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FACTORS INFLUENCING THE DISTRIBUTION OF THE LUSITANIAN AND THE MEDITERRANEAN PINE VOLES (*MICROTUS LUSITANICUS* AND *M. DUODECIMCOSTATUS*) IN PORTUGAL: A MULTISCALE APPROACH

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NOTA PRÉVIA

Nos termos do nº1 do Artigo 40, Capítulo V, do Regulamento de Estudos Pós-Graduados da Universidade de Lisboa, publicado no *Diário da República* – II Série Nº 153, de 5 de Julho de 2003, na elaboração desta dissertação foi efectuado o aproveitamento total de resultados de trabalhos já publicados ou submetidos para publicação em revistas internacionais indexadas e com arbitragem científica, os quais integram alguns dos capítulos da presente tese. Tendo em conta que os referidos trabalhos foram realizados em colaboração com outros autores, o candidate esclarece que, em todos eles, liderou e participou activamente na sua concepção, recolha, análise e discussão de resultados, bem como na escrita dos artigos.

Como esta tese integra diversos artigos científicos, o padrão de formatação apresentado em cada um varia de acordo com as normas de cada revista em que os artigos se encontram publicados ou submetidos para publicação.

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ABSTRACT

The main goal of this thesis was to determine the environmental factors influencing the distribution of two closely related voles in Portugal. More specifically the aim was to analyse and compare the environmental preferences of the Lusitanian pine vole (*Microtus lusitanicus* (Gerbe, 1879)) and the Mediterranean pine vole (*Microtus duodecimcostatus* Sélys-Longchamps, 1839), at different scales of analysis, and also to quantify the importance of spatial autocorrelation component in species distribution models.

At a national level the distribution pattern of pine voles was associated with climate variables, landscape units, and soil types. The environmental associations at a landscape scale reflected the importance of habitat connectivity and heterogeneity for the Lusitanian pine vole, and of open areas, free of shrubs, for the Mediterranean pine vole. At a local scale, the high importance of verges, especially for the Lusitanian pine vole, highlights the several advantages of these particular vegetation structures for small mammals.

The sympatric area of both pine voles was consistently narrow in national and regional distribution maps. Results indicated that the local coexistence of the two voles should be a rare event.

Vole distribution data were characterized by strong spatial autocorrelation even after accounting for several significant environmental effects. At the largest scale the spatial effects were the most important. At a finer scale, these effects, although still important, played a secondary role when explaining the species distributions and segregation.

Furthermore, novel information was provided on space use, social behaviour, activity patterns, and habitat selection of Lusitanian pine vole.

The discrimination of the two species in the field, and in a potential sympatric area, was possible using presence signs, namely the proportion of burrow openings.

Multiple factors may influence the distribution of pine voles in Portugal. Some of these factors gain importance at specific spatial scales and others can only be discussed using more than one scale approach.

Key-words: pine voles; distribution modelling; scale; spatial autocorrelation; sympatry; coexistence; space use.

RESUMO

Os pequenos mamíferos desempenham um papel fundamental nos ecossistemas mediterrânicos, sendo não só o recurso alimentar de base para várias espécies de mamíferos carnívoros e aves de rapina diurnas e nocturnas, mas também sendo eles próprios consumidores de plantas, frutos e pequenos invertebrados. As espécies com hábitos fossadores chegam mesmo a interferir na composição das comunidades florísticas, assim como na estrutura e composição do solo nos locais onde vivem.

A Península Ibérica, assim como as restantes penínsulas mediterrânicas, é conhecida por ter um elevado número de espécies endémicas de micromamíferos, contribuindo desta forma para a conhecida importância da bacia mediterrânica como "hotspot" de biodiversidade. Apesar deste facto, sabe-se muito pouco da ecologia de algumas destas espécies, em particular dos microtídeos endémicos destas penínsulas.

Uma dos aspectos importantes para o conhecimento ecológico das espécies é o estudo dos padrões da sua distribuição e das suas preferências ecológicas. Do ponto de vista da conservação de comunidades, este tipo de estudos é essencial quando se pretendem implementar planos de gestão e intervir nos habitats. Por outro lado, este conhecimento é também importante para os investigadores e técnicos que trabalham na área de controlo de pragas, uma vez que algumas destas espécies podem causar prejuízos na agricultura.

O rato-cego (*Microtus lusitanicus* (Gerbe, 1879)) e o rato-cego-mediterrânico (*Microtus duodecimcostatus* de Selys-Longchamps, 1839) apresentam características interessantes para o estudo comparativo dos padrões de distribuição e preferências ecológicas: são espécies-irmãs, de hábitos fossoriais e com distribuição restrita. O rato-cego ocorre apenas na Península Ibérica (incluindo os Pirinéus) e o rato-cego-mediterrânico ocorre na Península Ibérica e sul de França. Em Portugal, o rato-cego ocorre no norte e centro, enquanto o rato-cego-mediterrânico é encontrado no centro e sul do país. Contudo, apesar de numa escala nacional se definir uma área de simpatria para as duas espécies no centro do país, não é claro se, numa escala regional ou local, as espécies co-existem ou, pelo contrário, se excluem, em consequência de preferências ecológicas diferenciais ou competição interespecífica. Embora com uma distribuição europeia restrita, ambas as espécies de ratos-cegos podem atingir elevadas densidades populacionais em áreas agrícolas, principalmente em pomares de produção comercial, e provocar prejuízos consideráveis.

No que respeita à caracterização das preferências ecológicas, nomeadamente em áreas não agrícolas, é patente a falta de informação, principalmente sobre o rato-cego. Em particular, faltam estudos que comparem as duas espécies em termos dos factores ecológicos mais determinantes na sua distribuição. Por outro lado, a clarificação adicional dos padrões de distribuição das espécies seria importante não só para o controlo de pululações ocasionais em meios agrícolas, mas também determinante para distinguir situações de alopatria, parapatria ou simpatria, e fornecer bases para uma melhor compreensão dos processos biogeográficos e evolutivos de espécies próximas.

Uma vez que as diferentes espécies reagem ao ambiente envolvente a diferentes escalas espaciais e diferentes processos ecológicos podem tornar-se mais evidentes consoante a escala utilizada, é fundamental considerar os efeitos de escala em estudos de padrões de distribuição de espécies. Por outro lado, sendo a autocorrelação espacial um fenómeno com importantes consequências matemáticas e ecológicas, a sua inclusão (ou correcção) nesses mesmos estudos revela-se imprescindível.

O principal objectivo desta tese foi determinar os factores ambientais que influenciam a distribuição espacial de duas espécies de ratos-cegos em Portugal. Mais concretamente, pretendeu-se analisar e comparar as preferências ecológicas do rato-cego (*Microtus lusitanicus*) e do rato-cego-mediterrânico (*Microtus duodecimcostatus*), a diferentes escalas (nacional, regional e local) e incluir a componente de autocorrelação espacial presente. Adicionalmente, validou-se a utilização de indícios de presença como uma metodologia expedita de identificação das espécies no seu habitat natural.

Assim sendo, neste trabalho procurou-se responder a diversas questões numa abordagem decrescente de escala: *i*) Quais os factores ambientais que influenciam a distribuição dos ratos-cegos em Portugal? Quais os limites de distribuição para cada espécie em Portugal e para a área de simpatria? *ii*) Que factores ambientais influenciam a distribuição regional dos ratos-cegos no centro de Portugal? Qual a escala mais relevante na explicação da distribuição regional das espécies? Existirá uma área de simpatria regional ou ocorrerá segregação espacial entre as espécies? Que factores ambientais poderão explicar as diferenças nos padrões de distribuição regional entre cada espécie *iii*) Qual o padrão de ocupação do espaço e do tempo em populações de rato-cego (*M. lusitanicus*), a uma escala local?, e *iv*) Numa área de potencial simpatria, os indícios de presença poderão ser usados no campo para distinguir as duas espécies? Se sim, que características desses indícios permitem uma identificação mais precisa?

Para responder a estas questões recorreu-se a diferentes abordagens metodológicas, quer na obtenção de dados, quer na sua análise. Como métodos de amostragem, recorreu-se aos dados de dieta de um predador nocturno generalista (coruja-das-torres *Tyto alba*) para o estudo da distribuição nacional das espécies e factores ambientais associados; à armadilhagem de animais no campo para avaliação dos factores ambientais, dos padrões de distribuição regionais e discriminação de indícios de presença; e ao rádio-seguimento de vários animais para o estudo da ocupação do espaço e do tempo de *M. lusitanicus*. Nos métodos analíticos, recorreu-se a modelos lineares generalizados espacialmente explícitos (com integração de mapeamento espacial de vectores próprios ou regressão autologística para descrever a autocorrelação espacial) na modelação das associações ambientais e ecológicas dos estudos de escala nacional e regional; à partição de variâncias para quantificar a importância relativa dos vários grupos de variáveis explicativas, da autocorrelação espacial e das escalas que foram consideradas para os estudos de distribuição nacional (ambiental, solo e espacial) e regional (paisagem, local e espacial); ao "kriging" para obter mapas de distribuição regional dos ratos-cegos; à análise composicional para analisar a selecção de habitat do rato-cego a duas resoluções de escala no estudo de âmbito local; e a árvores de classificação para validar a utilização de indícios de presença na identificação das espécies.

No estudo de âmbito nacional foi considerada toda a extensão de Portugal Continental; o estudo de escala regional e de indícios de presença foi realizado no Alto Alentejo (distrito de Portalegre); e o estudo local, que focou apenas o rato-cego, teve lugar numa área de policultura mediterrânica do concelho de Tomar.

Os principais resultados indicaram que a distribuição nacional de ambas as espécies de ratos-cegos é influenciada principalmente por factores espaciais, i.e. por autocorrelação espacial, e em menor grau por factores ambientais ou relativos ao solo. O rato-cego ocorre no norte e centro do país, fora das regiões classificadas de campina, em áreas de elevada precipitação anual, geada frequente, abundância de cambissolos, em solos em geral ácidos, litossolos pouco abundantes e ausência de solonshaks. O rato-cego-mediterrânico, por outro lado, ocorre no centro e sul do país, em zonas de características opostas à da espécie anterior. Assim, esta espécie está presente em áreas de campina, com solos de acidez moderada, com abundância de lito- e luvissolos, baixa precipitação anual, geada menos frequente, e cambissolos pouco abundantes. Os resultados apresentados neste trabalho mostraram uma área de simpatria nacional no sudoeste e centro do país e de dimensão reduzida. Esta área encontra-se fragmentada em quatro sub-áreas e inclui uma área de parapatria no centro do país, o que sugere que, a escalas mais finas, as zonas de contacto entre as duas espécies serão reduzidas. Assim, a distribuição nacional do rato-cego corresponde às áreas de menor influência mediterrânica do país e estende-se desde o Minho e Trás-os-Montes até à serra de Monchique (Algarve) e a Elvas, a oeste e este, respectivamente. Esta área de distribuição é descontínua entre Grândola e Sines. A área de distribuição do rato-cego-mediterrânico estende-se desde o Algarve até Alcochete a oeste e Idanha-a-Nova a este, correspondendo às áreas de maior influência mediterrânica. Também para esta espécie, a área de distribuição é descontínua na região da serra de São Mamede (Alto Alentejo).

