlap values. The null hypothesis is that the empirical niche overlap is explained by the available habitat. The null hypothesis is rejected if the empirical value falls outside the 95% confidence interval of the simulated (null) distribution. A rejection of the null hypothesis suggests that the differences or similarities in the niche are due to a preference or suitability for that particular habitat (Warren et al., 2008).

This approach was later extended by applying the similarity significance test in environmental space (see details in Broennimann et al. 2012) and both are commonly used to study niche conservatism in invasive species (Petitpierre et al., 2012; Pellissier et al., 2013), sister pairs (Blair et al., 2013) and in species delimitation (Wielstra et al., 2012).

However, both techniques carry limitations which may confound our understanding of PNC. Such limitations include the use of reciprocal tests for each sister pair (Warren et al., 2008; Broennimann et al., 2012), the restriction to two environmental variables (Broennimann et al., 2012), the arbitrary selection of 'background' regions and the underlying assumptions when constructing traditional ecological niche models from correlative models (Warren et al., 2008; Broennimann et al., 2012).

Both tests also do not account for spatial autocorrelation in the environmental data, therefore not assessing the degree to which niche divergence may be influenced by spatial autocorrelation (McCormack et al., 2010). An approach that addressed this issue was later proposed by McCormack et al. (2010). In this method, niche divergence is tested independently for each axis that makes up the multivariate niche space, thus circumventing the need for ENMs. Niche divergence is found when the divergence between the mean niches of the two species is higher than the difference in the means between the species background. Niche conservatism is inferred when the difference in the means between the species background is higher than the difference in the mean of the niches of the two species (McCormack et al., 2010). Despite some advantages of this method, it is not as widely used as Warren et al. (2008) and Broennimann et al. (2012) and is often treated as a complement to the other tests (Mao and Wang, 2011; Wooten and Gibbs, 2012; Loera et al., 2012).

Recently, Blonder et al. (2014) developed an approach to build n-dimensional hypervolume niches. In this case, niche overlap is regarded as the intersection of the hypervolumes of two species but no significance test was proposed with this method. Comparisons between different studies are confounded by various uncertainties and assumptions among the techniques used (Warren et al., 2008). These tools are also particularly limiting for species with few occurrence records, which also tend to be the most endemic species (Platts et al., 2014). These techniques also tend to rely on pseudoabsence or absence data, despite the higher incidence of presence-only datasets (Anderson, 2003), which therefore would be best analysed using presence-only models.

1.2.2 Is PNC or PND more common in extant phylogenies?

Given that allopatry is considered the most common mode of speciation (Coyne and Orr, 2004), it is often assumed that niche conservatism is equally common. This is particularly supported by Peterson et al.'s (1999) study of allopatric speciation across the Isthmus of Tehuantepec in southern Mexico. In this study, taxa in one side of the geographical barrier had similar niches to its corresponding sister pair on the other side. This led to the proposal that speciation occurred in geographical dimensions with niche differentiation happening post-speciation (Peterson et al., 1999). A study on allopatric sister pairs of *Plethodon* salamanders, using ENMs, tested whether the niche models of each sister species predicted the niche of the other species better than the niche obtained from the intervening barrier (Kozak and Wiens, 2006). The study found that most allopatric pairs had risen from allopatric isolation due to an environmental barrier, thus supporting niche conservatism as a driver of allopatric speciation (Wiens, 2004; Kozak and Wiens, 2006). The lack of niche divergence in speciation was also found in *Aphelocoma* jays (McCormack et al., 2010), the Iris species complex in Louisiana (Hamlin et al., 2016), lineages of the Nile monitor (*Varanus niloticus*) (Dowell and Hekkala, 2016) and *Gynandropaa* frogs of the Qinghai-Tibetan plateau (Hu et al., 2016).

The support for niche conservatism is mixed as there is also evidence of niche shifts (or PND) among closely-related species (Peterson, 2011) (Table 1.1). These findings challenge the expected association between allopatric speciation and niche conservatism and provide support for the occurrence of ecologically-mediated speciation.

1.2.3 Are PNC lineages more morphologically similar?

The proposition that closely-related species may be ecologically more similar can also be explained by closely-related species also being more morphologically similar to each other than distantly related species (Blomberg and Garland, 2002; Blomberg et al., 2003; Losos, 2008; Cooper et al., 2010). Traditionally, phylogenetic relatedness is first assessed in terms of morphological similarity in both ancient and extant organisms (MacLeod, 2002; Jensen, 2003; Cardini and Elton, 2008). In fact, a great tend-

Table 1.1 Summary of studies testing for PNC/PND in closely-related species with allopatric or parapatric geographic distributions.

Geographic distributions	PNC/PND	Study
Allopatric and Parapatric	PND	Graham et al. (2004)
Allopatric	PNC and PND	Pyron and Burbrink (2009)
Allopatric and Parapatric	PND	Ahmadzadeh et al. (2013)
Allopatric	PND	Wooten and Gibbs (2012)
Allopatric and Sympatric	PND	Nakazato et al. (2010)
Allopatric	PND	Mao and Wang (2011)
Allopatric and Parapatric	PND	Cicero and Koo (2012)
Parapatric	PNC	Loera et al. (2012)

ency for phylogenetic dependence of morphological traits has been shown (Freckleton et al., 2002). However, this tendency is not ubiquitous across all clades (Losos, 2008; Luxbacher and Knouft, 2009) with incongruencies between molecular and morphological phylogenies becoming more common (Cardini and Elton, 2008). Differences in morphological conservatism among clades may be associated with differences in the speciation mechanisms driving diversification in those lineages.

Given that allopatric speciation is associated with niche conservatism, allopatric sister taxa are expected to have low phenotypic variation between them because they experience similar ecological conditions (Kozak et al., 2006; Stuart and Losos, 2013). Morphological divergence was minimal in eastern North American woodland salamanders (*Plethodon*) which have disjunct distributions (Kozak et al., 2006). Little morphological differentiation has also been found in allopatric clades of the Afrotemperate endemic daisy genus *Macowania* (Bentley et al., 2014). However, morphological divergence was observed in one allopatric sister pair (*M. hamata-M. corymbosa*) suggesting adaptive divergence in allopatric speciation (Bentley et al., 2014).

In parapatric and sympatric speciation, species diverge while co-occurring. In order for closely-related species to co-occur they must not directly compete with one another in order to avoid competitive exclusion (Böhning-Gaese et al., 2003; Stuart and Losos, 2013). Species may alleviate competition by changes in their behaviour or morphology (Ackerly et al., 2006). Character displacement is defined as a shift in resource use or phenotype driven by natural selection and interspecific competition, allowing closely related species to co-occur (Brown and Wilson, 1956; Stuart and Losos, 2013). As a result, parapatric and sympatric speciation are associated with cases of character displacement (Stuart and Losos, 2013).

The role of character displacement in speciation is two-fold. It can be a trigger for the start of the speciation process (Pfennig and Pfennig, 2009), thus allowing species to adapt to novel ecological niches and reduce gene flow between diverging populations (Nosil, 2008; Rice and Pfennig, 2010). This has been observed in the sympatric populations of Spadefoot toad (*Spea multiplicata*) where significant reduction in gene flow has been associated with character displacement and may lead to potential genetic isolation between these populations in the future (Rice and Pfennig, 2010). In regions of secondary contact between species that have evolved allopatrically, character displacement can develop due to interspecific competition, therefore maintaining species boundaries by reducing hybridization between species (Losos, 2000). An example of character displacement due to secondary contact is found in the scincid lizards *Niveoscincus microlepidotus* and *Niveoscincus greeni* (Melville, 2002). These alpine species diverged allopatrically due to vicariant events in the Pleistocene but have now partial overlapping distributions (Melville, 2002). It was found that body size differences between the species were higher in the overlapping regions, thus suggesting character displacement due to interspecific competition (Melville, 2002).

1.3 Niche Conservatism among the squamates of Madagascar

The island of Madagascar, the world's fourth largest island (Wollenberg et al., 2011), has long mystified scientists as it hosts an impressively high level of endemism (Yoder and Nowak, 2006). It is thus considered a global biodiversity hotspot (Myers et al., 2000) which is currently under severe anthropogenic threat (Townsend et al., 2009). All non-introduced species of terrestrial mammals and amphibians found in Madagascar are endemic to this island as well as 92% of reptile species (Goodman and Benstead, 2005). These high levels of endemicity are a result of an early geographical isolation from Africa around 183-158 million years ago (Mya) (Vences et al., 2009) and from India around at least 80 Mya (Dewar and Richard, 2012). However, the origins of restricted local endemism within the island are less well understood (Pearson and Raxworthy, 2009).

The majority of the literature supports an allopatric mode of speciation, either due to fragmentation of tropical forests (Raxworthy and Nussbaum, 1995), watersheds (Wilmé et al., 2006) or river barriers (Pastorini et al., 2003). However, Madagascar has a diverse range of distinct climatic regions, from subarid zones in the south to tropical forest in the north and a steep eastern escarpment dictating a stricking moist-dry gradient from east to west (Pearson and Raxworthy, 2009) (Figure 1.2). A contrasting alternative hypothesis is that speciation has been driven by climate gradients via a parapatric mechanism (Pearson and Raxworthy, 2009). Tests comparing the suitability of the watershed hypothesis, as an example of allopatry, and the parapatric climatic gradient hypothesis, suggest that not one single mechanism may explain local endemism, with several genera either showing a combination of both mechanisms or none (Pearson and Raxworthy, 2009). Recently, topographic complexity has also been proposed as a driver of allopatric speciation and niche conservatism in other regions (Hu et al., 2016; Steinbauer et al., 2016). Given the topographical diversity of Madagascar (Dewar and Richard, 2012) (Figure 1.2), I hypothesize that topographic complexity may also be a factor driving speciation patterns within this island.

Squamates are among the most diverse terrestrial vertebrates and thus are a common model system for many biological subdisciplines, in particular in ecology and evolution (Sites et al., 2011; Pyron et al., 2013). Squamates are also model organisms because they are abundant, easy to find, capture and manipulate (Camargo et al., 2010) Given the increasing interest and use of squamates in evolutionary research, there is substantial knowledge on the phylogenetic relationships between species (Sites et al., 2011) leading to complete phylogenetic trees of all recognised families and subfamilies (Pyron et al., 2013). As a result, the number of studies on phylogeography and speciation using squamates has grown

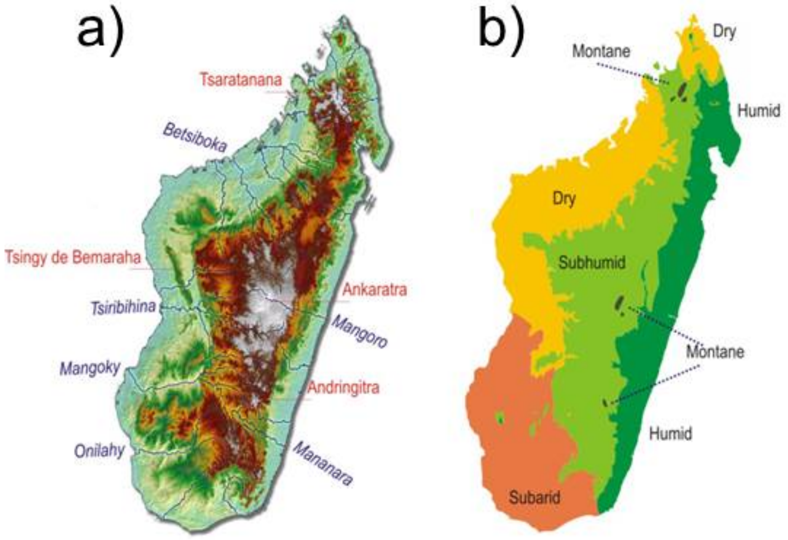

Figure 1.2 Maps showing the island of Madagascar and (a) topography with major mountain massifs (red) and rivers (blue), and (b) major bioclimatic zones (adapted from Brown et al. (2016)).

exponentially in the past 20 years (Camargo et al., 2010). In particular, squamates have been used in studies of PNC and PND and cases of both niche conservatism (Morales-Castilla et al., 2011) and niche divergence (Wooten and Gibbs, 2012) have been identified further making the squamates an interesting group to study the presence and absence of PNC and PND. The species-rich and highly endemic squamates of Madagascar are a particularly suitable model system for studies on speciation within the island because there is a large number of known sister pairs available thus allowing for robust statistical comparisons (Nagy et al., 2007).

The Malagasy squamates have been surveyed for a long time, providing a robust and comprehensive knowledge on the distribution of many species. This provides confidence to the inference of species ecological niches from their distributions. Additionally, recent phylogenetic work has also been applied to many of the reptile groups (Townsend et al., 2009; Pyron et al., 2013). These groups are also suitable for the study of PNC as they are relatively recent radiations emerging during the Cenozoic period (Raxworthy and Nussbaum, 1995; Yoder and Nowak, 2006), therefore having little potential for post-speciation range and ecological shifts than older radiations (Losos and Glor, 2003; Blair et al., 2013).These organisms are also suitable for studies of diversification due to most taxa having low dispersal capacity and strong sensitivity to environmental conditions (Wooten and Gibbs, 2012). The squamates in Madagascar have been often under phylogeographic studies in order to understanding speciation process and genetic differentiation among organisms (Boumans et al., 2007). The taxa are widely distributed within the entire island with regional and micro-endemism (Mezzasalma et al., 2017).

The presence of PNC in allopatric speciation is mixed (Peterson, 2011). Putting methodological differences aside, there is still no overwhelming tendency for niche conservatism or divergence among closely-related species. Thus it is necessary to explicitly test for the presence of niche conservatism and divergence when making inferences about the potential speciation mechanisms that influence diversification patterns on the island of Madagascar.

To study niche conservatism in Madagascar it is critical to have a test that can provide enough statistical power. Given that Madagascar is a presence-only dataset with often few occurrence records per species, more commonly used techniques (e.g., ENMs) may not be suitable for this kind of dataset as they carry assumptions on absence data and statistical limitations for small number of occurrence points (Pearson et al., 2007; Bukontaite et al., 2015).

The association between morphological conservatism and speciation mechanisms can be seen to be as equally debatable as the association between niche conservatism and allopatric speciation. Therefore robust associations between the two types of phylogenetic signal should not be assumed when conducting studies on speciation mechanisms in Madagascar. An explicit test of the occurrence of character displacement and its association with speciation and niche conservatism is also necessary in order to better understand the agents driving the emergence of new species and the maintenance of species boundaries after secondary contact within the island.

1.4 Thesis Overview

In this thesis I present and evaluate novel techniques for testing for PNC in pairs of species that have few known occurrence points. I conducted an explicit test for the presence of niche conservatism in the island of Madagascar, by applying these methods to the Malagasy squamates. I also address the degree of character displacement among these sister taxa, given the role of character displacement in driving and completing speciation processes. Testing for niche and morphological conservatism in the island of Madagascar will provide greater insight into the mechanisms of speciation that drove this radiation, and that also limits the current distribution of these species.

The limitations outlined above (Section 1.2.1 and 1.3) motivated the need to develop a novel methodology for testing niche conservatism. The new test is multi-dimensional, non-reciprocal, does not require 'background' points and includes an innovative null biogeographic model which is distinct from other significance tests.

First, in Chapter 2, I developed new techniques to measure niche conservatism. I present a novel measurement of niche overlap, the *MO* metric (for Multidimensional Overlap) and a novel null biogeographic test, the RTR significance test (for Random-Translation-and-Rotation). I compared the *MO* metric with three existing measurements of niche overlap using a simple 2-dimensional virtual scenario. I also used both the *MO* metric and the RTR significance test to analyse niche evolution in three real-case scenarios. I found that both tools are suitable for testing ecological speciation.

In Chapter 3, I further explored the characteristics of both tools by testing the sensitivity of these techniques to three main sources of error in spatial ecological modelling. This allowed a better interpretation of their outputs in future analysis. I applied these tools to 3 dimensional virtual landscapes which had differences in spatial autocorrelation, size of the extent and intensity in sampling bias of virtual species occurrence data. The ability to pick up a signal was mostly associated with the spatial autocorrelation in the environments within the landscape. I also found the test to be conservative in its ability to pick up a statistically significant signal for PNC.

In the following chapter (Chapter 4), I applied the *MO* metric and the RTR significance test to the Malagasy squamate sister pairs in order to answer biological questions on the associations between niche conservatism and current species distributions and topographic complexity

(Chapter 4). I tested for niche conservatism and divergence using both liberal and conservative two-tailed tests. PNC was found to have occurred more often than PND. I found cases of PNC and PND in both allopatric and parapatric sister taxa in the more liberal test, but PNC was only found in allopatric taxa and PND in parapatric taxa in the more conservative test. I also found a strong association between topographic complexity and niche conservatism.

Next, I looked at whether niche conservatism could also be associated with character displacement (Chapter 5). This analysis provided a more holistic understanding on the association between conservatism and speciation by looking at morphological conservatism alongside niche conservatism. I found no association between niche conservatism and morphological conservatism. Competition was found to be weakly associated with character displacement across all taxa. Topographic complexity was found to be strongly associated with allopatric taxa but not parapatric or sympatric taxa. These findings suggest that different factors may be driving different facets of conservatism among closely-related species.

In the final chapter of this thesis (Chapter 6), I discussed the broader implications of my findings to the literature of PNC/PND and specifically to our understanding of speciation in the island of Madagascar. I also outlined potential advancements to the methodological techniques developed in this thesis and future lines of enquiry with regards to speciation in Madagascar.

The work presented in this thesis aims to contribute to the study of niche ecology and biogeography by developing a new metric for quantifying the niche overlap between two species, and developing a novel null biogeographic of PNC for two populations or species. Chapter 2 will be a published 'Special Paper' in the *Journal of Biogeography* (Nunes and Pearson, 2016). In this publication, all the code used in the analysis has been made available (Appendix A in this thesis). The new methods presented in this thesis are not exclusive to Madagascar. Other biogeographic regions with similar evolutionary histories (i.e., large oceanic islands) and thus harbouring comparable levels of endemicity may also benefit from the approaches developed in this thesis. Chapter 3 is a supplement to Chapter 2 which is in preparation to be submitted to *Ecological Modelling*. The theories tested in Chapter 4 and 5, such as trends of ecological and morphological conservatism in allopatric speciation, are also of great relevance within the field of evolutionary ecology.

Chapter 2

A null biogeographic test for assessing ecological niche evolution

This work was conducted in collaboration with Richard G. Pearson and Mark Wilkinson.

Laura G. A. Nunes and Richard G. Pearson conceived the ideas; Laura G. A. Nunes designed, performed, collected and analysed the data; Laura G. A. Nunes led the writing with critical input from Richard G. Pearson and Mark Wilkinson.

This chapter is published as a ‘Special Paper’ in the *Journal of Biogeography* (Nunes and Pearson, 2016)

2.1 Abstract

Quantification of the degree to which ecological niches change over evolutionary timescales is important for deepening our understanding of evolutionary and ecological processes. Phylogenetic niche conservatism (PNC) is when closely related species differ less ecologically than expected by chance, whereas Phylogenetic Niche Divergence (PND) is when closely related species differ more ecologically than expected by chance. I present a new null model to test for PNC and PND (the RTR significance test), which I combine with a novel metric for quantifying niche overlap. The RTR null model comprises many thousands of replicates generated by randomly translocating and rotating the set of occurrence records for two populations (e.g., sister species) while maintaining the spatial configuration between all occurrences within each replicate. For each replicate, I calculate niche overlap as the proportion of the combined niche breadth that is shared by the two species, averaged over n environmental dimensions. This approach enables us to test whether the observed niche overlap is more or less than expected by chance given the environmental conditions present in the study area. I test the performance of my approach in comparison to other methods using both simulated and real case scenarios, including crested newts in Europe, pocket gophers in North America, and lemurs in Madagascar. I find that this measure of niche overlap performs better than other metrics in an artificial simulation scenario, and I find evidence for both PNC and PND in the case studies for Europe, North America and Madagascar. My results demonstrate that both the RTR significance test and the novel metric of niche overlap are consistent with evolutionary theory and are suitable methods to test for PNC and PND. I make available scripts to implement the RTR test and metric of niche overlap, and expect that the methods will prove useful for addressing a broad set of questions relating to ecological niche evolution and speciation, particularly for restricted-range species for which few known occurrence records are available.

2.2 Introduction

Knowledge of how species respond to environmental conditions over evolutionary timescales is important for understanding the causes of biodiversity proliferation, change and persistence (Barraclough, 1998; Wiens and Donoghue, 2004). In particular, there is interest in whether tolerance to environmental stressors tends to be conserved across a phylogeny (Wiens, 2004) or divergent from species to species (Losos, 2008; Ogburn and Edwards, 2015). Phylogenetic niche conservatism (PNC) is the tendency for closely related species to differ less ecologically than expected by chance, and phylogenetic niche divergence (PND) is the tendency for closely related species to differ more ecologically than expected by chance (Pyron et al., 2014). These concepts are of particular importance for understanding the biogeography of speciation; for instance, allopatric speciation has been inferred in cases of PNC (Peterson et al., 1999; Wiens, 2004) whereas PND is expected in cases of parapatric speciation (whereby ecological divergence along an environmental gradient results in species with distinct ecological niches). Studies to-date have revealed evidence both for PNC and PND but methodological difficulties have made the search for general patterns difficult (Graham et al., 2004; Wiens and Graham, 2005; Warren et al., 2008; Peterson, 2011).

A combination of newly available phylogenies, growing databases of species occurrence records, new fine-resolution environmental variables derived from remote sensing, and recently developed GIS-based statistical and machine-learning tools (e.g., ecological niche models, ENMs; also termed Species Distribution Models) provide opportunities to substantially advance understanding of PND and PNC (Soberón, 2007; McCormack et al., 2010). A general methodology for testing for PNC and PND is to: (i) collect geo-referenced occurrence records for populations with known phylogenetic relationships (e.g., sister species); (ii) couple the occurrence records with a set of geo-referenced environmental variables (e.g., temperature, precipitation, vegetation cover); and (iii) statistically compare the environments occupied by different populations. I note that this approach tests for differences in the niches currently occupied by the populations (the 'occupied niche', *sensu* Peterson et al. 2011) rather than the niches within which the populations could exist (either the 'full fundamental niche' or 'existing fundamental niche' *sensu* Peterson et al. 2011).

PNC and PND can be quantified in terms of niche overlap; that is, the proportion of an environmental niche that is shared between two species (Colwell and Futuyma, 1971). These measures of niche overlap range from 1 (niche equivalency) to 0 (niche divergence), with varying degrees of niche similarity in between (Warren et al., 2008). An important advance proposed by Warren et al. (2008) has been the use of null model tests to assess observed niche differences (or similarities) in the context of the environmental conditions available in the study area. Generating a suitable null model allows us to ask whether the observed similarity or difference between the niches of two populations is statistically meaningful given the available environments. Warren et al. (2008) proposed two null tests, and several other authors have proposed methods for testing niche similarity (see Appendix C for review of methods and their limitations).

Here I present a new method that takes an alternative approach to testing for PNC and PND and addresses some of the problems with other approaches. Specifically, I have devised a method to test whether two populations are currently distributed in such a way that niche overlap is higher (PNC) or lower (PND) than would be expected by chance. I introduce a new null model (the Random-Translation-and-Rotation (RTR) significance test) and a novel metric for quantifying niche overlap. The new methods are suitable for the study of range-restricted species with few known occurrence records, and were designed to aid the understanding of the landscape-scale ecological processes involved in speciation. I highlight that the current RTR approach is designed to test for PNC among closely related species and is not well suited to applications outside of phylogeography; for example, studies of niche differentiation among invasive species require tests that cover two geographic areas (native and invaded ranges) but the RTR test is limited to a single study area. I make available R scripts to implement the methods (see Appendix A).

2.3 Materials and Methods

I propose a methodology that involves four main steps: 1) Collection of geo-referenced occurrence data for two populations (e.g., sister species) and environmental variables for the region of interest; 2) Measurement of observed niche overlap; 3) Production of a null reference frequency distribution of niche overlap values; and 4) Comparison of the observed niche overlap value to the null reference distribution to make the decision of rejecting or accepting the null hypothesis. The null hypothesis is that the observed niche overlap is no different to that expected at random, as defined by my null model (see below). Rejection of the null hypothesis would suggest that environmental conditions have played an active role in defining distributions (i.e., populations are adapted to particular ecological niches). Failure to reject the null hypothesis would suggest that spatial rather than ecological processes have been dominant in defining present day distributions.

The two methods that I present below - the new metric and the RTR null model - can be used together (as I do here) but may also be used separately with existing metrics of niche overlap and significance tests (e.g., the RTR approach could form the basis for running ENMs and calculating the distribution of niche overlap using Schoener's D or Hellinger's I under the null hypothesis). All the analyses were conducted in R (R Core Team, 2014).

2.3.1 A metric for measuring multidimensional niche overlap

I quantify the overlap (x) along a given environmental axis (e) between two species (i and i') as:

$$x_{e(i,i')} = \frac{min(y^{max}_{e,i}, y^{max}_{e,i'}) - max(y^{min}_{e,i}, y^{min}_{e,i'})}{max(y^{max}_{e,i}, y^{max}_{e,i'}) - min(y^{min}_{e,i}, y^{min}_{e,i'})} \qquad x_{e(i,i')} \in [0,1]; i \neq i' \tag{2.1}$$

where $y^{max}_{e,}$ and $y^{min}_{e,}$ are the maximum and minimum values of e for species i or i' respectively and the overlap ($x_e(i,i')$) is a real-value ranging between 0 and 1. In instances where the environmental breadths of the species do not overlap, my equation will return a negative value which I replace by a value of 0 to indicate no overlap between the axes.

I next average all the axes overlap values across all of the dimensions used to define the niches of the two species (N) using a metric I term MO (for Multidimensional Overlap):

$$MO_{i,i'} = \frac{1}{N}\sum_{e=1}^{e=N} x_e(i,i') \qquad MO_{i,i'} \in [0,1]; i \neq i' \tag{2.2}$$

such that the overall overlap ($MO_{i,i'}$) is a real-value between 0 and 1 (see Appendix D for further details on the MO metric).

The MO metric is a presence-only approach that has similarities with the BIOCLIM method (Busby, 1991) in that it constructs simple climate envelopes around the occurrence records (Booth et al., 2014). One limitation of such methods is that they are sensitive to occurrence records that are ecological outliers, which can lead to overestimation of the ecological niche (Farber and Kadmon, 2003). Overestimation due to outliers can be addressed by selecting a 'core niche region', such as the 5-95% percentile of the niche (Carpenter et al., 1993). I therefore implemented a method to undertake an optional 'trimming' function to remove ecological outliers (see Appendix A) and I have tested sensitivity of results to removal of outliers (see Appendix D). A second limitation is that very limited sampling of the species' distribution (e.g., fewer than five occurrence records) is likely to lead to underestimation of the species' niche. However, all methods are limited by the availability of empirical data and my approach has the advantage of avoiding making unfounded extrapolations beyond the range of the available data. A third limitation of BIOCLIM-like methods is that all environmental variables are treated as equally important (there is no weighting of variable importance, unlike in methods such as MAXENT; Phillips et al. 2006). It is therefore important to apply a priori ecological knowledge (e.g.,Blair et al. 2013) and/or statistical assessment of variable importance (e.g., by jackknifing, Wielstra et al. 2012; Soto-Centeno et al. 2013) to select relevant environmental variables for the taxa under consideration. In my three case studies (see below) I used the same variables that were used in the original studies, each of which applied a priori statistical analysis or expert knowledge to identify important variables. A fourth limitation is that my approach examines only elements of niche evolution that affect the minimum and maximum values along each niche dimension. Unlike methods that fit response curves in ENMs, my method does not attempt to identify more subtle differences in niches that are reflected in the shape of the functional response to the environment. The benefit of this is that I avoid the many assumptions that go into fitting response curves to limited data and uncertainty over what form the curves should take (Elith and Graham, 2009).

The RTR test is suitable for populations (e.g., of sister species) that have restricted ranges and few occurrence records, and has several advantages over alternative tests, including: (i) there is no need for reciprocal tests because an individual 'background' region does not need to be defined for each species; (ii) it maintains the spatial autocorrelation of the point data; (iii) the test is based on overlap in n-dimensional niche space; (iv) there is no limit to the number of ecological dimensions or occurrence records that can be included; and (v) the test returns a p-value to assess statistical significance rather than two p-values from a reciprocal test due to the need for individual background regions.

2.3.2 RTR null biogeographic model

I have developed a novel test that uses what I term the Random Translocation and Rotation (RTR) null model. The RTR null model comprises many thousands of replicates that are generated by randomly translocating and rotating the pooled set of occurrence records for two populations while maintaining the spatial configuration between all occurrences (i.e., of both compared species together) within each replicate. Niche overlap is calculated for each replicate and the observed niche overlap is then compared to the distribution of overlap values from the null model (Figure 2.1). If the observed niche overlap falls outside a critical boundary, I reject the null hypothesis and infer that the niches are conserved (e.g., above 95% percentile of null distribution, PNC) or divergent (e.g., below 5% percentile of null distribution, PND). The significance threshold of this null model approach is not restricted to upper and lower 5% boundaries, thus the investigator is able to select the critical threshold of the model, as well as choose between a two-tailed or one-tailed test for PNC or PND (see Appendix A).

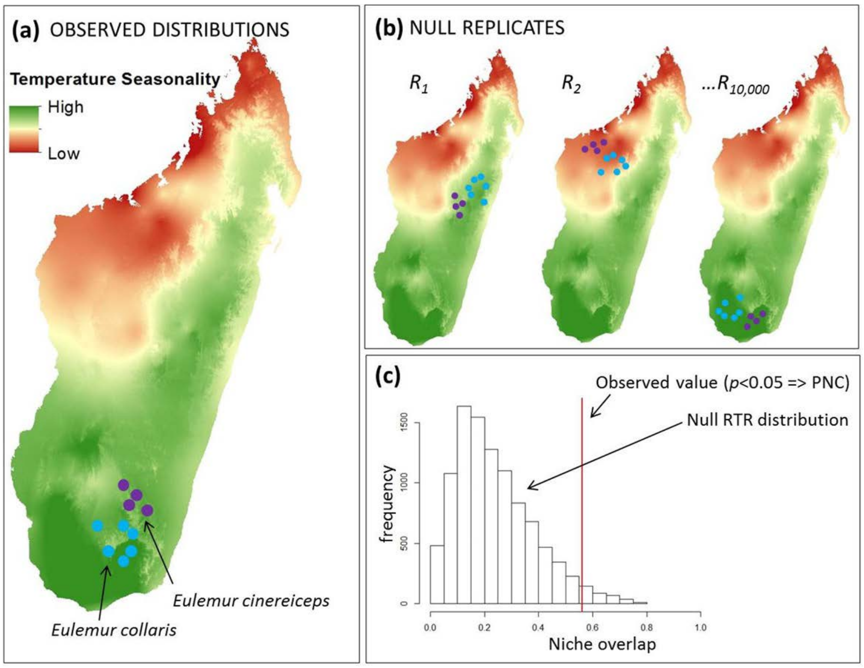

Figure 2.1 Random translation and rotation (RTR) null model test for phylogenetic niche divergence (PND) and phylogenetic niche conservatism (PNC), illustrated for a pair of sister lemur species in Madagascar (see Blair et al. 2013). (a) Occurrence records for the sister species, visualized on an example environmental layer (multiple environmental layers (e.g., temperature, precipitation) are used in the test to characterize the n-dimensional ecological niche space). (b) Thousands of null replicates are generated by randomly rotating and translating the set of occurrence records for the two species. (c) For the observed distributions and for all null replicates, the niche overlap is calculated and the observed overlap is compared against the null model. In this instance the observed niche overlap is in the highest 5% of the null distribution, so I infer PNC.

The RTR null model maintains the spatial configuration, and thus spatial autocorrelation, between all occurrences within each replicate (note somewhat comparable methods by Beale et al. 2008 and Chapman 2010). The method enables us to test whether the observed distributions are likely to be driven by environmental factors: I ask whether the niches occupied by two species are more or less similar than would be expected at random if the spatial configuration of the set of occurrences is maintained within a given landscape (background region). In effect, I keep the spatial configuration constant so I can ask whether there is something ecologically ‘special’ about the way that the two species are currently located on the landscape. Notice that here I am not assessing the present day spatial configuration of the two species (e.g., are the ranges adjacent?) but rather I am assessing whether the ecological niches currently occupied are more similar or different than expected by chance within the landscape.

As with other null models (e.g., Warren et al. 2008), results from the RTR approach are impacted by the extent of the landscape over which the replicates are run. I address sensitivity to selection of the study region below (see Testing sensitivity to extent of study region). However, an important difference to current methods is that I do not define separate background regions for each species; instead, I randomly translocate and rotate within a single region the set of occurrence records for the two species combined, thus maintaining the spatial configuration between species. I therefore make no assumptions about geographic constraints that might separate the distributions of the two populations. In some instances it may be appropriate to identify likely geographic constraints (e.g., the Isthmus of Tehuantepec in southern Mexico; Peterson et al. 1999; Warren et al. 2008); however, in most cases geographic constraints are less clear and I do not want to impose a priori any geographic separation when I am testing whether a single population might have split into separate populations (e.g., sister species) due to ecological divergence.

2.3.3 Comparing niche overlap metrics using simulated species

To assess the performance of different measures of niche overlap, I compared the MO metric against three alternatives: (i) Warren et al.'s (2008) I statistic, which is based on a comparison of ENMs and has been widely used; (ii) Broennimann et al.'s (2012) PCA-env framework with the D statistic, a more recent methodology that has been applied to invasive species; and (iii) Blonder et al.'s (2014) n-dimensional technique, in which niches are built as multidimensional hypervolumes.

I compared the metrics using simulated (artificial) species. Simulated species were preferable here to real case studies from nature because the degree of PND and PNC could be precisely defined, providing a 'known truth' against which the different metrics could be compared. I simulated the environmental niche overlap of two simulated species in a two-dimensional environmental domain of 100 x 100 grid cells (following Broennimann et al. 2012 and Colwell et al. 2009). I generated two opposing gradients across the artificial landscape to represent two uncorrelated environmental variables. I represented each species distribution as a square of 30x30 grid cells (see Appendix E for further information on methods and expected outcomes). One species' distribution was kept static in the bottom left corner of the environmental domain, while the other was initially placed in the bottom left corner but then moved one grid cell at a time in either direction (up or right) or diagonally away from the other species. The movement of one simulated species away from the other across the environmental gradient meant that there was increasing ecological divergence with increasing geographic separation (Figure E.1 in Appendix E).

To calculate the I statistic, I needed to construct ENMs. I used the R package 'DISMO' 1.0-5 (Hijmans et al., 2014) to build ENMs and the package 'SDMTOOLS' 1.1-221 (VanDerWal et al., 2014) to calculate I. I opted for ENMs constructed using MAXENT 3.3.3K (Phillips et al., 2006) as this method performs well in comparison with other ENM approaches (Elith et al., 2006) and was used in Warren et al.'s original paper (2008). I followed Warren et al. (2008) in maintaining default values for all program settings, including regularization and feature selection. To calculate D I used the R script for uncalibrated PCA-env functions provided by Broennimann et al. (2012). To build and measure the intersection of two hypervolumes following Blonder et al. (2014), I used their package 'HYPERVOLUME' 1.4.1 (Blonder, 2015) with 1,000 random numbers, a bandwidth of 0.1, a quantile of 0, and a reduction factor of 0.5, as recommended by Blonder et al. (2014).

2.3.4 Testing the null model using three case studies

I also tested this methodology (RTR null model combined with the new MO metric of niche overlap) using three real-world case studies. I selected case studies based on the following criteria: (i) a previous study has been published that includes estimates or hypotheses regarding PNC or PND; and (ii) occurrence records are available for the included sister species. Following each of the original studies, I used geo-referenced environmental layers from Worldclim at 30 arc-seconds resolution (Hijmans et al., 2005), in each case selecting the same set of variables as the original study (see Appendix F). I selected two case studies with a continental setting (the Balkan Peninsula and North America) and one island (Madagascar) since islands have more obvious natural boundaries and therefore raise different issues concerning the selection of a suitable study region:

i. Crested newts in the Balkans. The phylogeography of the crested newts has been extensively studied (Arntzen et al., 2007; Wielstra et al., 2010, 2012), which allows us to make some predictions about the potential for niche conservatism. There is evidence for vicariance among two sister pairs: (i) *Triturus karelinii* - *T. ivanbureschi*, which is thought to have split due to the uplift of the Armenian Plateau; and (ii) *T. carnifex* - *T. macedonicus*, which is thought to have split due to the formation of the Adriatic Sea (Wielstra et al., 2010).Wielstra et al. (2012) used ordination methods to assess niche evolution and found evidence for PNC. This finding is in line with the theoretical expectation that adaptation to new niches tends to be slower than extinction rates, leading to PNC in cases of allopatric speciation (Peterson et al., 1999). I aimed to test this using the RTR method.

Occurrence records for the crested newt species were obtained from Wielstra et al. (2012). I set the western and eastern boundaries of Europe (Figure 2.2a) based on the overall distribution of the *Triturus* clade in Europe (Wielstra et al., 2012). The environmental layers used (see Appendix F) were clipped to the same extent using the crop function from the 'RASTER' 2.5-2 package (Hijmans, 2015), and all layers were converted to a Lambert Conformal Conic projection (at central meridian 18°E, standard parallels 42°N and 46°N), which represents low spatial distortion for the Balkan region (Zagmajster et al., 2008).

ii. Pocket gophers in North America. Though studies of ecological divergence are commonly conducted at the species level, there is also interest in looking at a population level in order to understand biogeographic patterns of within-species divergence (Graham et al., 2004; Glor and Warren, 2011). *Geomys pinetis* is the only species of pocket gopher found in south-eastern USA and within this species there is a geographic and genetic subdivision between eastern (*G. pinetis*) and western populations (hereafter *G. mobilensis*) on either side of the Apalachicola River (Soto-Centeno et al., 2013). Soto-Centeno et al. (2013) used the 'identity' test and the 'blob' range-breaking test of ENMTools (Glor and Warren, 2011) and could not reject a hypothesis of niche similarity between the two populations across the river barrier. Here I aimed to test whether a signal for PNC or PND could be detected using the RTR method.

Occurrence records for *G. pinetis* and *G. mobilensis* were obtained from the MaNIS website (http://manisnet.org), following Soto-Centeno et al. (2013). I ran the RTR test for the south-eastern region of the country (Figure 2.2b) which is equivalent to the extent used in Soto-Centeno et al. (2013). The Lambert conformal conic projection (central meridian 96°E, standard parallels 20°N and 60°N) was used to transform the environmental layers (see Appendix F) because this has low spatial distortion for North America (Les et al., 2013).

iii. Lemurs in Madagascar. Blair et al. (2013) found that two sister pairs of *Eulemur* lemurs in Madagascar (*E. collaris* - *E. cinereiceps* and *E. rufus* - *E. rufifrons*) have clear riverine barriers; for one of these pairs (*E. rufus* - *E. rufifrons*) they found evidence of PNC, and for the other pair (*E. collaris* - *E. cinereiceps*) they found no significant signal, based on the null background tests of Warren et al. (2008). By contrast, two other sister pairs (*E. albifrons* - *E. sanfordi* and *E. flavifrons* - *E. macaco*) were found to have less well defined geographic barriers; for one of these pairs they found support for significant PND (*E. flavifrons* - *E. macaco*) and for the other pair (*E. albifrons* - *E. sanfordi*) they found no significant signal (Blair et al., 2013).

I obtained the *Eulemur* occurrence records from Blair et al. (2013) and restricted the analysis to the island of Madagascar (Figure 2.2c). The environmental layers used (see Appendix F) were transformed to an oblique Mercator projection for Madagascar (following Pearson and Raxworthy 2009).

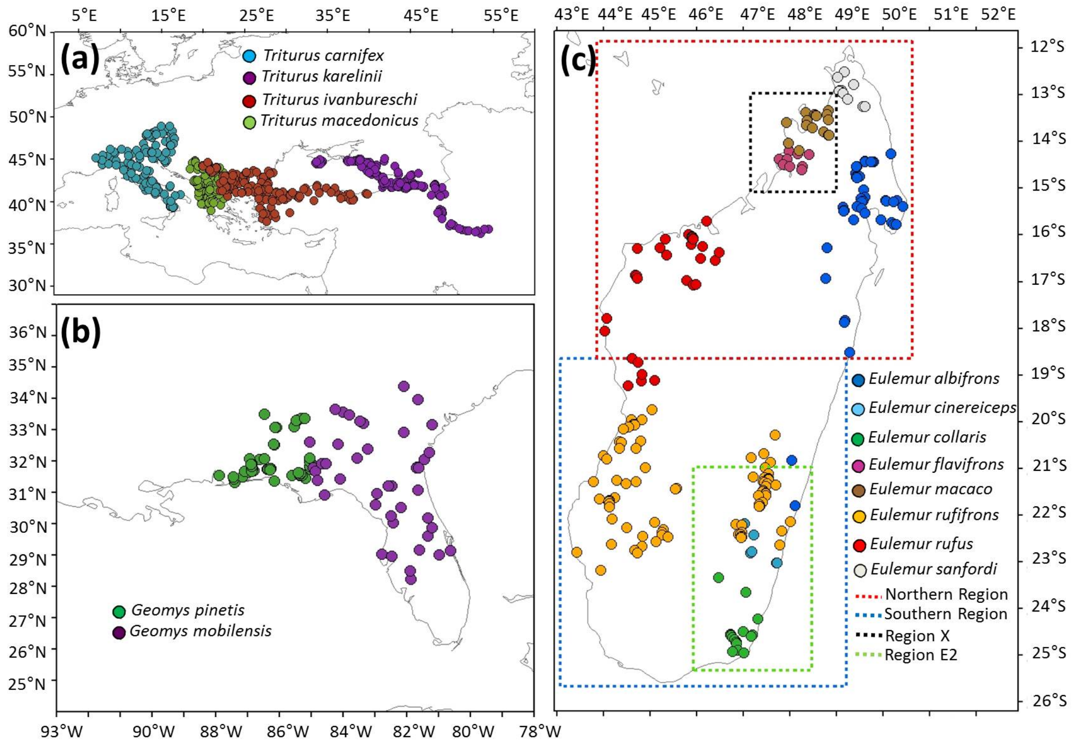

Figure 2.2 Species' occurrence records and extents of the study regions used in the three case studies. (a) Crested newts in the Balkans (*T. karelinii* - *T. ivanbureschi* and *T. carnifex* - *T. macedonicus*); (b) Populations of pocket gophers in south-eastern USA (*G.pinetis* - *G.mobilensis*); (c) Lemurs in Madagascar (*E. collaris* - *E.cinereiceps*; *E.flavifrons* - *E.macaco*; *E.albifrons* - *E. sanfordi* and *E.rufus* - *E.rufifrons*). Regions E2 and X refer to proposed areas of endemism for lemurs according to Pastorini et al. (2003) which coincide with the range-restricted sister pairs (see main text). The outlines (dashed lines in (c)) represent the alternative extents used to test sensitivity of RTR null model to differences in the extent of the study region.

2.3.5 Testing sensitivity to extent of study region

The RTR test requires selection of an overall study region within which the RTR replicates are generated. The selection of this overall background region will affect the model output and the interpretation of the findings (e.g., the observed niche overlap being unique in relation to a particular region but not to another). Selecting an extent that is too wide might include environments that are too different from what the species or clade are likely to experience thus biasing the null library to unrealistic measurements of niche overlap. Selecting a small extent will lead to a reduction in the available environmental heterogeneity being sampled which could result in the exclusion of such unrealistic environments, thus reducing irrelevancy in null library. However, a too narrow extent could mean that there is more similarity between the environments occupied by the observed distribution and the environments sampled within the study region due to higher likelihood of partial overlap within and between the simulated distributions and the observed distributions. Smaller study regions are therefore expected to return lower type I error rates (i.e., false rejection of the null hypothesis) than tests performed across larger areas. In general, a study region should be selected that bounds the landscape, and hence the set of environmental conditions, that the species could reasonably be expected to have had the opportunity to occur in. Thus, factors such as the dispersal capacity of the species, topographic features in the landscape (e.g., barriers to dispersal), and the distribution of major clades to which the species belong might be considered when selecting the study region.

I tested sensitivity of the RTR significance test to the extent of the study region by undertaking the *Eulemur* analysis for the whole island of Madagascar and also for alternative smaller extents. I did this for the two most range-restricted sister pairs: *E. collaris* - *E. cinereiceps* and *E. falvifrons* - *E. macaco.* I divided Madagascar latitudinally, approximately cutting the island in half, to generate sub-island extents that take into account the island's general north-south zonation (Blair et al., 2013). I also generated further restricted study regions by approximating areas of lemur endemism proposed by Pastorini et al. (2003). Thus, *E. collaris* - *E. cinereiceps* was tested with a southern extent and a more restricted south-eastern extent (corresponding to region E2 in Pastorini et al. 2003; Figure 2.2c) and *E. falvifrons* - *E. macaco* was tested with a northern extent and a smaller north-western extent (corresponding to region X in Pastorini et al. 2003; Figure 2.2c).

2.4 Results

2.4.1 Performances of different niche overlap metrics in a common simulated scenario

I found contrasting performance between niche overlap metrics when tested using simulated species, with some metrics having a tendency to overestimate niche overlap while others tend to underestimate niche overlap in relation to the 'known truth' scenario (Figure 2.3b).

The novel metric presented in this study, MO, captures the expected results more closely than the other three metrics (Figure 2.3), with the expected ranges for each region being matched precisely with the outcomes observed in the new metric (Figure 2.3c).

Warren's I metric over-predicted niche overlap in instances where no overlap should be observed in region D (Figure 2.3d). This metric also did not capture the expected gradient in niche overlap in region C (Figure 2.3d). However, it captured the expected range of niche overlap in region B (Figure 2.3d). Moreover, niche identity (complete niche overlap) was correctly predicted to occur only in Region A (Figure 2.3d).

Broennimann's D statistic also tended to over-predict niche overlap in region D (Figure 2.3e); however, this was to a lesser extent than Warren's I statistic, as it was able to identify some cases of complete niche divergence in that region. The expected gradient from 0 to 0.5 was observed in region C (Figure 2.3e). The metric was the only one to overestimate niche overlap (ranging from 0.2-1) in region B (Figure 2.3e). Broennimann et al.'s method also over-predicted the occurrence of identical niches, which was predicted beyond region A (Figure 2.3e). Nevertheless, overall it was able to capture the expected range of 0 to 1 (Figure 2.3e).

Blonder et al.'s (2014) hypervolume approach also captured the 0 to 1 range, though niche overlap was under-predicted in regions C and D, where only niche divergence was observed (Figure 2.3f). However, the approach accurately captured the expected range from 0 to 1 in region B, and complete niche overlap was correctly detected only in Region A.

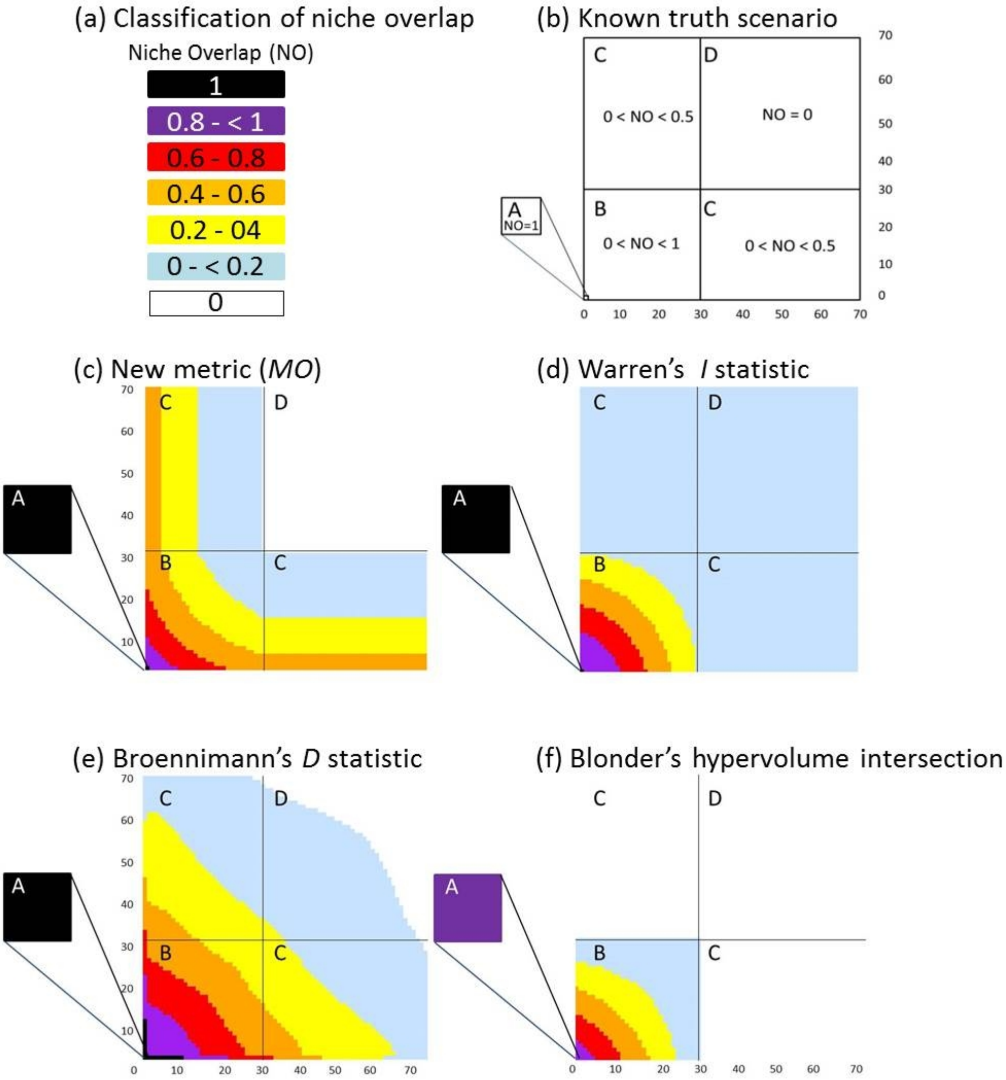

Figure 2.3 Niche overlap values generated by four alternative metrics for a simulated scenario. (a) I used the classification of niche overlap established by Rödder and Engler (2011) but also distinguished between completely divergent niches (niche overlap [NO]=0) and identical niches (NO=1). (b) The 'known truth' represents the expected ranges of niche overlap in each section of a 2-dimensional 70x70 grid (see Appendix E for more details). (c) The new niche overlap statistic (MO) introduced in this chapter. (d) Warren et al.'s (2008) background similarity test with the I statistic. (e) Broennimann et al.'s (2012) PCA uncalibrated technique with the D statistic. (f) Blonder et al.'s (2014) intersection of hypervolumes between two species.

2.4.2 Performance of the RTR null biogeographic model in real case scenarios

The RTR test supports a finding of PNC for both sister pairs of crested newts (Table 2.1). This is in agreement with previous findings (Wielstra et al., 2012).

For the populations of pocket gophers, I found no significant signal for either PNC or PND, which is consistent with the results of Soto-Centeno et al. (2013). However, the observed niche overlap was close to significant for PNC (observed $MO = 0.59$ and 95% threshold for significance $= 0.61$), indicating that further studies may be appropriate to test this finding (Table 2.1).

Results for lemurs show contrasting findings between the RTR null model and the null model of Warren et al. (2008), with agreement in the findings for only one out of four sister pairs. The RTR test finds support for PNC for the pair *E. collaris* - *E. cinereiceps* (Table 2.1). This is in contrast to the findings of Blair et al. (2013), who found no significant signal using the tests of Warren et al. (2008). For the pair *E. rufus* - *E.rufifrons*, I find evidence for PND. This is again in contrast to Blair et al. (2013), who found some support for PNC. For the other two *Eulemur* pairs, I find no significant signal using the RTR test. This is in agreement with Blair et al. (2013) for the *E. albifrons* - *E. sanfordi* pair, but in conflict for *E. flavifrons* - *E. macaco* (Blair et al. 2013 found evidence for PND).

Table 2.1 Observed niche overlap measured with the MO metric and outputs from the RTR null biogeographic model as a significance test for the observed niche overlap value against a null library for two sister pairs of crested newts in the Balkans (*T. karelinii* - *T. ivanbureschi* and *T. carnifex* - *T. macedonicus*), a sister pair of pocket gophers (*G.pinetis* - *G.mobilensis*) in North America and four sister pairs of lemurs from in Madagascar (*E. collaris* - *E.cinereiceps*; *E.flavifrons* - *E.macaco*; *E.albifrons* - *E. sanfordi* and *E.rufus* - *E.rufifrons*). PNC refers to Phylogenetic Niche Conservatism when the observed niche overlap is higher than expected by chance, and PND refers to Phylogenetic Niche Divergence for cases where the observed niche overlap is lower than expected by chance. No significant signal (NSS) refers to cases where the observed niche overlap does not occur less often than expected by random chance. Results from the RTR test are compared against expectations based on published papers.

Sister pair	Original Study	Observed Niche Overlap (MO metric)	5%, mean and 95% tails for Niche Overlap based on a null RTR distribution	Hypothesis from original study	RTR test result
T. karelinii - *T. ivanbureschi*	Wielstra et al. 2012	**0.61**	0.26;0.37;0.51	**PNC**	**PNC**
T. carnifex - *T. macedonicus*	Wielstra et al. 2012	**0.69**	0.23;0.42;0.65	**PNC**	**PNC**
G. pinetis - *G. mobilensis*	Soto-Centeno et al. 2013	**0.59**	0.31; 0.47 ;0.61	**NSS**	**NSS**

E. collaris - *E. cinereiceps*	Blair et al. 2013	**0.56**	0.05;0.24;0.51	**NSS**	**PNC**
E. flavifrons - *E. macaco*	Blair et al. 2013	**0.34**	0.09;0.31;0.57	**PND**	**NSS**
E. albifrons - *E. sanfordi*	Blair et al. 2013	**0.24**	0.09;0.25;0.45	**NSS**	**NSS**
E.rufus - *E. rufifrons*	Blair et al. 2013	**0.19**	0.27;.0.48;0.67	**PNC/NSS**	**PND**

Changing the extent of the study region for *Eulemur* pairs showed that the RTR test is sensitive to selection of the area over which the test is run (Table 2.2). For one sister pair (*E. collaris* - *E. cinereiceps*) I observed loss of significant signal as the extent of the study region was reduced; however this loss of signal was only observed when the background area was very close in extent to that of the two species. Thus, use of a small study extent produced a result consistent with the findings from Blair et al. (2013), who also used a small extent ('background' regions were selected by constructing minimum convex polygons around occurrence records). For the other sister pair, no significant signal was found regardless of the extent of background area used (Table 2.2).

Table 2.2 Observed niche overlap measured with the MO metric and outputs from the RTR null biogeographic model as a significance test for the observed niche overlap value against a null library for two *Eulemur* sister pairs in Madagascar using alternative study region extents. Regions E2 and X refer to proposed areas of endemism for lemurs according to Pastorini et al. (2003) which coincide with the range-restricted sister pairs (see main text). The results from the RTR null model are compared against hypotheses based on published papers. Warren et al.'s (2008) background similarity test is based on results from Blair et al. (2013). PNC refers to Phylogenetic Niche Conservatism when the observed niche overlap is higher than expected by chance, and PND refers to Phylogenetic Niche Divergence for cases where the observed niche overlap is lower than expected by chance. No significant signal (NSS) refers to cases where the observed niche overlap does not occur less often than expected by random chance.

Sister pair	Extent of study region	Observed Niche Overlap (MO metric)	5%, mean and 95% tails for Niche Overlap based on a null RTR distribution	P-value (obtained from RTR test)	Background similarity test	RTR test result
E. collaris - *E. cinereiceps*	Region E2	**0.56**	0.10 ; 0.40 ;0.70	0.26	**NSS**	**NSSl**
	Southern Region		0.06 ; 0.23 ;0.48	0.02	**NSS**	**PNC**
	Whole Island		0.05 ; 0.24 ;0.51	0.03	**PNC/NSS**	**PNC**
E. flavifrons - *E. macaco*	Region X	**0.34**	0.20;0.37;0.56	0.41	**PND**	**NSS**
	Northern Region		0.10;0.32;0.60	0.42		**NSS**

Whole Island	0.09;0.31; 0.57	0.40	**NSS**

2.5 Discussion

2.5.1 Performances of different niche overlap metrics in a common simulated scenario

My analysis showed contrasting outcomes across niche overlap metrics for the same simulated scenario. These findings highlight the importance of choosing an appropriate metric for answering specific ecological and/or evolutionary questions. I found that the statistic introduced in this chapter, the MO metric, performed better than other metrics by producing results closer to those expected from the 'known truth' scenario.

I found poorer performance of Warren's I statistic and Broennimann's PCA-env procedure with the D statistic for quantifying niche overlap when compared to other metrics. The two statistics tend to be correlated (Warren et al., 2008) and I have demonstrated a common tendency to overestimate the simulated niche overlap when no niche overlap is expected to be found and underestimate when one environmental variable overlaps but not the other. This tendency to overestimate the niche overlap has been reported previously in simulated environments (Broennimann et al., 2012) and is likely because the approaches measured the intersection of predictions from two ENMs, which are designed to estimate suitability and therefore tend to overestimate species distributions (because some suitable habitats will be unoccupied; Peterson et al. 2011).

I found a tendency for the n-dimensional hypervolume approach to underestimate niche overlap in my simulation. This is particularly noticeable in instances where the niches do not overlap on at least one axis. There are multiple aspects that could account for underestimation of niche overlap using the hypervolume approach (Blonder et al., 2014). In my simulations, the most likely explanation for underestimation is that the hypervolume method measures niche overlap as the intersection of two volumes such that the volumes will not intersect at all (niche overlap is measured as 0) if they have one or more non-overlapping variables. None of the other metrics I assessed make this strict assessment of niche overlap, either because they calculate niche overlap by doing a cell-by-cell comparison (e.g., Warren's I and Broennimann's D) or they calculate an averaged proportion of the niche breadth shared across all axis (the MO metric). However, I note that in cases when the volumes did overlap on both axes in my simulation, the performance of the hypervolume approach was comparable to that of the MO metric (which closely followed the 'known truth').

In comparison with the other metrics tested, I found that the new MO metric better captured niche overlap in the simple simulated scenario that I tested. There is, however, scope to refine this metric in light of limitations (see Methods). One limitation is the potential sensitivity to ecological outliers (see Appendix D). I have implemented a function to remove ecological outliers (see Appendix A) but removal of outliers is only advised when it is expected that some points may be erroneous (e.g., misidentifications) or there are likely sink populations that do not represent the niche. Removing true ecological extremes will lead to a misrepresentation of the species occupied niche. Although there are limitations, I have demonstrated in these comparisons that the conceptually simple MO metric is a useful approach for quantifying niche overlap.

2.5.2 Performance of the RTR null biogeographic model in real case studies

By applying the RTR test to real case studies, I have shown that the new test can provide comparable outcomes to expected and previously observed patterns from the literature. I have also shown differences in the outputs of the RTR method and Warren et al.'s (2008) background test, with agreement for only one out of four pairs, but congruency between the RTR method and ordination techniques and the 'blob' range-breaking test for the remaining pairs. Application of the MO metric and the RTR significance test supports a hypothesis of PNC for two allopatric sister pairs of crested newts in the Balkans (*T. carnifex* - *T. macedonicus* and *T. karelinii* - *T. ivanbureschi*). This finding is in line with previous work that has used ordination techniques to identify PNC in this study system (Wielstra et al., 2012) and offers a way of further testing the theoretical expectation that PNC is a key pattern emerging from allopatric speciation (Cooper et al., 2010; Losos, 2011; Crisp and Cook, 2012). For pocket gophers in south-eastern North America, I found no significant signal for either PNC or PND, which implies that observed ecological differences between the populations are not likely due to selection for a particular set of conditions. Rather, my results suggest that niche differences are a coincidental result of different environments available on each side of a geographic barrier (the Apalachicola river).

The endemic lemurs of Madagascar have been assessed previously in tests of which, if any, mode of speciation may have been most important in driving local endemism and speciation across the island (Pearson and Raxworthy, 2009; Blair et al., 2013). My measurements of niche overlap using the MO metric were congruent with Warren et al.'s I and D statistics, with my results consistently falling within the range of values presented by Blair et al. (2013). However, the RTR test identified significant statistical support for PNC in the pair *E. collaris* - *E. cinereiceps*, which is not congruent with the results using Warren et al.'s background test (Blair et al., 2013). My finding, combined with strong genetic support for the Mananara river acting as a barrier to gene flow (Wyner et al., 2002), suggests an allopatric mode of speciation for this pair, with niches failing to evolve on either side of a geographic divide.

My analyses find no significant support for either PNC or PND in two *Eulemur* sister pairs (*E. flavifrons* - *E. macaco* and *E. albifrons* - *E. sanfordi*). These results are in contrast to those of Blair et al. (2013), who found support for PND for one of the pairs (*E. flavifrons* - *E. macaco*). Closer analysis of the differences between the null libraries produced by Blair et al. (2013) using Warren's background test and the RTR significance test shows that the RTR test captured a broader null distribution for *E. flavifrons* - *E. macaco.* This explains the difference in statistical inference between the two null models for these sister pairs, despite comparable observed niche overlap values between the two studies. However, it is important to highlight that both *E. flavifrons* - *E. macaco* and *E. albifrons* - *E. sanfordi* have no complete geographical barriers and have potential hybrid zones (see Blair et al. 2013 and references therein). This suggests that other factors besides geographic or environmental separation may be responsible for speciation within these pairs (e.g., microhabitat selection, Rakotondranary and Ganzhorn 2011), which would account for the lack of signal found in my analysis.

I found support for PND for *E. rufus* - *E. rufifrons*, which opposes the hypothesis of PNC of Blair et al. (2013), who found weak support for PNC. However, there is limited spatial overlap between the potential distributions of the pairs (Blair et al., 2013), which suggests that the species have different ecological preferences and is consistent with my finding of PND.

One explanation for differences in results between the RTR test and Warren et al.'s (2008) null models is that the RTR approach focuses on the ecological dimension of niche evolution between species while Warren et al. (2008) focuses on the geographical dimension (i.e., the RTR method measures overlap in niche space, whereas Warren et al. 2008 measures the spatial overlap of ENMs). This difference likely accounts for different biological inferences from the alternative methods. I contend here that my approach of measuring overlap in niche space is most appropriate for robustly testing for PNC and PND, but further research is needed to decipher the different facets of niche evolution that are picked up by each of the approaches. Consequently, I expect that the new methods presented here will complement, rather than replace, existing methods.

I highlight that the RTR approach is best suited to study range-restricted species where many unique RTR replicates can be generated within a background region. Instances where a species is wide-ranging relative to the study region, or where the 'shapes' of the species' distribution and study region are such that RTR replicates can be located in only a limited number of ways, will result in few replicates and potential spatial biases. I illustrate that spatial biases are case specific in Appendix G by showing the locations of 10,000 RTR replicates for a pair of newts and a pair of lemurs. Spatial bias in the RTR null model acts to curtail the background region, and therefore the range of environments considered (note that this does not negate the meaning of the significance test for PNC or PND, but it does mean that the range of conditions over which the calculation is performed is only a subset of those in the selected background region). As with selection of the background region, spatial bias within the RTR null model will be an important area for future research. To facilitate this I provide R code in Appendix A for replicating the analyses I have done in Appendix G.

As with other tests for PNC and PND, the RTR test found instances with no significant signal. In addition to the actual absence of either

PNC or PND, there are a number of methodological factors that can explain non-significant results, including: (i) the choice of predictor variables (some key variables may be excluded from the analysis); (ii) the coarse resolution of analysis (niche differentiation within the 1 km^2 cells used will not be picked up); (iii) incomplete knowledge of occurrences (modern-day loss and fragmentation of habitats means I have an incomplete picture of the niches of the species), and (iv) inappropriate extent of the study region (explored in more detail below).

2.5.3 Testing sensitivity to extent of study region

I have shown that the RTR test is sensitive to the extent of the study region. My tests for Madagascar suggest that sensitivity to the selection of study region extent is low, unless the extent becomes very small (e.g., of similar size to the extent of the two species' distributions). Further exploration of this sensitivity, and of different strategies for selecting the study region (e.g., based on dispersal capacity, following Anderson and Raza 2010), is warranted.

Discordance between my results and those of Blair et al. (2013) may be due in part to the different sensitivities of the RTR test and Warren et al.'s (2008) test to the extent of the study region. Blair et al. (2013) defined the background area of each species based on a minimum convex polygon bound by the occurrence records of each species. A serious drawback of using minimum convex polygons around occurrence records for each species is the assumption that all locations within the polygon are suitable habitat for the species. This assumption is unlikely to hold in many cases (e.g., consider a species that occupies warm lowlands around the base of a mountain: drawing a minimum convex polygon around the species' occurrence records will encompass both the lowlands and also the cooler mountain top).

A result that is non-significant is not expected to become significant if the extent is made smaller. This was observed in my analysis of *E. flavifrons - E. macaco*. I thus find that a tight study region, such as those defined by Blair et al. (2013), is more prone to result in falsely accepting the null hypothesis. A wider extent, by contrast, is more likely to result in rejection of the null hypothesis.

2.6 Conclusions

Overall, I find that the new metric of niche overlap, the MO metric, and the new RTR significance test are suitable methods for testing for PNC and PND, particularly when applied to range-restricted species with few occurrence records. Given its novel approach to the study of niche dynamics between populations, the RTR method holds great promise for testing for PNC and PND across large phylogenies with many sister pairs, and thus shedding new light on evolutionary processes, in particular speciation.

Chapter 3

Interpreting the incidence of Phylogenetic Niche Conservatism from a null biogeographic model

This work was conducted in collaboration with Richard G. Pearson and Mark Wilkinson.

Laura G. A. Nunes conceived the ideas, designed the research, collected and analysed the data and led the writing, with critical input from Richard G. Pearson and Mark Wilkinson.

3.1 Abstract

Phylogenetic Niche Conservatism (PNC) is the tendency for closely related species to share similar ecological preferences. To date, multiple quantitative tools have been developed to test for PNC, with null models being the most common approaches. These null models are sensitive to a number of parameters, resulting in a need for cautious interpretation of results. The random-translation-and-rotation (RTR) significance test is a null model that, when combined with a measure of multidimensional overlap (the *MO* metric), is a new approach for testing PNC in a sister taxa. Here I explore three sources of uncertainty with this method: i) size of the background region; ii) sampling bias in occurrence records; and iii) environmental spatial autocorrelation. I test how these factors affect the ability of the RTR null model and the *MO* metric in testing for PNC using virtual species distributions and virtual environmental layers. I show that the approach is relatively insensitive to changes in the background region, due to the independence of the *MO* metric to background region, and is robust to sampling bias, as it does not rely on random point sampling. However, the approach is particularly sensitive to the strength of environmental spatial autocorrelation. These findings help to discern the uncertainties surrounding the RTR null model outputs for real case scenarios. Correct interpretation of tests for PNC is critical to enhance our understanding of speciation processes and the potential to identify robust evolutionary trends in extant phylogenies.

3.2 Introduction

There has been interest in identifying whether species preferences for certain environmental conditions, or a species ecological niche, are conserved through time (Wiens and Graham, 2005; Crisp and Cook, 2012). These niche-related patterns and processes may shed light on the factors that limit species distributional ranges and drivers of species divergence (Wiens and Graham, 2005). Phylogenetic niche conservatism (PNC) describes a scenario where ecological niches evolve slowly through time thus leading to closely-related species having similar environmental preferences (Wiens, 2004). Recent knowledge of species distributions and the development of geo-referenced environmental information have greatly enhanced studies on niche evolution (Soberón, 2007; McCormack et al., 2010). Attempts to identify PNC from extant species distributions have involved the application of different null models. Null models are hypothesis testing techniques used to distinguish significant observed interactions from random ones (Gotelli and Graves, 1996; Merckx et al., 2011; Fuller and Enquist, 2012) by eliminating environmental or taxonomical constraints (Storch et al., 2008). However, different null models often result in incongruent outcomes (Graham et al., 2004; Wiens and Graham, 2005; Warren et al., 2008; Peterson, 2011; Nunes and Pearson, 2016).

When testing for ecological niche evolution, generating a suitable null model allows us to ask whether the observed similarity or difference between the niches of two populations is statistically meaningful given the available environments. PNC is identified when closely related species differ less ecologically than expected by chance (Chapter 2, Nunes and Pearson 2016). I define niche overlap as the degree of overlap in the set of environmental conditions occupied by two species (or two populations) (Colwell and Futuyma, 1971).

I recently introduced a novel null biogeographic model, the random-translation- and- rotation approach (herein RTR significance test) (Chapter 2, Nunes and Pearson 2016). In this approach, the locality data points of a species pair are randomly rotated around the centroid of the combined data points of both species within a sister pair and randomly translated to another region within a defined background extent. The simulated niche overlap value is derived from that random geographic distribution, while maintaining the original geographic configuration and distance between the locality points. This process is repeated many times (e.g., 10,000) to build a library of null replicates against which the observed overlap can be compared. This approach has been shown to give results consistent with evolutionary theory when applied to sister pairs of *Tristurus* newts in Europe and *Geomytis* gophers in North America, though findings were less congruent to those using other tests for *Eulemur* lemurs in Madagascar (Chapter 2, Nunes and Pearson 2016).

It is critical to determine whether methods are capable of correctly distinguishing between species with similar and dissimilar ecological preferences (Godsoe, 2010). To ensure the correct interpretation of the results within and between null models it is important to understand possible sources of error (Cariboni et al., 2007). Three main sources of uncertainty have been identified when using null biogeographic tests for PNC: i) defining the background region within which the test will be performed (Anderson and Raza, 2010); ii) not accounting for spatial autocorrelation in point data (endogenous autocorrelation); and iii) not accounting for uncertainties in the interpolated values of environmental layers, which may lead to high environmental spatial autocorrelation (exogenous autocorrelation) (Bahn et al., 2006).

i) Size of background region. Selecting an appropriate background region is a major step in ecological niche research because it can greatly impact model outputs (Anderson and Raza, 2010; Saupe et al., 2012; Nunes and Pearson, 2016). There is no general consensus on what criteria is best to delimit a background region. Approaches that could be important in delimiting the background landscape include i) accounting for the proportion of the background area that is occupied by the species (the relative occurrence area, ROA) (Lobo and Tognelli, 2011) and ii) implementing knowledge on dispersal ability, thus delimiting an area that describes the regions accessible to a species (Anderson and Raza, 2010; Barve et al., 2011; Saupe et al., 2012). Although incorporating knowledge of dispersal ability has been advocated, studies rarely take this into account and instead use political and biogeographic divisions (Meyer and Thuiller, 2006; Acevedo et al., 2012) or minimum convex polygons enclosing the occurrences of species (Blair et al., 2013; Ahmadzadeh et al., 2015).