É de salientar a provável presença de uma população de rato-cego na serra de Monchique, o que a confirmar-se, deverá constituir um isolado populacional com elevado interesse de conservação.

Na área de estudo de âmbito regional (Alto Alentejo), verificou-se que são os factores ambientais de escala local que têm maior peso na distribuição regional de cada uma das espécies, quando comparados a factores ambientais de escala paisagística, sendo este resultado mais notório no rato-cego. No entanto, os factores espaciais também tiveram um peso relevante na explicação dos padrões de distribuição, sobretudo do rato-cego-mediterrânico.

Verificou-se que os locais ocupados pelo rato-cego se caracterizaram localmente pela presença de bermas, maior humidade e teor de areia no solo; e, em termos de paisagem, por elevadas abundâncias de habitats lineares na envolvência. A presença do rato-cego-mediterrânico está associada igualmente à presença de bermas, mas também a uma maior cobertura de árvores e elevada biomassa de plantas herbáceas a uma escala local; e, numa escala de paisagem, à ausência de zonas de matos nas áreas circundantes.

As semelhanças que foram encontradas entre as duas espécies reflectem a importância da abundância dos estratos de vegetação como protecção de predadores ou condições climáticas, da abundância de uma componente vegetal que forneça alimento e de condições do solo que facilitem a construção de túneis e actividades subterrâneas.

Para a mesma área de estudo regional foi obtido um mapa que revela uma faixa estreita de simpatria e uma linha de parapatria que, no geral, vem reforçar e precisar espacialmente os resultados do mapa nacional de simpatria.

As diferenças nos padrões de distribuição regional das duas espécies de ratos-cegos foram mais evidentes a uma escala local, quando comparada à escala paisagística que teve uma influência reduzida na explicação desses padrões. Existe, no entanto, um importante efeito espacial (gradiente norte-sul) que também explica o padrão observado de uma espécie "do norte" e outra espécie "do sul". Deste modo, os locais de ocorrência do rato-cego diferenciam-se dos locais de rato-cego-mediterrânico por terem altitudes mais elevadas, maior cobertura de arbustos, solos menos argilosos, e menor cobertura arbórea, em termos de escala local; ao nível da paisagem, o rato-cego ocupa áreas com presença de manchas florestais e ausência de montados, enquanto o rato-cego-mediterrânico ocorre nas áreas de características opostas. Estas características deverão traduzir diferenças no comportamento e fisiologia entre as duas espécies, uma vez que o rato-cego é menos fossorial do que a espécie do sul e, deste modo, poderá ter maior necessidade de cobertura vegetal junto ao solo para movimentos superficiais, solos mais brandos para maior facilidade de construção de túneis e maior proximidade à serra com verões mais amenos. Uma elevada heterogeneidade ao nível do habitat poderá potenciar a ocorrência de condições locais para a co-existência das duas espécies.

Embora a co-ocorrência das duas espécies seja possível, dada a separação de vários aspectos do seu nicho ecológico, a reduzida extensão da área de simpatria estimada e a ausência de capturas de ambas as espécies para o mesmo local indica que esse acontecimento deverá ser bastante raro.

Foram registadas áreas vitais relativamente extensas para o rato-cego, para a área de estudo de âmbito local: 1042 m^2 para os machos e 862 m^2 para as fêmeas (método do Polígono Mínimo Convexo; método kernel 95% com valores de 229 m^2 para machos e 159 m^2 para fêmeas). Não foi registado qualquer efeito do sexo ou da condição reprodutora nos valores das áreas vitais.

Verificou-se a existência de sobreposição de áreas vitais (incluindo centros de actividade) e de laços sociais, nomeadamente entre machos e fêmeas, e entre fêmeas e subadultos. Estes resultados, quando comparados com outros microtídeos, sugerem a ocorrência de formação de pares monógamos entre machos e fêmeas. Faltam, no entanto, dados para classificar o sistema reprodutor da espécie como monógamo, mesmo que facultativo.

Os animais estudados não mostraram uma clara preferência pelo período diurno ou nocturno no seu ritmo de actividade ou movimentos realizados. No entanto, este resultado esteve dependente de interacções entre o sexo, condição reprodutora e período do dia: os machos foram mais activos durante o dia comparado com as

fêmeas e ambos os sexos foram igualmente activos durante a noite; os machos não-reprodutores percorreram maiores distâncias do que os machos reprodutores e as fêmeas não-reprodutoras; as fêmeas percorreram maiores distâncias durante a noite quando comparado com o período diurno; os animais reprodutores (ambos os sexos) percorreram distâncias maiores durante a noite do que durante o dia.
Os animais seguidos seleccionaram as unidades de habitat a duas escalas de percepção. Na definição das áreas vitais dentro da área de estudo, foram seleccionadas positivamente as bermas, vinha e olival; e negativamente os incultos, pastagem, hortas e urbano. No grau de utilização das várias unidades dentro de cada área vital, os animais usaram mais intensamente as bermas, em comparação com olival, vinha e incultos. Estes resultados vêm reforçar as inúmeras vantagens das bermas para esta espécie, fornecendo abrigo contra predadores, condições metereológicas e intervenções agrícolas no solo, e produzindo também recursos alimentares.

O estudo realizado sobre a utilização de indícios de presença revelou que estes podem ser utilizados com elevada fiabilidade na distinção dos locais de ocorrência das duas espécies de ratos-cegos, quando em área potencial de simpatria. A característica que revelou maior precisão na distinção das espécies foi a proporção de entradas para os túneis subterrâneos. Assim, por cada 10 indícios encontrados no campo (montículos de terra e/ou entradas superficiais), se mais de 8 tiverem entrada visível, o local será ocupado por rato-cego (i.e. maioritariamente entradas à superfície com poucos ou nenhum montículos); se o número de entradas superficiais não exceder 8, a espécie correspondente será o rato-cego-mediterrânico (i.e. montículos mais frequentes com poucas ou nenhuma entradas). Estes resultados indicam que este método poderá ser uma ferramenta muito útil em projectos que necessitem de avaliações ou monitorizações rápidas no campo. A utilização de indícios de presença na identificação destas espécies tem menores custos e logística simplificada, permitindo a sua aplicação por pessoal não-técnico. Embora a informação de presença/ausência possa ter menor relevância na aplicação em problemáticas agrícolas, quando recolhida em número suficiente de unidades de amostragem (i.e. transectos ou quadrats), a informação simples de presença pode ser convertida em índices de frequência e utilizados como estimativas indirectas de abundância.

Os resultados obtidos na presente tese de doutoramento realçam a importância de distintos fenómenos ecológicos nas diferentes escalas utilizadas. Deste modo, a distribuição dos ratos-cegos em Portugal é regulada por múltiplos factores que

podem ganhar diferente protagonismo nas diversas escalas espaciais em que são estudados.

Palavras-chave: ratos-cegos; modelos de distribuição; escala; autocorrelação espacial; simpatria; coexistência; utilização do espaço.

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Chapter 1

General introduction

1. GENERAL INTRODUCTION

1.1. Understanding species distributions

One of the major aims in ecology is to understand species distributions (Krebs 1994). Why do animals live where they do? Why does the Pine marten live all across Europe, but not in southern Iberia? Why is the Cabrera vole restricted to the Iberia? And why do they occur at grasslands and not in forests?

There are two different kinds of answers to these questions: the ecological explanation and the geographical/historical explanation (Myers and Giller 1988). The ecological explanation involves specific traits like reproductive rate, behaviour, mobility, and so on. All these characters influence the way populations respond to the environment (Cox and Moore 2000, Huggett 2004). This environment, in turn, may be abiotic (physical factors such as temperature, topography, water, or soil) or biotic (presence of other species, like predators, competitors, parasites, and humans; Myers and Giller 1988). Thus, each species can tolerate a specific range of environmental conditions which limits the choices made by species: it can only live where environmental conditions are within its tolerance limits (Myers and Giller 1988). The historical explanation involves the notion that species have a centre of origin and their range is spread through dispersal mechanisms, while the geographical explanation considers the importance of geologic and climatic changes that splits one population in two or more isolated groups in that range spread (Brown and Lomolino 1998, Huggett 2004).

Accordingly, species distributions may range in size from one hundred square meters to almost the entire terrestrial world. The limits of species distributions are, thus, determined by physical, biotic and historical factors, which originate diverse distribution patterns, from restricted and fragmented ranges to widespread and continuous ones (Myers and Giller 1988).

1.2. Coexistence of species

From the ecological point of view, species are often characterized in terms of their ecological niche: the habitat where a species lives and the position it occupies in the food chain. A distinction is draw, however, between the fundamental niche and the realized niche (Hutchinson 1957, Myers and Giller 1988). The fundamental niche corresponds to the set of optimal physical conditions where a species would live in the absence of competitors, predators, parasites, etc., whereas the realized niche is a smaller fragment of the fundamental niche, and corresponds to a "real world" situation where a species is constrained by abiotic and biotic elements (Hutchinson 1957).

According to this, a niche reflects how an individual, species or population interacts and responds to its environment, and involves an adaptation to that environment (Myers and Giller 1988). Most of the times, this environment supports other coexisting species that may or may not have similar ecological niches. However, the competitive exclusion principle states that two species cannot occupy identical niches (Hutchinson 1957, 1959). So, coexisting species may use the same type of ecological resource, although exploiting it in different ways or different times, thus avoiding competition (Hutchinson 1959, Douglass 1976, Cox and Moore 2000, Kronfeld-Schor and Dayan 2003, Spaeth 2009).

1.3. Species habitat models

Species distribution modelling has received an increasing interest in the past ten years (Guisan and Zimmermann 2000, Rushton *et al.* 2004, Guisan *et al.* 2006). Major ecological use has been in explaining and predicting species distributions and abundances (e.g. Orrock *et al.* 2000, Fleishman *et al.* 2001, Miller and Franklin 2002, Pearson *et al.* 2004, Monestiez *et al.* 2006), but also in developing important tools for conservation and management (Gibson *et al.* 2004, Muñoz *et al.* 2005, Stokes and Cunningham 2006), evolutionary ecology (Anderson *et al.* 2002, Arif *et al.* 2007, Rissler *et al.* 2007, Martínez-Freiria et al. 2008), or global climate change (Bussche *et al.* 2008, De Marco *et al.* 2008). Some of the popularity of distribution modelling arose as remotely sensed data, environmental digital information and Geographic Information Systems (SIGs) became more available, as well as an increase in computer power (Johnston 1998, Wadsworth and Treweek 1999, Rushton *et al.* 2004). Thus, new insights into species distributions and new modelling techniques have been presented at diverse extents and scales that summarize relationships between species occurrence and environmental variability (Guisan and Zimmermann 2000, Peres-Neto 2004).

Species distribution modelling has the capacity to improve our understanding of the pattern of endemism and speciation processes, by allowing species range delimitation, the recognition of areas of endemism, or erroneous presence localities (Raxworthy *et al.* 2007). Distribution modelling has considerable utility in improving our understanding of closely related species (Raxworthy *et al.*

2007), as ecological niches can be more easily compared when projected in a geographic space. When modelling the potential geographic distribution of sister species, GIS-models can provide insights into the role of ecological factors in driving speciation processes (Kozak *et al.* 2008).

1.4. The importance of scale

Different species respond to their environment at different spatial and temporal scales, and, at each scale, different aspects of the habitat become more or less important in regulating biological processes (Kotliar and Wiens 1990, Suárez-Seoane and Baudry 2002). Information on which scale most influences the relations between species and the landscape can help us predict how animals will respond to modifications of their environment, understand the mechanisms animals use, and, thus, provide crucial information for guidelines aiming landscape management and habitat mapping (Suárez-Seoane and Baudry 2002). However, the scale at which the behavioural processes between landscape structure and habitat selection operates is species dependent, and identification of the appropriate scale can only be achieved with a multiscale approach (Cushman and McGarigal 2002, Johnson *et al.* 2004). So, scale is an important consideration when studying patterns in species distribution.

The concept of scale has been largely discussed in the ecological literature (Wiens 1989, O'Neill and King 1998, Peterson and Parker 1998, Schneider 2001, Turner *et al.* 2001). Two different concepts are encompassed in the term scale: extent and resolution (Wiens 1989). While the extent refers to the dimension of the study area, the resolution corresponds to the dimension of the sampling unit used in that study area (Wiens 1989, Turner *et al.* 2001). Typically, studies over a large extent have low resolution, and studies of high resolution only cover small extents, although it may not always be the rule.

1.5. The importance of spatial autocorrelation

Spatial autocorrelation is a phenomenon where the closer two locations are, the more similar are their measured values (Legendre 1993, Legendre and Legendre 1998). Autocorrelation in biotic and abiotic resources is known for a long time and has originated the first rule of geography: "…everything is related to everything else, but near things are more related than distant things" (Tobler 1970). It is expected that the distribution of a species that depends on these resources (biotic and abiotic) shows also spatial autocorrelation (Legendre 1993).

Thus, if we are measuring presence-absence of one species, we expect that nearby locations of presence sites have a higher probability of being also presence sites. The patterns in species distributions may be spatially autocorrelated also because of contagious population processes (e.g. growth, reproduction) and historical factors, besides of the presence of spatial structure among environmental factors (Legendre 1993, Segurado *et al.* 2006). In fact, species and environment may share a spatial structure because of the effect of spatially structured environmental variables and non-environmental processes, which may or may not be interrelated themselves (Borcard *et al.* 1992).

When modelling species distributions, the occurrence of spatial autocorrelation may have a positive and a negative side. The most common reported is the negative side. The spatial autocorrelation leads to dependence among observations, decreasing with distance among them (Lennon 2000), which violates the assumption of independence of residual errors in correlation and regression models (Legendre 1993). When autocorrelation is present, this assumption is violated, leading to an underestimation of variance and overestimation of the significance of explanatory variables effect (Legendre 1993, Lennon 2000). On the positive side, spatial autocorrelation can be incorporated in spatially explicit models, without increasing much model complexity. It also presents an opportunity to increase our understanding of contagious biotic processes such as population growth, dispersal, or competition (Griffith and Peres-Neto 2006). A variety of methods have been developed and reviewed to include or correct the effects of spatial autocorrelation (Keitt *et al.* 2002, Dormann *et al.* 2007, Miller *et al.* 2007). These methods include autocovariate regression (Augustin *et al.* 1996, Keitt *et al.* 2002), spatial eigenvector mapping (Dray *et al.* 2006, Griffith and Peres-Neto 2006), spatial generalized least squares (Diniz-Filho *et al.* 2003), autoregressive models (Keitt *et al.* 2002, Lichstein *et al.* 2002), generalised linear mixed models (Venables and Ripley 2002), generalised estimating equations (Omar *et al.* 1999, Carl and Kühn 2007), variation partitioning of the spatial component (Borcard *et al.* 1992, Méot *et al.* 1998), kriging (Legendre 1993, van Horssen *et al.* 2002), or classification and regression trees with spatial dependence (Miller and Franklin 2002). Besides the fulfilment of model assumptions, the inclusion of the spatial autocorrelation has the advantage of incorporating the values of the neighbourhood, which increases the prediction power of distribution models (Knapp *et al.* 2003, Overmars *et al.* 2003, Vaugham and Ormerod 2003). Moreover, spatial models may improve variable selection (Keitt *et al.* 2002). In fact, non-spatial models cannot account for spatial autocorrelation and thus may incorrectly select explanatory variables just because they have a similar autocorrelation as the dependent variable, and not because they are good predictors of the ecological pattern under study (Legendre 1993, Lennon 2000, Segurado *et al.* 2006).

Besides these spatial effects, it is also important to consider the scale of the data, as the resolution and the extent of spatial data influence the pattern that can be observed (Overmars *et al.* 2003). Thus, species habitat models should reflect the influence of spatial structure (Legendre 1993, Augustin *et al.* 1996) and spatial scale in ecological patterns and processes (Wiens 1989).

1.6. Pine voles as focal species

Small mammals are a key group in Mediterranean ecosystems as staple prey of several species of carnivores and birds of prey (López-Gordo *et al.* 1976, Delibes *et al.* 1984, Veiga 1986), and also as consumers of plants, fruits or invertebrates (Gurnell 1985, Blondel and Aronson 1999). Those species with fossorial and burrowing habits may even interfere with soil structure and vegetation composition in the occupied sites (Huntly and Reichman 1994, Borghi and Giannoni 1997, Inouye *et al.* 1997, Gomez-García *et al.* 1995, 1999, Campos *et al*. 2001, Zhang *et al*. 2003).

The Iberian Peninsula is known for large numbers of rare and endemic small mammal species when compared to the central European regions (Bilton *et al.* 1998, Baquero and Telleria 2001), resulting from global tectonic and mostly climatic events that conducted to a geographical isolation for long periods of time during the last glacial phase (Bilton *et al.* 1998, Maridet *et al.* 2007). This might have allowed accumulation of new mutations and adaptive changes which enabled the formation of high numbers of endemic species in the Iberian Peninsula (Bilton *et al.* 1998). Nevertheless, knowledge on some of these species is still scarce, especially on what concerns the endemic microtines occurring in this region.

The Lusitanian pine vole (*Microtus lusitanicus* (Gerbe, 1879)) and the Mediterranean pine vole (*M. duodecimcostatus* Sélys-Longchamps, 1839), as sister species (Jaarola *et al*. 2004), represent a promising system to study ecological differences and similarities in microtines. Both voles show similar and marked burrowing behaviour and a restricted European distribution, with main distribution ranges concentrated in the Iberian Peninsula (Giannoni *et al*. 1993, Cotilla and Palomo 2007; Mira and Mathias 2007). In Portugal, these species have a general allopatric distribution pattern, with a narrow area of potential sympatry in the centre of the country (Madureira 1984).

Most studies concerning these two pine vole species have focused mostly on aspects of distribution records (Saint-Girons 1971, Niethammer 1982, Borghi *et al.* 1991, Brunet-Lecomte 1991), taxonomy and morphology (Herrera 1973, Madureira 1982b, 1982c, 1984, Niethammer 1982, Brunet-Lecomte *et al.* 1987), physiology (Madureira 1984, Mathias 1989, Mira 1999), population dynamics (Guédon *et al.* 1992, Guédon and Pascal 1993, Paradis *et al.* 1993, Paradis 1995), microhabitat conditions (Soriguer and Amat 1980, Borghi *et al.* 1994, Mira and Mathias 1994), reproduction (Claramunt 1976, Winking 1976, Palomo *et al.* 1989, Guédon *et al.* 1991a, 1991b, Mira 1999), burrowing and swimming behaviours (Giannoni *et al.* 1992, 1993, 1994), communication (Giannoni *et al.* 1997), and interactions with plants and environment (Borghi *et al.* 1990, Borghi and Giannoni 1997, Gómez-García *et al.* 1999, 2004). However, a large number of studies report only to the Mediterranean pine vole and to agricultural areas. Indeed, first major advances in the knowledge of this species were accomplished with a French research program in the 80's which aimed to study the reproductive biology and population dynamics of this pine vole species and to develop integrated control methods in agricultural areas (Guédon *et al.* 1992). In Portugal, important advances on the knowledge of these species were made with the research of Madureira (1984) who provided first insights on national distribution, systematic, morphology and biometry, and haematology of the Lusitanian and Mediterranean pine voles. Following this work, Mira (1999) focused on the Mediterranean pine vole and clarified aspects of growth, reproduction, physiology, population dynamics and aspects of microhabitat in an orange orchard. On what concerns the characterization of ecological preferences, namely outside agricultural areas, a lack of studies is notorious, especially for the Lusitanian pine vole (Mira and Mathias 2007). In particular, studies comparing the ecological determinants in the distribution of the two species of pine voles are almost absent. Moreover, the additional clarification of both species distributional patterns is, not only useful for the national agencies of pest control, but it is also crucial to differentiate situations of allopatry, parapatry or sympatry (Bull 1991) and to give insights on biogeography and evolutionary processes of closely related species (Arif *et al.* 2007). Integrative ecological studies of closely related *taxa* are required to explore the causative factors that determine species distributions, and might offer insight into the selective forces that may have driven evolutionary divergence of species. Differing ecological (and physiological) tolerance is probably an important factor facilitating coexistence of these sister-species when in sympatry, and is likely to have been a factor in their speciation. Furthermore, the analysis of the degree of overlap and coexistence between the two species can reveal clues on important ecological processes, such as interspecific competition

(Grant 1972). Therefore, the above pine voles represent good candidate species for the investigation of correlates on the distributional patterns.

1.7. Some background information on pine voles

Voles of the genus *Microtus* Schrank (1798) are usually the dominant herbivorous small mammals in the Northern Hemisphere, being an ecologically diverse group. Most species prefer open habitats, such as grasslands, meadows or pastures, although some species are also associated to forested areas (Getz 1985). This genus represents one of the best known cases of rapid mammalian radiation that originated ca. 65 extant species distributed across the Paleartic and Neartic regions (Chaline et al 1999). The genus *Microtus* is derived from the fossil genus *Allophaiomys*, which descended from the genus *Mimomys* (Chaline *et al.* 1999, Van Kolfschoten and Markova 2005). Most extant *Microtus* species that appear in the fossil records date to no more than 0.7-0.5 Mya, being included in the ages following the Middle Pleistocene (Brunet-Lecomte and Chaline 1991, Chaline et al 1999, Van Kolfschoten and Markova 2005). It is suggested that most of these European lineages arose by allopatric speciation (Chaline *et al.* 1999).

The Lusitanian pine vole (*Microtus lusitanicus* (Gerbe, 1879)) and the Mediterranean pine vole (*Microtus duodecimcostatus* Sélys-Lonchamps, 1839), as stated before, are fossorial voles occurring in the Iberian Peninsula (Giannoni *et al.* 1993, Cotilla and Palomo 2007, Mira and Mathias 2007). Although these voles spend most of their time in underground tunnels and burrows, they do not show any major morphological adaptation to a subterranean lifestyle as those observed in moles (genus *Talpa*) or other "truly" subterranean rodents (e.g. genera *Spalax*, *Cryptomys*, *Heterocephalus*).These voles are considered sister *taxa* that represent widely accepted species although showing low genetic divergence (Jaarola *et al.* 2004). The Lusitanian pine vole appears to be derived from the Mediterranean pine vole (Brunet-Lecomte *et al.* 1987), although both species have a recent origin of less than 0.1 Mya (Chaline *et al.* 1999). In Portugal, two subspecies of *M. lusitanicus* have been recognized: *M.l.mariae* in the extreme north of the country (Gerês and Montesinho mountains) and *M.l.lusitanicus* in the centre-north of the country (Douro basin downwards; Madureira 1982b). In contrast, only one subspecies of *M. duodecimcostatus* has been referred to our country: *M.d.centralis* Miller, 1908 (Madureira 1984).