The ROA is a useful metric to describe whether a species has a wide or narrow distribution in relation to the study region (value close to 1 or close to 0 respectively) (Chefaoui et al., 2011). In this chapter, I test the usefulness of ROA for selecting a background region with the RTR significance test. I expect that as the ROA tends toward 1 (i.e., as the study region becomes more restricted) the RTR significance test becomes less informative and with a higher probability of falsely not detecting a signal as the simulated distributions are too geographically and environmentally similar to each other.

ii) Sampling bias in occurrence records. A common characteristic of spatial data is the presence of spatial auto correlation, that is the tendency for spatial data at nearby locations not to be independent from each other (Fortin and Jacquez, 2000; Dormann et al., 2007). This is a common characteristic of species occurrence data due to preferential sampling in some locations versus others (Merckx et al., 2011; Fourcade et al., 2014). Model outputs based on empirical data that has high sampling bias are expected to be significantly different to outputs based on randomly sampled data (Phillips, 2009). Sampling bias is one of the main factors that has confounded relationships between niche-breadth and range size (Slatyer et al., 2013). Sampling bias has also been recognized as a problem in species distribution models, which are often used in studies of PNC (Kramer-Schadt et al., 2013). A null biogeographic model that assumes spatial independence between random point data does not account for the sampling biases in the observed point data. As a result, such a model is more likely to find significant differences between the observed data and the randomly sampled data, thus falsely rejecting the null hypothesis (type I error) (Raes and ter Steege, 2007; Merckx et al., 2011; Wagner and Dray, 2015). The RTR test maintains the same spatial structure (and hence autocorrelation) in the null model as in the observed data, so I expect that the test is less prone to errors arising from sampling biases.

iii) Strength of environmental spatial autocorrelation. Global climatic datasets have biases and uncertainties because of data sparsely collected from weather stations (Deblauwe et al., 2016) and strong interpolations in areas with no actual data (Daly et al., 2007; Fernández et al., 2013). The degree of independence between grid cells, or spatial autocorrelation, due to different interpolating methods may affect our assessment of niche dynamics between species (Fernández et al., 2013) but these differences are rarely thoroughly tested (Bedia et al., 2013). I expect the RTR significance test to more often pick up a signal for PNC in environments which are not very spatially autocorrelated because the heterogeneity of the landscapes will be higher and therefore the observed climatic signal will often be more distinct from null replicates.

In this chapter I conduct the first comprehensive analysis of the sensitivity of the RTR significance test to the limitations outlined above. This provides a better understanding of the performance of the RTR significance test, leading to better interpretation of test results.

3.3 Methods

All analyses were carried out using R version 3.1.2 (R Core Team, 2014). I constructed controlled virtual scenarios that involved virtual landscapes and virtual species distributions. These scenarios addressed the sensitivity of the RTR method to size of background region, sampling bias and environmental spatial autocorrelation. To mimic actual geographical dimensions, I used the extent of the island of Madagascar (Figure 3.1a).

I used the circulant embedding algorithm from the R package 'RandomFields' 3.1.1(Schlather et al., 2015) to build virtual landscapes with a known mean, variance and amount of spatial autocorrelation. Each scenario involved a set of landscapes build from a set random field algorithm. The mean and variance of the virtual layers were the same as a real bioclimatic variable for Madagascar (temperature seasonality at a 1 km^2 resolution from WorldClim, Figure 3.1a) (Hijmans et al., 2005). I selected this layer because it exhibits a clear environmental gradient from north to south and is an important abiotic factor for the fauna in Madagascar (Blair et al., 2013). Each scenario involved a set of three virtual layers: One layer with no spatial autocorrelation to represent fine-scale environmental variation where the value of each grid cell is independent from each other (Godsoe, 2010). The other two layers had the same degree of spatial autocorrelation that represented environmental gradients across a landscape (Godsoe, 2010). These two virtual layers were set by two different random field algorithms, therefore being orthogonal and thus reducing inflation of niche estimates from collinearity (Estrada-Peña et al., 2013). Each scenario was repeated with both these layers having equal levels of spatial autocorrelations set to either low, medium or high (i.e., the environmental gradient extended over 100,000, 300,000, 600,000 grid cells respectively, Figure 3.1d-f). I measured the spatial autocorrelation within the bioclimatic layer for Madagascar using the 'RFempiricalvariogram' function from the 'RandomFields' 3.1.1 package (Schlather et al., 2015). Medium spatial autocorrelation was set to be the same as the spatial autocorrelation of the actual bioclimatic variable. I also cropped all virtual layers to two additional extents: one that would include only the southern part of Madagascar and another that represented only the south-east region of the island (Figure 3.1b).

The virtual species distributions were modelled based on niche responses to the two spatially autocorrelated virtual landscapes. I simulated virtual species distributions using the R package 'virtualspecies' 1.1 (Leroy et al., 2015), which constructs species distributions based on predefined environment-occupancy responses (Meynard and Kaplan, 2013). I constructed two virtual species with the same environmental preferences to simulate PNC. The environment-occupancy response (or species niche) was defined by a normal distribution around the mean environmental conditions found in the smallest extent of the virtual layer (Figure 3.1b) and a standard deviation of 200 because a narrower niche variance would often fail to give a large number of suitable occurrences within the species extent. A suitability map based on probability of occurrence was built using the virtual layers and the species niche. To ensure that the distributions of the species were allopatric (i.e., non-overlapping in space) I drew random points for each species in separate but adjacent regions within the spatially autocorrelated virtual layers (Figure 3.1b). This hard boundary acts as a proxy for a biogeographic barrier to dispersal, such as a river or mountain range (Godsoe, 2010).

To test for PNC I used the *MO* metric, which measures multidimensional niche overlap by calculating the proportion shared between the niche limits of two species which are obtained from their locality data (Nunes and Pearson 2016, Appendix D). The RTR significance test was then used to measure the statistical significance of the observed niche overlap between the two virtual species in relation to the available landscape.

For each scenario, I ran the following steps (Figure 3.2): i) I created 3 virtual layers, one with no environmental spatial autocorrelation and two with equal levels of environmental spatial autocorrelation; ii) I constructed habitat suitability maps for each species based on pre-defined environment-occupancy responses; iii) I drew 100 random points from each adjacent region in the suitability map, sampling grid cells that had a suitability equal or above 0.9; iv) I applied 4 levels of sampling bias to northern regions by separately selecting 30 points from these 100 points thus creating 4 distributions for each virtual distribution with different bias. v) For each distribution pair, I measured the observed niche overlap using the *MO* metric; vi) I used the RTR significance test to build null replicates of niche overlap within the full extent of virtual layer; viii) After 1,000 RTRs, I tested whether the observed niche overlap value was significant against the null generated library of niche overlap values using the 'RTRsignificance' function and 3 significance levels (0.1, 0.05 and 0.01) (Nunes and Pearson 2016, Appendix A); ix) For each species distribution bias treatment, I re-ran steps vi)-viii) of the analysis with a medium extent and a smaller extent, thus maintaining the distribution of points for each scenario and bias treatment (Figure 3.2). x) I also re-ran the entire analysis for landscape with two other levels of environmental spatial autocorrelation.

To have statistical robustness and reduce biases, I repeated this process for 100 different random field scenarios. In each scenario I ensured that the same 100 random fields were used for all combinations of treatments and that the same rotations and translations were performed for each distribution. I ran 1,000 RTRs, which was sufficient to reach stability in the output (i.e., there was no change in p-value to 4 decimal points with increasing number of repeats). I recorded the statistical signal for PNC at 3 significance levels (0.1, 0.05 and 0.01) to account for changes in the confidence of statistical signal due to different treatments. I measured the range size occupied by the two species in relation to the total area of background region (i.e., the relative occurrence area, ROA). I assessed how the ROA changed with different treatments of extent size, spatial autocorrelation and sampling bias and whether it could account for differences in the incidence of PNC across treatments. I also measured the standard deviation in the environmental values captured by the spatially autocorrelated virtual layers to account for changes in variability in the layers due to changes in size of background region and environmental spatial autocorrelation.

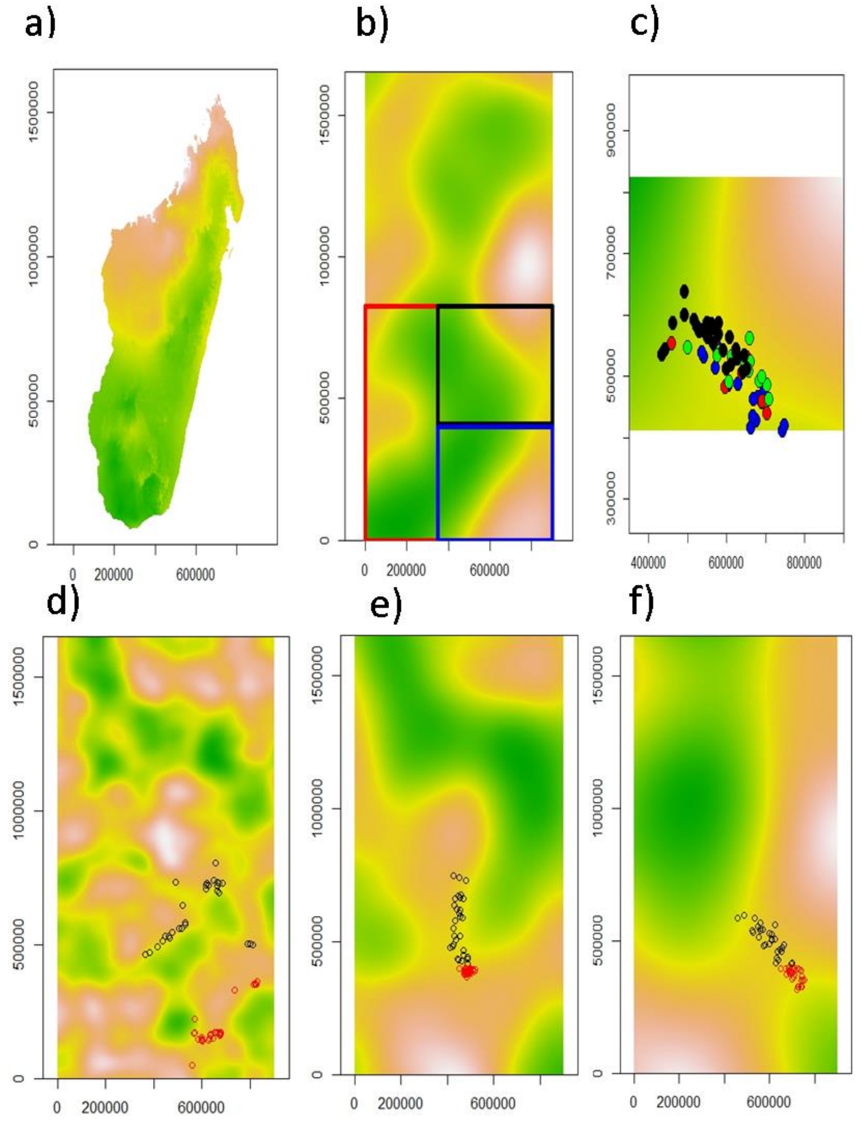

Figure 3.1 Virtual layers and treatments used in virtual scenarios a) Temperature seasonality at a 1 km^2 resolution, for Madagascar from WorldClim (Hijmans et al., 2005) b) Different extents of background region used: Medium (red box) and sampling areas for species A (black box) and species B (blue box). The small extent was the combination of the two sampling areas. c) Four different sets of observations for the same virtual species distribution with different degrees of sampling area bias: no sampling bias (blue), low latitudinal sampling bias (red), medium bias (green) and high bias intensity for northern distributions (black). Panels d, e, and f show three virtual layers with low, medium and high spatial autocorrelation (100,000 grid cells, 300,000 grid cells and 600,000 grid cells respectively). Panels d, e and f also have 2 virtual species distributions with unbiased sampling plotted, black circles for species A and red circles for species B.

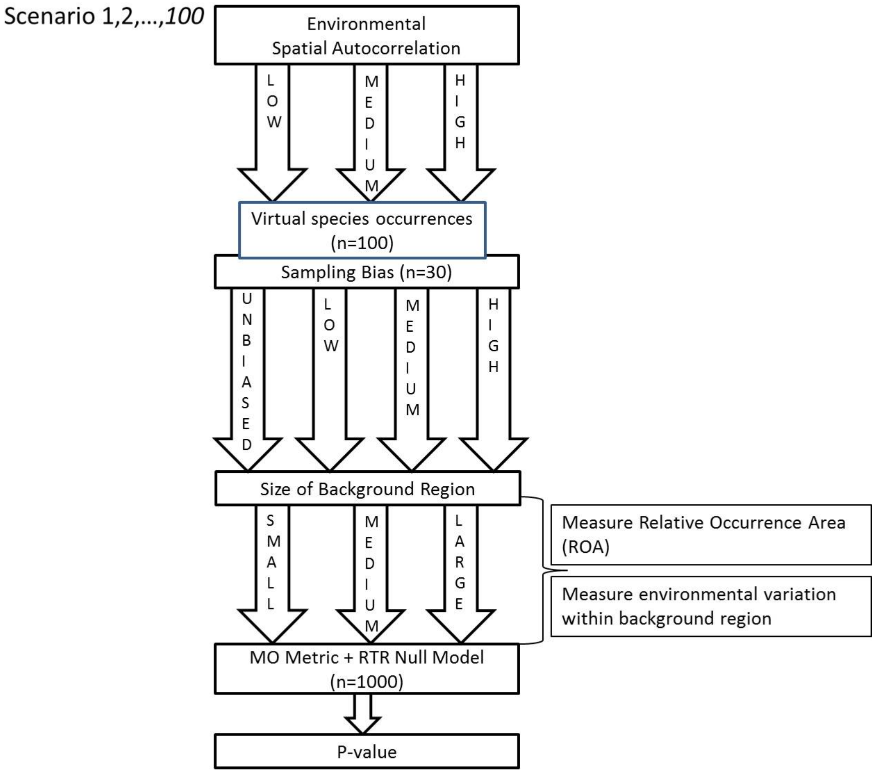

Figure 3.2 Schematic flow chart showing the sequence of steps of each random field scenario

3.3.1 Effects of size of background region

The RTR significance test can be sensitive to the choice of background region because the amount of geographical region being sampled will reflect the amount of environmental variability included in the null model. It has been shown that the RTR significance test is less likely to pick up a significant signal when conducted within a small extent in relation to the size of the species distributions (Chapter 2, Nunes and Pearson 2016); however, how often this change in signal occurs is yet to be determined. To test the sensitivity of the RTR approach to size of background region, I re-ran the simulations with a medium and a small background region (Figure 3.1b). I expect that by reducing the extent, a signal for PNC is less likely to be detected because the environments being randomly sampled by the RTR significance test will often be more similar to each other due to higher incidence of partially overlapping RTR replicates (note that completely overlapped RTRs, duplicates, are removed from the simulation). As the *MO* metric is not dependent on the extent of the background region, the observed niche overlap will not be affected by changes in the extent.

3.3.2 Effects of sampling bias in occurrence records

The RTR significance test maintains the spatial bias in the distribution data by not using random point data to build its null library. Instead, the test maintains the geographic configuration of the observed point data and randomizes where those points may be found by randomly rotating and translating the points to anywhere else in the available landscape. To test the sensitivity of the *MO* metric and the RTR significance test to sampling biases, I simulated the virtual distributions by adding a latitudinal sampling bias so that northern occurrences were more likely to be picked up (Fourcade et al., 2014). The sampling bias was tested using the three intensity levels described above (low, medium and high; Figure 3.1c). I tested whether a highly biased occurrence dataset was more likely to detect a significant signal, as autocorrelation in point data may influence statistical inference (Wagner and Dray, 2015). Higher sampling bias causes the occurrences of each species to be more clumped together in my simulation, thus the species are more geographically distant from each other. Given the spatial autocorrelation in the environment, distant populations will more likely experience different environmental conditions. I therefore expect a smaller overlap between the observed niches of the two species with higher sampling bias. I also expect a decrease in ROA with higher sampling bias given that occurrences will be more clumped together.

3.3.3 Effects of environmental spatial autocorrelation

I aimed to address the issue of environmental spatial autocorrelation by re-running the simulations with environmental layers that have different degrees of spatial autocorrelation in their environmental gradients. Low environmental spatial autocorrelation describes an environment where environmental conditions in a given grid cell are weakly dependent on the conditions found in nearby cells (Figure 3.1d). This results in a landscape composed of small patches of similar size. A layer with medium environmental spatial autocorrelation has less patch density of larger and more variable sizes (Figure 3.1e). A landscape with high spatial autocorrelation has only one or two homogeneous patches with an environmental gradient extending from one extremity of the region to the other (Figure 3.1f). In landscapes with high autocorrelation, environmental conditions are only found at particular regions while in landscapes with less spatial correlation the environmental conditions are found repeatedly within the extent. (Figure 3.1d-f). I therefore predict that PNC will be more commonly detected in landscapes with lower environmental spatial autocorrelation due to the environmental spatial heterogeneity of the environmental conditions.

In the RTR significance test, there is one common background region where the null model runs as opposed to separate regions for each species (it is common in other methods, including the background similarity test of Warren et al. (2008), to have separate regions for each species). Separate species-specific regions in landscapes with high spatial autocorrelation often results in the non-detection of PNC because the species are found in regions with different environments despite having similar niche preferences (Godsoe, 2010). I therefore expect the RTR significance test to be less prone to erroneously not pick up a PNC signal in landscapes with high spatial autocorrelation. This is because the RTR null model is measuring the niche similarity between the species in relation to the entire region rather than against the environments within their observed distributional range.

The observed niche overlap captured by the MO metric is expected to decrease in environments which are highly spatially autocorrelated as species are found to be occupying different environments. I also expect the ROA to be smaller in scenarios with high environmental spatial autocorrelation compared to scenarios with low autocorrelation because species occurrences are expected to be more clumped together as the environments they respond to are less spread across the landscape.

When the virtual layers have high environmental spatial autocorrelation, I expect the range in the environmental conditions to be reduced as the extent of the background region becomes smaller. This is due to certain regions having particular conditions not found across the entire landscape. When the background region is smaller, these particular conditions may no longer be present and will therefore not be sampled with the RTR significance test.

3.4 Results

I observed no influence of changes in the size of the extent on the rates of detecting PNC with any treatment (Figure 3.3). I found that the rate of detecting PNC using the RTR test was lower in cases with low environmental spatial autocorrelation and higher for scenarios with medium spatial autocorrelation (Figure 3.3). I also found a consistent increase in incidence of PNC with increase of sampling bias landscapes with low environmental spatial autocorrelation, but this trend was not observed in medium or high spatial auto correlated environments (Figure 3.3).

I did observe a steep decline in the incidence of PNC with increasing threshold of significance in simulations with low environmental spatial autocorrelation (Figure 3.3). This decline was less pronounced in the other two treatments, indicating that tendency for PNC was stronger in these landscapes (Figure 3.3).

Reductions in ROA due to increases in sampling bias were significant in all landscapes with low environmental spatial autocorrelation regardless of extent (Figure 3.4, Appendix H). But in medium and high spatial auto correlated environments, sampling bias only caused a significant reduction in ROA for high sampling bias, regardless of extent (Figure 3.4, Appendix H). ROA also reduced significantly with increasing environmental spatial autocorrelation regardless of sampling bias or extent, but less significantly between medium and high environmental autocorrelation treatments (Figure 3.4, Appendix H.). With regards to extent, ROA increased significantly with decreases in extent, regardless of sampling bias or environmental spatial autocorrelation (Figure 3.4, Appendix H.).

I found that the standard deviation from the mean conditions of the environmental conditions within the virtual layers were only significantly reduced when compared medium and high extents to small extents in all environmental spatial autocorrelation treatments (Figure 3.5, Appendix H.).

Finally, I demonstrated that the observed niche overlap remained relatively high across all treatments, averaging in the 0.6-0.8 range (Figure 3.6). It remained stable in environments with low spatial autocorrelation, irrespective of sampling bias treatment (Figure 3.6, Appendix H) but significantly decreased with increasing sampling bias in landscapes with medium and high environmental spatial autocorrelation (Figure 3.6, Appendix H). I also found that observed niche overlap significantly decreased with increase in environmental spatial autocorrelation irrespective of sampling bias treatment (Figure 3.6, Appendix H).

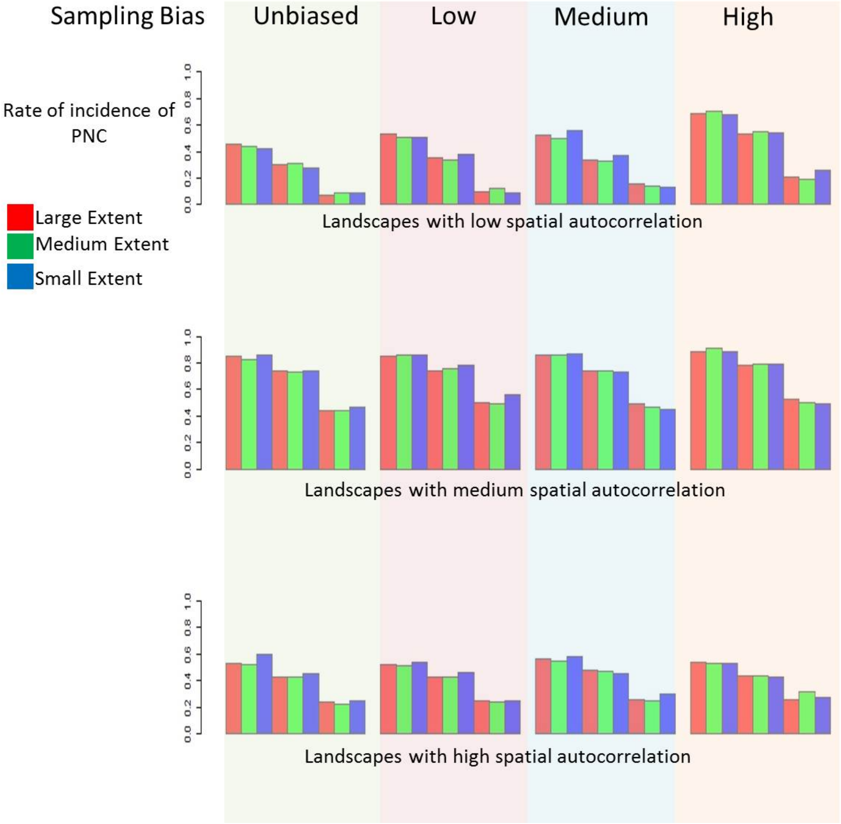

Figure 3.3 Barplot showing the rate of incidence of PNC under different treatments for size of background region, sampling bias and strength of environmental spatial autocorrelation. Large extents (red), medium extent (green) and small extent (blue).Rate is measured for three significance levels a=0.10; 0.05; 0.01 from left to right

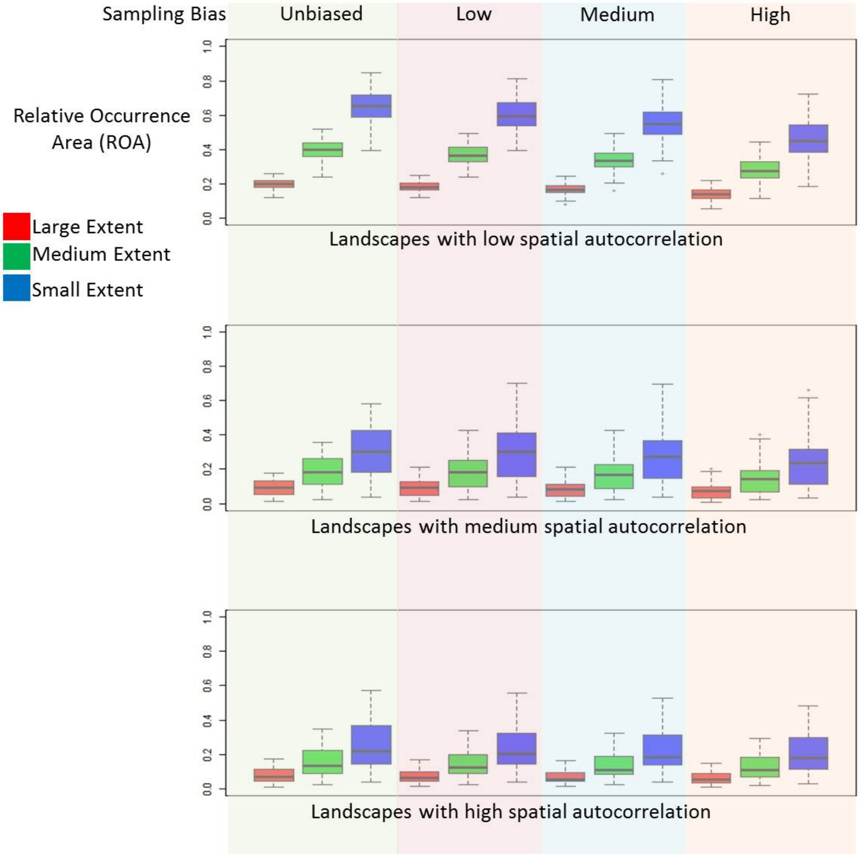

Figure 3.4 Boxplot showing the variation in relative occurrence area (ROA) between the distributions and the available environment given different treatments for size of background region, sampling bias and strength of environmental spatial autocorrelation.

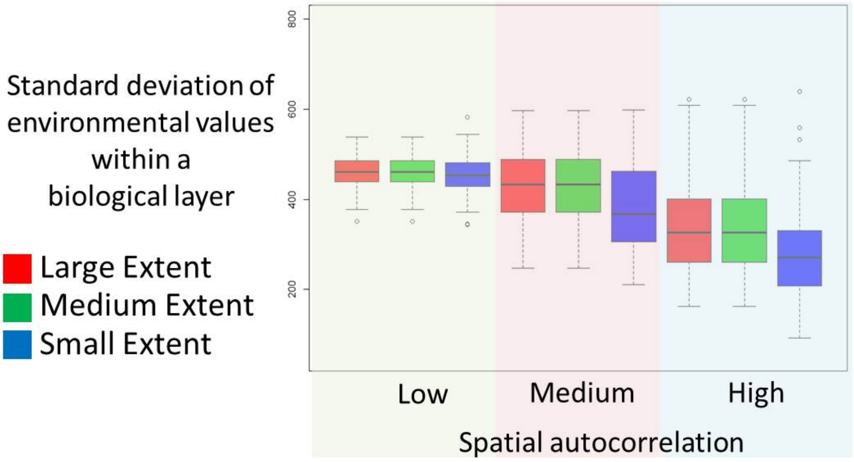

Figure 3.5 Boxplot showing the variation in the environmental conditions of the virtual layers with different extents and treatment for spatial autocorrelation.

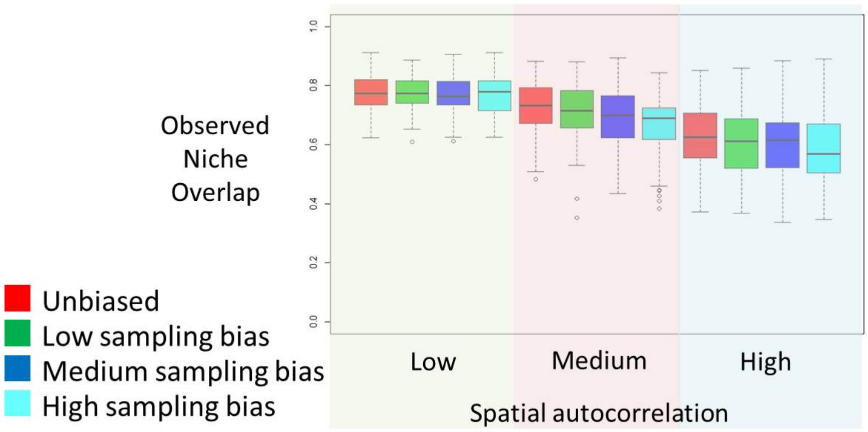

Figure 3.6 Boxplot showing the observed niche overlap for different treatments of spatial autocorrelation and sampling bias.

3.5 Discussion

3.5.1 Sensitivity of RTR outputs to size of background region

I found that the outputs of the RTR null model did not vary significantly with size of extent. This is contrary to my initial expectation that a smaller extent would lead to fewer chances of detecting a statistically significant signal. Failing to find this trend can be interpreted in two ways: i) The RTR model is generally insensitive to changes in extent, unless the extent size is too close to the extent covered by the species distributions (Chapter 2, Nunes and Pearson 2016); or ii) this study's protocol is too simplistic to capture robust trends. The simplicity is due to the use of only three environmental axes, two which had similar environmental parameters (i.e., mean, variance and spatial autocorrelation). The RTR null model has been shown to be sensitive to choice of extent in more complex, real case scenarios (Chapter 2, Nunes and Pearson 2016); thus, despite the apparent insensitivity to size of extent in these simulated scenarios, I highlight the possibility that for more complex cases there needs to be careful consideration of the appropriate choice of extent.

When assessing the validity of statistical outputs, it may be challenging to distinguish between statistical artefacts and true biological phenomenons (McPherson et al., 2004). In this case, are sister taxa with smaller ROA more likely to pick up a significant statistical signal because the null library is more heterogenous (i.e., statistical artefact) or because the taxa are occupying distinct environmental conditions (i.e., biological phenomenon). In this analysis, significant changes in the ROA did not significantly impact the chances of picking a significant statistical signal. It could be argued that in these simulations as the significant signal is not influenced by ROA, the outputs are describing true biological interactions.

The *MO* metric is a presence-only statistic as it does not require calibration using a background region (Chapter 2, Nunes and Pearson 2016, Appendix D), unlike other commonly used metrics (e.g., *D* and *I* metrics of Warren et al. 2008). Because the *MO* metric is not affected by the choice of background region, re-running the RTR null model with different extents will only affect the significance of the observed value against the null library rather than the estimations of the observed niche overlap. This means that it is more tractable to test the robustness of model outputs to different extents because the observed niche overlap is not a confounding factor. I therefore advise the use of different extents when running the RTR null model in order to test the robustness of the outputs.

As expected, I reported smaller variation in the environmental conditions within the biological layers with decrease in extent size. This shows that choosing a small extent might remove important variability in the environment which may affect the output of the RTR significance test. On the other hand, it may be necessary to choose a smaller extent in order to remove environments that are too different to be useful for inclusion in the null library of the RTR null model (e.g., northern biomes are not as informative when testing species living in southern regions).

3.5.2 Sensitivity of RTR outputs to intensity of sampling bias

The RTR null model was found to not be very sensitive to intensity of sampling bias. As expected, I demonstrated how high sampling bias has a tendency to reduce the ROA. This tendency may not be common in other types of sampling bias which were not simulated in this study (see Fourcade et al. 2014 for examples). However, the reduction in ROA due to sampling bias did not affect the chances of picking up a PNC signal.

I found that the *MO* metric was sensitive to the intensity of sampling bias in medium and high environmental regional gradients. This is because the *MO* metric is a presence-only statistic which therefore takes the occurrence records as being the true representation of the complete niche of a species. I demonstrated that the *MO* metric is sensitive to non-detection sampling bias, where true occurrences are not observed and therefore not included in the model (Hefley et al., 2013). Correction for non-detection sampling bias is challenging and requires auxiliary information (e.g., information on sampling effort) which may be lacking for many species (Hefley et al., 2013). I therefore highlight the need for careful consideration of the limitations of the dataset when applying the *MO* metric and, when possible, the need to correct for sampling bias in the dataset before applying the *MO* metric.

3.5.3 Sensitivity of RTR outputs to strength of environmental spatial autocorrelation

In my landscapes with low spatial autocorrelation there is low specificity in environmental conditions to particular geographical areas. In my simulation, species are distributed according to their niche preferences within a specific geographic region within a larger background region. These simulations could describe real case scenarios where species distributions are not limited by their ecological niche but by geographical barriers to dispersal. In this case, a species potential distribution is not matched by its actual distribution (Soberón and Peterson, 2005; Munguía et al., 2008). I found low incidence of PNC in these scenarios when using the RTR significance test. This may be explained by the distributions of the species not being limited by unique ecological conditions found within the distributions but by geographical barriers to dispersal.

Simulations with high spatial autocorrelation describe landscapes which have homogeneous environmental gradients over large expanses across the landscape. In this study, given that species are restricted to particular non-overlapping regions, they may often be occupying regions which have distinct environmental conditions, despite having the same fundamental niches. This is demonstrated by the significantly lower niche overlap observed in virtual layers with high spatial autocorrelation in relation to the other two treatments. Methods testing for PNC in allopatric pairs which are found in different ecological regions may falsely result in a signal for niche divergence instead of niche conservatism (Godsoe, 2010). Virtual layers with high environmental spatial autocorrelation may describe landscapes that while the environmental conditions are specific to particular regions, the breadth of environmental conditions are not unique to those regions (e.g., a range of 10 degrees of temperature stretched per 100 grid cells consistently across the landscape). Similarly, the niche breadth captured by the species distributions may often be found in other regions in the landscape due to the homogeneous environmental configuration of the landscape and the maintenance of the configuration of the point data when measuring null replicates with the RTR significance test. For this reason, measures of niche overlap with the MO metric in randomly translocated distributions are likely to be similar to the observed niche overlap. I found the RTR test to be less likely to detect a signal for PNC in these environments. The lack of statistical signal for PNC could be due to the maintenance of the spatial configuration of the point data when running the null replicates thus maintaining the climatic signal between the observed distributions and the null replicates (Peterson et al., 2009).

Landscapes with medium spatial autocorrelation have more environmental heterogeneity than the landscapes with high spatial autocorrelation (Figure 3.1e-f). Under these conditions, the environments that a species occupies are less likely to be found by random chance. This can be observed in real case scenarios of highly heterogeneous environments. In these environments, there may be opportunities for isolation due to environmental disruptions to a species range, with populations remaining isolated due to niche conservatism (Wiens, 2004). Cases of ecological conservatism are expected to be picked up by the RTR significance test, due to the uniqueness of the observed niche conditions in relation to random niche overlaps. In this chapter, I observed higher detection of a signal for PNC in these landscapes when compared to the other two environmental spatial autocorrelation treatments. I have thus demonstrated the ability of the RTR null model to detect the specificity of the environments occupied by the sister pair in relation to the available environments across the whole landscape.

3.5.4 Sensitivity of other factors to endogenous and exogenous autocorrelation

It is unknown whether endogenous (e.g., spatial biases in point data) or exogenous autocorrelation (e.g., environmental spatial autocorrelation) have the greater impact on model outputs (Bahn et al., 2006). Here I demonstrated that exogenous autocorrelation may influence model outputs more than endogenous autocorrelation in the RTR significance test. Studies have shown that the geographic extent of the analysis may determine which autocorrelation affects outputs the most. Analyses conducted in small to medium extents may require more accuracy in endogenous spatial information (Bahn et al., 2006). I found no noticeable differences in the incidence of PNC with small or medium extents with higher sampling bias or environmental spatial autocorrelation. This suggests that the observed differences in the chances of picking up a PNC signal with the RTR significance test due to exogenous autocorrelation may be scale independent.

A decrease in the environmental variability within the biological layers due to reduction in size of background region was more pronounced in the simulations with higher environmental autocorrelation. This can be explained by the fact that environmental distances between grid cells are more correlated with geographic distances. In these cases, reducing the extent will eliminate more extreme environments because they are only found in more distant regions. In highly spatial autocorrelated environments, a reduction in the background region may result in less incidence of picking up a significant signal with the RTR null model because there is less environmental variability available. In this chapter, I did not find this trend, which I attributed to the lack of complexity found in these simulations in comparison to real case scenarios. Testing the degree of environmental spatial autocorrelation within bioclimatic layers can be a critical step in choosing an appropriate size of the background region prior to running the RTR null model.

The *MO* metric was also shown to be sensitive to the strength of environmental spatial autocorrelation. I observed a decreasing trend in observed niche overlap from low to high spatial autocorrelated environments. With increasing environmental spatial autocorrelation, the niches occupied by the two species may become almost distinct subsets of a common fundamental niche, thus giving an observed niche overlap estimate which is smaller than the ones observed in less homogeneous landscapes (i.e., with low environmental spatial autocorelation). This has been observed in the performance of other niche overlap statistics (Godsoe, 2010) and highlights the inability of these tests to discern between niche preferences (fundamental niche) from niche availability (occupied niche) (Warren et al., 2014).

3.5.5 Caveats and concluding remarks

I used virtual scenarios to have full control over spatial and environmental parameters that may affect model outputs. The simplistic approach that I used here, whereby the analyses were restricted to three environmental axes (only two of which the species responded to), enables us to have a clear interpretation of the results. But because these simulations lack the complexity which is typically found in real scenarios (e.g., more environmental variables), trends observed in real case studies may not be captured in this study. This is the case of the lower likelihood of detecting a significant pattern in small background regions compared to larger regions (Chapter 2, Nunes and Pearson 2016), which was undetected in my simulations. If more environmental layers with different degrees of spatial autocorrelation were included, it is likely that the tendency for loss of a significant signal would be observed more often. The tendency for not obtaining a significant signal has been reported in another null model that also retained spatial autocorrelation (Beale et al., 2008). One of the interpretations was that the environmental conditions described in those geo-referenced layers were not important in defining the distribution of the species (Beale et al., 2008). Others have suggested that, given the high spatial autocorrelation within environmental layers and the maintenance of the spatial autocorrelation between point data under these null models, the observed distributions will often have the same climate signal as the null distributions (Peterson et al., 2009). As I have highlighted above, this will result in the observed niche overlap often not being significantly distinct from the null replicates.