The two species can be discriminated in the field according to measures of the hind feet length (HFL) and body length (BL): the Lusitanian pine vole has HFL < 16 mm and BL < 105 mm; while the Mediterranean pine vole has HFL > 16 mm and BL > 89 mm (Madureira 1982c, 1984). Regarding bone material, several discriminant functions were developed that can be applied to skulls (Madureira 1981) or mandibles (Madureira 1982a) allowing to differentiate the species from each other. While the first method allows quick species identification in the field, the second one is more suitable for specimens collected from predators' diet or museum collections.

Both species are western European endemisms (Cotilla and Palomo 2007, Mira and Mathias 2007) and occupy the same type of habitat, such as damp and easily movable soils, with high vegetation cover (Madureira 1984), and both feed on roots, tubers and fruits (Madureira 1982c, Vinhas 1993). In addition, both species are common rodent pests in vegetable crops and orchards (Vinhas 1993, Cotilla and Palomo 2007, Mira and Mathias 2007), although main complaints of damage started after the set up of intensive irrigation systems in the 70's (Sezinando 1982, Bäumler *et al.* 1984, Madureira 1984, Fernandes and Grilo 1989, Guédon 1991, Vinhas 1993, Mira 1999). In recent years, with the increasing number of young oak plantations in Portugal, reports of damage by these species (namely bark consumption) have also increased (Bäumler *et al.* 1984, A. Vinhas, pers. comm.). Accordingly, in favourable conditions, such as in agricultural areas, they can be reproductively active all year (Cotilla and Palomo 2007, Mira and Mathias 2007) and reach high densities.

As fossorial species, their presence in the field is suggested by surface signs of burrow and tunnel activities, such as soil mounds and burrows openings at the surface (Borghi *et al.* 1994, Vinhas 1999). However, animals should also have frequent surface activity, once they are frequent prey of owls (Campos 1977, Veiga 1978, Madureira 1979).

1.7.1. The Lusitanian pine vole

The Lusitanian pine vole occurs in the northwest region of Iberian Peninsula and in the French Pyrenees (Mira and Mathias 2007). In Spain, its geographical range was defined from Navarra and Huesca at north, and then through La Rioja, Soria, and mountains of Guadarrama, Gredos, Gata, while in Portugal its range was considered to cover an area extending from the mountain of Estrela to Setúbal in the Atlantic coast (Mira and Mathias 2007; see figure 1). The species occupies a wide range of habitats, from meadows, pastures, riversides, woods of oaks and sweet chestnuts, to agricultural areas (Mathias 1999, Mira and Mathias 2007).

It is a small herbivorous vole of 14-19 g of body mass (Mira and Mathias 2007). During winter and spring, its diet includes mostly leaves and stems, while during summer and autumn it consumes mainly subterranean parts of herbaceous plants, with high prevalence of geophytes (Mathias 1999, Mira and Mathias 2007).

Information on social behaviour of Lusitanian pine vole is scarce, but it has been suggested that animals live in small groups of one couple and their offspring (Mira and Mathias 2007). The sexual maturation of males is reached at 50 days of age, and females are mature at 35 days of age, producing 1 to 5 embryos (Madureira 1984), but usually 2-3 embryos per litter (Mira and Mathias 2007).

The species is a frequent prey of Barn owl (*Tyto alba*) and Tawny owl (*Strix aluco*), as well of several terrestrial carnivores (Mira and Mathias 2007).

Occasionally it reaches high densities in agricultural areas, with major incidence in orchards (apples, pear, cherry), but also in vegetable crops (carrots and potatoes), and melon plantations. Orchards damages can lead to a loss of 10-15% of fruit trees (Bäumler *et al.* 1984; Mira and Mathias 2007). In intensive production orchards there are reports of 100 to 200 animals/ha, with a maximum of 300 animals/ha, although no cycling populations are known across the entire geographical range of the species (Mira and Mathias 2007).

1.7.2. The Mediterranean pine vole

The Mediterranean pine vole is distributed across most of the Iberian Peninsula, except in the northwestern area and some scattered areas in Spain, occurring also in southern France (Cotilla and Palomo 2007). There is an estimated region of overlap in Spain (in northern Navarra, Basque country, western La Rioja, and Castilla-Leon), and in Portugal (Castelo Branco to Setúbal) (Madureira 1984, Cotilla and Palomo 2007; see figure 1).

This vole occupies open habitats, with Mediterranean characteristics, natural or agricultural (Cotilla and Palomo 2007), and the species presence and abundance is usually associated with high herbaceous vegetation cover (Mira and Mathias 1994, Paradis 1995, Mira 1999).

Although considered also a small vole, it is larger than the Lusitanian pine vole: 19-32g of body mass (Cotilla and Palomo 2007). As an herbivore, it consumes mostly subterranean plant parts (e.g. bulbs, roots, rhizomes, etc.), although aerial parts are sometimes also eaten (Borghi and Giannoni 1997, Cotilla and Palomo 2007). There are also reports of high consumptions of geophytes (Soriguer and Amat 1980), namely the subterranean parts of *Oxalis pes-caprae* (Bäumler *et al.* 1984, Mira 1999) and *Merendera montana* (Gomez-Garcia *et al.* 2004).

Morphological and biometric studies suggest that this species is more adapted to underground life, as revealed by a stronger neck musculature and higher proodonty of the upper incisors (Madureira 1982c, Mathias 1990).

The mating system appears to be monogamy with formation of small family groups of 3.1 animals per family, on average (Paradis and Guédon 1993, Cotilla and Palomo 2007). Animals became sexually mature at 60-70 days of age and produce 2.13 embryos per litter (1-5; Mira 1999). Several authors defend that the Mediterranean pine vole follows a K-strategy type (Guédon *et al.* 1991b, Guédon and Pascal 1993). However, individuals can be reproductively active all year (Paradis and Guédon 1993), but with maximum values of sexual activity in winter and spring (from November to May with maximum in February/March) (Mira 1999).

The species is also a frequent prey of the Barn owl, especially dispersing juveniles, as well as of other small and medium birds of prey and carnivores (Cotilla and Palomo 2007).

The Mediterranean pine vole can also reach high densities in agricultural areas, namely orange orchards in Algarve and Setúbal (Vinhas 1993) leading to 5 to 10% losses of the annual production (Bäumler *et al.* 1984, Cotilla and Palomo 2007). Data on population dynamics indicate that they can reach high densities in orchards (100 to 400 animals/ha in southern France) and in irrigated crops (390 animals/ha in southern Spain) (Cotilla and Palomo 2007). In spite of that, no cyclic fluctuations are known for populations within the geographical range (Guédon and Pascal 1993, Paradis and Guédon 1993, Cotilla and Palomo 2007). Paradis *et al.* (1993) suggest that the regional abundance of the species is determined by "source-sink" dynamics and that stable population dynamics may be related to a discontinuous spatial distribution and intense local dispersal of the species (Paradis 1995).

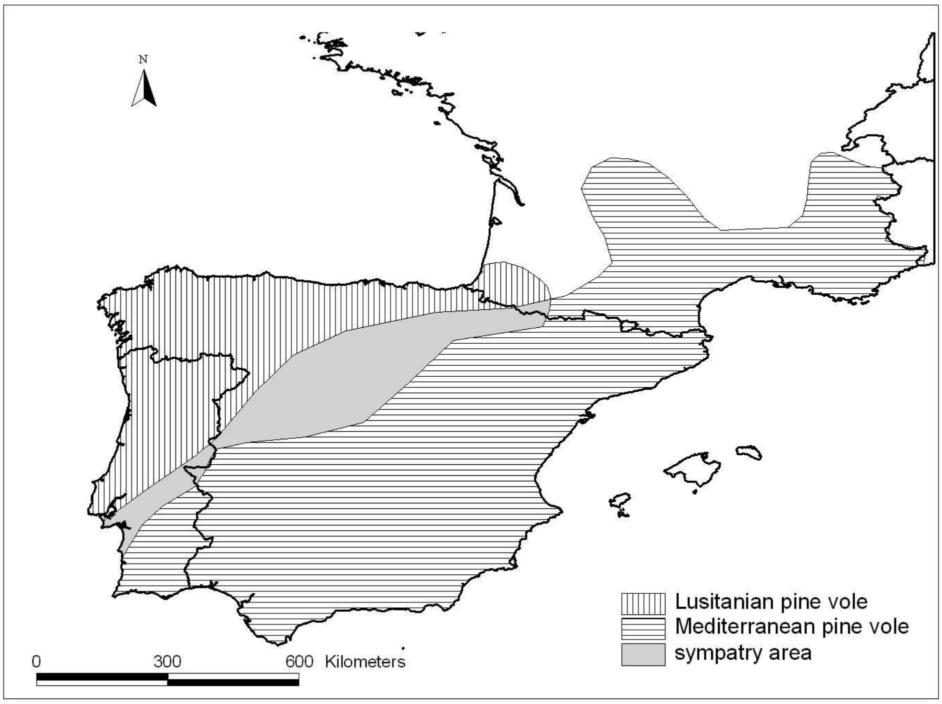

Figure 1. Distribution area of the Lusitanian pine vole (*Microtus lusitanicus*) and Mediterranean pine vole (*Microtus duodecimcostatus*) in western Europe and estimated sympatry area (adapted from Madureira 1984, Mitchell-Jones *et al.* 1999, Cotilla and Palomo 2007, Mira and Mathias 2007).

1.8. Objectives and thesis organization

1.8.1. Objectives

The present thesis is focused on the role of environmental factors regulating the spatial distribution of the Lusitanian pine vole (*Microtus lusitanicus*) and Mediterranean pine vole (*Microtus duodecimcostatus*) in Portugal, at different scales: national, regional and local. Coarse scales tend to reveal generalized ecological patterns, whereas mechanistic explanations are better determined from fine-scale studies (Wiens 1989). Specifically the aim is to explain and compare the patterns in both species distributions at each scale approach, incorporate the spatial autocorrelation component, and present new data on the ecological niche of the two species.

Accordingly, this thesis aimed to answer specific questions according to the scale of the approach:

1) National-scale: which environmental factors influence the distribution of the two species of pine voles in Portugal? What are the distribution limits for each species in Portugal, and what is the extent of the sympatric area between the two species?

2) Regional-scale: Which environmental factors influence the distribution of the two pine voles in central Portugal? Which resolution scale is most relevant in explaining the distribution of the two pine voles in the centre of the country? Is there a regional sympatric area between the two species? Which environmental factors explain the differences in distributions patterns of the two species? At what resolution scale are these factors most evident?
3) Local-scale: what are the main patterns of space and temporal use by the Lusitanian pine vole in a Mediterranean polyculture?

Several other questions were raised during this study, so I included additional questions regarding methodological issues: 4) Can we use presence signs in the field for discrimination of the two species in an area of sympatry? If so, which signs achieve best classification accuracy?

1.8.2. Thesis organization

This dissertation consists of five chapters of results (Chapters 3 to 7). Each one of these chapters comprises one scientific paper, published (Chapters 3, 6, 7) or submitted for publication (Chapters 4, 5) in international peer reviewed journals. The present **Chapter 1** includes a short review about the main issues and goals of the thesis, and in **Chapter 2** the study areas are described. In **Chapter 3**, the large scale distribution of the two pine voles is analysed and the relative effects of macro-scale environmental, soil, and spatial characteristics on species distribution are evaluated. This chapter also includes national predictive maps for the occurrence of each species and for sympatry areas. To achieve this, new tools were used such as the spatial eigenvector mapping (SEVM) to describe the spatial autocorrelation in species data, and variance partition techniques to quantify the relative effects of environmental, soil, and spatial characteristics on species distributions. **Chapter 4** deals with the relative contributions of local vs. landscape scale factors, and of spatial structure to the regional distribution of each species. Results aim to identify basic ecological preferences of each species, at different resolutions of the spatial scales. This study was conducted on a smaller area extent (161 300 ha), in a Mediterranean landscape of central Portugal (Portalegre disctrict), and the three sets of explanatory variables are analysed with generalised linear models and a variance partitioning procedure. In **Chapter 5**, kriging methods are used to present the potential regional distribution of each species and sympatric areas for central Portugal. Habitat niche differences between the two pine voles are also explored to infer the degree of ecological segregation and the possible coexistence in the field. The relative importance of local and landscape scales, and spatial factors are evaluated with a generalised linear model and variance partitioning. In **Chapter 6**, the spatial and temporal ecology of the Lusitanian pine vole is investigated in a Mediterranean polyculture, using radio-telemetry methods. Within a small scale study area extent (3 ha), answers are provided on how sex and reproductive factors can influence home range size and movements of the Lusitanian pine vole; what type of social system is present; the daily activity pattern of the species, the periodicity of short-term activity rhythms; and the habitat preferences at different spatial resolution scales. In **Chapter 7**, a classification tree analysis is applied to assess the validity of using presence signs in the field for the discrimination of the two pine voles in an area of sympatry, and to determine the type of presence signs that best discriminate them. The results obtained in this chapter, although very promising for future field sampling, were not applied in our identification methods. Finally, in **Chapter 8** the main findings presented in the previous five chapters are summarized and integrated, and main conclusions and implications of these results for the understanding of the ecology and life history traits of the studied pine voles are presented. This chapter also emphasizes areas where further studies are needed.

The papers are listed below, each corresponding to a Chapter, from 3 to 7:

Paper I (Chapter 3): Santos, S.M., Mira, A.P., Mathias, M.L. Factors influencing large scale distribution of two sister species of pine voles (*Microtus lusitanicus* and *Microtus duodecimcostatus*): the importance of spatial autocorrelation. Published in Canadian Journal of Zoology (a) (2009), 87(12): 1227-1240 (doi: 10.1139/Z09-108).

Paper II (Chapter 4): Santos, S.M., Mathias, M.L., Mira, A.P. The influence of local, landscape and spatial factors on the distribution of the Lusitanian and the Mediterranean pine voles in a Mediterranean landscape. Submitted to Mammalian Biology.

Paper III (Chapter 5): Santos, S.M., Mathias, M.L., Mira, A.P. Local coexistence and niche differences between the Lusitanian and Mediterranean pine voles (*Microtus lusitanicus* and *M. duodecimcostatus*). Submitted to Ecological Research.

Paper IV (Chapter 6): Santos, S.M., Lourenço, R.F., Mathias, M.L., Mira, A.P. Spatial and temporal ecology of the Lusitanian pine vole (*Microtus lusitanicus*) in a Mediterranean polyculture. Published in Animal Biology (b) (in press).

Paper V (Chapter 7): Santos, S.M., Mira, A.P., Mathias, M.L. Using presence signs to discriminate between similar species. Published in Integrative Zoology (c) (2009), 4: 258-264 (doi: 10.1111/j.1749-4877.2009.00163.x).

The leading author of the papers comprised in this thesis was responsible for sample collection, data analyses, and manuscript writing.

Note:

(a) Can J Zool – Published by NRC Reseach Press and indexed in Thomson Scientific's Journal Citations Report.

(b) Animal Biology – Published by Brill and indexed in Thomson Scientific's Journal Citations Report.

(c) Integrative Zool – Published by Wiley-Blackwell and indexed in ISI Web of Knowledge SM.

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Chapter 2

Study areas

2. STUDY AREAS

This thesis was conducted on three different study areas, according to the extent defined for each spatial scale considered. In the chapter concerning the national species distribution (Chapter 3), the entire continental area of Portugal was considered, representing an extent of 89 015 km^2. Data was gathered through compilation of information of institutional databases on pine voles distribution. Data for the chapters related to the regional scale approach (Chapters 4, 5 and 7) were obtained through fieldwork in Northern Alentejo, in an area of 1 613 km^2, and in the chapter concerning the local scale approach (Chapter 6) a small area of 0.03 km^2 was defined, near the city of Tomar (Santarém district). The Northern Alentejo area was chosen because it is located within the potential sympatric area of the Lusitanian and Mediterranean pine voles (Madureira 1984) and previous studies have confirmed the occurrence of both species in the area (Mira 1995, Santos 1998). This area also holds a high diversity of landscapes and land uses. On the other hand, the area near Tomar was chosen due to its high abundance of voles within a non-intensive agricultural system, and also due to logistical reasons.

The following paragraphs concern the descriptions of the Northern Alentejo and Tomar study areas, where intensive fieldwork was conducted (figure 2).

Figure 2. Location of study areas in Portugal (study area A: Northern Alentejo; study area B: Tomar).

2.1. Study area A: Northern Alentejo

The study concerning the regional scale approach was conducted in the Northern Alentejo (- 07º26'03'' W, 39º17'39'' N, Portalegre district, Portugal) from September 2006 to May 2007, over an area of 1 613 km^2 (figures 3 and 4).

2.1.1. Climate

The climate is Mesomediterrananean with a mean temperature of 7.4 ºC in January (3.1 – 11.7 ºC) and 24.7 ºC in July (16.8 – 33.3ºC), and an average annual rainfall of 717 mm (Rivas-Martínez and Arregui 1999, Ninyerola *et al.* 2005).

2.1.2. Landscape and land uses

The study area includes a landscape unit of mountain characteristics (São Mamede mountain) in the northeast and a unit of lowlands in the south and west (Cancela d'Abreu *et al.* 2002). At the lowlands the landscape is dominated by open fields with low fragmentation, scattered oak trees with varying densities, called "montado" (open forest of cork/holm/pyrenean trees with an agro-silvo-pastoral use; Pinto-Correia 1993), sometimes interspaced with polycultures close to the villages (figure 4A, B). The relief has a smooth shape (altitude of 200 to 400 m) and human settlements are located, and spaced almost uniformly in the landscape (Cancela d'Abreu *et al.* 2002). The mountain of São Mamede has contrasting characteristics when compared to surrounding lowlands (figure 4C). It presents a marked relief with 1025 m a.s.l. at the highest point and extensive areas above 600 m a.s.l.. The high geologic and microclimatic diversity of the mountain has conducted to higher heterogeneity in the land uses when compared to the lowlands. The agriculture is more pronounced in the valleys, perennial cultures in the plains and woods are dominant in the steep slopes (Cancela d'Abreu *et al.* 2002). This landscape unit is included in the Natural Park of São Mamede Mountain, corresponding to about 30% of the study area.

Land uses in the study area are markedly of Mediterranean-type, including a combination of agricultural, forestry and cattle production activities. There is a marked presence of "montados" of holm oaks, but also of cork and Pyrenean oaks, today, most frequently associated to cattle production. In the surroundings of human settlements, areas of polycultures are common, with olive groves, orchards, vineyards, vegetable gardens, and arable lands; Cancela d'Abreu *et al.* 2002, IGeoE 2003). The mountain slopes are mostly occupied by forests: plantations of pine (*Pinus pinaster*), eucaliptus (*Eucalyptus* spp.), and chestnut trees (*Castanea sativa*), and small fragments of Pyrenean oak woods (Cancela d'Abreu *et al.* 2002).

The main urban center is Portalegre (district main city) holding almost 26 000 inhabitants. The study area includes also other cities such as Castelo de Vide and Marvão in the north, Crato and Alter do Chão in the west, and Arronches in the south. However, the generality of the study area has low human density, with scattered human settlements across the landscape (Cancela d'Abreu *et al.* 2002).

2.1.3. Biogeographic units and vegetation series

According to the phytosociologic classification of Costa and collaborators (1998), the study area is entirely included in the Luso-Extremadurense Province (figure 3). The Cacerense Superdistrict (Hurdano-Zezerense Subsector; Toledano-Tagano Sector) occurs at the northwestern half of the study area, with abundance of schist, granitic and quartzy soils. The most representative vegetation of this superdistrict belongs to series of holm-oak woods (*Pyro bourgaenae-Querceto rotundifoliae*), with broom shrubs (Cytiso multiflori-Retametum shpaerocarpae), oak shrubs (*Rhamno fontqueri-Quercetum cocciferae*), and rock-roses (*Genisto hirsutae-Cistetum ladaniferi*). The rosemary shrubs (*Scillo-Lavanduletum sampaioanae*) are common at the granitic rocky areas (Costa *et al.* 1998). Distric Cambisols dominate in this superdistrict (Instituto do Ambiente 2007).

The mountain of São Mamede belongs to the Oretano Subsector, which results in vegetation elements with strong Atlantic characteristics, such as *Quercus robur*, *Quercus pyrenaica*, *Ulex minor*, *Drosera intermedia*, *Castanea sativa*, *Cytisus multiflorus*. Most representative phytosociologic units in this subsector are oak woods (*Arbuto-Quercetum pyrenaicae*), white-broom shrubs (*Cytisetum multiflori-eriocarpi genistetosum falcatae*) and gorse (*Halimio umbellate-Ulicetum minoris*), but also cork-oak woods (*Sanguisorbo-Quercetum suberis*) (Costa *et al.* 1998). Distric Cambisols dominate in this subsector, although Humic Cambisols are also present in the top of the mountain (Instituto do Ambiente 2007).

The southern half of the study area is represented by the Alto Alentejano Superdistrict (Araceno-Pacense Subsector; Mariânico-Monchiquense Sector). Schist and granitic soils dominate in this area of lowlands. Here, the "montados" of holm-oak (*Pyro-Quercetum rotundifoliae*) and cork-oak woods (*Sanguisorbo-Quercetum suberis*) are very common in the landscape (Costa *et al.* 1998). The soils classes present in this superdistrict include mostly Ortic Luvisols, but also Ferric Luvisols and Chromic Vertisols (at the southwestern corner) (Instituto do Ambiente 2007).