Another parameter that may impact my results is the niche breadth of the species. For this analysis, I selected a niche breadth that ensured that I always obtained 100 grid cells with suitability higher than 0.9 when extracting virtual occurrence records, but with no basis on typical niche breadth of endemic species. For example, a narrower niche breadth may result in higher incidence of PNC and may increase the detectability of robust trends of how background region, sampling bias and environmental gradients affect model outputs. I have outlined potential caveats and further lines of enquiry regarding the performance of the RTR null biogeographic model but I highlight the usefulness of this analysis in highlighting potential sources of uncertainty surrounding the model outputs.

Overall, I show the usefulness of null models in testing for PNC and a clear protocol to test how different parameters may cloud our interpretation of the results. Knowledge of the intricacies of null models and their outputs will greatly enhance our understanding of how PNC is observed in real scenarios and how to better develop and refine null models to more accurately capture these evolutionary trends. This will assist with making more confident predictions of past evolutionary histories and future responses of species to environmental pressures.

Chapter 4

Topographic complexity promotes Phylogenetic Niche Conservatism among sister pairs of squamates in Madagascar

This work was conducted in collaboration with Richard G. Pearson and Mark Wilkinson.

Laura G. A. Nunes and Richard G. Pearson conceived the ideas; Laura G. A. Nunes designed the research, collected and analysed the data; Figure 4.1 was first drafted by Richard G. Pearson; Laura G. A. Nunes led the writing with critical input from Richard G. Pearson and Mark Wilkinson.

4.1 Abstract

Phylogenetic niche conservatism (PNC) or divergence (PND), whereby closely-related species retain or depart from their ancestral ecological preferences respectively, may play a role during speciation processes, however robust drivers of PNC have not been identified. Here, I test whether the prevalence of PNC and PND is associated with sister species with particular spatial distributions (i.e., non-overlapping or allopatric pairs and overlapping or parapatric pairs), time since species divergence and between sister pairs found in regions of high or low topographic complexity. I test for these hypotheses in sister pairs of squamates in Madagascar as they are a case study that may be generalised to other regions. I combined occurrence data for 28 range-restricted sister pairs and data on climatic and topographic conditions to test for PNC or PND in each sister taxa using the RTR significance test. First, I tested whether PNC or PND were more prevalent. Then I test for an association between cases of PNC and PND and spatial distributions using conservative and liberal two-tailed tests. I also looked for associations between tendency for niche conservatism and either i) spatial distributions, ii) time since divergence and iii) topographic complexity. In this analysis, I found cases of PNC to be twice as common as PND. Therefore niche conservatism may play an important role in the speciation processes on Madagascar. I found an exclusive association between PNC and allopatric distributions and PND with parapatric sister taxa using a conservative two-tailed test. In a liberal test, I found cases of both conserved and divergent ecological speciation for both allopatric and parapatric sister pairs. I found no association between tendency for niche conservatism and spatial distributions or time since divergence, but taxa with higher tendency for conserved niches were found in regions of higher topographic complexity. Regions of high topographic complexity may explain incidence of niche conservatism among sister taxa. High unevenness in surface terrain may create barriers between geographic distributions thus promoting small-scale species isolation. These disjunct distributions are maintained as species retain their ancestral climatic conditions, given that niches are conserved through time.

4.2 Introduction

For many decades there has been an increasing understanding of the genetic mechanisms which cause ancestral populations to become distinct species (Coyne, 1992; Barraclough and Nee, 2001; Butlin and Ritchie, 2009). This has resulted in an extensive record of how speciation events may have shaped the extant tree of life (Barraclough and Nee, 2001). However, these studies lack an understanding of the role of non-genetic processes in driving speciation events (Schluter, 2009; Nosil et al., 2012; Anacker and Strauss, 2014). Recently there has been a rise in finely resolved phylogenetic data, comprehensive distribution data and global geo-referenced ecological data which has made tests for speciation events based on geographic and ecological processes possible for the first time (Soberón, 2007; McCormack et al., 2010).

Three main scenarios have been proposed to describe lineage divergence due to geographical and ecological isolating patterns: 1) 'Hard allopatry' refers to cases when an ancestral species distribution becomes divided due to the formation of a geographic barrier (vicariance or physical disruption) (Pyron and Burbrink, 2010); 2) 'Soft allopatry' describes cases when environmental change causes the ancestral population to split its distribution in order to retain its ancestral environmental conditions (ecological disruption) (Wiens, 2004; Pyron and Burbrink, 2010); 3) 'Parapatry' describes cases when speciation occurs along an environmental gradient with partial range overlap, leading to isolation in ecological rather than geographical space (ecological divergence in parapatric speciation) (Figure 4.1.). In parapatry, there is gene flow between the populations occurring during the speciation process but ceasing with time (Pyron and Burbrink, 2010; Sousa and Hey, 2013). The tendency for closely-related species to retain their ancestral ecological preferences (or niche) is referred to as phylogenetic niche conservatism (PNC), while the tendency for closely-related species to depart from their ancestral niche is termed phylogenetic niche divergence (PND).

Here I aim to test the assertion that current ranges reveal the mechanisms described in Figure 4.1: if we assume that post-speciation range shifts have not removed all signal of the speciation process, then I anticipate that PNC will be found in pairs with allopatric distributions

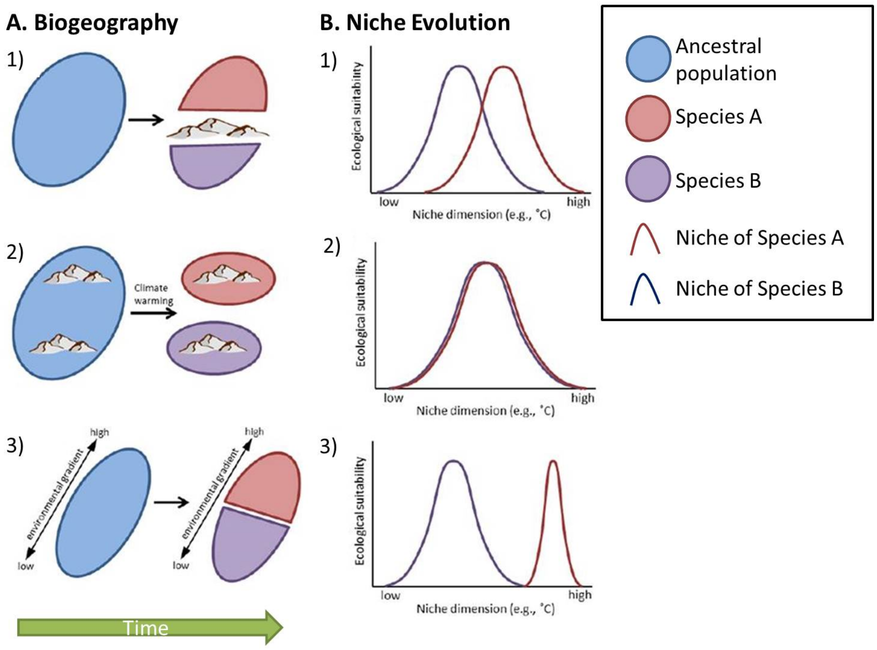

Figure 4.1 Alternative mechanisms of speciation illustrated in terms of biogeography and ecology. (A1) 'Hard' allopatric speciation in which a geographic barrier leads to spatial isolation. (A2) 'Soft' allopatric speciation in which environmental change (e.g., warming) causes incipient species to become geographically isolated due to failure to adapt to new environments. (A3) Parapatric speciation in which ecological divergence along an environmental gradient is enabled by disruptive selection and assortative mating. (B1) Some niche lability is observed but there is no statistically significant signal of either PND or PNC. (B2) PNC, in which lack of adaptation to new environments means that niches remain similar. (B3) PND, in which adaptation to different environments leads to distinct ecological niches between two closely related species.

(Wiens 2004; Figure 4.1A2,B2) and PND will occur among pairs with parapatric distributions (Florio et al. 2012; Figure 4.1A3,B3).

Sister taxa are useful for assessing speciation events because due to their recent divergence there is less time for range and niche shifts to have occurred (Losos and Glor, 2003; Anacker and Strauss, 2014). Combining data on recently diverged taxa with fine resolution data on geo-referenced species occurrences and ecological conditions allows scientists to measure and compare ecological preferences between closely-related species. In this chapter, I describe the species niche as the set of ecological conditions in which species are found ('occupied niche' *sensu* Peterson et al. 2011).

Comparing the niches of two sister species will thus inform on whether the speciation in a given pair could be attributed to PNC, PND or other non-ecological mechanisms (e.g., sexual selection, Lande 1981).

It has been proposed that speciation is usually assisted by ecological conservatism (Wiens, 2004; Peterson, 2011). This is particularly supported by a study of allopatric speciation in the Isthmus of Tehuantepec in Mexico, where niche conservatism was found between sister pairs distributed in either side of the barrier (Peterson et al., 1999). Other studies have also found potential correlations between allopatry and ecological niche similarity in sister pairs (Kozak and Wiens, 2006; Warren et al., 2008). However, there has also been evidence of niche shifts among closely related species (e.g., Graham et al. 2004; Blair et al. 2013). Phylogenetic niche conservatism is also more commonly found in recent speciation events but less pronounced in older lineages (Peterson, 2011). I therefore expected younger sister pairs to have higher tendency for niche conservatism than older lineages.

The degree of similarity between two niches is traditionally quantified by the degree of overlap between the niches of two species (Colwell and Futuyma, 1971). PNC and PND are detected when the niche overlap between a sister pair is higher or lower than expected by chance respectively (Chapter 2, Nunes and Pearson 2016). In these cases, speciation is likely to be explained due to ecological processes. There are many reasons why a significant ecological signal might not be found: i) Non-niche-based mechanisms (e.g., reproductive isolation) were involved in the speciation of the pair (Svensson, 2012), ii) Ecological niches may have continued to change (e.g., random drift) post-speciation (Kozak and Wiens, 2006), thus causing the loss of the original signal, iii) Post-speciation range shifts may mask the original ecological signal (Losos and Glor, 2003), iv) Resolution of the environmental variables is too coarse (Pearson and Dawson, 2003; Anderson, 2013), and v) the environmental variables selected do not reflect the key ecological mechanism (Blair et al., 2013). Finding cases of significant signal are therefore interesting and in need of explanation. These cases are important because they may describe ecological processes that have been retained through time and been critically involved in past speciation events.

Madagascar, the world's fourth largest island (Wollenberg et al., 2011) is a global biodiversity hotspot (Myers et al., 2000). The island has been subject to substantial interest in the evolutionary mechanisms underlying the unique patterns of endemism observed (Wilmé et al., 2006; Pearson and Raxworthy, 2009; Brown et al., 2014). The majority of the literature supports an allopatric mode of speciation, either due to fragmentation of tropical forests (Raxworthy and Nussbaum, 1995), river barriers (Pastorini et al., 2003) or watersheds (Wilmé et al., 2006). The hypothesis of watersheds defining centres of endemism predicts that quaternary fluctuations in climate lead to allopatric isolation between lowland retreat-dispersion watersheds (Wilmé et al., 2006). This scenario fails to explain other patterns of endemism such as in mountains (Pearson and Raxworthy, 2009). A contrasting alternative, the climate gradient hypothesis outlines a parapatric mechanism of speciation that might explain patterns of endemism at higher elevations (Pearson and Raxworthy, 2009). Tests comparing the suitability of the watershed hypothesis and the parapatric current climatic gradient hypothesis, suggest that a single mechanism may not explain speciation, with several genera either showing a combination of mechanisms or neither (Pearson and Raxworthy, 2009).

Topographic complexity in mountainous regions has recently emerged as an alternative potential explanation for lineage diversification (Xu et al., 2010; Bentley et al., 2014; Verboom et al., 2015; Hu et al., 2016). Mountain uplifts generate topographic complexity within a small geographical region which may result in barriers to gene flow, ecological stratification and environmental heterogeneity (Xu et al., 2010; Gillespie and Roderick, 2014; Hu et al., 2016). This small scale geographic isolation, referred to as 'topography-driven isolation', may confine species to particular zones without the presence of more noticeable geographical barriers (e.g., rivers) (Steinbauer et al., 2016). As a result, elevation has been associated with higher species richness, such as plants in the Andes (Bentley et al., 2014), and niche conservation, as illustrated for amphibians on the Qinghai-Tibetan Plateau (QTP) in China (Hu et al., 2016).

The altitudinal gradients found in Madagascar have not been directly assessed against speciation mechanisms in the island. Higher species richness is found at regions of higher elevation in Madagascar (Wollenberg et al., 2008). I hypothesize that regions of high elevations may be more topographically complex. I predict that regions with higher topography complexity provide isolated patches between distributions thus promoting the occurrence of allopatric speciation and the presence of niche conservatism among sister pairs of squamates in Madagascar. I expect pairs with higher tendency for niche conservatism to be found in regions of higher topographic complexity due to the potential role of 'soft allopatry' and niche conservatism in driving speciation in these areas.

To date, there has been no assessment of the drivers of niche conservation or divergence in closely related pairs which would further elucidate the speciation patterns more commonly found in the island. The Malagasy squamates are suitable for testing speciation processes because: i) ectothermic organisms are more sensitive to climatic conditions (Hu et al., 2016) therefore being more susceptible to ecologically-mediated processes; ii) they are relatively young due to radiations emerging during the Cenozoic period (Yoder and Nowak, 2006), therefore having less potential for post-speciation range and ecological shifts compared to sister pairs of older radiations (Blair et al., 2013); iii) there are almost resolved phylogenetic trees (Townsend et al., 2009; Pyron et al., 2013), with many sister pairs; iv) there is geo-referenced locality data available for most species; and v) most species are range-restricted.

4.3 Materials and Methods

4.3.1 Species distribution, selection of sister pairs and data on divergence times

Species occurrence data were compiled from open-sourced databases and available literature (see Appendix I for full list of references for each species). Selection of species pairs was based on Pyron et al.'s (2013) phylogeny of Magalasy squamata which included all recognised families and subfamilies and Townsend et al.'s (2009) phylogeny of *Brookesia* that included all named species but 2. Both trees were constructed using mitochondrial and nuclear data (Townsend et al., 2009; Pyron et al., 2013). I collected occurrence records from a total of 73 sister pairs across all Malagasy squamates. All geo-referenced data were transformed to an Oblique Mercator projection for Madagascar following Pearson and Raxworthy (2009) and all the analyses were conducted in R (R Core Team, 2014).

From this data set, I selected the sister taxa which were more range-restricted. The degree to which the sister pair was range-restricted was measured by calculating the relative occupancy area (ROA) of the combined distribution of the sister taxa in relation to the whole island of Madagascar. Sister taxa that occupied more than half of the area of the island were not included because they were considered too widespread in order for the RTR significance test to be applied. In total I had 28 sister pairs, including 14 pairs of chameleons, 6 pairs of geckos, 3 pairs of skinks, 1 snake sister pair, 1 iguana sister pair and 3 other lizard sister pairs. I had a total of 667 unique occurrence records, with a minimum of 2 records per species, and average 12 occurrences records per species, and a maximum of 29 occurences per species, with the exception of *Brookesia superciliaris* which had 78 unique occurrences.

Data on median divergence times for each sister pair was obtained from the website Timetree (http://www.timetree.org) (Hedges et al., 2006), except for the estimated time of divergence for the *Furcifer verrucosus* species complex which was obtained from Florio and Raxworthy (2016). Timetree is a knowledgebase online resource that contains a global time tree of life (TTOL) (Hedges et al., 2015). This is a data-driven synthesis of published time-calibrated phylogenetic trees and mapped timetrees and divergence data based on community consensus (Hedges et al., 2015).

I classified the type of geographic distribution for each sister pair based on the amount of range overlap: allopatric pairs had no range overlap (i.e., range overlap equal to 0), sympatric pairs had complete range overlap (range overlap equal to 1) and parapatric pairs had partial range overlap (i.e., range overlap higher than 0 but less than 1). Species range estimates were based on minimum convex polygons. Range overlap was equal to the area occupied by both species divided by the area of the smaller range species, so that sympatric species had complete overlapping ranges or the range of the smaller species completely nested within the range of the sister species (Anacker and Strauss, 2014). Using this classification I had 13 allopatric pairs, 11 parapatric pairs and 4 sympatric pairs.

4.3.2 Environmental and topographic data

I compiled geo-referenced climatic data from the WorldClim database at a 1 km^2 resolution (Hijmans et al., 2005). From the available 19 bioclimatic variables, I selected 7 which are more biologically relevant to reptiles (Pearson et al., 2014): (1)Variables which may have a direct physiological impact on reptiles such as extremes of temperature (Maximum Temperature of Warmest Month (BIO5) and Minimum Temperature of Coldest Month (BIO11)); (2) Variables which are measures of habitat suitability due to water availability and water stress (Annual Precipitation (BIO12) and Precipitation of Driest Quarter (BIO14); (3) Variables that are indicators of local climatic adaptations in reptiles (Temperature Seasonality (BIO4) and Precipitation Seasonality (BIO15)) and (4) a variable which is associated with maximum reptile activity (Mean Temperature of Wettest Quarter (BIO8)).

Topographic information can also be informative of reptile niche preferences, as they tend to bask on south facing slopes with intermediate steepness (Pearson et al., 2014), so I obtained aspect and slope data at 1 km^2 resolution from the MadaClim website (http://madaclim.org). Additionally, species affinity for water bodies can also be important to their distribution (Raxworthy et al, 2003), therefore I created a layer on the distance to main rivers. I used the MODIS water mask data set (MOD44W, Carroll et al. 2009) for Madagascar, rescaled to 1 km^2 resolution. Euclidean distance to rivers was calculated, in order to create a continuous geo-referenced layer on proximity to rivers.

To test for whether topographic complexity is associated with niche conservatism, I collected data on elevation and terrain roughness. Georeferenced elevation data was obtained from the Shuttle Radar Topography Mission (SRTM) at a 1 km^2 resolution (Farr and Kobrick, 2001). The degree of unevenness of the land surface, or terrain roughness, was measured from the elevation layer by calculating the Terrain Roughness Index (TRI). This index calculates the elevational differences between a cell and its adjacent cells (Riley, 1999). Mean elevation and mean terrain roughness of each species pair was measured by calculating the mean elevation and terrain roughness from all known occurrences of each taxa in a pair and then averaged for each sister taxa (Verboom et al., 2015). Mean values were preferred to median values to include all possible variation occupied by the species.

4.3.3 RTR significance test

The RTR significance test (for Random Translation and Rotation) is unique compared to other available methods in its test of landscape-scale ecological processes involved in speciation (Chapters 2-3, Nunes and Pearson 2016). It evaluates whether the observed distribution of a pair of species or populations is found in a particular set of environmental conditions which are not found anywhere else in the landscape. In this way, if a pair is distributed within a landscape in a particular shape which is associated with the environments in that region, it may be proposed that the pair's current geographic distributions may exhibit an ecological pattern for either PNC or PND (Chapter 2, Nunes and Pearson 2016).

The known distribution of many Malagasy squamates is restricted to a few occurrence records. Some commonly applied tests of PNC/PND rely on ecological niche models (ENMs) which are less suitable for species with few data points (Pearson et al., 2007; Bukontaite et al., 2015). The *MO* metric (for Multidimensional Overlap) and the RTR significance test are also more suitable methods for range-restricted sister pairs with few occurrence records (Chapter 2, Nunes and Pearson 2016) and thus more appropriate to the study of PNC and PND in Malagasy squamates.

I measured niche overlap for sister taxa using the *MO* metric, which is a presence-only approach for multidimensional overlap (Nunes and Pearson, 2016). The axes breadths occupied by each taxon was measured and, using the *MO* metric, the overlap between the breadths of each axis was calculated and averaged across all axes (Appendix D, Nunes and Pearson 2016). The overlap ranges from 0 (no overlap) to 1 (identical niches). I used the RTR significance test (Chapter 2, Nunes and Pearson 2016) to test whether the observed niche overlap occurs more or less often than expected by chance. In this approach the combined distribution of the sister pair was randomly translocated and rotated to another area within a background region. In this chapter, I defined the whole of the island of Madagascar as the background region in order to include all the available ecological conditions found in the island. With each repeat, the *MO* metric was used to measure the niche overlap observed between the simulated distributions. The observed niche overlap was compared to this null library of simulated niche overlap values. In this chapter I repeated this process 1,000 times as this was found to be sufficient to reach stability of the null model output (e.g., no change in p-value to 4 decimal places with increasing number of replicates). I defined a signal for PNC when the observed niche overlap was higher than 95% of the niche overlaps in the null library and PND when it was smaller than 5%. I also tested the statistical signal using a conservative two-tailed test, with significant levels at the 97.5% and 2.5% boundaries to test for robustness of model outputs. I used this conservative approach as it provides more support for taxa that in fact have an observed distribution with distinct ecological signal from its replicates, further reinforcing the potential presence of ecological speciation in these pairs. A drawback of using hard boundaries for distinguishing significant ecological signals is the non-detection of taxa with a statistical signal that is close to significant. In this chapter, I described the use of the relative frequency ranking of the observed niche overlap in relation to the null library as a measure of the tendency of a pair's niche conservatism (i.e., the tendency for niche conservatism). This ranking can be used as an indicator of the strength of the statistical signal for PNC or PND in a sister taxa and important information from these pairs may be obtained which would not be possible if these pairs were not considered under hard statistical boundaries.

While allopatric species are expected to have a signal for PNC and parapatric species a signal for PND, sympatric species are not expected to carry any signal for ecological conservatism or divergence due to spatial speciation processes as they are explained by other evolutionary mechanisms (e.g., sexual selection, Lande 1981). However I highlight the importance of including these pairs in this analysis to demonstrate whether the RTR significance test is capable of discerning between pairs which are expected to have ecological speciation and pairs that do not. I expect the RTR test to not detect a signal of ecological speciation for all sympatric pairs.

4.4 Results

Out of the 13 allopatric pairs, the RTR test revealed a significant signal for PNC for 2 pairs and no PND in the conservative two-tailed test, but 3 cases of PNC and 1 case of PND in the liberal two-tailed test. Out of the 11 parapatric pairs, the RTR test revealed a significant signal for PND for 1 pair and none for PNC in the conservative two-tailed test but found 2 cases of PNC in the liberal two-tailed test. As expected none of the sympatric pairs showed a significant signal for either PNC or PND (Table 4.1).

The RTR test was not sensitive to the relative occupancy area of the sister pair in relation to the whole island of Madagascar. I can therefore infer that the likelihood of picking up a significant signal was not influenced by a statistical artefact of small sized distributions being more susceptible to a significant signal.

I found allopatric pairs to have on average higher tendency for niche conservatism than parapatric pairs but sympatric pairs had on average higher tendency for niche conservatism than either of the other two spatial distributions (0.530 ± 0.367, 0.431 ± 0.383 and 0.629 ± 0.311 respectively), though no differences were statistically significant (Figure 4.2.). Pairs with earlier time since speciation did not have more conserved niches than pairs with older divergence times (Table 4.2; Figure 4.3b.). Elevation was correlated with topographic complexity suggesting that regions of higher elevations are also more topographic complex (Figure 4.3a.). Both topographic features were correlated with tendency for niche conservatism (Table 4.2.), with pairs with high niche conservatism being found in areas with higher elevation and terrain roughness (Figure 4.3c-d.).

Table 4.1 Hypotheses of PNC/PND from the RTR significance test for each sister pair with classification of geographic distribution as allopatric, parapatric or sympatric. Allopatric pairs have no range overlap between their distributions, parapatric pairs have partial overlap between their ranges and sympatric pairs have one range completely within the range of the other. Tendency for niche conservatism was measured as the ranking of the observed niche overlap in relation to the highest simulated value of niche overlap within its null library. I tested significance at a liberal two-tailed distribution of 10% (significance levels set at the lower and upper 5% boundaries) and a conservative 5% two-tailed distribution (significance at the lower and upper 2.5% boundaries). Significance at the upper boundaries (i.e., higher than 0.95 or 0.975) were considered Phylogenetic Niche Conservatism and at the lower boundaries (i.e. lower than 0.05 or 0.025) were considered Phylogenetic Niche Divergence. * for significant p-value at liberal significant levels and ** at conservative significant levels. NSS for non-significant statistical signal, PNC for significant statistical signal for Phylogenetic Niche Conservatism and PND for significant statistical signal for Phylogenetic Niche Divergence.

Sister Pair	Type of Distribution	Tendency for Niche Conservatism	p-value	Signal for Ecological Speciation (10% significance level)	Signal for Ecological Speciation (5% significance level)	Mean Elevation (in metres a.s.l)	Mean terrain roughness	Time Since Divergence (Mya)
Brookesia nasus-Brookesia lolontany	Allopatric	0.80	0.20	NSS	NSS	1516.22	105.09	46.1
Brookesia ambreensis-								

Brookesia antakarana	Parapatric	0.84	0.16	NSS	NSS	969.17	58.48	0.9
Brookesia griveaudi- *Brookesia valerieae*	Allopatric	0.97	0.03*	PNC	NSS	581.17	115.90	12.4
Brookesia betschi- *Brookesia lineata*	Parapatric	0.54	0.46	NSS	NSS	1200.92	162.85	13.5
Brookesia thieli- *Brookesia vadoni*	Parapatric	0.966	0.034*	PNC	NSS	954.19	73.92	15.8
Brookesia superciliaris- *Brookesia therezieni*	Sympatric	0.18	0.18	NSS	NSS	816.21	42.85	19
Brookesia minima *Brookesia tuberculata*	Sympatric	0.755	0.245	NSS	NSS	794.79	73.30	32.9
Brookesia dentata- *Brookesia exarmata*	Allopatric	0.327	0.327	NSS	NSS	296.26	18.98	27.6
Brookesia karchei- *Brookesia peyrierasi*	Allopatric	0.416	0.416	NSS	NSS	501.93	118.76	26.7
Uroplatus alluaudi- *Uroplatus pietschmanni*	Allopatric	0.913	0.087	NSS	NSS	902.12	47.98	41.3
Furcifer antimena- *Furcifer labordi*	Parapatric	0.002	0.002**	PND	PND	72.65	5.57	5.6
Furcifer petteri-								

Furcifer willsii	Parapatric	0.966	0.034*	PNC	NSS	671.95	40.57	26.1
Furcifer verrucosus A- *Furcifer verrucosus B*	Parapatric	0.071	0.071	NSS	NSS	128.20	15.61	1.9
Phelsuma berghofi- *Phelsuma malamakibo*	Allopatric	0.54	0.46	NSS	NSS	646.73	76.53	12.1
Lygodactylus mirabilis- *Lygodactylus pictus*	Allopatric	0.527	0.473	NSS	NSS	1691.94	68.32	25.7
Lygodactylus arnoulti- *Lygodactylus pauliani*	Sympatric	0.686	0.314	NSS	NSS	1694.57	50.55	23.7
Oplurus fierinensis- Oplurus grandidieri	Parapatric	0.713	0.287	NSS	NSS	440.39	23.98	6
Pygomeles braconnieri- *Pygomeles petteri*	Allopatric	0.159	0.159	NSS	NSS	106.06	17.99	15.8
Tracheloptychus madagascariensis- *Tracheloptychus petersi*	Allopatric	0.033	0.033*	PND	NSS	88.13	8.87	11.8
Zonosaurus quadrilineatus- *Zonosaurus trilineatus*	Allopatric	0.061	0.61	NSS	NSS	53.94	7.57	2.5
Paroedura karstophila- *Paroedura oviceps*	Parapatric	0.164	0.164	NSS	NSS	282.12	38.78	28

Paroedura androyensis- *Paroedura picta*	Parapatric	0.082	0.082	NSS	NSS	139.19	23.70	43.8
Compsophis albiventris- *Compsophis boulengeri*	Allopatric	0.986	0.014**	PNC	PNC	1049.12	83.88	10.8
Amphiglossus mandokava- *Amphiglossus tanysoma*	Parapatric	0.082	0.082	NSS	NSS	546.58	56.99	7
Calumma brevicorne- *Calumma tsaratananense*	Sympatric	0.894	0.106	NSS	NSS	1339.15	99.84	10.2
Calumma guibei- *Calumma hilleniusi*	Allopatric	0.99	0.01**	PNC	PNC	1547.12	85.62	14
Voeltzkowia lineata- *Voeltzkowia rubrocaudata*	Allopatric	0.172	0.172	NSS	NSS	198.99	12.24	7.4
Trachylepis aureopunctata- *Trachylepis dumasi*	Parapatric	0.359	0.359	NSS	NSS	500.94	37.22	21.5

Table 4.2 Linear regressions between degree of niche conservatism and geographic and topographic variables.

Degree of Niche Conservatism $*Variable$	p-value	Significance Level
Relative Occupancy Area	0.5866	n.s
Mean Elevation	8.734x10^{-4}	<0.001
Mean Terrain Roughness	6.975x10^{-3}	<0.01
Time Since Divergence	0.4001	n.s

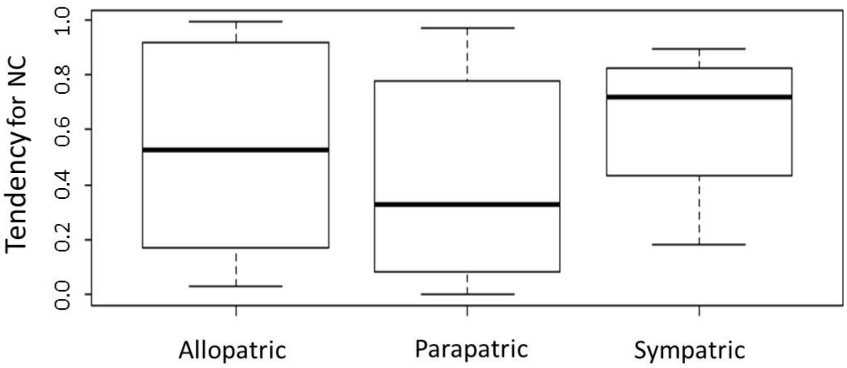

Figure 4.2 Boxplot showing the mean, variance and upper boundaries on tendency for niche conservatism (NC) between allopatric, parapatric and sympatric pairs. Distributions were not significantly different.

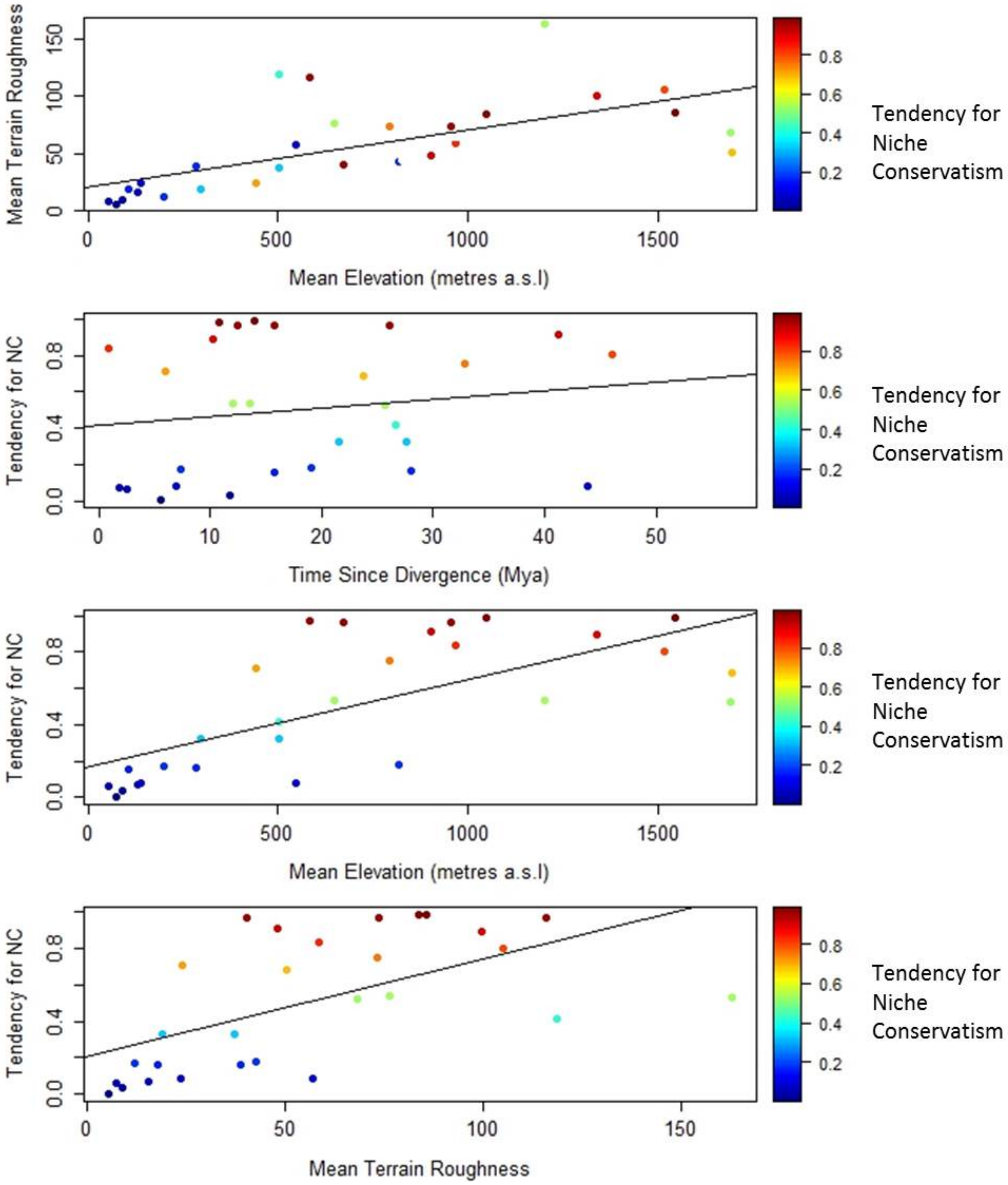

Figure 4.3 Correlations between elevation with terrain roughness (a), between tendency for niche conservatism (NC) and time since divergence (b), elevation (c) and terrain roughness (d). No significant relationship was found between time since divergence and niche conservatism (R^2= 0.0274, p-value=0.4001). Correlations were positively significant between elevation and terrain roughness, between elevation and tendency for niche conservatism and between terrain roughness and tendency for niche conservatism (R^2=0.4668; 0.3489 and 0.484, p-value=1.307x10^{-9}; 8.734x10^{-4} and 6.975x10^{-3} respectively). Colours refer to tendency for niche conservatism, from low (blue) to high (red).

4.5 Discussion

4.5.1 Phylogenetic niche conservatism and divergence among squamates in Madagascar

Phylogenetic niche conservatism has been found to have different strengths in different clades (Kubota et al., 2016). I found an overwhelming absent pattern for both PNC and PND among the sampled taxa. In the few sister taxa where significant signals were detected, PNC did occur twice as often as PND. These results support the notion that most species do not diverge due to ecological innovation (Peterson, 2011). In a conservative test, I found cases of PNC to only occur in two sister taxa with allopatric distributions and the only case of PND was found in a sister pair with a current parapatric distribution. These findings support the association of PNC with allopatric speciation (Wiens, 2004) and PND with parapatric speciation (Florio et al., 2012). However, I found cases of PNC and PND in both allopatric and parapatric species when the statistical significance thresholds were more liberal.

In the liberal test, 3 allopatric pairs were found to have a signal for PNC (*B. griveaudi - B. valerieae*; *C. albiventris - C. boulengeri* and *C. guibei - C.hilleniusi*). The best documented case among these pairs is *B. griveaudi - B.valerieae* where the ancestral distribution of *B.griveaudi - B. valerieae* became disjunct due to aridification (Townsend et al., 2009). Thus this sister taxa could be a case of 'soft allopatry', where climatic conditions form a barrier to dispersal between two populations (Pyron and Burbrink, 2010). Given the pair's tendency for niche conservation, the populations likely were unable to adapt to the novel climatic conditions and maintain connectivity between the two ranges (Wiens, 2004). Parapatric pairs are more often associated with PND (Florio et al., 2012). From my 11 parapatric pairs, only the sister taxa *F.antimena - F.labordi* was consistent with this hypothesis in both types of two-tailed test. Niche divergence in this pair is due to *F.labordi* having a wider distribution than *F.antimena*, showing a higher adaptability of this species to different environments.

Pairs with no significant signal may suggest: i) other environmental variables could have been more important in their speciation (Blair et al., 2013), ii) ecological speciation may have taken place at a finer spatial scale, iii) speciation is not related to broad environmental conditions or geographic barriers (e.g., sexual selection, Lande 1981). I thus recognise the limitations of my analysis in using global geo-referenced layers which may fail to account for differentiation of the species niches at a small scale (Fišer et al., 2015; Hu et al., 2016).

I highlight the high tendency for not capturing a significant signal when using the RTR significance test (Chapter 3). The RTR significance test, contrary to other null models such as the background similarity test of Warren et al. (2008), maintains the spatial configuration of the occurrence data when randomising the null replicates. This approach thus maintains the spatial autocorrelation of the climatic conditions captured in the observed distributions in the null replicates. Null replicates will therefore have similar climatic signals to the observed data, making statistically significant observations less common than if null replicates were based on randomly sampled points (Peterson et al., 2009).

4.5.2 Topographic complexity as a promoter of niche conservatism

I found no significant trends for allopatric sister taxa to have higher tendency for niche conservatism than parapatric or sympatric sister taxa. I also did not find a significant effect of time since divergence on the signal of niche conservatism. I did find taxa with higher tendency for niche conservatism to be found in regions of higher topographic complexity. I thus propose ‘topography-driven isolation’ (Steinbauer et al., 2016) as a likely driver of niche conservatism for the squamates. Squamates in Madagascar are poor dispersers thus having a micro endemic phenotype (Wollenberg et al., 2011). They are also ectothermic, therefore having high sensitivity to changes in climatic conditions and a strong inability to adapt to these climatic fluctuations (Hu et al., 2016). Low dispersal ability and niche conservatism could result in populations being isolated by local scale topographic changes, such as elevational uplifts and terrain roughness. The mountainous regions of Madagascar harbour high species richness and endemism for many groups such as palm tree species (Rakotoarinivo et al., 2013), reptiles and amphibians (Brown et al.,

2016) due to high speciation rates (Wollenberg et al., 2008). Thus high topographic complexity could explain the higher speciation rates and endemism in these region of the island.

In this chapter, I found PNC to be more commonly found in regions of higher elevation and uneven terrain, that is higher topographic complexity. Other studies have also demonstrated PNC to be more common than PND in regions of higher topographic complexity (Hu et al., 2016). This pattern may be explained by small scale barriers to dispersal, leading to locally adapted gene pools (Xu et al., 2010). Mountainous regions are also characterised by patchy habitats and steep climatic gradients, therefore being potential sources for local-scale climatic refugia (Dobrowski, 2011). Microrefugia are small, isolated regions that harbour local climatic conditions which are decoupled from regional climatic averages and therefore may be favourable microhabitats for populations or species during periods of fluctuating climatic conditions (e.g., the Quaternary period) (Dobrowski, 2011). Regions of higher topographic complexity may have higher prevalence of sites of microrefugia while lowland regions may have lesser potential for microrefugia (Valencia et al., 2016). During periods of fluctuating climatic conditions, taxa found in mountainous regions may have the opportunity to conserve their ancestral climatic niches through time due to the availability of sites of microrefugia, therefore retaining a signal for PNC. Conversely, taxa found in lowland regions need to be capable of diverging from their ancestral niches due to lack of sites microrefugia, therefore having a signal for PND.

4.5.3 Caveats and future work

In the liberal two-tailed test, I found cases of PNC in parapatric sister taxa and of PND in allopatric taxa, which are contradictory to the expectation of PNC in allopatric speciation and PND in parapatric speciation. One contingency when using current geographic distributions is that geographical ranges may not have remained stable through time but still maintain the signal for PNC or PND (Losos and Glor, 2003). This could result in sister taxa having a signal for PNC or PND which is opposite to the expectation based on their current spatial distributions. For example, the two cases of parapatric sister taxa with a signal for

PNC (*B.thieli - B. vadoni; F. petteri - F. willsii*) could be explained if the current overlap in the ranges is due to secondary contact post-speciation (Coyne and Orr, 2004). *B.thieli - B.vadoni* is an example of a cryptic sister taxa, a common feature among reptiles and amphibians (Rissler and Apodaca, 2007; Florio et al., 2012; Wielstra et al., 2012). Cryptic species are species which are morphologically similar to their close relatives which may blur significant genetic divergence (Rissler and Apodaca, 2007; Wielstra et al., 2012). I found that the niches between this cryptic sister taxa are more similar than expected but it is debatable whether cryptic species are ecological 'clones' (Fišer et al., 2015). I propose that further work looking at niche differences at a small scale may explain their genetic divergence and speciation mechanism (Fišer et al. 2015, see Chapter 5). Small scale niche shifts may include morphological variation related to differences in resource use (Johnson et al., 2006; Goodman et al., 2008). Likewise, the allopatric sister pair which was found to have a PND signal (*Trachylepis aueropunctata - Trachylepis dumasi*) could be a result of a loss of a contact zone due to geographic or climatic disruptions post-speciation. Identifying sister taxa with discrepancies in these two components of speciation can be useful for selecting candidate taxa for assessments of whether speciation took place with or without gene flow (parapatric or allopatric respectively) using genomic techniques (Martin et al., 2013; Seehausen et al., 2014).

It is possible that species pairs which are more sensitive to statistical thresholds may be cases whose distributions may have been less stable through time (e.g., due to range shifts caused by paleoclimatic fluctuations, see Losos and Glor 2003 and references therein). Thus, I recognise that potential shifts in the distributions post-speciation could mask true speciation processes (Losos and Glor, 2003). However, due to the very low number of statistically significant cases found in this study, the reason for the loss of statistical signal is not fully understood. Further research with an increased sample size may further elucidate on the factors affecting the statistical stability of these outputs.

Chapter 5

Character displacement is associated with topographic complexity among allopatric sister taxa of squamates in Madagascar

This work was conducted in collaboration with Richard G. Pearson and Mark Wilkinson.

Laura G. A. Nunes conceived the ideas, designed the research, collected and analysed the data and led the writing, with critical input from Richard G. Pearson and Mark Wilkinson.

5.1 Abstract

Character displacement, whereby closely-related species diverge in their morphology to alleviate competition and enhance reproductive isolation, plays an important role in species divergence and the maintenance of species boundaries in contact zones. Here I test for multiple hypotheses on potential drivers of character displacement among the squamates in Madagascar and test for associations between character displacement and patterns of niche conservatism on the island. Body size is an adaptive morphological trait and therefore body size differences between species is a suitable proxy for measuring character displacement. I collected data on body size and time since divergence for 61 sister pairs of squamates in Madagascar and data on tendency for niche conservatism for 28 out of the 61 sister pairs. To test for a role of phylogenetic relatedness in body size differences, I tested whether body size asymmetries between sister pairs were statistically more or less pronounced when compared with body size asymmetries between non-sister pairs. I tested whether pairs with allopatric distributions have smaller and less variable body size asymmetries than pairs with overlapping ranges (parapatric and sympatric pairs). I then tested for associations between body size asymmetries and i) time since divergence, ii) strength of competition, iii) similarity in climatic niches, iv) differences in topographic complexity and v) tendency for niche conservatism between sister taxa. For each sister taxa, I quantified the amount of range overlap, as a measure of strength of competition, and amount of climatic niche overlap, as a measure of climatic similarity. I measured the amount of topographic complexity that a sister pair is found to account for degree of microhabitat diversity, which may impact morphological variation between species, and also because it has been previously associated with niche conservatism in Madagascar. I found no significant tendency for sister pairs to have lower or higher character displacement than non-sister pairs. Character displacement in allopatric pairs was not significantly smaller but was significantly less variable than in pairs with overlapping ranges. The only factors to be associated with character displacement were found to be competition and topographic complexity. Higher topographic complexity was strongly associated with higher character displacement in allopatric pairs but not in parapatric or

sympatric sister pairs. Character displacement in allopatric pairs may be influenced by topographic complexity due to local adaptation in small, isolated regions. Given that niche conservatism is also more commonly found in regions of higher topographic complexity, I propose that while conservatism of ecological niches in these regions may reflect evolutionary conservatism of beta (β) niches at broader macro scales, character displacement may account for evolutionary lability in the alpha (α) niche due to micro-scale adaptive pressures.

5.2 Introduction

Character displacement may play a significant role during the speciation process, by either initiating divergence or maintaining species boundaries due to decreased potential for hybridization between divergent species (Pfennig and Pfennig, 2009). There has been a long standing view that closely-related species are more morphologically similar than distantly related species because closely-related species will exploit similar resources (Harvey and Pagel, 1991; Blomberg et al., 2003; Cooper et al., 2010; Naisbit et al., 2011). This is more pronounced at coarse phylogenetic scales such as across genera. At fine phylogenetic scales, such as between sister taxa, the trend of morphological conservatism is less well defined. While some cases find closely-related species to have more conserved morphological traits than distantly related species, other studies found no support for this trend (Luxbacher and Knouft, 2009). As a result, there has been growing interest in understanding the associations between character displacement and different speciation mechanisms.

Speciation could be either due to geographical isolation and/or ecological divergence. If we assume that current species distributions retain an indication of the initial geographical patterns at the time of speciation (Losos and Glor, 2003; Pearson and Raxworthy, 2009), species with non-overlapping ranges are assumed to have diverged through geographic separation (vicariance or allopatric speciation) while species with partial or complete range overlap are thought to have diverged due to environmental or behavioural differences (parapatric and sympatric speciation respectively) (Coyne and Orr, 2004; Fitzpatrick et al., 2009; Pyron and Burbrink, 2010). While phylogenetic niche conservatism (PNC), whereby closely-related species share similar ecological niches, is commonly associated with allopatric speciation (Wiens, 2004), phylogenetic niche divergence (PND), in which closely related species have less similar niches due to ecological divergence, is consistent with parapatric speciation (Florio et al., 2012).

The potential role of niche conservatism in promoting allopatric speciation suggests that allopatric species may have lower character displacement than parapatric species (Kozak et al., 2006). The conservatism of phenotypic traits among allopatric sister pairs could be due to the species being exposed to similar climatic conditions and the lack of direct competition between the species as they diverge in isolation. In contrast, higher levels of character displacement would be found in species with parapatric or sympatric ranges to alleviate the pressures of direct competition (Brown and Wilson, 1956; Peers et al., 2013; Beans, 2014) or due to adaptations to diverging ecological conditions (Gvoždík et al., 2008). The amount of character displacement could also be related to the frequency of co-occurrence; thus, parapatric species with more overlap in their range are expected to have higher phenotypic differences (Pfennig and Pfennig, 2009). In this scenario, levels of character displacement are expected to be greater in species with overlapping ranges than with allopatric ranges (Adams et al., 2007). Competition between species would result in increasing dissimilarity in morphological traits through time, therefore older lineages are expected to have higher character displacement than more recent lineages (Pfennig and Pfennig, 2009). Regions of high topographic complexity result in high climatic heterogeneity and barriers to dispersal (Verboom et al., 2015; Steinbauer et al., 2016; Berardi et al., 2016). These regions are important in promoting allopatric speciation (Britton et al., 2014) and niche conservatism (Chapter 4). Sister taxa found in regions of higher topographic complexity may have less character displacement because allopatric speciation will involve little ecological divergence and no direct competition between the species (Table 5.1.).

There has been increasing interest in the drivers of speciation among taxa of the island of Madagascar (Wilmé et al., 2006; Pearson and Raxworthy, 2009; Brown et al., 2014). Despite hypotheses of allopatric or parapatric speciation being applicable to some species (e.g., allopatric speciation for lemurs, Wilmé et al. 2006), there is still a lot of uncertainty

Table 5.1 Hypotheses regarding character displacement among sister taxa due to phylogenetic relatedness, time since divergence, competition or climate-induced phenotypic variation, topographic complexity and phylogenetic niche conservatism.

Factor driving character displacement	Hypothesis
Phylogenetic relatedness	Sister taxa have lower character displacement than non-sister taxa because species with shared ancestry will have similar functional traits as they tend to exploit similar resources.
Time since divergence	Older lineages have higher character displacement than recent lineages because closely-related species become morphologically more dissimilar through time.
Competition-induced phenotypic variation	Sister taxa with higher incidence of co-occurrence (i.e., higher range overlap) will have higher character displacement in order to alleviate competition for resources and maintain species boundaries.
Climate-induced phenotypic variation	Sister taxa with greater differences in occupied ecological niches will have larger character displacement because morphological traits are adapted to particular ecological conditions.
Topographic complexity	Sister taxa in regions of higher topographic complexity have lower character displacement because of incidences of allopatric speciation and niche conservatism.
Phylogenetic Niche Conservatism	Sister taxa with higher tendencies for phylogenetic niche conservatism will have lower character displacement because of lower plasticity in adapting to different niches.

regarding other taxa (Pearson and Raxworthy, 2009). One group which is still subject to much speculation over their main mode of speciation is the squamates, which include chameleons, geckos and snakes. There is also no robust trend of niche conservatism or divergence in explaining speciation patterns in these taxa (Chapter 4). Tests of morphological divergence may shed additional light on the mechanisms responsible for driving speciation and maintaining species boundaries in these taxa which are not explained by ecological conservatism or divergence. Given that character displacement may be affected by different factors, it is important to account for several explanatory variables when performing assessments of character displacement in order to reject any alternative hypotheses that may have also influenced character displacement (Beans, 2014).

Body size has an impact on an organism's ecology, life history traits and sensitivity to changes in climate (Cooper and Purvis, 2010; Naisbit et al., 2011; Rainford et al., 2016) and is therefore a useful trait in testing for both competition-induced and climate-induced phenotypic variation. Body size is an adaptive morphological trait in squamates (Sistrom et al., 2012) and is often a proxy for a species resource use (Adams et al., 2007; Camargo et al., 2010). Body size is phylogenetically conserved in some squamates but not others (Luxbacher and Knouft, 2009). Species pairs with overlapping ranges (parapatric or sympatric) are expected to have higher and more variable amounts of competition, thus the higher and more variable differences in body size between allopatric pairs and pairs with overlapping ranges are also expected (Pfennig and Pfennig, 2009; Sistrom et al., 2012). I expect higher body size asymmetry to be explained by less overlap in climatic variables experienced by the sister pair due to climate-induced phenotypic variation (Gvoždík et al., 2008).

While PNC was found to be more common in regions of higher topographic complexity (Chapter 4), there has not been an analysis of whether body size between sister taxa in these regions is also conserved. In this chapter I also test for the hypothesis of decreased body size differences with higher topographic complexity. I expect sister pairs in regions of higher topographic complexity to have lower differences in body size due to higher incidence of allopatric speciation and niche conservatism. These comparisons will elucidate on the drivers of character displacement and consequent reproductive isolation which gave rise to the sister pairs.

5.3 Materials and Methods

Species occurrence data were compiled from online databases and available literature (see Appendix D for full list of references for each species). I selected a total of 73 Malagasy sister pairs based on Pyron et al.'s (2013) phylogeny of squamates and Townsend et al.'s (2009) phylogeny of *Brookesia*. These phylogenies were constructed from both mitochondrial and nuclear data and include all currently recognised families and subfamilies (Townsend et al., 2009; Pyron et al., 2013) From these, I selected the sister taxa which had more than one occurrence record per species. I obtained a total of 2950 unique occurrence records, with a minimum of 2 occurrence records per species, a maximum of 160 records per species and an average of 24 records per species. In total I had 61 sister pairs, including 19 pairs of chameleons and 19 pairs of geckos, 8 pairs of snakes, 7 pairs of skinks, 2 pairs of iguanas and 6 others pairs of lizards.

To measure differences in body size between closely-related species, I obtained data on maximum known male snout-to-vent length (SVL) from Meiri (2008), Rocha et al. (2010) and Brown et al. (2016). SVL is often used as a proxy for body size in squamates (Losos, 2000; Brown et al., 2016) and is a readily available variable which is commonly used in biogeographical and macroecological studies (Brown et al., 2016) and is the most commonly reported size index for squamates (Meiri, 2008). Body size asymmetry within a sister pair was calculated proportionally as the SVL of the larger sister divided by that of the smaller sister in the pair, so that higher values were indicative of taxa with more dissimilar body sizes. All geo-referenced data were transformed to an Oblique Mercator projection for Madagascar following Pearson and Raxworthy (2009) and all the analyses were conducted in R (R Core Team, 2014)

5.3.1 Phylogenetic relatedness

I tested whether body size asymmetry was phylogenetically conserved using clades with higher species richness. I thus tested for phylogenetic conservatism among *Amphiglossus* (4 sister pairs from 22 species), *Brookesia* (9 sister pairs from 32 species), *Calumma* (5 sister pairs from 38 species), *Furcifer* (5 sister pairs from 19 species), *Lygodactylus* (4 sister pairs from 21 species), *Phelsuma* (4 sister pairs from 30 species), *Uroplatus* (5 sister pairs from 13 species) and *Zonosaurus* (4 sister pairs in 17 species). I calculated the body size differences between all non-sister pairs within each genus. In this way, non-sister pair comparisons were phylogenetically controlled so that there were no comparisons of body size across genus which might result in unrealistic body size asymmetries. Each sister pair comparison was compared against this distribution of non-sister pair comparisons and the body size asymmetry of a sister taxa was considered phylogenetically conserved or divergent if it fell within the lower or upper 5% boundary respectively. I used Bonferroni correction (0.1 significance level divided by 40 which is the number of sister pairs across all genera) to reduce chances of falsely rejecting the null hypothesis (Type I error).

5.3.2 Time since divergence

I obtained data on median divergence times for each sister pair from the website Timetree (http://www.timetree.org) (Hedges et al., 2006), except for the estimated time of divergence for the *Furcifer verrucosus* species complex which was obtained from Florio and Raxworthy (2016).Timetree is an online database on dated phylogenetic trees from either published literature or based on community consensus (Hedges et al., 2015). I tested for a linear association between time since divergence and character displacement.

5.3.3 Competition-induced phenotypic variation

To test for differences in body size due to competition, I measured the range overlap between species in a pair, with higher range overlap being indicative of higher competition, with the underlying assumption that as species direct competition would increase with increasing co-occurrence between species due to increasing range overlap. The range size of each species was measured using minimum convex hulls around the occurrence data (Verboom et al., 2015). Range overlap was equal to the area occupied by both species divided by the area of the smaller range species, so that sympatric species had complete overlapping ranges or the range of the smaller species completely nested within the range of the sister species (Anacker and Strauss, 2014; Verboom et al., 2015). I classified the types of distributions of sister taxa based on amount of range overlap: allopatric pairs had no range overlap, (i.e., range overlap equal to 0), sympatric pairs had complete range overlap (range overlap equal to 1) and parapatric pairs had partial range overlap (i.e., range overlap higher than 0 but less than 1). Using this classification I had 16 allopatric pairs, 40 parapatric pairs and 5 sympatric pairs. I tested whether the mean body size asymmetry of allopatric pairs was smaller than between pairs with overlapping ranges (parapatric and sympatric pairs). I assessed if the variability in body size asymmetry (the variance around the mean) was significantly higher in pairs with overlapping distributions than allopatric pairs. I also analysed for competition-induced phenotypic variation by testing for a linear correlation between the amount of range overlap and amount of character displacement.

5.3.4 Climate-induced phenotypic variation

To test for associations between body size differences and climatic differences between species within a pair, I compared the amount of niche similarity between sister pairs to their corresponding amount of body size asymmetry. I compiled geo-referenced climatic data from the WorldClim database at 1 km^2 resolution (Hijmans et al., 2005). I selected three bioclimatic variables which have direct physiological impact to squamates (Maximum Temperature of Warmest Month (BIO5), Mean Temperature of Wettest Quarter (BIO8) and Minimum Temperature of Coldest Month (BIO11)), two variables which are associated with local climatic adaptations in squamates (Temperature Seasonality (BIO4) and Precipitation Seasonality (BIO15)) and two variables that are measures of habitat suitability due to water availability and water stress (Annual Precipitation (BIO12) and Precipitation of Driest Quarter (BIO14) respectively).

I measured the ecological similarity between sister pairs using the *MO* (for Multidimensional Overlap) metric for niche overlap (Nunes and Pearson, 2016). The *MO* metric is a presence-only approach for multidimensional overlap. This statistic measures the overlap between the values within the environmental breadth for each environmental axis separately for the two species within a sister pair and then averages the overlap across across all axes (Nunes and Pearson, 2016). The metric ranges from 0 (no overlap) to 1 (identical niches) (for further details, see Appendix D). I tested for linear relationships between the overlap of each climatic axis and body size asymmetry and between overall niche overlap and body size asymmetry.

5.3.5 Topographic complexity

To test for differences in body size due to topographic complexity, I collected data on elevation and terrain roughness. Geo-referenced elevation data was obtained from the Shuttle Radar Topography Mission (SRTM) at a 1 km^2 resolution (Farr and Kobrick, 2001). Topographic complexity was calculated from the degree of unevenness of the land surface, or terrain roughness, from the elevation layer by calculating the Terrain Roughness Index (TRI). This index describes the elevational differences between a cell and its adjacent cells (Riley, 1999). The mean topographic complexity of a sister taxa was measured by calculating the mean terrain roughness of all known occurrences of each species and averaged for each sister taxa (Verboom et al., 2015). I use mean values rather than medians to account for all possible variation captured by the species' occurrences. I tested for a linear relationship between mean topographic complexity and body size asymmetry. I did separate analyses for allopatric pairs and pairs with overlapping ranges because an assessment of character displacement in non-overlapping pairs removes the potential influence of competition in character displacement.

5.3.6 Phylogenetic niche conservatism

The RTR (for Random Translation and Rotation) significance test is novel null model which tests for landscape-based ecological processes in speciation between a pair of populations or species (Chapter 2, Nunes and Pearson 2016). It tests whether the ecological processes captured by the distributions of the pair are unique to those regions and therefore are not expected to occur anywhere else in the landscape. This test is designed to identify cases of PNC, PND and non-significant niche conservatism or divergence between a pair (Chapters 2-4, Nunes and Pearson 2016). Species pairs which are widespread across a landscape are less suitable for the RTR significance test because they are less likely to result in enough unique null replicates. The most range-restricted pairs included in this analysis have been previously tested for the tendency of their niches to be conserved or divergent with the RTR significance test (tendency for niche conservatism, Chapter 4). Here I measured for potential linear relationships between tendency for niche conservatism and body size asymmetry between these range-restricted pairs.

5.4 Results

Body size asymmetry was significantly lower between sisters than non-sister pairs for 4 out of 40 sister pairs (Table 5.3.). One case of body size asymmetry was significantly greater than non-sister pairs (Table 5.4.). However, the body size asymmetries of all sister taxa were not statistically significant from non-sister comparisons when the analysis was corrected with Bonferroni (corrected significance level = 0.0025). Allopatric pairs were found to have on average less and significantly less variable body size asymmetry compared to sister pairs with overlapping ranges (1.166 ± 0.167, 1.296 ± 0.451 respectively) (Figure 5.1.). The significance was lost with the removal of the highly morphologically dissimilar sympatric pair *C. brevicorne* - *C. tsaratananense* (1.238 ± 0.228, p-value= 0.193).

I did not find a significant association between time since divergence and character displacement (Table 5.4, Figure 5.2.). The association between competition and character displacement was close to significance (Table 5.4, p-value= 0.0614), while no climatic variable or overall ecological niche similarity was statistically associated with character displacement (Table 5.4, Figure 5.2). Higher tendency for niche conservatism was also not statistically associated with lower character displacement (Table 5.4.).

There was a positive relationship between topographic complexity and character displacement (Table 5.4, p-value= 0.065). The strength of the statistical signal for range overlap and topographic complexity was lost with the removal of *C. brevicorne* - *C. tsaratananense* (p-value=0.274 and 0.422 respectively). When taking into account pairs with no range overlap (allopatric pairs) separately from pairs with overlapping ranges, allopatric pairs in regions of higher topographic complexity had significantly greater body size differences than allopatric pairs in regions of lower topographic complexity (Figure 5.3a). Pairs with overlapping ranges (parapatric and sympatric distributions) had no significant differences in body size associated with topographic differences (Figure 5.3b).

Table 5.3 Range overlap, niche overlap, body size asymmetry and tendency for phylogenetic conservatism or divergence for each sister pair. A value below 0.05 is considered to be significant for morphological conservatism and above 0.95 is considered to be significant for morphological divergence. Data on the tendency for niche conservatism was obtained from Chapter 4. Pairs with no tests of niche conservatism are n/a and pairs which were not tested for phylogenetic morphological conservatism or divergence are n/a

Sister Pair	Range Overlap	Niche Overlap	SVL asymmetry	Ranking of SVL of sister pairs against non-sister pairs	Tendency for Niche Conservatism	Time since divergence (Mya)
Brookesia nasus- *Brookesia lolontany*	0	0.111	1.531	0.582	0.802	46.1
Brookesia ambreensis- *Brookesia antakarana*	0.474	0.847	1	0.031	0.836	0.9
Brookesia griveaudi- *Brookesia valerieae*	0	0.201	1.038	0.081	0.97	12.4
Brookesia betschi- *Brookesia lineata*	0.147	0.276	1.324	0.431	0.535	13.5
Brookesia thieli- *Brookesia vadoni*	0.640	0.690	1.360	0.453	0.966	15.8
Brookesia superciliaris-						

Brookesia therezieni	1	0.355	1.019	0.046	0.18	19
Brookesia minima						
Brookesia tuberculata	1	0.115	1.056	0.116	0.755	32.9
Brookesia dentata-						
Brookesia exarmata	0	0.055	1.196	0.302	0.327	27.6
Brookesia karchei-						
Brookesia peyrierasi	0	0.069	1.333	0.441	0.416	26.7
Uroplatus fimbriatus-						
Uroplatus giganteus	0.912	0.545	1.119	0.209	n/a	14.1
Uroplatus alluaudi-						
Uroplatus pietschmanni	0	0.403	1.025	0.0533	0.913	41.3
Uroplatus ebenaui-						
Uroplatus phantasticus	0.614	0.553	1	0.031	n/a	51.9
Uroplatus henkeli-						
Uroplatus sikorae	0.626	0.317	1.309	0.418	n/a	28.4
Uroplatus guentheri-						
Uroplatus malahelo	0	0.124	1	0.0306	n/a	56.8
Furcifer antimena-						
Furcifer labordi	0.283	0.135	1.232	0.351	0.192	5.6
Furcifer oustaleti-						

Furcifer verrucosus	0.960	0.885	1.072	0.143	n/a	12.2
Furcifer angeli- *Furcifer pardalis*	0.509	0.287	1.563	0.602	n/a	5.25
Furcifer petteri- *Furcifer willsii*	0.149	0.635	1.184	0.294	0.608	26.1
Furcifer verrucosus A- *Furcifer verrucosus B*	0.125	0.261	1.195	0.307	0.071	1.9
Phelsuma breviceps- *Phelsuma mutabilis*	0.374	0.273	1.042	0.093	n/a	48.8
Phelsuma dubia- *Phelsuma ravenala*	0.503	0.224	1.115	0.202	n/a	10.7
Phelsuma quadriocellata- *Phelsuma antanosy*	0.375	0.198	1.271	0.394	n/a	16.1
Phelsuma berghofi- *Phelsuma malamakibo*	0	0.057	1.052	0.107	0.538	12.1
Lygodactylus verticillatus- *Lygodactylus heterurus*	0	0.085	1.08	0.148	n/a	1.3
Lygodactylus guibei- *Lygodactylus miops*	0.531	0.549	1.152	0.253	n/a	19.6
Lygodactylus mirabilis-						

Lygodactylus pictus	0	0.005	1.310	0.426	0.527	25.7
Lygodactylus arnoulti- *Lygodactylus pauliani*	1	0.031	1.027	0.062	0.686	23.7
Zonosaurus aeneus- *Zonosaurus rufipes*	0	0.460	1.158	0.262	n/a	14.2
Zonosaurus quadrilineatus- *Zonosaurus trilineatus*	0	0.053	1.086	0.157	0.061	2.5
Zonosaurus haraldmeieri- *Zonosaurus madagascariensis*	0.119	0.236	1.102	0.185	n/a	3
Zonosaurus anelanelany- *Zonosaurus laticaudatus*	0.477	0.267	1.570	0.603	n/a	3.7
Amphiglossus melanurus- *Amphiglossus ornaticeps*	0.971	0.663	1.806	0.704	n/a	10.3
Amphiglossus mandokava- *Amphiglossus tanysoma*	0.017	0.119	1.437	0.512	0.082	7
Amphiglossus frontoparietalis- *Amphiglossus punctatus*	0.819	0.758	1.041	0.087	n/a	10
Calumma nasutum- *Calumma boettgeri*	0.594	0.651	1.146	0.247	n/a	10.9
Calumma furcifer-						

Calumma gastrotaenia	0.979	0.549	1.028	0.064	n/a	15.4
Calumma globifer-Calumma parsonii	0.993	0.276	1.735	0.673	n/a	10.9
Calumma brevicorne-Calumma tsaratananense	1	0.299	3.864	0.985	0.894	10.2
Calumma guibei-Calumma hilleniusi	0	0.159	1.273	0.399	0.99	14
Paroedura karstophila-Paroedura oviceps	0.112	0.225	1.255	n/a	0.164	28
Paroedura androyensis-Paroedura picta	0.797	0.533	1.915	n/a	0.082	43.8
Compsophis albiventris-Compsophis boulengeri	0	0.061	1.443	n/a	0.986	10.8
Oplurus fierinensis-Oplurus grandidieri	0.377	0.474	1.122	n/a	0.713	6
Pygomeles braconnieri-Pygomeles petteri	0	0.062	1	n/a	0.159	15.8
Tracheloptychus madagascariensis-Tracheloptychus petersi	0	0.108	1	n/a	0.033	11.8

Voeltzkowia lineata-Voeltzkowia rubrocaudata	0	0.373	1.132	n/a	0.172	7.4
Trachylepis aureopunctata-Trachylepis dumasi	0.284	0.365	1.491	n/a	0.359	21.5
Blaesodactylus antongilensis-Blaesodactylus sakalava	0.016	0.594	1.072	n/a	n/a	15.2
Geckolepis maculata-Geckolepis typica	0.936	0.748	1.111	n/a	n/a	30.4
Hemidactylus mabouia-Hemidactylus mercatorius	0.921	0.838	1.607	n/a	n/a	2.9
Thamnosophis lateralis-Thamnosophis stumpffi	0.974	0.582	1.073	n/a	n/a	7
Thamnosophis epistibes-Thamnosophis martae	0.018	0.099	1.163	n/a	n/a	9.5
Compsophis infralineatus-Compsophis laphystius	0.899	0.619	1.260	n/a	n/a	11.8
Leioheterodon modestus-Leioheterodon madagascariensis	0.999	0.860	1.25	n/a	n/a	11
Oplurus cuvieri-Oplurus cyclurus	0.394	0.613	1.040	n/a	n/a	13.5

Trachylepis elegans- *Trachylepis madagascariensis*	1	0.563	1.169	n/a	n/a	10.4
Liopholidophis dolicocercus- *Liopholidophis sexlineatus*	0.619	0.500	1.185	n/a	n/a	11.5
Dromicodryas bernieri- *Dromicodryas quadrilineatus*	0.384	0.794	1.081	n/a	n/a	9.5
Paroedura bastardi- *Paroedura tanjaka*	0.419	0.276	1.5	n/a	n/a	35.8
Madagascarophis colubrinus- *Madagascarophis meridionalis*	0.918	0.402	1.207	n/a	n/a	8.5

Table 5.4 Linear regressions between body size asymmetry and niche conservatism, topographic complexity and climatic variables among all sister pairs.