The Pacense Superdistrict (Araceno-Pacense Subsector; Mariânico-Monchiquense Sector) is present in the extreme southeastern corner of the study area. The area corresponds also to lowlands with gorse (*Ulici eriocladi-Cistetum ladaniferi*) and broom (*Retamo sphaerocarpae-Cistetum bourgaei*) which are a result of degradation of holm-oak woods (*Pyro-Quercetum rotundifoliae*) (Costa *et al.* 1998). Ortic Luvisols also dominate in this superdistric (Instituto do Ambiente 2007).

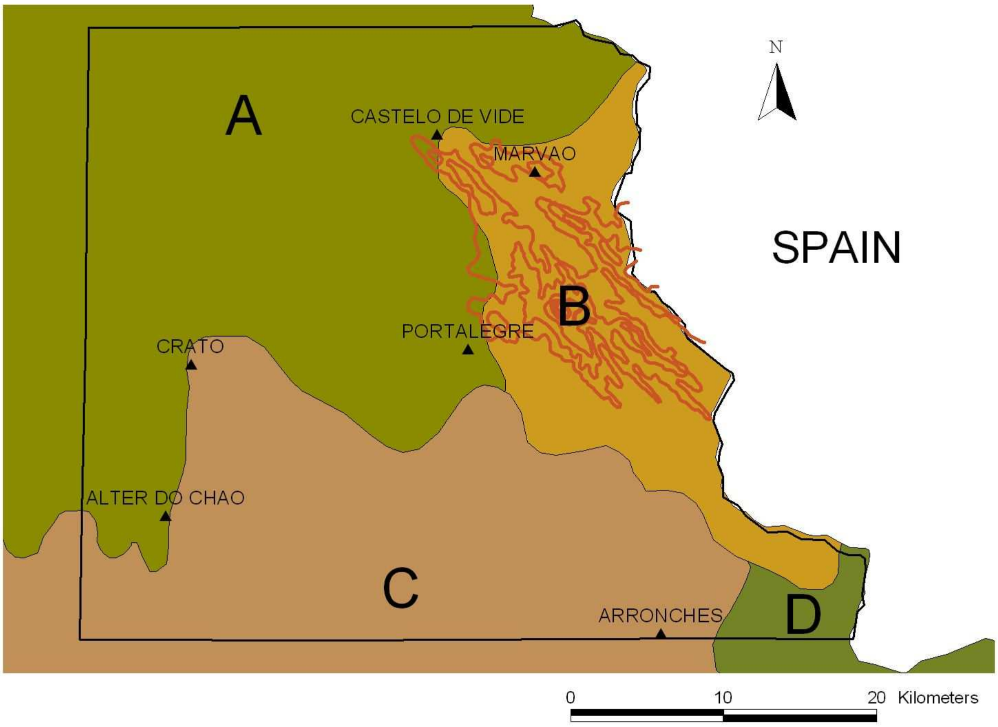

Figure 3. Study area A (Northern Alentejo): main urban settlements, main biogeographic units (A: Cacerense Superdistrict; B: Oretano Subsector; C: Alto Alentejano Superdistrict; D: Pacense Superdistrict), and altitude isolines above 500 m a.s.l. (the mountain landscape corresponds to the biogeographic unit B, and the lowlands landscape corresponds to units A, C, and D; adapted from Costa *et al.* 1998, Instituto do Ambiente 2007).

Figure 4. Examples of the lowlands landscape in the north (A) and in the south (B), and of the mountain landscape (C) of the Alto Alentejo study area.

2.2. Study area B: Tomar

The local-scale study was conducted on a 3 ha-area, localized in Central Portugal, Tomar district (-08º18’29’’ W, 39º37’47’’ N), from January to May 2008. The nearest cities to the study area are Tomar (ca. 10 km east) and Ferreira do Zêzere (ca. 8 km north; figures 5, 6 and 7).

2.2.1. Climate

The climate is Mediterranean with mild winters (9.3 ºC in January) and hot summers (24.5 ºC in July). The altitude values ranges between 150 and 300 m a.s.l.. Annual precipitation averages 922 mm, concentrated in Autumn and Spring months (Rivas-Martínez and Arregui 1999, Ninyerola *et al.* 2005).

2.2.2. Landscape and land uses

Globally, the study area is included in a landscape of smooth undulating relief with continuous and monotonous presence of pine and gum forest plantations (*Pinus pinaster*, *Eucalyptus* spp.; figure 5). The high frequency of summer fires during the last decade has increased the replacement of pines by *Eucalytptus* plantations (Cancela d’Abreu *et al.* 2002). Main economic activities in this region are thus associated with forestry, while agriculture and pasture activities are very localized (Cancela d’Abreu *et al.* 2002).

On a regional scale, the land uses are more diversified with the occurrence of mosaics with olive groves, vineyards and polycultures, concentrated near to small villages. These small-scale mosaics, still managed traditionally and extensively, are often surrounded by intensive production and mixed forests (Cancela d’Abreu *et al.* 2002). Agricultural activities are sustained by part-time farmers, who manage these mosaics for self-consumption (figures 6 and 7).

2.2.3. Biogeographic units and vegetation series

According to the phytosociological classification of Costa and collaborators (1998), the study area is included in the Gaditano-Onubro-Algarviense Province, Ribatagano-Sadense Sector and Ribatagano Superdistrict. The native vegetation of this superdistrict is usually reduced to very small fragments which includes cork-oak woods (*Oleo-Quercetum suberis* and *Asparago aphylli-Quercetum suberis*), myrtles (*Asparago aphylli-Myrtetum communis*), and shrubs of oaks (*Erico-Quercetum lusitanicae*) (Costa *et al.* 1998). Sandstone soils are common in this area (Costa *et al.* 1998), although the study location is included in a large patch of Eutric Litosols associated to Luvisols (Instituto do Ambiente 2007).

At the study area, the fruit trees and crop species dominate, intermixed in small mosaics (figures 6 and 7). The olive tree (*Olea europaea*) and the vine (*Vitis vinifera*) are the most common species, but other fruit trees are also present: pear- (*Pyrus communis*), apple- (*Malus domestica*), quince- (*Cydonia oblonga*), peach- (*Prunus persica*), orange- (*Citrus* spp.), limon- (*Citrus limon*), fig- (*Ficus carica*). The herbaceous vegetation is mainly composed by ruderal and nitrophilous species, typical of agro-ecosystems (*Calendula arvensis*, *Hordeum murinum*, *Bromus* spp., *Avena barbata*, *Coleostephus myconis*, *Echium plantagineum*, *Parentucellia viscosa*, *Raphanus raphanistrum*, *Daucus carota*, *Plantago* spp., *Sonchus oleraceus*, *Rumex* spp., *Geranium mo*lle, *Vicia sativa*, *Digitalis purpurea*, *Lavatera cretica*, *Urtica* sp., *Scrophularia scorodonia*).

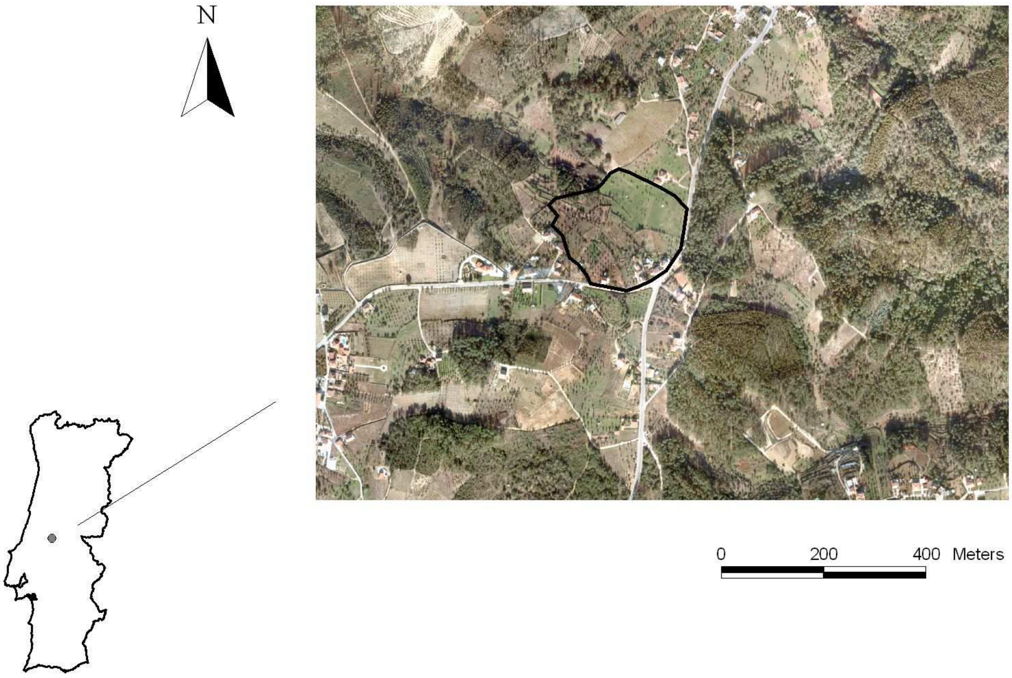

Figure 5. Aerial view showing the landscape matrix of study area B (Tomar; orthophoto, 2004 flight, resolution of 0.5 m, IGeoE).

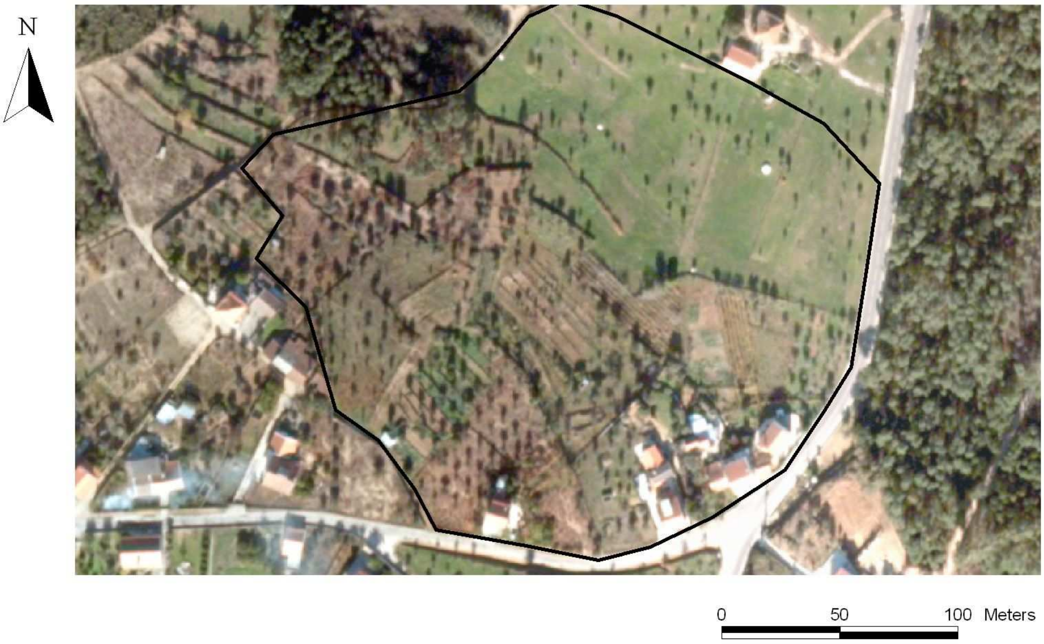

Figure 6. Detailed aerial view of study area B (Tomar) illustrating the mosaic of the Mediterranean polyculture (orthophoto, 2004 flight, resolution of 0.5 m, IGeoE).