Body Size Asymmetry $*Variable$	p-value
Tendency for Niche Conservatism	0.4106
Time Since Divergence	0.6855
Niche Overlap	0.926
Temperature Seasonality (BIO4) Overlap	0.747
Maximum Temperature of Warmest Month (BIO5) Overlap	0.95
Mean Temperature of Wettest Quarter (BIO8) Overlap	0.895
Minimum Temperature of Coldest (BIO11) Overlap	0.528
Annual Precipitation (BIO12) Overlap	0.475
Precipitation of Driest Quarter (BIO14) Overlap	0.722
Precipitation Seasonality (BIO15) Overlap	0.691
Range Overlap	0.0614
Mean Terrain Roughness	0.0656

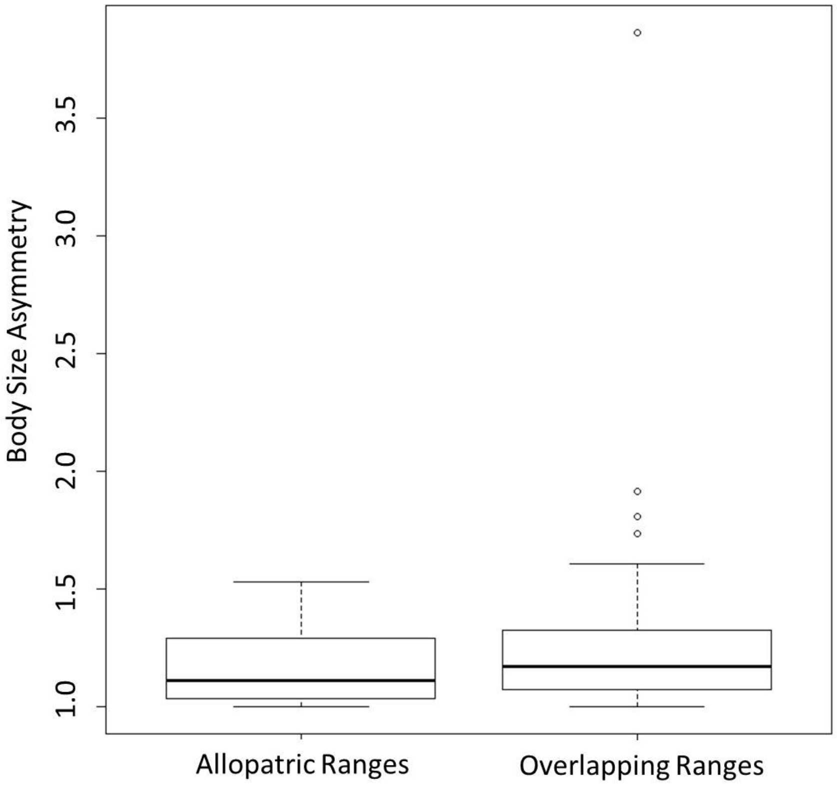

Figure 5.1 Boxplot showing the tendency for body size asymmetry between sister taxa with allopatric and overlapping ranges. Allopatric sister pairs had a range overlap of 0, overlapping ranges had an overlap higher than zero and less or equal to 1. The body size asymmetry in sister pairs with overlapping ranges is significantly more variable than in allopatric sister pairs ($F_{(15,44)}$=0.1368, p-value=1.453x10^{-3}) but there were no significant differences in mean body size asymmetry between allopatric and overlapping sister pairs (1.166 ± 0.167 and 1.296 ± 0.451 respectively)

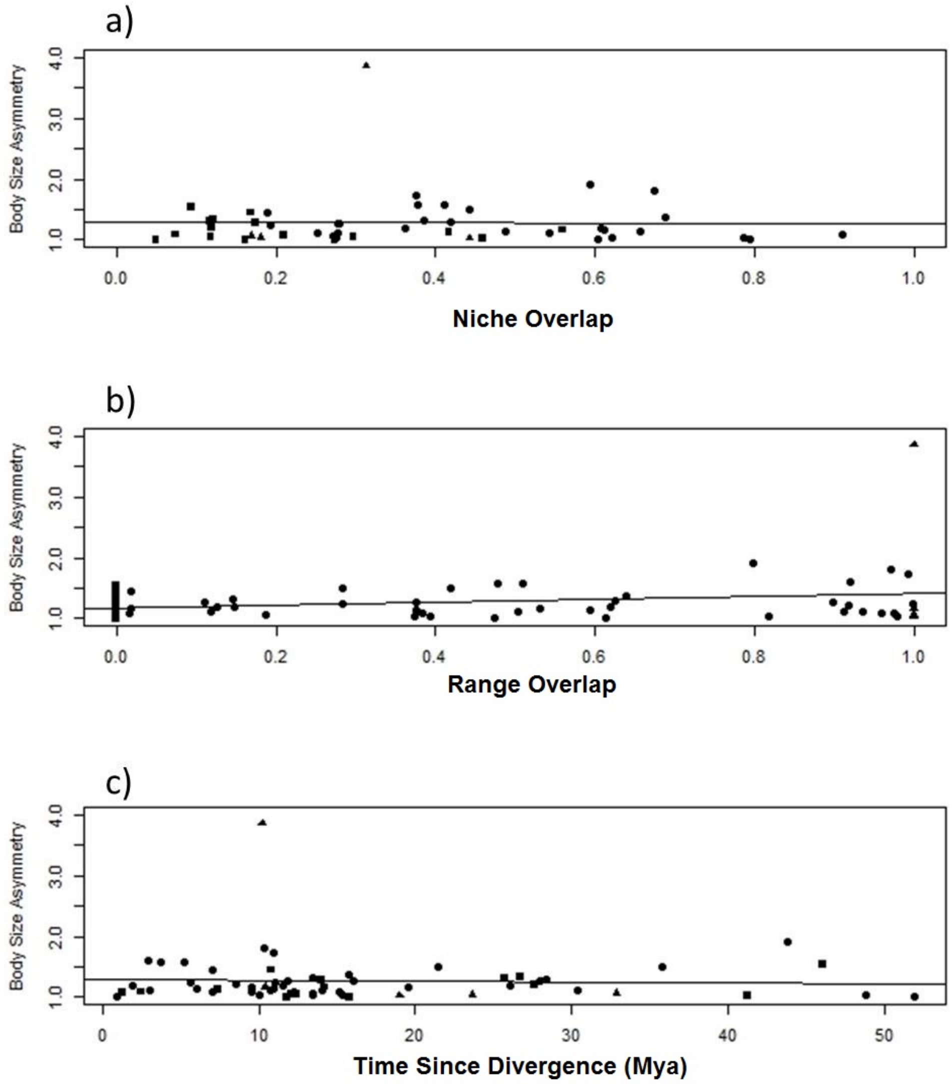

Figure 5.2 Linear relationships between body size asymmetry and niche overlap (a), range overlap (b) and time since divergence (c) between sister pairs. Squares for Allopatric pairs (Range Overlap = 0), circles for Parapatric pairs (0 <Range Overlap <1) and triangles for Sympatric pairs (Range Overlap = 1). Correlation was close to significance between body size asymmetry and range overlap (R^2=0.055, p-value=0.069). Correlations were not significant between body size asymmetry and niche overlap or time since divergence (R^2=1.902x10^{-4} and 2.799x10^{-3}; p-value= 0.926 and 0.686 respectively)

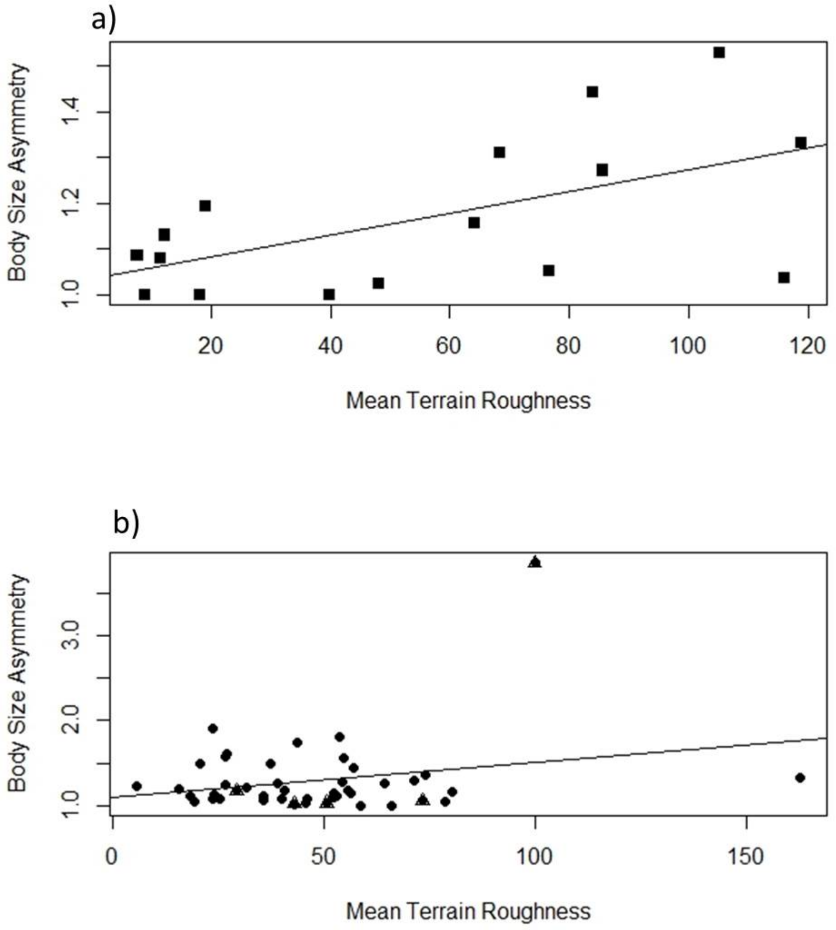

Figure 5.3 Linear relationship between mean topographic complexity and body size differences in allopatric pairs (a) and overlapping (parapatric and sympatric) sister pairs (b). Squares for Allopatric pairs (Range Overlap = 0), circles for Parapatric pairs (0 <Range Overlap <1) and triangles for Sympatric pairs (Range Overlap = 1). Allopatric pairs in regions of higher topographic complexity had significantly higher differences in body size (R^2=0.3271; p-value=0.0206). Parapatric and sympatric species had no significant differences in body size due to topographic differences (R^2=0.0594; p-value=0.1067).

5.5 Discussion

In this chapter I looked at whether phylogeny, climate, competition and topographic complexity were statistically associated with character displacement among closely-related species. Among my sampled taxa, there was not a strong tendency for sister pairs to have smaller body size differences than non-sister pairs. The tendency for closely related species to have more similar body size in relation to distant-related species has been supported in some cases but not others (Luxbacher and Knouft, 2009). Body size is highly variable among lizards (Sears and Angilletta, 2004; Luxbacher and Knouft, 2009), and my findings support the notion that phylogenetic conservatism of body size is not ubiquitous among squamates. I also did not find a tendency for younger sister taxa to have smaller character displacement than older sister taxa. This is counter to the hypothesis that character displacement increases with time (Pfennig and Pfennig, 2009).

I did not find a significant tendency for allopatric pairs to have smaller body size asymmetry in comparison to pairs with overlapping ranges. However I did find co-occurring pairs to have significantly more variable differences in body size than allopatric pairs. This supports the hypothesis that allopatric pairs may have less variation in body size as they have no direct competition, due to geographic isolation (Pfennig and Pfennig, 2009), and less climate-induced phenotypic variation due to phylogenetic niche conservatism (Kozak et al., 2006; Verboom et al., 2015).

In this chapter, I used the amount of range overlap between a sister pair as a measure of direct competition between species and found a weak association between body size differences and range overlap. The highest case of character displacement was found in the sympatric pair *C. brevicorne - C. tsaratanense.* This can be explained by the amount of contact between the two species species which may reflect the amount of direct competition between the species, thus higher competition resulting in higher body size differences. These findings support the hypothesis of higher character displacement in sympatric pairs due to competition-induced selection (Pfennig and Pfennig, 2009).

I found cases of no character displacement in *B. ambreensis - B. antakarana*, 2 *Uroplatus* sister pairs (*U. guentheri - U. malahelo* and *U.ebenaui - U.phantasticus*) and the pairs *P. braconnieri - P. petteri* and *T. madagascariensis - T. petersi.* Among these 5 pairs, *U. guentheri - U. malahelo*, *P. braconnieri - P. petteri* and *T. madagascariensis - T. petersi* have allopatric distributions. This fits with the association between geographic isolation and morphological conservatism (Kozak et al., 2006;

Verboom et al., 2015).

The remaining 2 pairs (*B. ambreensis* - *B. antakarana* and *U.ebenaui* - *U. phantasticus*) have parapatric distributions, thus the lack of character displacement displayed in the pairs contradicts my initial hypothesis of greater character displacement with competition. This discordance is not uncommon in closely-related species and may be explained by other morphological traits or a multivariate combination of traits driving species divergence (Anacker and Strauss, 2014). For example, I found no differences in body size in the pair *B.ambreensis* - *B.antakarana* though it is thought to be morphologically distinct (Glaw et al., 2012). This pair is also a problematic species complex due to the low genetic divergence between the species (Nagy et al., 2012) with a relatively young divergence time when compared to the rest of the *Brookesia* phylogeny (Townsend et al., 2009; Tolley and Menegon, 2013). The lack of character displacement together with a recent divergence with some hybridization may suggest incomplete speciation in this pair (Nosil et al., 2009). The pair *U.ebenaui* - *U. phantasticus* also have no differences in body size despite having overlapping ranges. Other morphological or behavioural traits may be responsible for the reproductive isolation in this pair, thus allowing co-occurrence without hybridization (Pfennig and Pfennig, 2009). For example, *U.ebenaui* has a smaller neck triangle and interorbital ridge length and *U.phantasticus* has a smaller head than its sister pair (Ratsoavina et al., 2012).

Analysis of the correlation between body size differences with climatic variables among all sister pairs found non-significant associations. I also did not find niche conservatism to be associated with morphological conservatism. Therefore, my findings do not support the hypothesis of climate-induced phenotypic variation in body size in these taxa (Gvoždík et al., 2008).

5.5.1 Topographic complexity as a promoter of character displacement

Topographic complexity is also known to influence phenotypic variation (Berardi et al., 2016). I found allopatric species to have higher character displacement at regions of higher topographic complexity though this trend was not observed among species with overlapping ranges (parapatric and sympatric species). I have previously found that sister pairs in regions of higher topographic complexity have more conserved niches than pairs in regions with less terrain unevenness (Chapter 4). My finding of higher character displacement in allopatric pairs in regions of higher topographic complexity is opposed to the initial hypothesis of allopatric speciation leading to both niche and morphological conservatism between sister pairs. Morphological divergence in regions of high topographic complexity may be due to local adaptation to small isolated regions (Gillespie and Roderick, 2014). These small isolated patches may represent regions with specific microhabitat characteristics. These local micro-scale biotic or abiotic conditions may lead to diversification through character displacement thus facilitating speciation (Massatti and Knowles, 2014). Microhabitat diversity may result in morphological variation between populations or closely-related species (Vitt et al., 1997). Closely-related species have been found to vary greatly in their morphology due to differences in habitat use (Goodman et al., 2008). Regions of higher topographic complexity may harbour greater diversity in microhabitat therefore resulting in sister pairs with higher body size differences.

Character displacement in taxa with overlapping ranges was not strongly influenced by topographic complexity. The absence of a strong association between these pairs and topographic differences may be due to competition also influencing character displacement. Therefore I described testable hypotheses on the drivers of character displacement between allopatric pairs and pairs with overlapping ranges. While sister taxa with overlapping ranges have morphological divergence generated by competition, allopatric species diverge morphologically due to differences in microhabitat preferences within their small isolated ranges (Massatti and Knowles, 2014).

In this chapter I have identified a mismatch between the conservatism of ecological niches and morphological variation between closely-related species, with sister pairs with more conserved ecological niches not having more similar body sizes. This is attributed to traits capturing species adaptations at different scales. While body size is a trait related to local adaptations, climatic niche traits refer to broader-scale adaptations. Traits that describe local scale interactions, such as body size, are associated with the alpha (α) niche of a species, while traits describing the ecological niche of a species, are related to the beta (β) niche of a species (Ackerly et al., 2006; Silvertown et al., 2006). My findings support previous studies which have also demonstrated a tendency for α niche traits to be more labile and β niche traits to be more conservative among closely-related species (Silvertown et al., 2006).

5.5.2 Caveats and future work

Studies looking at speciation mechanisms through present-day species distributions do not account for potential changes in the ranges since the time of divergence (Losos and Glor, 2003). Cases where distributions have become separated or re-connected post-speciation may mask the true speciation mechanisms involved in their divergence. Phylogenetic niche conservatism is associated with allopatric speciation because it may maintain isolation between species without the presence of a strong geographical barrier (Wiens, 2004). Discrepancies between PNC/PND and current species distributions could be explained by shifts in the distributions post-speciation. Allopatric species may have secondary contact due to favourable environmental changes within the barrier. When species re-connect, they may or may not hybridize depending on the strength of their genetic isolation and reproductive incompatibility (Coyne and Orr, 2004). Character displacement may maintain species boundaries by reducing hybridization (Pfennig and Pfennig, 2009). Allopatric species are expected to have similar morphological traits but character displacement may be observed in regions of secondary contact (Melville, 2002; Kozak et al., 2006). This may be the case for the pairs *B. thieli* - *B. vadoni* and *F. petteri* - *F. willsii*, which have current parapatric distributions with significantly conserved niches (Chapter 4). In this chapter, I have demonstrated that these two pairs also have character displacement. Thus it is possible that these pairs may have diverged allopatrically, thus exhibiting phylogenetic niche conservatism, but are able to maintain their species boundaries and co-occur because of character displacement under secondary contact.

Another example of possible distribution shifts post-speciation is the pair *T. madagacariensis* - *T. petersi.* This pair has an allopatric distribution with no differences in body size but was found to have divergent niches (Chapter 4). There are at least two mechanisms which could explain this pattern: i) the pair diverged parapatrically, producing a signal for PND, with posterior loss of its hybrid zone. In this scenario, morphological divergence might have occurred over morphological traits other than body size (Martin and Pfennig, 2011) or ii) the species diverged allopatrically, therefore exhibiting morphological conservatism, with the geographical barrier creating environmental differences between the two distributions (Glor and Warren, 2011), therefore the test picking up significant niche divergence in this pair.

I emphasize that my analysis is limited by the use of one morphological trait (body size) which may or may not always be strongly associated with resource use (Peers et al., 2013). Character displacement can also take place through a combination of many traits (Martin and Pfennig, 2011). Other morphological traits not tested in this chapter have been identified as proxies for character displacement and speciation (e.g., head shape in salamanders, Adams et al. 2007) and therefore inclusion of other traits can further elucidate the presence or absence of competition within my sample. Examples of traits that better explain divergence in co-occurring sister pairs also include reproductive traits (Qualls and Shine, 1998) or behavioural traits such as species recognition signals (Losos and Leal, 2013) and mating signals (Boughman, 2002). An example of reproductive isolating traits in reptiles is the differences in locomotor speed between gravid females of neighbouring populations of scincid lizards (*Lamphropholis guichenoti*) (Qualls and Shine, 1998). These local scale differences in traits may not be evidenced by more superficial traits, such as body size (Qualls and Shine, 1998) or not captured by species-wide mean values (Anacker and Strauss, 2014).

I also highlight that the method outlined in this analysis is not able to make robust inferences regarding the stability of species distributions through time. The most conclusive approach to identify cases of secondary contact or loss of a hybrid zone is to test whether gene flow took place at time of divergence (Martin et al., 2013; Seehausen et al., 2014). However, these approaches are very cost-demanding and to this date have only been applied to few species pairs (e.g., 3 species of *Heliconius* butterflies,Martin et al. 2013). In this chapter, I present an approach which tests for the likely presence of gene flow between species due to spatial distributions, niche conservatism and character displacement. This approach may be more easily applied to a larger sample size and thus be capable of identifying candidate pairs for future genomic work.

Chapter 6

Discussion

6.1 Overview

In this thesis I examined the incidence of phylogenetic niche conservatism and divergence (PNC and PND respectively) among the squamates of Madagascar. PNC and PND are important factors for understanding speciation between closely-related species. Character displacement is another contributing factor to the on-set of speciation and maintenance of species boundaries but its association with niche conservatism is unclear.

The Malagasy squamates are of interest due to their high endemicity and the unresolved understanding of the factors driving their speciation patterns. These squamates are useful for studies on speciation due to their recent divergence times, and thus less chance for post-speciation shifts in distributions and niches unlike older lineages. To date, there has not been a study of PNC and PND for a large number of sister taxa across the Malagasy squamates.

I began by assessing the methods currently available to test for PNC and PND which motivated the development of the new methodologies presented in this thesis. I compared the new methodologies to existing methods using virtual and empirical studies to test for congruence between test outputs. After a thorough assessment of the sensitivities of the new methods, I applied these tools to the dataset of Malagasy squamate sister taxa to test for PNC/PND in this group. I also measured the degree of character displacement within these sister taxa in order to draw comparisons between niche conservatism and character displacement and to provide a more complete understanding on the drivers of speciation within the island of Madagascar.

In Chapter 2, I developed two new tools to test of PNC/PND. The first tool is a new measure of niche overlap, the *MO* metric (for Multidimensional Overlap). The second tool is a novel null biogeographic model, the RTR significance test (for Random-Translation-and-Rotation). These tools may be used together or in combination with existing methods. I compared the ability of the *MO* metric in measuring niche overlap with 3 other existing methods using simple virtual scenarios. I also used the *MO* metric and the RTR significance test to analyse PNC/PND in real sister taxa which had been previously tested with other methods. Overall the new methodologies performed well in both types of scenarios.

In Chapter 3, I used more complex virtual scenarios to further assess the performance of the new methodology (*MO* metric combined with the RTR significance test) in identifying PNC under different conditions. I tested the methodology's ability to detect a significant signal for PNC for a virtual sister taxa when presented with changes in environmental spatial autocorrelation, size of background region and intensity of sampling bias of occurrence data. The method was shown to be more sensitive to environmental spatial autocorrelation than to size of background region or sampling bias. I also observed a tendency for the method to fail to find a significant signal for PNC. This is a common feature of tests which retain the spatial autocorrelation between the occurrence data, thus being a more conservative test for PNC/PND than other available tests which are less constrained (e.g., background test of Warren et al. 2008).

Having a good understanding of the performance of the *MO* metric and the RTR significance test from Chapters 2 and 3, I applied these tools to the Malagasy dataset in Chapter 4. Out of 28 pairs, I found few cases of significant PNC and PND which is in agreement with the findings on the conservative nature of the RTR significance test of Chapter 3. In this chapter, I also looked at the associations between PNC/PND and current distributions between sister taxa. The hypotheses were that allopatric speciation would be associated with PNC (Wiens, 2004) while parapatric speciation would be associated with PND (Florio et al., 2012). I assume that current species distributions have not suffered from range or niche shifts post-speciation (Losos and Glor, 2003) thus allopatric distributions retain the original signal of allopatric speciation and parapatric distributions for parapatric speciation. I only found an exclusive association between PNC and allopatric sister taxa and PND with parapatric sister taxa when a more conservative two-tailed test was used. I further tested whether topographic complexity, a recently proposed driver of allopatric speciation and niche conservatism (Steinbauer et al., 2016; Hu et al., 2016), could explain the observed patterns of PNC/PND in the island. This hypothesis was strongly supported in these taxa due to a significantly positive relationship between niche conservatism in sister taxa and regions of higher elevations and terrain roughness.

The incidence of PNC/PND was also investigated with regards to character displacement in Chapter 5. A relationship between niche conservatism and morphological conservatism was not found but character displacement was found to increase with increasing levels of competition within these taxa. Given the importance of topographic complexity in speciation found in Chapter 4, I also tested for topographic complexity in explaining patterns of character displacement. Allopatric pairs in regions of higher topographic complexity were found to have higher character displacement while no relationship was found for co-occurring pairs (parapatric and sympatric pairs). Given that niche conservatism was also associated with topographic complexity (Chapter 4), this finding suggests that character displacement may not be a strong correlate for broad scale ecological differences between closely-related species. Morphological differences between closely-related species may instead represent differences in local adaptations to non-abiotic stressors such as biotic interactions.

6.2 Comparisons to the literature

The quest for quantifying the incidence of PNC in extant lineages has greatly developed due to the emergence of quantitative statistical tools such as ecological niche models (ENMs), null background tests and multivariate analyses (Warren et al., 2008; Broennimann et al., 2012; Blonder et al., 2014). In this thesis I presented a novel methodology which aims to complement the current plethora of tests available. This methodology is not only unique in its statistical approaches, but it also aims to answer distinct evolutionary questions from other approaches. In Chapter 2, I have presented the *MO* metric which is a presence-only measure of niche overlap which is multidimensional and circumvents some of the assumptions and limitations of ENMs. This statistic may be particularly suitable for data sets with few occurrence records whereby ENMs (Pearson et al., 2007) and kernel density approaches are less suitable (Blonder et al., 2014). I demonstrated in Chapter 2 how the *MO* metric can provide comparable estimates of niche overlap to other commonly used approaches thus being an equally strong contender for measuring niche overlap in multi-dimensions. In Chapter 2, I also introduced a novel null model, the RTR significance test, which is also distinct from other currently available methods, in particular the background test of Warren et al. (2008). This test stands out from other available tools due to two features: i) it maintains the spatial autocorrelation of the observed occurrence data; ii) it tests for landscape-based ecological processes involved in speciation. The null model presented in Chapter 2 is introduced not as a replacement of other available tools but rather as a more suitable approach when asking questions on whether the distribution of a species or sister pair is capturing unique environmental signals not found anywhere else in a landscape, thus suggesting that the sister taxa may be constrained by ecological rather than geographical factors.

The literature has highlighted a number of sources of error which may influence a test's ability to distinguish between PNC from non-significant ecological signals (e.g., size of background region, intensity of sampling bias in occurrence data and spatial autocorrelation within environmental layers). In Chapter 3 I found the approach introduced in Chapter 2 to not be very sensitive to these factors except for differences in the spatial autocorrelation within the environmental layers. The test was also found to be conservative in that a statistically significant signal was rarely recovered in the virtual simulations. Other null tests that also maintain signal in the occurrences when performing the null replicates have also been found to rarely detect a significant statistical signal (Beale et al., 2008; Peterson et al., 2009).

Niche conservatism has been proposed as a common driver of allopatric speciation (Peterson et al., 1999; Wiens, 2004). However, my results suggest that speciation is not always explained by a lack of niche differentiation. In Chapter 4, I found only a few cases of PNC in my sample, with some PNC signal being captured in parapatric sister pairs. Recent studies have proposed topography as a cause of fragmentation of species' ranges and thus a promoter of allopatric speciation (Steinbauer et al., 2016). Results from Chapter 4 found that terrain roughness, as a proxy for topographic complexity, was strongly associated with PNC, where sister taxa with higher tendency for niche conservatism were more often found in regions of higher topographic complexity. These findings agree with other studies on topographic complex regions, such as the Qinghai-Tibetan Plateau (QTP) in China (Hu et al., 2016), where niche conservation was also observed.

The tendency for closely-related species to have similar morphological traits has been supported in some clades but not others (Losos, 2008;

Luxbacher and Knouft, 2009). In Chapter 5, I did not find a strong tendency for closely-related species to be more morphologically similar when compared to non-sister species as judged by body size. Morphological divergence has been observed in regions of geographic overlap between species (parapatry) in order to alleviate pressures of direct competition (Böhning-Gaese et al., 2003; Rice and Pfennig, 2010). My results show that the degree of character displacement may be associated with intensity of competition between sister taxa, thus the findings from Chapter 5 are in agreement with the hypothesis of competition-induced phenotypic variation (Brown and Wilson, 1956; Peers et al., 2013; Beans, 2014). Morphological conservatism is often assumed in allopatric species (Kozak et al., 2006; Stuart and Losos, 2013). Results from Chapter 5 did not show character displacement to be smaller in allopatric pairs than co-occurring pairs but character displacement was significantly less variable. To date, the association between character displacement and topographic complexity has not been directly analysed in squamates. I find the variation in character displacement among allopatric pairs to be associated with topographic complexity, where allopatric pairs found in regions of higher terrain unevenness having higher character displacement than allopatric pairs found in regions of more even surface terrain.

6.3 Future directions

6.3.1 Methodological advancements to the *MO* metric and the RTR significance test

6.3.1.1 Future work on measuring niche overlap

The choice of predictors to explain an abiotic environment should have biological meaning (Randolph, 2002). A major limitation when constructing ecological niches is the choice and number of predictors required to accurately describe the niche of species (Jackson et al., 2009). For instance, Broennimann's PCA approach limits the niche to only two axes (Broennimann et al., 2012) but the use of as many environmental variables as possible is often impractical (Jackson et al., 2009). In this thesis I used variables which had been previously used in other case studies

(Chapter 2) and that I had *a priori* hypotheses of their biological importance to squamates (Chapter 4 and 5).

Metrics for niche modelling are prone to a trade-off between number of occurrences and number of predictors used (Stockwell and Peterson, 2002; Coudun et al., 2007) though the use of rules-of-thumb has been contested (Rodda et al., 2011). In Chapter 2, I have presented a novel measure of niche overlap, the *MO* metric, which is not susceptible to this trade-off. This meant I could use the same number of environmental axis for all taxa regardless of the number of occurrences available. However, the *MO* metric may be susceptible to the risk the inflation of the niche overlap value if the predictors are too correlated (Estrada-Peña et al., 2013).

Future work could focus on techniques for variable reduction such as the use of jack-knife perturbations, PCA approaches and the use of variance inflation factors (VIF) and their impact on outputs of the *MO* metric. The issues with dimensionality have also been addressed in ecological networks (Eklöf et al., 2013). In this case, the use of boxicity was recently applied to define the minimum number of species interactions needed to accurately describe an ecological network (Eklöf et al., 2013). A similar approach could potentially be applied to the study of niches, in order to find the minimum number of predictors required to accurately describe species' niches.

6.3.1.2 Potential constraints of the RTR significance test

The RTR significance test acts by randomising the null replicates in their rotation and position in a given landscape. However, as mentioned in Chapter 2, it is possible to run the test in only one of these transformations. For example, there may be interest in testing whether a particular geographic barrier may have a significant impact in the niche differences between two flanking populations (Glor and Warren, 2011; Soto-Centeno et al., 2013). The RTR significance test may be adapted to only rotate but not translate a pair of allopatric distributions around a geographic barrier. Future work may look into the sensitivity of these approaches in detecting for PNC using both virtual and empirical studies. In Chapter 2 I have also demonstrated that spatial biases in null replicates could occur in some cases. While I have provided tools to test for the incidence of this bias, future work may look into providing a technique to reduce the impact of such bias in the outputs of the null model.

Null models that account for spatial autocorrelation are referred to as restricted randomization techniques (Fortin and Jacquez, 2000). The RTR significance test is one of such techniques because the null replicates are restricted by the spatial configuration of the empirical observations. On the other hand, the test is unrestricted in the lack of selection for particular null replicates (e.g., null replicates with similar environmental properties to empirical observations). The degree of restrictiveness in a model is relative to the level of understanding of the system and thus there is flexibility in how much we would like to restrict our randomisations. In Chapter 3, I looked at the sensitivity of the RTR significance test in picking up PNC under different scenarios. Future work may involve looking at how constraints to this test may affect its ability to pick up a statically significant signal. For instance, the RTR approach could be further refined by only keeping RTRs which fall in particular environments (e.g., a preference for lower elevations), at the cost of increasing computing time (Fortin and Jacquez, 2000) and chances of falsely accepting the null hypothesis (Type II error). Consequently, it could be relaxed by allowing the model to draw random points within the RTR, at the risk of also increasing computing time and chances of falsely rejecting the null hypothesis (Type I error).

My analysis in Chapter 3 also did not include an assessment of how spatial resolution may affect model output. A significant advantage of the RTR modelling approach is the possibility of including environmental variables at different resolutions because they are independent of each other. This is particularly important for species-environment responses which are scale-dependent. For instance, this type of dependency has been observed in the distribution of elephants, which respond to forage features at a coarser resolution than surface water (De Knegt et al., 2010). Future work may focus on the geographic scale at which PNC can be picked up using both virtual scenarios as well as tests on empirical studies where species-environment responses are known to be scale-dependent.

I have presented the RTR significance test as an approach to test for niche evolution within a geographic space. It is also possible to adapt this null model to run a similar analysis within an environmental space. For instance, Chapman (2010) tested the association between climate variables and species distributions by generating simulated climate gradients that have similar properties to those in real landscapes. Taking a similar approach whereby the environment is randomized, instead of the species distributions (as I do here), might prove fruitful for exploring other patterns of niche evolution. However, I highlight that such analysis may produce environmental regions which are not found in nature (Warren et al., 2008). Therefore, by keeping the RTR to geographical space the empirical landscape-based approach to the analysis of niche evolution is preserved.

6.3.2 Further insight into phylogenetic conservatism among squamates in Madagascar

6.3.2.1 Other factors which may explain PNC/PND patterns

In Chapter 4 I looked at topographic complexity as a potential driver of PNC in the island of Madagascar. Other mechanisms have been proposed but were not explicitly tested in this thesis. Future work may look into testing for PNC/PND under other proposed scenarios of speciation, such as river barriers (Pastorini et al., 2003), allopatric isolation between lowland retreat-dispersion watershed (Wilmé et al., 2006) or parapatric speciation along current climatic gradients (Pearson and Raxworthy, 2009).

The tropical niche conservatism hypothesis expects higher species richness and niche conservatism in regions with higher climate stability, such as tropical regions (Qian, 2014). One approach to climate stability is the use of past precipitation anomalies. Paleo-precipitation was found to drive endemism of palm trees in Madagascar (Rakotoarinivo et al., 2013). Higher anomalies between past and current precipitation are associated with the maintenance of the tropical forest during the last glacial maximum. These regions may have acted as refugia for species by providing climatically stable environments (Rakotoarinivo et al., 2013). In Chapter 4 I tested for niche conservatism in the island of Madagascar but further work may look into explicitly testing whether niche conservatism is more commonly found in regions of higher climatic stability.

6.3.2.2 Character displacement in multivariate morphological space

In Chapter 5 I analysed morphological conservatism for sister taxa of squamates in Madagascar using only body size differences as a proxy for character displacement. However, this analysis was only limited to one morphological trait, while morphological divergence may take place in other traits or a combination of traits (Martin and Pfennig, 2011). Future work may focus on including a large number of traits which will enable the analysis of morphological conservatism in multi-dimensions. Techniques such as the MO metric and Blonder et al. (2014)'s n-hypervolume approach are both suitable approaches to test for multivariate morphological overlap between taxa.

6.3.2.3 Genomics on sister pairs to test for speciation with and without gene flow with PNC/PND

In this thesis, I assumed that current geographic distributions have not shifted post-speciation, therefore retaining the original geographic distributions at the time of speciation (Losos and Glor, 2003). However, if range shifts have occurred, then parapatric or allopatric speciation are more difficult to pick up from current geographic distributions. The biggest distinction between allopatric and parapatric speciation is the absence of gene flow in allopatric speciation. Recent developments in genomics have returned promising results on the ability to estimate ancestral gene flow between species (Renaut et al., 2013; Martin et al., 2013; Seehausen et al., 2014). Genomics can thus discern between speciation with and without gene flow. In this thesis I have identified sister taxa which, given their inconsistencies between the expected signal for ecological speciation (PNC or PND) and their current distributions (allopatric or parapatric distributions), they may have had range shifts post-speciation. These pairs can be candidates for genomic work on the occurrence of gene flow to explicitly test whether they have diverged with or without gene flow. Moreover, knowledge on the presence or absence of gene flow between sister taxa would be critical when testing for the association between PNC and allopatric speciation (i.e., speciation without gene flow) and PND with parapatric speciation (i.e., speciation with gene flow).

6.4 Conclusions

In this thesis I have presented a novel approach to study ecological processes involved in speciation using the Malagasy squamates as a case study. The new approach was assessed with virtual and empirical scenarios and holds great promise when addressing questions on endemism and landscape-based ecological conservatism or divergence. When applied to Madagascar, my studies suggest a role of topography in driving speciation patterns in the squamates of Madagascar. I have found topographic complexity to be associated with niche conservatism, thus regions at high elevations, which are also regions of higher topographic complexity, may have higher incidence of allopatric speciation. These findings could explain the higher species richness and rates of endemism found in the mountainous regions of Madagascar. I have found character displacement to be associated with topographic complexity but not with niche conservatism, suggesting species with similar climatic niches may be morphologically adapted to local biotic stressors post-speciation. The code to run MO metric and the RTR significance test has been made available so it may be applied to other case studies in order to further investigate the incidence of PNC in other regions and better test associations between PNC and speciation mechanisms.

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Appendix A

Appendix: R code used in the analysis of Chapter 2

A.1 R code used in Chapter 2

This is the R code used to run all the analyses presented in Chapter 2. To use it, simply copy and paste it into a R environmnent.

```
library('doParallel')
library('doRNG')

library('raster')
library('dismo')
library('rgeos') #for convexhull and centroid
library('maptools')

### Trimming occurences, remove occurences which represent
    outliers in environmental hyperspace (Farber and Kadmon,
    2003)
trim.occ<-function(bios,occ, plot=TRUE){
  S=matrix(c(extract(bios,occ)),ncol=nlayers(bios),nrow=nrow(
     occ))
  C=cov(S) #covariance matrix
  M=apply(S,2,mean) #mean conditions of each climate indice

  D<-rep(NA,nrow(S))
  for(i in 1:nrow(S)){

    Tr=t(S[i,]-M) #transpose opeerator
    D[i]=Tr%*%((C)^-1)%*%(S[i,]-M) #malahanobis distance
  }

  D<-cbind(c(1:nrow(S)),D) #add locality index to D distances

  qD<-D[c(which(D[,2]>quantile(D,0.05))),] #trim 5%
  qD<-qD[c(which(qD[,2]<quantile(D,0.95))),] #trim 95%

  occ_trim<-occ[qD[,1],] #keep locality points within 5-95th
     percentile
  if(plot==TRUE){
    plot(D[,2], xlab='', ylab='Mahalanobis Distance')
    abline(h=quantile(D,0.95), col='red')
    abline(h=quantile(D,0.05), col='red')
    plot(bios[[1]], col='grey')
    points(occ)
    points(occ_trim,col='red')
```

```
  }
  return(occ_trim)
}
########## COMPARE NICHE
#### new metric - niche overlap function ###
mo.metric<-function(sp1,sp2,bios){

  e_sp1<-na.omit(extract(bios,sp1)) #extract climatic
      variables for species 1
  e_sp2<-na.omit(extract(bios,sp2)) #extract climatic
      variables for species 1

  e1_max<-apply(e_sp1,2,max) #max of species 1
  e1_min<-apply(e_sp1,2,min) #min of species 1

  e2_max<-apply(e_sp2,2,max) #max of species 2
  e2_min<-apply(e_sp2,2,min) #min of species 2

  overlap<-rep(NA,ncol(e_sp1)) #to store  overlap of
      enviromental varialble (Axis overlap, Fig. 1)
  for(n in 1:ncol(e_sp1)){
    overlap[n]<-(min(e1_max[n],e2_max[n])-max(e1_min[n],e2_
        min[n]))/(max(e1_max[n],e2_max[n])-min(e1_min[n],e2_
        min[n]))
    if(overlap[n]<0){overlap[n]<-0} #there is no overlap
        between the axis if overlap is negative
  }

  sumoverlap<-sum(overlap)/ncol(e_sp1) # Cumulative sum of
      axis overlap, relative to potential overal
  return(list(overlap,sumoverlap))
}


################# end function ###########

################ rtr function
rtr<-function(sp1,sp2,bios,rotation=TRUE,translation=TRUE){
  library('raster')
  library('maptools')
  library('dismo')
  library('rgeos') #for convexhull and centroid
  sp3<- rbind(unique(sp1),unique(sp2)) # unify both clouds of
       points to maintain spatial configuration
```

```
poly<-SpatialPoints(sp3)
poly<-gConvexHull(poly) #make minimum convex polygon
cent<-gCentroid(poly) #find centroid of polygon

x_centre<-as.vector(extent(cent))[1]  #find x- centre of
    polygon
y_centre<-as.vector(extent(cent))[3]  #find y- centre of
    polygon

x<-sp3[,1] #x-coordinates
y<-sp3[,2] #y-coordinates

cat(paste('',i,'-RUN  ',sep=''))
repeat{
  ###### rotation
  if(rotation==TRUE){ ##option to rotate the points, no
      translation
    deg<-runif(1,0,360) #random angle in degrees

    rad<-deg*0.0174532925 #coverts degrees to radian

    R<-matrix(c(cos(rad),sin(rad),0,-sin(rad),cos(rad)
        ,0,0,0,1),byrow=T,ncol=3,nrow=3) #clockwise

    a<-matrix(c(1,0,0,0,1,0,x_centre,y_centre,1),nrow=3,
        ncol=3,byrow=T) #rotation at the centre of origin

    cn<-matrix(c(1,0,0,0,1,0,-x_centre,-y_centre,1),nrow=3,
        ncol=3,byrow=T) #rotation at the centre of origin

    R<-cn%*%R%*%a

    x<-sp3[,1] #x-coordinates
    y<-sp3[,2] #y-coordinates

    rot<-matrix(rep(NA),ncol=3,nrow=length(x))
    for(j in 1:length(x)){
      rot[j,]<-R%*%matrix(c(x[j],y[j],1),ncol=1,nrow=3)
          #apply rotation to points
    }

    #apply rotation matrix to the original matrix
    repmat<-matrix(rep(c(x_centre,y_centre)),ncol=2,nrow=
        nrow(sp3),byrow=T)
```

```
    s<-sp3-repmat
    s<-cbind(s,1)
    s<-matrix(unlist(s),ncol=3,byrow=F)
    so<-tcrossprod(s,round(R,5))

    newpool1<-so[,1:2]+repmat

  x<-newpool1[,1]
  y<-newpool1[,2]
  }
  if(translation==TRUE){ #option to translate the points, no rotation
  #### translation
  xmin=extent(bios)[1];xmax=extent(bios)[2];ymin=extent(bios)[3];ymax=extent(bios)[4]

  x_trans<-runif(1,-(xmax-xmin),(xmax-xmin)) #random long trans
  y_trans<-runif(1,-(ymax-ymin),(ymax-ymin)) #random lat trans

  x<-round(x+x_trans) #add translation vector

  y<-round(y+y_trans) #add translation vector


  newpool1<-cbind(x,y) #combine new coordinates
  }

 # plot(bios[[1]])
  #points(newpool1)

  climate_niche = extract(bios, newpool1) #if NA, then a point is in the ocean/outside study region - no variables
  if(isTRUE(length(which(is.na(climate_niche)==FALSE))==length(climate_niche))){  #if TRUE no NAs then break loop, keep RTR polygon
    break}}

gridcells<-extract(bios, newpool1,cellnumbers=TRUE)[,1] #store gridcells to check for duplicates
```

```
  sim.niche<-mo.metric(newpool1[1:nrow(sp1),],newpool1[(nrow(sp1)+1):nrow(climate_niche),],bios)[[2]] #measure niche overlap of simulated polygon
  print(noquote(paste('Simulated Niche Overlap:',sim.niche))) #print measured niche overlap

  return(list(sim.niche,as.vector(gridcells)))} #return niche overlap of RTR and the gridcells occupied by the simulated points

####################### end functions ########################

################### PLOT SIGNIFICANCE
RTRsignificance<-function(sp1,sp2,bios,rtrs, tails=TRUE, divergence=TRUE){

  climate_sp1 = na.omit(extract(bios, sp1)) #extract niches
  climate_sp2 = na.omit(extract(bios, sp2)) #extract niches

  observed<-mo.metric(sp1,sp2,bios)[[2]] #observed niche overlap
  meaniche<-mean(rtrs)

  ### is PNC, one-tailed test

  nineperc<-as.numeric(quantile(rtrs,0.95)) #define critical value limits for up
  top<-observed>as.numeric(quantile(rtrs,0.95)) #niche conserved
  Fn<-ecdf(as.numeric(rtrs))
  location<-Fn(observed)
  pvalue<-min((sum(rtrs <= observed)/length(rtrs)),(sum(rtrs >= observed)/length(rtrs))) #one tailed each time
  bottom<-NA
  fiveperc<-NA

  if(tails==TRUE){ ##two-tailed test
  fiveperc<-as.numeric(quantile(rtrs,0.025)) #define critical value limits for bottom
  nineperc<-as.numeric(quantile(rtrs,0.975)) #define critical value limits for up
  bottom<-observed<as.numeric(quantile(rtrs,0.025)) #niche diverge
```

```
  top<-observed>as.numeric(quantile(rtrs,0.975)) #niche
      conserved
  Fn<-ecdf(as.numeric(rtrs))
  location<-Fn(observed)
  pvalue<-min((sum(rtrs <= observed)/length(rtrs)),(sum(rtrs
     >= observed)/length(rtrs)))*2 #two tailed p-value
}

  if(divergence==TRUE){ ## is PND, one tailed test
    fiveperc<-as.numeric(quantile(rtrs,0.05)) #define
        critical value limits for bottom
    bottom<-observed<as.numeric(quantile(rtrs,0.05)) #niche
        diverge
    Fn<-ecdf(as.numeric(rtrs))
    location<-Fn(observed)
    pvalue<-min((sum(rtrs <= observed)/length(rtrs)),(sum(
        rtrs >= observed)/length(rtrs))) #one tailed
  top<-NA
  nineperc<-NA
  }

  hist(rtrs, xlab='Niche Overlap Value', ylab='Frequency',
      main=NULL, xlim=c(0,1))
  legend('topright',legend=c('Observed Niche Overlap Value'),
       lty=c(1),lwd=c(2),col='red')
  abline(v=observed, col='red', lwd=2)
  return(data.frame(observed,bottom,top,meaniche,fiveperc,
      nineperc,pvalue,location))}

################## end function

#### function to store grid cells to test for spatial bias
rtr.bias<-function(sp1,sp2,bios,rotation=TRUE,translation=
   TRUE){
  library('raster')
  library('maptools')
  library('dismo')
  library('rgeos') #for convexhull and centroid
  sp3<- rbind(unique(sp1),unique(sp2)) # unify both clouds of
       points to maintain spatial configuration
  poly<-SpatialPoints(sp3)
  poly<-gConvexHull(poly) #make minimum convex polygon
  cent<-gCentroid(poly) #find centroid of polygon
```

```
x_centre<-as.vector(extent(cent))[1]  #find x- centre of
    polygon
y_centre<-as.vector(extent(cent))[3]  #find y- centre of
    polygon

x<-sp3[,1] #x-coordinates
y<-sp3[,2] #y-coordinates

repeat{
  ###### rotation
  if(rotation==TRUE){ ##option to rotate the points, no
      translation
    deg<-runif(1,0,360) #random angle in degrees

    rad<-deg*0.0174532925 #coverts degrees to radian

    R<-matrix(c(cos(rad),sin(rad),0,-sin(rad),cos(rad)
        ,0,0,0,1),byrow=T,ncol=3,nrow=3) #clockwise

    a<-matrix(c(1,0,0,0,1,0,x_centre,y_centre,1),nrow=3,
        ncol=3,byrow=T) #rotation at the centre of origin

    cn<-matrix(c(1,0,0,0,1,0,-x_centre,-y_centre,1),nrow=3,
        ncol=3,byrow=T) #rotation at the centre of origin

    R<-cn%*%R%*%a

    x<-sp3[,1] #x-coordinates
    y<-sp3[,2] #y-coordinates

    rot<-matrix(rep(NA),ncol=3,nrow=length(x))
    for(j in 1:length(x)){
      rot[j,]<-R%*%matrix(c(x[j],y[j],1),ncol=1,nrow=3)
          #apply rotation to points
    }

    #apply rotation matrix to the original matrix
    repmat<-matrix(rep(c(x_centre,y_centre)),ncol=2,nrow=
        nrow(sp3),byrow=T)

    s<-sp3-repmat
    s<-cbind(s,1)
    s<-matrix(unlist(s),ncol=3,byrow=F)
    so<-tcrossprod(s,round(R,5))
```

```
      newpool1<-so[,1:2]+repmat

      x<-newpool1[,1]
      y<-newpool1[,2]
    }
    if(translation==TRUE){ #option to translate the points,
       no rotation
      #### translation
      xmin=extent(bios)[1];xmax=extent(bios)[2];ymin=extent(
          bios)[3];ymax=extent(bios)[4]

      x_trans<-runif(1,-(xmax-xmin),(xmax-xmin)) #random long
           trans
      y_trans<-runif(1,-(ymax-ymin),(ymax-ymin)) #random lat
          trans

      x<-round(x+x_trans) #add translation vector

      y<-round(y+y_trans) #add translation vector


      newpool1<-cbind(x,y) #combine new coordinates
    }

    # plot(bios[[1]])
    #points(newpool1)

    climate_niche = extract(bios, newpool1) #if NA, then a
        point is in the ocean/outside study region - no
        variables
    if(isTRUE(length(which(is.na(climate_niche)==FALSE))==
        length(climate_niche))){  #if TRUE no NAs then break
        loop, keep RTR polygon
      break}}

  gridcells<-extract(bios, newpool1,cellnumbers=TRUE)[,1] #
      store gridcells to check for duplicates or spatial bias

  return(as.vector(gridcells))} #return the gridcells
      occupied by the simulated points
#### end function ####

#### function to measure and visualise spatial bias ###
```

```
rtr.bias.map<-function(sp1,sp2,bios,iter,plot=TRUE){

  r<-raster(ncol=ncol(bios[[1]]),nrow=nrow(bios[[1]]))
  extent(r)<-extent(bios[[1]])
  res(r)<-res(bios[[1]])
  projection(r)<-projection(bios[[1]])
  values(r)<-0
  r[which(is.na(values(bios[[1]])))]<-NA
  r[rtr_bias(sp1,sp2,bios[[1]])]<-1
  r3<-r
  for(i in 1:iter){
    cat(paste('',i,'-RUN  ',sep=''))
    values(r)<-0
    r[rtr_bias(sp1,sp2,bios[[1]])]<-1
    r2<-r
    r3<-mosaic(r3,r2,fun=sum)
    removeTmpFiles(1) #remove temp files from 1 hour ago - to save memory
  }
  r3[which(is.na(values(bios[[1]])))]<-NA
  r4<-r3
  if(plot==TRUE){
    plot(r3)
  }
  return(r4)}
#### end function


############ RTR script for plotting percentile and stability througout simulations
library('doParallel')
library('doRNG')

cluster<-3 #number of CPUs to run simulation -advice: always leave one CPU free to not overlaod the computer
cl<-makeCluster(cluster) # add number of CPUs
registerDoParallel(cl)
seeded=10 #set seed for Mesenne-Twister, same seed= repeatability; NA = always different seeds,non-repeatable

trials=5  #number of RTRs per batch, (e.g do 100 RTRs each time, to measure percentile, stability, duplicates etc)
cutoff=79  # stop running when reaches this number+1
```

```r
sample=2 #define how many percentiles necessary to measure stability

start.time<-Sys.time() # record start time

observed<-mo.metric(sp1,sp2,bios)[[2]] #observed niche overlap

set.seed(seeded, kind="Mersenne-Twister") #set seed

RTRs<-foreach(i=1:trials, .combine='cbind', .errorhandling='remove') %dorng% {
  rtr(sp1,sp2,bios,rotation=TRUE, translation=TRUE)}

## check for duplicates
if(length(which(duplicated(RTRs[2,])==TRUE))>0){ #is number of duplicates > 0 ?
  non.dup<-RTRs[,-(which(duplicated(RTRs[2,])==TRUE))]### if YES then remove duplicates to keep unique NOs (niche overlap)
}else{non.dup<-RTRs} #if no duplicates then no removal


null.no<-c(non.dup[1,]) ##store NO values only for building null library


Fn<-ecdf(as.numeric(null.no)) #Empirical Cumulative Distribution Function
perc<-Fn(observed) #Note: this is not p-value, it is the location of the observed niche overlap in the null distribution (the percentile)

plot(1, type="n", xlab="Number of Iterations", ylab="Percentile", xlim=c(0,cutoff+1), ylim=c(0, 1))
points(length(null.no),perc, pch=19)
abline(0.05,0,col='red')
abline(0.95,0,col='red')
abline(0,0,col='green')
legend(cutoff/2,0.8, c('Percentile','Stability','Significance Threshold','Stability Threshold'), pch=c(19,19,NA,NA),lty = c(0, 0, 1,1),lwd = c(0, 0, 1,1),bty = "n",col = c("black","blue","red","green"))
```

```
#_prev ==stands for previous value

null.no_prev<-length(null.no) #number of unique NOs of
    previous step

perc_prev<-perc #store percentile of previous step

stability_prev<-c(1) #start of stability string

#checkpoints
print(noquote(paste('Number of Unique RTRs:','',length(null.
    no))))#print number of Iterations (number of unique NOs)

end.time<-Sys.time() #Register end of loop
time.taken<-end.time-start.time ##Register time it took to
    run the batch
print(noquote(paste('Duration of Analysis:',time.taken))) #
    print duration of batch so can estimate how long it will
    take to finish all the repeats

repeat{
  RTRs<-foreach(i=1:trials, .combine='cbind', .errorhandling=
      'remove') %dorng% {
    rtr(sp1,sp2,bios,rotation=TRUE, translation=TRUE)}

  RTRs<-cbind(non.dup,RTRs) ##bind new null NO with previous
      batch of unique NOs

  ## check for duplicates
  if(length(which(duplicated(RTRs[2,])==TRUE))>0){ #is number
      of duplicates > 0 ?
    non.dup<-RTRs[,-(which(duplicated(RTRs[2,])==TRUE))]###
        if YES then remove duplicates to keep unique NOs
  }else{non.dup<-RTRs} #if no duplicates then no removal


  null.no<-c(non.dup[1,]) ##store NO values only for building
      null library


  Fn<-ecdf(as.numeric(null.no)) #Empirical Cumulative
      Distribution Function
```

```
perc<-Fn(observed) #Note: this is not p-value, it is the
    location of the observed niche overlap in the null
    distribution (the percentile)

points(length(null.no),perc,pch=19) # plot percentile

null.no_prev<-c(null.no_prev,length(null.no)) # #number of
    combined unique null nicheo verlap values

perc_prev<-c(perc_prev,perc) #store all percentile
    locations


df<-data.frame(null.no_prev,perc_prev) #table showing
    numble of RTRs and percentile of observed niche overlap
colnames(df)<-c('Iterations','Percentile')


lines(df[,1],df[,2]) #plot line showing number of NOs and
    percentile
if(isTRUE(length(null.no_prev)>sample)){
points(length(null.no),round(sd(df[(nrow(df)-sample):nrow(
    df),2]),2),col='blue',pch=19) #plot stability of
    percentile

stability<-c(round(sd(df[(nrow(df)-sample):nrow(df),2]),2))
     #calculate stability of percentile => standard
    deviation of percentile of the last *sample* batches
stability_prev<-c(stability_prev,stability) #store
    stability measure
}
#checkpoint for stability
print(noquote(paste('Stable:',isTRUE(abs(df[nrow(df),2]-df
    [(nrow(df)-1),2])<0.01)))) #measure of stability
#checkpoints
print(noquote(paste('Number of Unique RTRs:','',length(null
    .no))))#print number of Iterations (number of unique NOs
    )

end.time<-Sys.time() #Register end of loop
time.taken<-end.time-start.time ##Register time it took to
    run the batch
print(noquote(paste('Duration of Analysis:',time.taken))) #
     print duration of batch so can estimate how long it
```

```
      will take to finish all the repeats

  if(isTRUE(length(null.no)>cutoff)) break } #if number of
     NOs reaches cutoff, stop the simulation
stopCluster(cl)

stability<-stability_prev[-1] #print measure of stability at
    the end of the analysis

final.table<-cbind(df,c(rep('NA',sample),stability)) #table
    showing number of NOs, percentile and stability of
    percentile
colnames(final.table)<-c('Iterations','Percentile','Stability
    ')
final.table #print table
```

Appendix B

Appendix: Description of the R methods involved in the RTR significance test

B.1 Description of the methods involved in the R code of the RTR test

The RTR test is implemented as follows: First, a minimum convex polygon (MCP) is drawn around the points of both populations and the centroid of this MCP is found. We achieve this using the functions SpatialPoints, gConvexHull and gCentroid from the R packages 'sp' (Pebesma and Bivand, 2005) and 'rgeos' (Bivand and Rundel, 2014). Second, a random number generator (we use the Mersenne Twister (Matsumoto and Nishimura, 1998) from the R function 'runif') is used to generate an angle of rotation, a latitude and a longitude. These random values are then used to rotate the set of occurrence records around the centroid of the MCP, and to translate the centroid to another location in the study region. If one or more occurrence records now fall outside the study area (e.g., in the sea) the replicate is rejected. Third, the niche overlap for the replicate set of occurrences is calculated. Fourth, the second and third steps are then repeated many times so that a null distribution of niche overlap values across the study region is generated, from which statistical significance of the observed niche overlap value against the null distribution can be inferred (Fig. 2.1).

In this chapter we ran 10,000 replicates for each RTR test (Pearson and Raxworthy, 2009) and we tested if the observed niche overlap ranked in the bottom 5% or if it ranked the top 5% of the null model values using the quantile function in R. This proved to be enough replicates to test for significance, with models taking between 10 minutes and 78 hours to run on a desktop PC using 3 CPUs. However, in other studies more or less replicates may be required to reach a conclusion of whether or not a test is significant. Also, in some instances it is likely that relatively few replicates can be generated in reasonable computing time (in particular, if the MCP covers a large proportion of the study area). To explore such instances in future applications, the implementation that we make available (see Appendix A) enables the user to easily view in run time the number of replicates being generated, the number of those replicates that are duplicates, and changes in the p-value. Thus, the user can monitor stability in the p-value (once there is little change no new replicates are required (see Appendix C) and the number of unique replicates generated (few unique replicates indicates that the distribution may be too widespread for the test).

Appendix C

Appendix: Brief review of existing methods for PNC testing

C.1 Brief review of existing methods and their limitations

Warren et al. (2008) adapted two metrics to quantify niche overlap: Schoener's D and Hellinger's I, which they used to quantify the spatial overlap between distributions generated by ENMs. An important advance proposed by Warren et al. (2008) has been the use of null model tests to assess observed niche differences (or similarities) in the context of the environmental conditions available in the study area. Generating a suitable null model allows us to ask whether the observed similarity or difference between the niches of two populations is statistically meaningful given the available environments. Warren et al. (2008) proposed two null tests: (i) the 'background similarity test', in which observed niche overlap is compared against a null distribution created by comparing the ENM of one species to an ENM generated from random points within the potential geographic range of the other species (commonly defined by a minimum convex polygon around occurrence records Warren et al. 2010; Blair et al. 2013, though improved approaches that make better estimates of the accessible area for each species have been proposed Barve et al. 2011); and (ii) the 'identity test', in which null replicates are simulated by randomly reassigning known occurrence records to each of the populations. Other methods for testing niche similarity include the kernel smoothing approach of Broennimann et al. (2012), which was designed principally to test for niche differences between populations of invasive species in different geographic regions (Broennimann et al., 2012; Petitpierre et al., 2012), and the n-dimensional hypervolume approach of Blonder et al. (2014) whereby niche overlap is calculated as the intersection of the hypervolumes of two populations.

Current methods have several limitations. These include, for Warren et al's background and identity tests: (i) the need to arbitrarily define a separate 'background' region for each species, which can affect model outputs (Anderson and Raza, 2010) and result in conflicting findings that are difficult to interpret (e.g., Warren et al. 2008; McCormack et al. 2010; Blair et al. 2013) due to reciprocal testing (i.e., species A to species B, and also species B to species A); (ii) reliance on the assumptions that underlie ENMs, which introduce uncertainties such as over the choice of algorithm

(Pearson et al., 2007); and (iii) use of a test based on spatial overlap of suitable conditions across the landscape, rather than of overlap in n-dimensional niche space. Other tests also have limitations, including: being limited to two ecological dimensions (Broennimann et al's kernel smoothing approach); and being limited to a minimum of 10 occurrence records per environmental variable and having no associated significance test (Blonder et al's hypervolume method).

Appendix D

Appendix: Details on the *MO* metric and removal of environmental outliers from a presence-only dataset

D.1 Details on the *MO* metric and removal of environmental outliers from a presence-only dataset

The *MO* metric is a presence-only statistic which gives the same weight to all the occurrences. In practice, the environmental conditions at each occurrence record are plotted separately for each population on a real-value continuous vector bound by the maximum and minimum values of the combined niche breadth of the two entities for each variable (Figure D.1). The overlap for each variable (niche axis) is calculated as the proportion of the combined niche breadth that is shared between the two populations (Figure D.1). The proportions are then averaged over all of the dimensions in order to calculate an overall measure of niche overlap (Figure D.1). Thus the metric ranges from 1, when there is complete niche overlap on all axes, to 0 when there is no niche overlap on any axis.

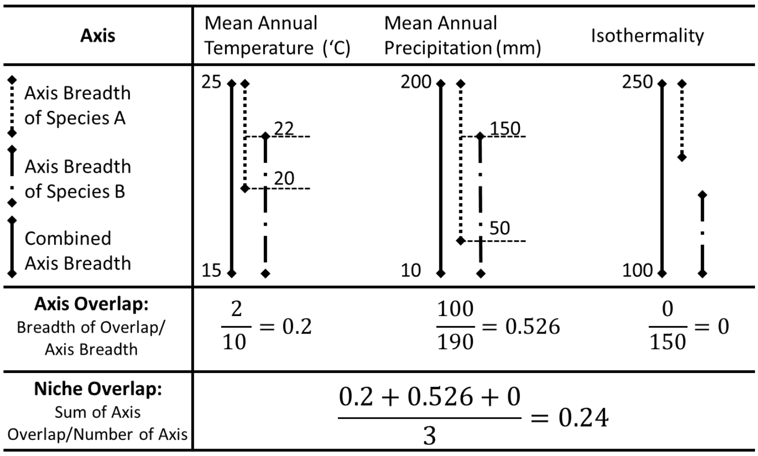

Figure D.1 A theoretical representation of how the *MO* metric of niche overlap is calculated for two species (A and B) using three bioclimatic variables. The environmental breadth of each axis for each species is obtained by extracting the environmental conditions from a presence-only dataset for the two species. In this case, the observed niche overlap is 0.24.

This means that occurrences which are in extreme environments, because they are either erroneous points or sink populations, may inflate our measure of niche overlap. In order to reduce this error, we have developed a function ('trim.occ') that identifies the points that represent extremes within the niche space of all occurences and removes the occurrences that are outside the $5-95^{th}$ range in the niche space.

We identify these outliers by using a multi-dimensional approach based on Mahalanobis distances (Farber and Kadmon, 2003). In this method, the climatic combinations found in the sites where the species is known to occur are given a Mahalanobis distance in relation to a vector describing the mean conditions found within the dataset, assuming that it describes the 'optimal' climatic niche of the species (Farber and Kadmon, 2003). In this way, any sites which have environmental conditions that are very different from the mean climatic niche can be identified and removed. This approach has several advantages over rectilinear approaches such as BIOCLIM because it accounts for correlations between variables, better addresses the concept of central tendency of niche theory, and uses the whole dataset (Farber and Kadmon, 2003). We replicated the methodology outlined by Farber and Kadmon (2003) in order to identify the points whose Mahalanobis distances from the mean niche conditions are outside the $5-95^{th}$ percentile of all distances.

We tested the effect of trimming the dataset for a pair of newts, which represents a sister pair with a high number of occurrences with potentially low data quality, and a pair of lemurs, which is a carefully curated dataset with few occurrence points of high quality. We repeated the RTR significance test with (i) trimming of the dataset, and (ii) the original dataset as outlined in the main text. We ran these tests for 1,000 null replicates, which were sufficient to reach stability of model outputs (i.e., no significant change in p-value) (Figure D.3b-d; Figure D.4b-d). We find no significant differences in the outputs (Figure D.3; Figure D.4a-c) and highlight the usefulness of using both approaches to increase confidence in the interpretation of the results.

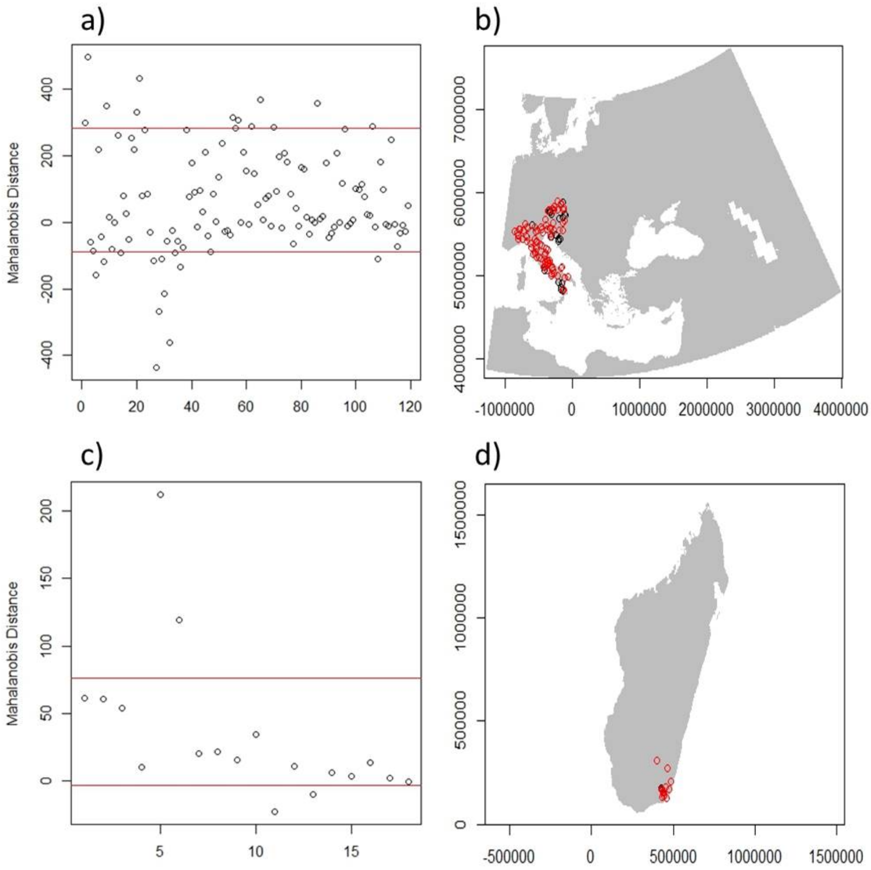

Figure D.2 Examples of trimmed datasets using$5-95^{th}$ percentiles for Mahalanobis distances in environmental hyperspace for the crested newt *Triturus carnifex* (a-b) and the lemur *Eulemur collaris* (c-d). Red lines indicates the 5^{th} and 95^{th} thresholds and environmental outliers are annotated with black dots in the maps.

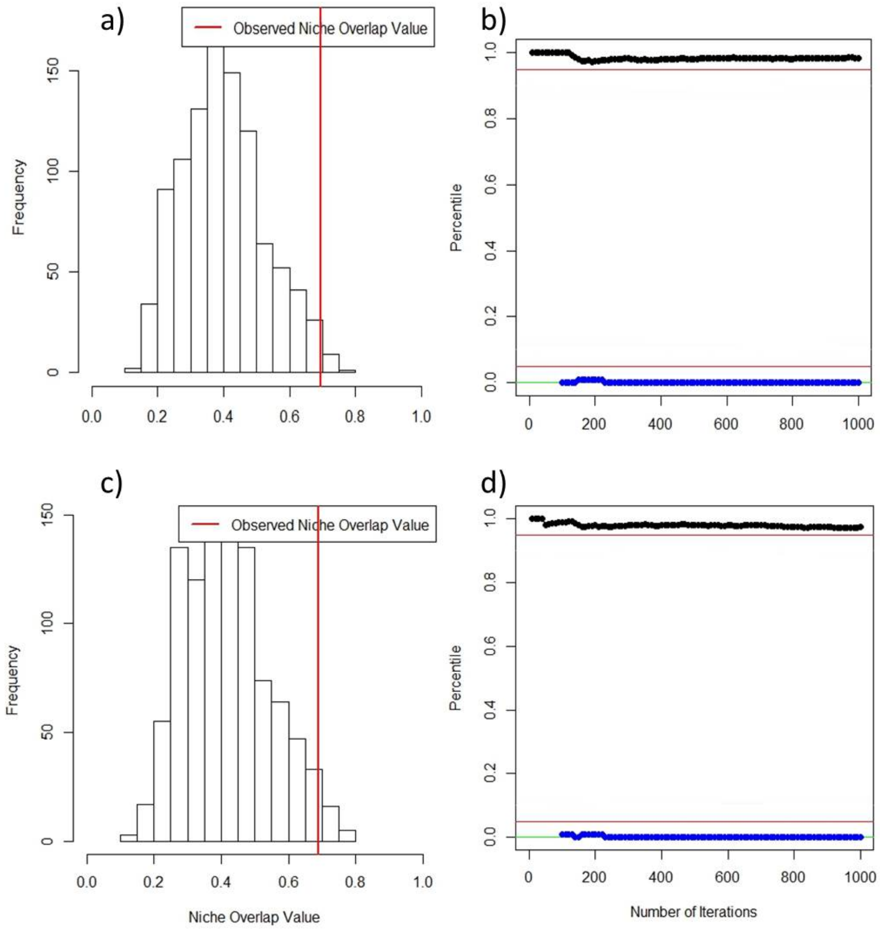

Figure D.3 Comparison of outputs from a trimmed occurrence dataset (a-b) and a non-trimmed occurrence dataset (c-d) for the pair of newts *T. carnifex - T.macedonicus.* For the trimmed dataset, the observed niche overlap is 0.695308 with a 95^{th} quantile threshold of 0.636262 and a p-value of 0.015. For the non-trimmed dataset, the observed niche overlap is 0.6901551, with a 95^{th} quantile threshold of 0.6561191 and a p-value of 0.026. Black circles refer to location of observed value in relation to null library in 10 repeat increments. Blue circles measure the differences in the p-value as a measure of output stability. Green line refers to no change in p-value. Red line indicates the threshold of significant for phylogenetic niche conservation or divergence at the upper or lower 5% tail of the null library respectively.

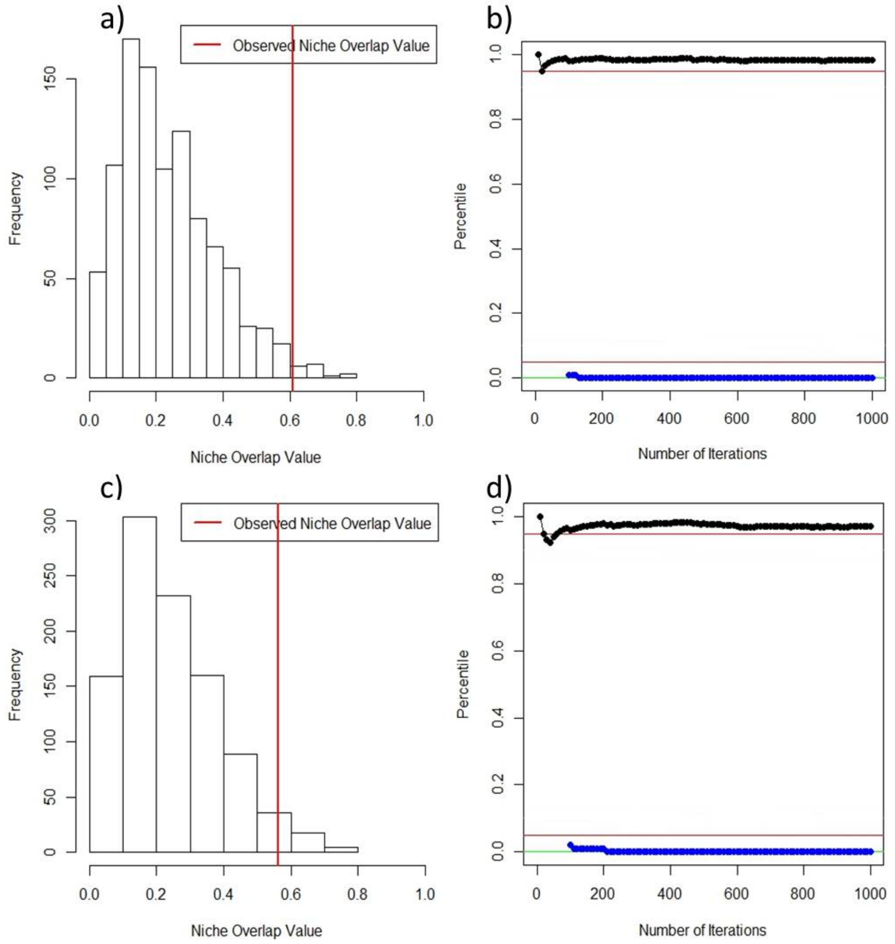

Figure D.4 Comparison of outputs from a trimmed occurrence dataset (a-b) and a non-trimmed occurrence dataset (c-d) for the pair of lemurs *E. collaris - E.cinereiceps.* For the trimmed dataset, the observed niche overlap is 0.6080928 with a 95^{th} quantile threshold of 0.5103819 and a p-value of 0.015. For the non-trimmed dataset, the observed niche overlap is 0.5617278 with a 95^{th} quantile threshold of 0.5143705 and a p-value of 0.028. Black circles refer to location of observed value in relation to null library in 10 repeat increments. Blue circles measure the differences in the p-value as a measure of output stability. Green line refers to no change in p-value. Red line indicates the threshold of significant for phylogenetic niche conservation or divergence at the upper or lower 5% tail of the null library respectively.

Appendix E

Appendix: Constructing virtual scenarios for testing the performance of niche overlap metrics

E.1 Constructing virtual scenarios for testing the performance of niche overlap metrics

We compared the metrics using simulated (artificial) species for which the degree of PND and PNC can be precisely defined (Broennimann et al., 2012). We used simulated species because case studies from nature are likely to generate a biased comparison of the performance of different approaches due to sampling errors and other biases (Broennimann et al., 2012). We compared the different metrics by assessing the performance of each test for the exact same ecological niche and species distributions. As we had full control over the virtual climatic layers, we assembled a 'known truth' scenario against which we could compare the actual outcomes from the metrics. The aim is to test the ability of each metric to correctly distinguish the properties of the known scenario.

We simulated the environmental niche overlap of two species in a two dimensional gridded domain of 100 x 100 grid cells (following Broennimann et al. 2012 and Colwell et al. 2009) (Fig SE.1). We restricted the analysis to two environmental variables with an opposing environmental gradient (layer 1 increasing gradient from left to right, layer 2 increasing gradient from the bottom to top, Fig. E.1). The simulations follow this method:

1) Create two virtual environmental layers of 100x100 gird cells with opposing environmental gradients (Fig. E.1)

2) Create two equal squared distributions of 30x30 grid cells each (entities)

3) Place both entities on top of each other located in the bottom left corner of the virtual layers, so that the bottom left corner of each entity are on top of each other (Fig. E.1a);

4) Move one entity step-wise (bottom left corner of one entity is moved to the right or to the top or both (i.e., diagonally) one or more grid cell) while the other remains fixed at the bottom left corner of the virtual layers (Fig. E.1b-d)

5) At each step, in either direction (right or up) or both, the observed niche overlap was measured using the 4 metrics outlined in the main text.