Figure 7. View of part of the Tomar study area in February 2008.

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Chapter 3

Large scale distribution of pine voles

Paper I

Factors influencing large scale distribution of two sister species of pine voles (*Microtus lusitanicus* and *Microtus duodecimcostatus*): the importance of spatial autocorrelation.

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Factors influencing large-scale distribution of two sister species of pine voles (*Microtus lusitanicus* and *Microtus duodecimcostatus*): the importance of spatial autocorrelation

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ABSTRACT

The Lusitanian pine vole (*Microtus lusitanicus* (Gerbe, 1879)) and the Mediterranean pine vole (*Microtus. duodecimcostatus* de Selys-Longchamps, 1839) are sister species with burrowing habits and a restricted European distribution. Our aim was to assess the relative effect of environmental, soil and spatial characteristics on the distribution of these species in Portugal, and obtain predicted occurrence maps for each species, particularly to identify areas of sympatry. We used spatial eigenvector mapping (SEVM) to describe the spatial autocorrelation in species data, and we partitioned the variance in species distributions to quantify the relative effects of environmental, soil and spatial characteristics. The spatial variables explained the major part of variability in both species distributions and were more important than environmental or soil variables. The Lusitanian pine vole occurs in areas outside landscape units of grassland, higher rainfall, frost, and cambisols, with mostly acid soils, lower abundance of litosols, and presence of solonshaks. The Mediterranean pine vole is distributed in grassland areas within intermediate values of soil pH, dominated by litosols and luvisols, and lower rainfall, frost, and cambisols. Our results showed disjunct sympatric areas of small size and a parapatry boundary for the centre of Portugal, suggesting that contact zones are probably narrow.

RÉSUMÉ: Le campagnol basque (*Microtus lusitanicus* (Gerbe, 1879)) et le campagnol provençal (*Microtus duodecimcostatus* de Sélys-Longchamps, 1839) sont des espèces-soeurs qui construisent des terriers et qui possèdent une répartition restreinte en Europe. Notre objectif est d'évaluer les effets relatifs des caractéristiques environnementales, pédologiques et spatiales sur la répartition de ces espèces au Portugal, de produire des cartes de l'occurrence prédite pour chaque espèce et, en particulier, d'identifier les zones de sympatrie. Nous utilisons l'analyse spatiale par vecteurs propres (SEVM) pour décrire l'autocorrélation spatiale dans les données des deux espèces et nous partitionnons la variance dans les répartitions des espèces afin de mesurer les effets relatifs dês caractéristiques environnementales, pédologiques et spatiales. Les variables spatiales expliquent la plus grande partie de la variabilité des répartitions des deux espèces et elles sont plus importantes que les variables de l'environnement ou du sol. Le campagnol basque se retrouve à l'extérieur des unités de paysage de prairies, dans des zones à pluviosité, gel et cambisols plus fréquents, surtout avec des sols acides, une quantité réduite de lithosols et une présence de solonchaks. Le campagnol provençal se répartit dans les zones de prairies, avec un pH intermédiaire du sol, dominé par des lithosols et des luvisols et avec pluviosité, gel et cambisols moins importants. Nos résultats montrent l'existence de zones disjointes de sympatrie de petite taille et une frontière de parapatrie au centre du Portugal, ce qui laisse croire que les zones de contact sont probablement étroites.

INTRODUCTION

Study of species distribution patterns at large spatial scales has received much attention from ecologists (Osborne and Tigar 1992; Venier et al. 1999; Anderson et al. 2002; Silva et al. 2002; Muñoz et al. 2005). From a conservation perspective, the knowledge about species distributions and species-habitat relationships at large or regional scales is crucial for biodiversity management (Bustamante 1997; Osborne et al. 2001; Anderson and Martínez-Meyer 2004; Muñoz et al. 2005; Mira et al. 2008). On the other hand, this information is also important for biologists that aim to control pests and diseases (Madison et al. 1981; Delattre et al. 1996; Silva et al. 2002; Peterson and Robins 2003; Venturi et al. 2004; Collinge et al. 2005). Despite their relevance, large-scale surveys of many mammals are difficult because of their secret nature, nocturnal activity, or even their subterranean life (Sutherland 1996).

The pine voles from Europe (genus *Microtus*, subgenus *Terricola*) are characterized by a burrowing behaviour (Madureira 1984; Mathias 1990; Giannoni et al. 1993) and generally a restricted distribution range (Mitchell-Jones et al. 1999). The Lusitanian pine vole (*Microtus lusitanicus* (Gerbe, 1879)) is distributed in the northwestern part of the Iberian Peninsula (including the French Pyrenees), whereas the Mediterranean pine vole (*Microtus duodecimcostatus* de Selys-Longchamps, 1839) occurs in the southern-central Iberian Peninsula and in southern France (Mathias 1999; Cotilla and Palomo 2007; Mira and Mathias 2007). In addition, they are sister species, derived from a common and unique ancestor (Jaarola et al. 2004) with several documented localities of co-occurrence for the Iberian Peninsula (e.g. Veiga 1978; Madureira 1984; Brunet-Lecomte et al. 1987; Borghi et al. 1994). Although detailed and quantitative studies are lacking, both species preferentially occupy the same type of habitat (damp and easily movable soils with high vegetation cover; Madureira 1982) and feed on roots, tubers, and fruits (Vinhas 1993; Mathias 1999). In Portugal, *M. lusitanicus* occurs in the north and *M. duodecimcostatus* occurs in the south. There is a predicted area of overlap of both species in the centre of the country, obtained from national records of each species overlapped (Madureira 1984). Although the general geographic ranges are known for the voles, these are based solely on dot maps (e.g. documentation of scattered locations plotted on a map), which lack precise information in several areas including at the distribution boundaries. This scarcity of information is mainly due to difficult trapping techniques required for these voles (Guédon et al. 1992; Mira 1999) and of the morphological similarity of the two species, especially in the overlap area (Madureira 1982, 1984). However, reliable identification based on bone material (skull and mandible metrics) was developed to discriminate both species (Madureira 1981, 1982).

Both species are rodent pests, mainly in orchards and vegetable crops (Vinhas 1993; Mira and Mathias 1994; Mira 1999), but also in cork-oak plantations (A. Vinhas, personal communication (2006)). Demographic impacts of control measures such as habitat and vegetation management are expected to vary according to the habitat preferences and behaviour of the target species, which seem to be different for each of the species (Vinhas 1993). As such, knowledge of the geographical extent of both species is also urgent for the national agricultural institutes responsible for pest control (Vinhas 1993).

The clarification of species distributional patterns is also crucial to differentiate situations of allopatry, parapatry, or sympatry (Bull 1991), as well as to provide insights on biogeography and evolutionary issues of closely related species (Arif et al. 2007). Moreover, the examination of the degree of coexistence between two species can reveal additional clues on important ecological processes, like interspecific competition (Grant 1972). Thus, pine voles represent good candidate species for studies on these issues.

One way of overcoming the geographical information gaps of species distributions is to use the available occurrence data to develop statistical models relating species distribution with existing mapped environmental and habitat variables. The integration of modelling with geographical information systems (GIS) technologies makes the extrapolation of species distribution across large regions much easier and reliable. This approach has gained much popularity and several modelling techniques are now available (Guisan and Zimmermann 2000; Guisan et al. 2002; Segurado and Araújo 2004).

The goal of our study was to access the role of different types of variables on the large-scale distribution of two species of pine voles in Portugal, and to obtain predictions for each species distribution and for sympatric areas. We tested whether species occurrence is predicted better by environmental, soil, or spatial variables. Considering that *M. lusitanicus* is known as the northern species and *M. duodecimcostatus* as the southern species (Madureira 1984), we predict that spatial autocorrelation, defined by spatial variables, play an important role in explaining the species distributions, at least as a shared effect within environmental and soil variables.

MATERIALS AND METHODS

Data collection

Both species of pine voles, *M. lusitanicus* and *M. duodecimcostatus*, are highly frequent prey items (5%-20% occurrence) in the diet of the Barn Owl *(Tyto alba* (Scopoli, 1769)) in the Iberian Peninsula (Campos 1977; Veiga 1978; Madureira 1979; Alegre et al. 1989). Based on this assumption, vole presence and absence information was obtained through the analysis of Barn Owl pellets from 339 sampling sites across all country with a total sample size of 53 081 mammal prey (for similar methods see Mira et al. 2008). Species identification was based on mandible biometrics following Madureira (1982). Most samples (92%) were collected and analysed after 1990.

The minimum number of pellets per sample site needed to define a true absence of each species at 95% confidence level was estimated with a Poisson distribution where the mean represents the mean proportion of mandibles of each species in all the samples collected (Green and Young 1993). According to these results, a true absence was dependent on a minimum number of pellets, whereas the definition of a presence was accepted in every sample (independent of number of pellets). Absence sites of *M. lusitanicus* were thus defined after a minimum of four pellets (or 14 mammal prey) per sample; and absence of *M. duodecimcostatus* was defined after eight pellets (or 27 mammal prey) per sample (mean value of 3.38 mammal prey per pellet).

After sample validation, two matrices were built and mapped on Universal Transverse Mercator (UTM) 10 km x 10 km grid cells: one for *M. lusitanicus* with 80 presence cells and 70 absence cells (n=150), and another for *M. duodecimcostatus* with 71 presence cells and 95 absence cells (n=166; Fig. 1).

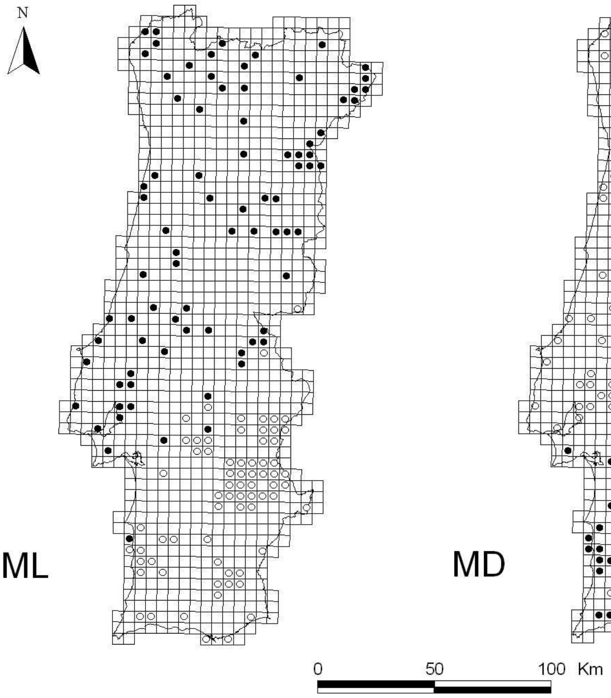

Fig. 1. Presence and absence of the Lusitanian pine vole (*Microtus lusitanicus*) and the Mediterranean pine vole (*Microtus duodecimcostatus*) at the Barn Owl (*Tyto alba*) samples (ML: *M. lusitanicus*, MD: *M. duodecimcostatus*, ●: pine vole presence, ○: pine vole absence). Absence samples were defined after a minimum of four pellets (or 14 mammal prey) for *M. lusitanicus* and eight pellets (or 27 mammal prey) for *M. duodecimcostatus* (see Materials and methods).