The translocation of one entity away from the other across the environmental gradient meant that there was increasing ecological divergence with increasing geographical separation, so in our ‘known truth’ scenario (Fig. E.2), the two entities occupy identical niches only when the two ranges completely overlap, both geographically and environmentally (no shift in distribution, Fig. E.1a, Fig. E.2). A range of niche overlap values >0 and <1 is expected when they partially overlap geographically and environmentally in both layers (30 or less steps to the right and to the top, Fig. E.1b, Fig. E.2) and between 0.5 and 0 when the entities only overlap in one environmental axis but with no geographic overlap (30 or more steps to the right or to the top, Fig. E.1c; Fig. E.2). This is because the maximum cumulative overlap will be 1 over 2 environmental axis (complete overlap in one axis but not the other), thus maximum overall niche overlap is 0.5 (see Fig. E.2). The two entities occupy completely different ecological niches (i.e., divergent niches) when their ranges do not overlap both geographically and environmentally (i.e., 30 or more steps to the right and to the top: Fig. E.1d, Fig. E.2).

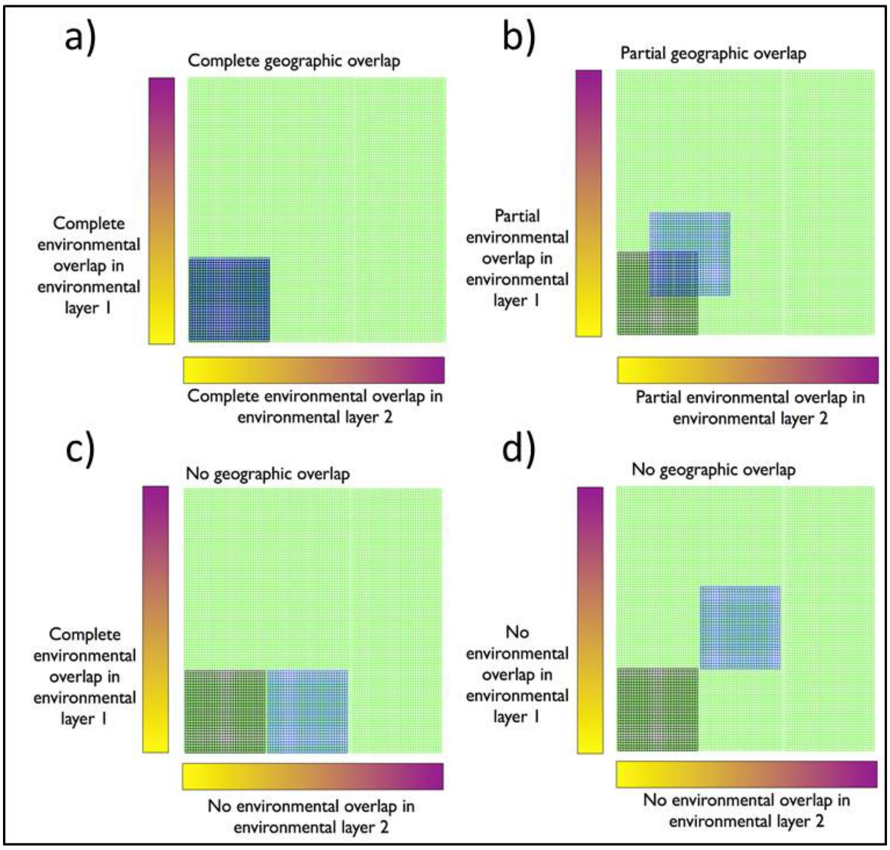

Figure E.1 Different outcomes expected from the 'known truth' scenario. The entities are both 30x30 grid cells, with one (blue square) moving either to the right, to the top or both in a 100x100 grid environment (green square), while the other entity (black square) remains stationary. The axes indicate the environmental gradients for layers 1 and 2, with layer 1 showing a vertical (latitudinal) gradient and layer 2 demonstrating a horizontal (longitudinal) gradient.

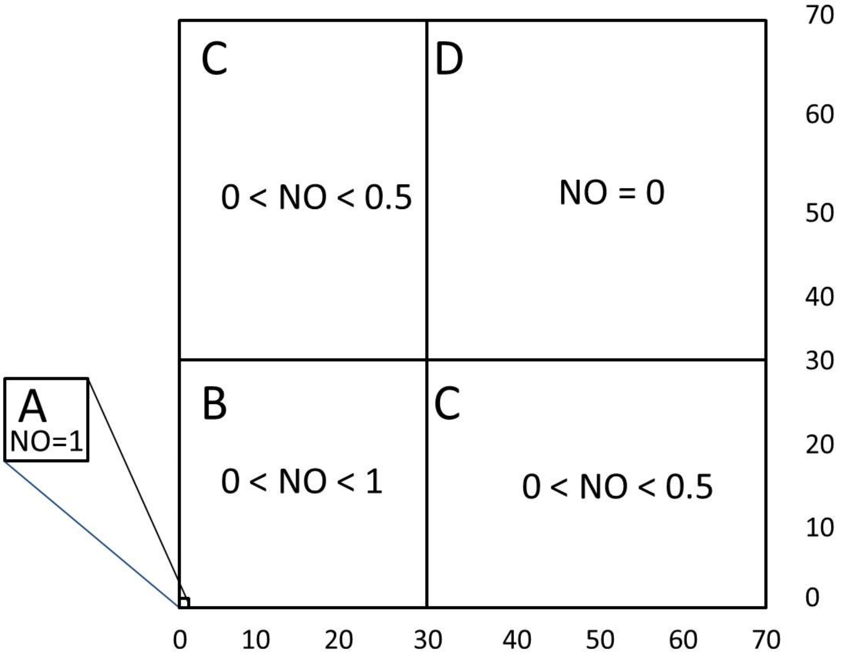

Figure E.2 Expected ranges of niche overlap (NO) in each possible scenario described in Figure E.1, plotted spatially in a 2-Dimensional 70x70 grid representing the 'known truth' scenario (100 grid cells minus 30 grid cells of the entity, as the translocated entity cannot move beyond the 100x100 boundary). Region A represents the scenario of complete niche overlap, as illustrated in Fig. E.1a. Region B represents all scenarios of partial geographic and environmental overlap, exemplified in Fig. E.1b. Regions C represent all scenarios of no geographic overlap but complete overlap in one environmental axis, exemplified in Fig. E.1c. Region D represents all possible scenarios of no geographic and no environmental overlap, thus complete niche divergence as exemplified in Fig. E.1d. The same outline is presented for the actual results from the analysis of niche overlap. Observed results for the four alternative metrics tested are presented in Fig. 2.3

Appendix F

Appendix: Environmental variables for real case studies

F.1 Environmental variables used in the real case studies

Table F.1 Environmental variables obtained from the WorldClim database version 1.4 (Hijmans et al., 2005) at a resolution of 30 arc-seconds (±1 km^2). The variables used in each case study are marked with an X.

Bioclimatic variable	(Wielstra et al., 2012)	(Soto-Centeno et al., 2013)	(Blair et al., 2013)
BIO 1 = Annual Mean Temperature			
BIO 2 = Mean Diurnal Range (Mean of monthly (max temp - min temp))		X	
BIO 3 = Isothermality (BIO2/BIO7) (x 100)			X
BIO 4 = Temperature Seasonality (standard deviation x 100)			X
BIO 5 = Max Temperature of Warmest Month		X	
BIO 6 = Min Temperature of Coldest Month			X
BIO 7 = Temperature Annual Range (BIO5-BIO6)			X
BIO 8 = Mean Temperature of Wettest Quarter		X	
BIO 9 = Mean Temperature of Driest Quarter		X	
BIO 10 = Mean Temperature of Warmest Quarter	X		
BIO 11 = Mean Temperature of Coldest Quarter	X		
BIO 12 = Annual Precipitation		X	
BIO 13 = Precipitation of Wettest Month		X	
BIO 14 = Precipitation of Driest Month			
BIO 15 = Precipitation Seasonality (Coefficient of Variation)	X	X	
BIO 16 = Precipitation of Wettest Quarter	X	X	
BIO 17 = Precipitation of Driest Quarter	X		X
BIO 18 = Precipitation of Warmest Quarter			
BIO 19 = Precipitation of Coldest Quarter			

Appendix G

Appendix: Assessing potential spatial bias in null replicates from the RTR significance test

G.1 Assessing potential spatial bias in null replicates from the RTR significance test

The RTR significance test is distinct from alternative methods due to its ability to maintain the spatial autocorrelation within the replicates. This however might come at a cost of the replicates only being able to fit certain parts of the background region (e.g., in the case of the crested newts, high sampling in the northern regions of the Balkans because of the Aegaean Sea in the south). This potential sampling bias will therefore bias the environments being sampled in the null library. For range-restricted species, however, this sampling bias is less likely to occur as there is more surface area which the replicates can fit in. We therefore argue that the problem of spatial bias is case specific.

We tested the presence of spatial sampling bias by annotating which grid cells were being sampled at each repeat. This was done by introducing two functions to our R code ('rtr.bias' and 'rtr.bias.map'). 'rtr.bias' annotates the grid cells being sampled for each repeat (as measurements of niche overlap are taken directly from the grid cells) while 'rtr.bias.map' stores all the grid cells and produces a heatmap illustrating the frequency of the grid cells being sampled using the function 'levelplots' from the R package 'rasterVis' (Perpiñán and Hijmans, 2016). In this way it is possible to visualise which areas are more often being included in the null replicated library. We tested for spatial sampling bias for a pair of crested newts, representing a wide-ranging species living in a topographically unbalanced background region due to the presence of a large body of water in the southern parts of its background region. We also tested a range-restricted pair of lemurs, which we suspect may have fewer tendencies for spatial bias given the absence of problematic regions (e.g., large bodies of water) within the island of Madagascar. For each case study, we ran for 10,000 null replicates.

We find some spatial bias in the case of the newts, with northern environments being more sampled than southern environments (Fig. G.1a). In the case of the lemurs, the entire background region is sampled evenly, with a slight mid-domain effect (Fig. G.1b).

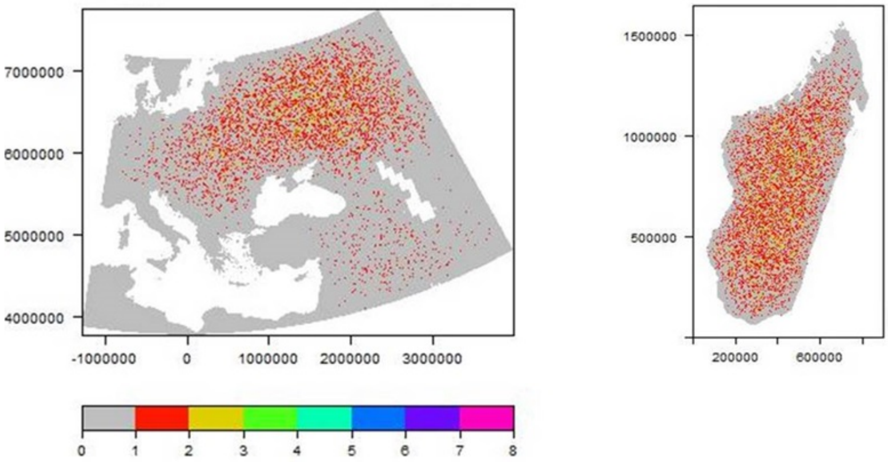

Figure G.1 Spatial bias in null replicates (n=10,000) for a sister pair of crested newts (*T. carnifex -T. macedonicus*) (a) and for a sister pair of lemurs (*E. collaris - E.cinereiceps*) (b). Colour legend represents the sampling frequency of a grid cell.

Appendix H

Appendix: T-test results for changes in ROA, variability within virtual layers and observed niche overlap due to different treatments

H.1 T-test result for ROA

Table H.1 Results from T-test on changes in ROA due to differences in sampling bias

Strength of Environmental Spatial Autocorrelation	Size of Background Region	Strength of Sampling Bias	t	df	p-value
Low	Large	Unbiased:Low	2.9761	197.456	0.003284
Low	Large	Unbiased:Medium	6.658	196.669	<0.0
Low	Large	Unbiased:High	11.8833	190.463	<0.0
Low	Large	Low:Medium	3.9709	194.498	0.0001007
Low	Large	Low:High	9.4781	186.456	<0.0
Low	Large	Medium:High	5.4698	195.254	<0.0
Low	Medium	Unbiased:Low	2.9761	197.456	0.003284
Low	Medium	Unbiased:Medium	6.658	196.669	<0.0
Low	Medium	Unbiased:High	11.8833	190.463	<0.0
Low	Medium	Low:Medium	3.9709	194.498	0.0001007
Low	Medium	Low:High	9.4781	186.456	<0.0
Low	Medium	Medium:High	5.4698	195.254	<0.0
Low	Low	Unbiased:Low	2.9761	197.456	0.003284
Low	Low	Unbiased:Medium	6.658	196.669	<0.0
Low	Low	Unbiased:High	11.8833	190.463	<0.0
Low	Low	Low:Medium	3.9709	194.498	0.0001007
Low	Low	Low:High	11.6618	186.456	<0.0
Low	Low	Medium:High	5.4698	195.254	<0.0
Medium	Large	Unbiased:Low	0.7338	197.172	0.4639
Medium	Large	Unbiased:Medium	1.593	197.595	0.1128
Medium	Large	Unbiased:High	3.5125	197.994	0.0005499
Medium	Large	Low:Medium	0.8247	197.924	0.4105
Medium	Large	Low:High	2.6605	197.03	0.008445
Medium	Large	Medium:High	1.8346	197.494	0.06807
Medium	Medium	Unbiased:Low	0.7338	197.172	0.4639
Medium	Medium	Unbiased:Medium	1.593	197.595	0.1128

Medium	Medium	Unbiased:High	3.5125	197.994	0.0005499
Medium	Medium	Low:Medium	0.8247	197.924	0.4105
Medium	Medium	Low:High	2.6605	197.03	0.008445
Medium	Medium	Medium:High	1.8346	197.494	0.06807
Medium	Low	Unbiased:Low	0.7338	197.172	0.4639
Medium	Low	Unbiased:Medium	1.593	197.595	0.1128
Medium	Low	Unbiased:High	3.5125	197.994	0.0005499
Medium	Low	Low:Medium	0.8247	197.924	0.4105
Medium	Low	Low:High	2.6605	197.03	0.008445
Medium	Low	Medium:High	1.8346	197.494	0.06807
High	Large	Unbiased:Low	0.8789	197.244	0.3805
High	Large	Unbiased:Medium	1.5167	194.824	0.131
High	Large	Unbiased:High	2.7534	190.099	0.006469
High	Large	Low:Medium	0.6321	197.134	0.5281
High	Large	Low:High	1.885	193.991	0.06092
High	Large	Medium:High	1.2765	196.796	0.2033
High	Medium	Unbiased:Low	0.8789	197.244	0.3805
High	Medium	Unbiased:Medium	1.5167	194.824	0.131
High	Medium	Unbiased:High	2.7534	190.099	0.006469
High	Medium	Low:Medium	0.6321	197.134	0.5281
High	Medium	Low:High	1.885	193.991	0.06092
High	Medium	Medium:High	1.2765	196.796	0.2033
High	Low	Unbiased:Low	0.8789	197.244	0.3805
High	Low	Unbiased:Medium	1.5167	194.824	0.131
High	Low	Unbiased:High	2.7534	190.099	0.006469
High	Low	Low:Medium	0.6321	197.134	0.5281
High	Low	Low:High	1.885	193.991	0.06092
High	Low	Medium:High	1.2765	196.796	0.2033

Table H.2 Results from T-test on changes in ROA due to differences in environmental spatial autocorrelation

Strength of Environmental Spatial Autocorrelation	Size of Background Region	Strength of Sampling Bias	t	df	p-value

Low:Medium	Large	Unbiased	20.0073	175.617	<0.0
Low:High	Large	Unbiased	22.6941	174.544	<0.0
Medium:High	Large	Unbiased	2.4397	197.976	0.01558
Low:Medium	Medium	Unbiased	20.0073	175.617	<0.0
Low:High	Medium	Unbiased	22.6941	174.544	<0.0
Medium:High	Medium	Unbiased	2.4397	197.976	0.01558
Low:Medium	Low	Unbiased	20.0073	175.617	<0.0
Low:High	Low	Unbiased	22.6941	174.544	<0.0
Medium:High	Low	Unbiased	2.4397	197.976	0.01558
Low:Medium	Large	Low	18.0153	164.05	<0.0
Low:High	Large	Low	22.6816	175.466	<0.0
Medium:High	Large	Low	2.5305	195.399	0.01218
Low:Medium	Medium	Low	18.0153	164.05	<0.0
Low:High	Medium	Low	22.6816	175.466	<0.0
Medium:High	Medium	Low	2.5305	195.399	0.01218
Low:Medium	Low	Low	10.4962	149.686	<0.0
Low:High	Low	Low	15.9808	137.379	<0.0
Medium:High	Low	Low	2.5305	195.399	0.01218
Low:Medium	Large	Medium	15.4202	179.174	<0.0
Low:High	Large	Medium	19.8284	192.316	<0.0
Medium:High	Large	Medium	2.3347	192.984	0.02059
Low:Medium	Medium	Medium	15.4202	179.174	<0.0
Low:High	Medium	Medium	19.8284	192.316	<0.0
Medium:High	Medium	Medium	2.3347	192.984	0.02059
Low:Medium	Low	Medium	15.4202	179.174	<0.0
Low:High	Low	Medium	19.8284	192.316	<0.0
Medium:High	Low	Medium	2.3347	192.984	0.02059
Low:Medium	Large	High	12.5212	192.756	<0.0
Low:High	Large	High	15.6523	197.887	<0.0
Medium:High	Large	High	1.6301	191.234	0.1047
Low:Medium	Medium	High	12.5212	192.756	<0.0
Low:High	Medium	High	15.6523	197.887	<0.0
Medium:High	Medium	High	1.6301	191.234	0.1047
Low:Medium	Medium	High	12.5212	192.756	<0.0
Low:High	Medium	High	15.6523	197.887	<0.0
Medium:High	Medium	High	1.6301	191.234	0.1047

Table H.3 Results from T-test on changes in ROA due to differences in size of background region

Strength of Environmental Spatial Autocorrelation	Size of Background Region	Strength of Sampling Bias	t	df	p-value
Low	Low:Medium	Unbiased	22.0628	163.894	<0.0
Low	Low:Large	Unbiased	44.1569	117.326	<0.0
Low	Medium:Large:	Unbiased	29.7343	145.588	<0.0
Medium	Low:Medium	Unbiased	7.1272	163.894	<0.0
Medium	Low:Large	Unbiased	14.2644	117.326	<0.0
Medium	Medium:Large:	Unbiased	9.6053	145.588	<0.0
High	Low:Medium	Unbiased	5.9104	163.894	<0.0
High	Low:Large	Unbiased	11.8292	117.326	<0.0
High	Medium:Large:	Unbiased	7.9655	145.588	<0.0
Low	Low:Medium	Low	21.8182	163.894	<0.0
Low	Low:Large	Low	43.6672	117.326	<0.0
Low	Medium:Large:	Low	29.4046	145.588	<0.0
Medium	Low:Medium	Low	6.3456	163.894	<0.0
Medium	Low:Large	Low	12.7002	117.326	<0.0
Medium	Medium:Large:	Low	8.552	145.588	<0.0
High	Low:Medium	Low	5.8626	163.894	<0.0
High	Low:Large	Low	11.7336	117.326	<0.0
High	Medium:Large:	Low	7.9011	145.588	<0.0
Low	Low:Medium	Medium	17.3132	163.894	<0.0
Low	Low:Large	Medium	34.6509	117.326	<0.0
Low	Medium:Large:	Medium	23.3332	145.588	<0.0
Medium	Low:Medium	Medium	6.0804	163.894	<0.0
Medium	Low:Large	Medium	12.1695	117.326	<0.0
Medium	Medium:Large:	Medium	8.1947	145.588	<0.0
High	Low:Medium	Medium	5.9579	163.894	<0.0
High	Low:Large	Medium	11.9243	117.326	<0.0

High	Medium:Large:	Medium	8.0296	145.588	<0.0
Low	Low:Medium	High	12.9414	163.894	<0.0
Low	Low:Large	High	25.9011	117.326	<0.0
Low	Medium:Large:	High	17.4413	145.588	<0.0
Medium	Low:Medium	High	5.5128	163.894	<0.0
Medium	Low:Large	High	11.0334	117.326	<0.0
Medium	Medium:Large:	High	7.4296	145.588	<0.0
High	Low:Medium	High	5.8198	163.894	<0.0
High	Low:Large	High	11.6479	117.326	<0.0
High	Medium:Large:	High	7.8435	145.588	<0.0

H.2 T-test results: Variability within virtual layers

Table H.4 Results from T-test on changes in the variability within the virtual layer due to differences in size of background region and environmental spatial autocorrelation

Strength of Environmental Spatial Autocorrelation	Size of Background Region	t	df	p-value
Low	Low:Medium	3.1321	777.986	0.001801
Low	Low:Large	3.1321	777.986	0.001801
Low	Medium:Large:	0	798	1
Medium	Low:Medium	7.4571	774.282	<0.0
Medium	Low:Large	7.4571	774.282	<0.0
Medium	Medium:Large:	0	798	1
High	Low:Medium	8.3279	797.401	<0.0
High	Low:Large	8.3279	797.401	<0.0
High	Medium:Large:	0	798	1
Low:Medium	Large	7.6616	543.657	<0.0
Low:High	Large	22.6305	487.51	<0.0
Medium:High	Large	13.7687	750.644	<0.0
Low:Medium	Medium	7.6616	543.657	<0.0
Low:High	Medium	22.6305	487.51	<0.0

Medium:High	Medium	13.7687	750.644	<0.0
Low:Medium	Low	13.7692	539.647	<0.0
Low:High	Low	32.2635	526.426	<0.0
Medium:High	Low	14.9444	795.819	<0.0

H.3 T-test results: Observed niche overlap

Table H.5 Results from T-test on changes in observed niche overlap due to differences in sampling bias and environmental spatial autocorrelation

Strength of Environmental Spatial Autocorrelation	Strength of Sampling Bias	t	df	p-value
Low	Unbiased:Low	0.1362	597.507	0.8917
Low	Unbiased:Medium	1.4323	589.667	0.1526
Low	Unbiased:High	1.0519	590.773	0.2933
Low	Low:Medium	1.2859	593.147	0.199
Low	Low:High	0.9097	593.993	0.3634
Low	Medium:High	0.3658	597.958	0.7146
Medium	Unbiased:Low	1.9898	586.806	0.04707
Medium	Unbiased:Medium	4.6004	588.402	<0.0
Medium	Unbiased:High	7.8454	576.379	<0.0
Medium	Low:Medium	2.4341	597.933	0.01522
Medium	Low:High	5.5718	596.057	<0.0
Medium	Medium:High	3.2367	595.277	0.001277
High	Unbiased:Low	2.6811	590.092	0.007542
High	Unbiased:Medium	2.3515	596.919	0.01902
High	Unbiased:High	6.089	596.047	<0.0
High	Low:Medium	0.4133	594.78	0.6795
High	Low:High	3.1329	595.932	0.001816
High	Medium:High	3.6794	597.87	0.0002549
Low:Medium	Unbiased	8.7168	526.464	<0.0
Low:High	Unbiased	21.0661	466.162	<0.0
Medium:High	Unbiased	12.0166	574.338	<0.0
Low:Medium	Low	9.9891	493.629	<0.0

Low:High	Low	22.2328	442.946	<0.0
Medium:High	Low	11.6703	579.011	<0.0
Low:Medium	Medium	11.7009	523.808	<0.0
Low:High	Medium	21.6213	488.125	<0.0
Medium:High	Medium	9.48	589.592	<0.0
Low:Medium	High	15.2172	501.096	<0.0
Low:High	High	26.1563	481.395	<0.0
Medium:High	High	9.8664	595.337	<0.0

Appendix I

Appendix: Reference list for occurrence records obtained for each species used in this thesis

I.1 Reference list for occurrence records obtained for each species used in this thesis

Table I.1 Reference list for occurrence records obtained for each species used in this thesis

Species	References
Brookesia nasus	Brown et al. 2014, GBIF(www.gbif.org)
Brookesia lolontany	Raxworthy and Nussbaum 1995; Carpenter and Robson 2005; Brown et al. 2014
Brookesia ambreensis	Raxworthy and Nussbaum 1995; Brown et al. 2014, GBIF(www.gbif.org)
Brookesia antakarana	Raxworthy and Nussbaum 1995; Brown et al. 2014
Brookesia griveaudi	Raxworthy and Nussbaum 1995; Schimmenti and Jesu 1996; Carpenter and Robson 2005; Brown et al. 2014, GBIF(www.gbif.org)
Brookesia valerieae	Carpenter and Robson 2005; Brown et al. 2014
Brookesia betschi	Raxworthy and Nussbaum 1995; Andreone and Randrianirina 2000; Brown et al. 2014
Brookesia lineata	Raxworthy and Nussbaum 1995; Carpenter and Robson 2005; Andreone et al. 2009; Brown et al. 2014
Brookesia thieli	Jenkins et al. 1999, 2003; Carpenter and Robson 2005; Brown et al. 2014, GBIF(www.gbif.org)
Brookesia vadoni	Brown et al. 2014,GBIF(www.gbif.org)
Brookesia superciliaris	Raxworthy and Nussbaum 1995; Andreone and Randrianirina 2000; Andreone et al. 2003; Carpenter and Robson 2005; Bora et al. 2007; Rabearivony et al. 2007; Gehring et al. 2010; Brown et al. 2014, GBIF(www.gbif.org)
Brookesia therezieni	Andreone and Randrianirina 2000; Brown et al. 2014, GBIF(www.gbif.org)
Brookesia minima	Raxworthy and Nussbaum 1995; Jenkins et al. 2003; Carpenter and Robson 2005; Andreone et al. 2009; Brown et al. 2014

Brookesia tuberculata	Carpenter and Robson 2005; Brown et al. 2014, GBIF(www.gbif.org)
Brookesia dentata	Brown et al. 2014
Brookesia exarmata	Schimmenti and Jesu 1996; Randrianantoandro et al. 2008; Brown et al. 2014, GBIF(www.gbif.org)
Brookesia karchei	Carpenter and Robson 2005; Brown et al. 2014
Brookesia peyrierasi	Carpenter and Robson 2005; Randrianantoandro et al. 2008; Gehring et al. 2010; Brown et al. 2014, GBIF(www.gbif.org)
Uroplatus fimbriatus	Bauer and Russell 1989; Andreone et al. 2001; Pearson and Raxworthy 2009; Gehring et al. 2010; Ratsoavina et al. 2013; Brown et al. 2014, GBIF(www.gbif.org)
Uroplatus giganteus	Ratsoavina et al. 2013; Brown et al. 2014
Uroplatus alluaudi	Bauer and Russell 1989; Pearson and Raxworthy 2009; Ratsoavina et al. 2013; Brown et al. 2014
Uroplatus pietschmanni	Ratsoavina et al. 2013; Brown et al. 2014, GBIF(www.gbif.org)
Uroplatus ebenaui	Bauer and Russell 1989; Andreone and Randrianirina 2000; D'Cruze et al. 2007; Andreone et al. 2009; Bora et al. 2009; Pearson and Raxworthy 2009; Brown et al. 2014, GBIF(www.gbif.org)
Uroplatus phantasticus	Bauer and Russell 1989; Nussbaum and Raxworthy 1994, 1995; Ramanamanjato et al. 2002; Andreone and Aprea 2006; Pearson and Raxworthy 2009; Ratsoavina et al. 2013; Brown et al. 2014, GBIF(www.gbif.org)
Uroplatus henkeli	Bauer and Russell 1989; Bora et al. 2009; Pearson and Raxworthy 2009; Ratsoavina et al. 2013; Brown et al. 2014, GBIF(www.gbif.org)
Uroplatus sikorae	Bauer and Russell 1989; Pearson and Raxworthy 2009; Gehring et al. 2010; Ratsoavina et al. 2013; Brown et al. 2014, GBIF(www.gbif.org)
Uroplatus guentheri	Bauer and Russell 1989; Ratsoavina et al. 2013; Brown et al. 2014

Uroplatus malahelo	Pearson and Raxworthy 2009; Ratsoavina et al. 2013; Brown et al. 2014, GBIF(www.gbif.org)
Furcifer antimena	Karsten et al. 2009; Brown et al. 2014, GBIF(www.gbif.org)
Furcifer labordi	Pearson and Raxworthy 2009; Brown et al. 2014, GBIF(www.gbif.org)
Furcifer oustaleti	Pearson and Raxworthy 2009; Brown et al. 2014, GBIF(www.gbif.org)
Furcifer verrucosus	Pearson and Raxworthy 2009; Brown et al. 2014, GBIF(www.gbif.org)
Furcifer angeli	Brown et al. 2014, GBIF(www.gbif.org)
Furcifer pardalis	Pearson and Raxworthy 2009; Brown et al. 2014, GBIF(www.gbif.org)
Furcifer petteri	Pearson and Raxworthy 2009; Brown et al. 2014, GBIF(www.gbif.org)
Furcifer willsii	Pearson and Raxworthy 2009; Brown et al. 2014, GBIF(www.gbif.org)
Furcifer verrucosus A	Florio and Raxworthy 2016
Furcifer verrucosus B	Florio and Raxworthy 2016
Phelsuma breviceps	D'Cruze et al. 2007; Pearson and Raxworthy 2009; Rakotondravony and Goodman 2011; Brown et al. 2014, GBIF(ww.gbif.org)
Phelsuma mutabilis	Andreone et al. 2001; Ramanamanjato et al. 2002; D'Cruze and Sabel 2005; Bora et al. 2009; Pearson and Raxworthy 2009; Gehring et al. 2010; Glaw et al. 2010; Labanowski and Lowin 2011; Brown et al. 2014, GBIF(www.gbif.org)
Phelsuma dubia	Raxworthy and Nussbaum 1994; Andreone et al. 2001; Pearson and Raxworthy 2009; Brown et al. 2014, GBIF(www.gbif.org)
Phelsuma ravenala	Pearson and Raxworthy 2009; Dubos et al. 2014
Phelsuma quadriocellata	Raxworthy and Nussbaum 1993; Ramanamanjato et al. 2002; Pearson and Raxworthy 2009; Gardner and Jasper 2010; Gehring et al. 2010; Brown et al. 2014, GBIF(ww.gbif.org)

Phelsuma antanosy	Ramanamanjato et al. 2002; Lehtinen et al. 2003; Pearson and Raxworthy 2009; Rakotondravony and Goodman 2011; Brown et al. 2014
Phelsuma berghofi	Pearson and Raxworthy 2009; Brown et al. 2014, GBIF(www.gbif.org)
Phelsuma malamakibo	Pearson and Raxworthy 2009; Brown et al. 2014
Lygodactylus verticillatus	Brown et al. 2014, GBIF(www.gbif.org)
Lygodactylus heterurus	Brown et al. 2014, GBIF(www.gbif.org)
Lygodactylus guibei	Brown et al. 2014, GBIF(www.gbif.org)
Lygodactylus miops	Brown et al. 2014, GBIF(www.gbif.org)
Lygodactylus mirabilis	Brown et al. 2014, GBIF(www.gbif.org)
Lygodactylus pictus	Brown et al. 2014, GBIF(www.gbif.org)
Lygodactylus arnoulti	Brown et al. 2014
Lygodactylus pauliani	Brown et al. 2014
Zonosaurus aeneus	Brown et al. 2014, GBIF(www.gbif.org)
Zonosaurus rufipes	Brown et al. 2014, GBIF(www.gbif.org)
Zonosaurus quadrilineatus	Brown et al. 2014, GBIF(www.gbif.org)
Zonosaurus trilineatus	Brown et al. 2014, GBIF(www.gbif.org)
Zonosaurus haraldmeieri	Brown et al. 2014
Zonosaurus madagascariensis	Brown et al. 2014, GBIF(www.gbif.org)
Zonosaurus anelanelany	Brown et al. 2014
Zonosaurus laticaudatus	Brown et al. 2014, GBIF(www.gbif.org)
Amphiglossus melanurus	Brown et al. 2014, GBIF(www.gbif.org)
Amphiglossus ornaticeps	Brown et al. 2014, GBIF(www.gbif.org)
Amphiglossus mandokava	Brown et al. 2014, GBIF(www.gbif.org)
Amphiglossus tanysoma	Brown et al. 2014
Amphiglossus frontoparietalis	Brown et al. 2014, GBIF(www.gbif.org)
Amphiglossus punctatus	Brown et al. 2014, GBIF(www.gbif.org)
Calumma nasutum	Brown et al. 2014, GBIF(www.gbif.org)
Calumma boettgeri	Brown et al. 2014, GBIF(www.gbif.org)
Calumma furcifer	Brown et al. 2014, GBIF(www.gbif.org)
Calumma gastrotaenia	Brown et al. 2014, GBIF(www.gbif.org)
Calumma globifer	Brown et al. 2014, GBIF(www.gbif.org)
Calumma parsonii	Brown et al. 2014, GBIF(www.gbif.org)
Calumma brevicorne	Brown et al. 2014, GBIF(www.gbif.org)
Calumma tsaratananense	Brown et al. 2014

Calumma guibei	Brown et al. 2014
Calumma hilleniusi	Brown et al. 2014, GBIF(www.gbif.org)
Paroedura karstophila	Brown et al. 2014
Paroedura oviceps	Brown et al. 2014
Paroedura androyensis	Brown et al. 2014
Paroedura picta	Brown et al. 2014
Compsophis albiventris	Brown et al. 2014, GBIF(www.gbif.org)
Compsophis boulengeri	Brown et al. 2014, GBIF(www.gbif.org)
Oplurus fierinensis	Brown et al. 2014, GBIF(www.gbif.org)
Oplurus grandidieri	Brown et al. 2014, GBIF(www.gbif.org)
Pygomeles braconnieri	Brown et al. 2014
Pygomeles petteri	Brown et al. 2014
Tracheloptychus madagascariensis	Brown et al. 2014, GBIF(www.gbif.org)
Tracheloptychus petersi	Brown et al. 2014, GBIF(www.gbif.org)
Voeltzkowia lineata	Brown et al. 2014
Voeltzkowia rubrocaudata	Brown et al. 2014
Trachylepis aureopunctata	Brown et al. 2014, GBIF(www.gbif.org)
Trachylepis dumasi	Brown et al. 2014
Blaesodactylus antongilensis	Brown et al. 2014, GBIF(www.gbif.org)
Blaesodactylus sakalava	Brown et al. 2014, GBIF(www.gbif.org)
Geckolepis maculata	Brown et al. 2014, GBIF(www.gbif.org)
Geckolepis typica	Brown et al. 2014, GBIF(www.gbif.org)
Hemidactylus mabouia	GBIF(www.gbif.org)
Hemidactylus mercatorius	Brown et al. 2014, GBIF(www.gbif.org)
Thamnosophis lateralis	Brown et al. 2014, GBIF(www.gbif.org)
Thamnosophis stumpffi	Brown et al. 2014, GBIF(www.gbif.org)
Thamnosophis epistibes	Brown et al. 2014, GBIF(www.gbif.org)
Thamnosophis martae	Brown et al. 2014, GBIF(www.gbif.org)
Compsophis infralineatus	Brown et al. 2014, GBIF(www.gbif.org)
Compsophis laphystius	Brown et al. 2014, GBIF(www.gbif.org)
Leioheterodon modestus	Brown et al. 2014, GBIF(www.gbif.org)
Leioheterodon madagascariensis	Brown et al. 2014, GBIF(www.gbif.org)
Oplurus cuvieri	Brown et al. 2014, GBIF(www.gbif.org)
Oplurus cyclurus	Brown et al. 2014, GBIF(www.gbif.org)
Trachylepis elegans	Brown et al. 2014, GBIF(www.gbif.org)

Trachylepis madagascariensis	Brown et al. 2014, GBIF(www.gbif.org)
Liopholidophis dolicocercus	Brown et al. 2014, GBIF(www.gbif.org)
Liopholidophis sexlineatus	Brown et al. 2014, GBIF(www.gbif.org)
Dromicodryas bernieri	Brown et al. 2014, GBIF(www.gbif.org)
Dromicodryas quadrilineatus	Brown et al. 2014, GBIF(www.gbif.org)
Paroedura bastardi	Brown et al. 2014
Paroedura tanjaka	Brown et al. 2014
Madagascarophis colubrinus	Brown et al. 2014, GBIF(www.gbif.org)
Madagascarophis meridionalis	Brown et al. 2014