Explanatory variables

Three sets of explanatory variables were defined: environmental (ENV), soil (SOIL) and spatial (SPAT) (Table 1). The environmental and soil sets were obtained from free digital sources available in "Atlas do Ambiente" (http://www.iambiente.pt/atlas/est/index.jsp; assessed 7th April 2007). Each variable was superimposed over a UTM 10 km x 10 km grid square layer in a GIS (ArcView version 3.2; Environmental Systems Research Institute, Inc. 1999), and the mean value (or modal for categorical predictors) was extracted for each UTM square unit. The soil set was considered a separate group in order to quantify the influence of its covariates and relate it to the weight of the environmental component, because soil properties are recognised as one of main determinants in the distribution of fossorial and subterranean rodents (Miller 1964; Busch et al. 2000; Romañach et al. 2005).

Table 1. Independent variables, their definitions, and transformations performed on the variables in our analysis of factors influencing large-scale distribution of Lusitanian pine voles (*Microtus lusitanicus*) and Mediterranean pine voles (*Microtus duodecimcostatus*).

Code	Description	Units	Range	Transformations[a]
Environmental				
alti	Mean elevation	m above sea level.	(0 – 1322)	sqroot
humid	Mean relative air humidity	% at0900 GMT	(32.5 – 92.5)	log
rain	Total annual rainfall	mm / year	(227.6 – 3000.0)	log
temp	Mean annual air temperature	ºC / year	(5.1 – 18.3)	
evapo	Mean annual evapotranspiration	mm	(200 – 1000)	
frost	Mean number of days per year with frost	days	(0.5 – 85.0)	sqroot
insola	Mean number of days per year with sun	h	(1043.9 – 3150.0)	
radiat	Mean solar radiation	kcal/cm^2	(70.0 – 167.5)	
grass	Presence of grassland unit		(0 – 100)	0–1
mont	Presence of "montado"[b] unit		(0 – 100)	0–1
Soil				
acid	Mean pH of water in the soil	pH	(4.0 - 8.0)	
cambi	Cambisol type	%	(0 – 100)	
lito	Litosol type	%	(0 – 100)	sqroot
fluvi	Fluvisol type		(0 – 61.3)	0–1
luvi	Luvisol type	%	(0 – 100)	sqroot
solon	Solonshak type		(0 – 100)	0–1
podz	Podzol type		(0 – 100)	0–1
verti	Vertisol type		(0 – 77.5)	0–1
Spatial				
vec150	Spatial eigenvector for *M. lusitanicus* data		(-0.17 to 0.08)	
vec148	Spatial eigenvector for *M. lusitanicus* data		(-0.16 to 0.24)	
vec166	Spatial eigenvector for *M. duodecimcostatus* data		(-0.09 to 0.18)	
vec164	Spatial eigenvector for *M. duodecimcostatus* data		(-0.18 to 0.17)	
vec162	Spatial eigenvector for *M. duodecimcostatus* data		(-0.16 to 0.20)	

[a]: The transformations used are as follows: sqroot, square root transformation; log, natural logarithm transformation; 0-1, binary transformation.

[b]: The unit "montado" corresponds to an open forest of cork and holm trees with an agro-silvo-pastoral use, see Pinto-Correia (1993).

The spatial set corresponded to a group of variables (eigenvectors) obtained through the spatial eigenvector mapping (SEVM) (Dray et al. 2006; Dormann et al. 2007). The SEVM approach removes spatial autocorrelation from regression models by treating it as a missing variable effect (Griffith 2004). Each eigenvector, obtained through a geographic weights matrix, exhibits a distinctive spatial pattern and represents a given spatial autocorrelation level (Griffith 2004). The SEVM adds a minimally sufficient set of eigenvectors as new variables to the set of the linear predictors, and in doing so eliminates spatial autocorrelation among the observations (Griffith 2004; Griffith and Peres-Neto 2006). The first extracted eigenvectors represent very broad-scale spatial structure and following eigenvectors correspond in turn to increasingly finer scale spatial structure (Borcard et al. 2004; Griffith and Peres-Neto 2006). Eigenvectors with positive eigenvalues represent positive autocorrelation, while eigenvectors with negative eigenvalues represent negative autocorrelation (Dray et al. 2006; Dormann et al. 2007). The extracted eigenvectors were tested by a 999-permutation procedure on Moran's I and only significant eigenvectors ($p<0.05$) were included in the regression models (Dray et al. 2006). Accordingly, two eigenvectors were selected for the *M. lusitanicus* model and three eigenvectors were selected for the *M. duodecimcostatus* model.

Ignoring spatial autocorrelation when developing species distribution models can result in the selection of model predictors that, in fact, do not correspond to statistically significant relationships (Legendre 1993; Segurado et al. 2006).

Data analysis

Generalised linear models (GLMs) have been used frequently in species distribution modelling (Bustamante 1997; Guisan and Zimmermann 2000; Muñoz et al. 2005) which allow the user to specify more flexible relationships and is better suited for analysing ecological relationships, in the form of link functions (Guisan et al. 2002). The three sets of explanatory variables were first analysed independently using a GLM with a binomial error structure (e.g. Guisan et al. 2002) to select the most parsimonious models to be used in further analyses. After that, models of each variable set were combined in joint (each pair of two sets) and full (all three sets) models, using the variance partitioning method (Borcard et al. 1992; Legendre 1993; Cushman and McGarigal 2002).

Model building

Preliminary screening and reduction of variables were undertaken with exploratory plots and univariate logistic regressions. Linearity in the logit was verified by scatter output graphs for continuous variables (Zuur et al. 2007). Logarithmic and square-root transformations were performed on several explanatory variables to achieve normality. Zero-inflated variables were transformed into binary classes (Table 1). Only variables with univariate significance <0.25 were used in posterior analyses (Hosmer and Lemeshow 2000; Tabachnick and Fidell 2001).

Pairwise Pearson correlations were calculated among all predictors to check for multicolinearity. Variables showing correlation values > 0.7 (Tabachnick and Fidell 2001) were excluded from further analysis, retaining the strongest predictor.

Multiple logistic regression models were built for each of the three sets of predictors (ENV, SOIL, and SPAT). For the SPAT set, all the eigenvectors previously selected ($p<0.05$) were used. For ENV and SOIL sets, we used the information-theoretical model comparison approach (ITMC; Burnham and Anderson 2002), which is more efficient than stepwise selection when building predictive models (Stephens et al. 2005; Whittingham et al. 2006). For these sets, models with all possible combinations of remaining variables (after univariate screening) were developed for each variable set and compared with the Akaike's information criterion corrected for small samples (AIC_c; Burnham and Anderson 2002). Models with $\Delta AIC_{c,\ i} < 4$ ($\Delta AICc$ = difference between each model AIC_c and $AIC_{c,\ min}$) are considered to have substantial support as candidate models (Burnham and Anderson 2002). The variance partitioning procedure implies building a full model with all variables selected in each variable set, besides the development of joint models. However, to avoid overffiting of full models, we followed the rule of 10 cases for each explanatory variable included in the model (Harrell et al. 1996). Thus, only variables with more than 90% of importance value within the best models ($\Delta AIC_c < 4$) of each variable set were considered for variation partitioning calculations. Before these calculations, interactions and quadratic terms were tested on best models (within each set) and retained if a decrease in the AIC_c was verified.

Three joint models (ENV + SOIL, ENV + SPAT, SOIL + SPAT) and one full model (ENV + SOIL + SPAT) were estimated with the previously selected predictors of all the three sets and an adaptation of the variation partitioning method was performed (Borcard et al. 1992; Legendre 1993; Cushman and McGarigal 2002). This procedure is designed to specify how much of the variation in species distribution explained by each variable set corresponds to its pure effect and which percentage is explained by interactions among sets (Borcard et al. 1992; Legendre

1993). Seven components of explained variation were obtained through the partitioning method (Borcard et al. 1992): (1) pure effects of ENV; (2) pure effects of SOIL; (3) pure effects of SPAT; (4) shared effects of ENV + SOIL; (5) shared effects of ENV + SPAT; (6) shared effects of SOIL + SPAT; and (7) shared effects of ENV + SOIL + SPAT. The explained deviance D^2 was used as a measure of variance explained by each logistic model (Guisan and Zimmermann 2000). As an example, the ENV pure effects can be interpreted as variation explained by environmental variables independently of any soil or spatial structure; the shared effects of ENV and SOIL correspond to variation explained by a confounding effect between environmental and soil variables; and the shared effects of ENV and SPAT can be interpreted as spatial structure in species distribution that is shared by environmental variables (Borcard et al. 1992; Borcard and Legendre 1994).

There are several advantages associated with the choice of the variation partition method. For example, when fraction (1) is very small, a false significant coefficient of determination can result if the common parts (4), (5), and (7) are not partialled out. Causality can be falsely attributed to the environmental predictors, when, in fact, the correlation observed derives from a common structure (spatial and (or) soil) that may be present in both dependent and independent variables (Legendre 1993).

Model validation

To evaluate the models goodness of fit, the explained deviance D^2 (Guisan and Zimmermann 2000) and validation graphs were considered. Detection of influential observations was achieved through Cook's distance diagnostics (problematic observations for values > 3; Guisan et al. 2002; Zuur et al. 2007). Model accuracy was measured with receiver operating characteristics (ROC) curves. The area under the ROC curve (AUC) is a single index of classification accuracy that is independent of species prevalence and threshold effects (Manel et al. 2001; Osborne et al. 2001). We also looked at the overall rate of correct classification and the proportion of presences (sensitivity) and absences (specificity) correctly classified, using the prevalence (0.53 for *M. lusitanicus* and 0.43 for *M. duodecimcostatus*) as the cut point (Jiménez-Valverde and Lobo 2007). The predictive power of the full models was assessed by 10-fold cross-validation.

Predicted maps

The spatial eigenvectors had to be estimated for the entire study area so that probabilistic maps could be produced. This was obtained for the entire country by an interpolation technique: ordinary kriging to a resolution of 10 km x 10 km (adapted from Miller and Franklin 2002; Fortin et al. 2005).

Given the several factors that can influence the actual species distributions, maximizing the fit between predicted and observed presences could result in an underestimation of the extent of the species potential distributions. One way to overcome this is to minimize the number of cells with observed presences that are predicted as absences by the model (Pearson et al. 2004). For map production, the potential occurrence areas were obtained with two threshold values: major presence areas were defined with the prevalence threshold (0.53 for *M. lusitanicus* and 0.43 for *M. duodecimcostatus*), while minor presence areas were defined with a lower value that maximised sensivity (0.30 for both species). This decision intends to highlight a gradient in species boundaries, as distribution limits are rarely lines or sharp borders (Fortin et al. 2005).

The potential distributions generated by full models of both species were overlaid to obtain regions of potential sympatry with the predicted presence of both species (Anderson and Martínez-Meyer 2004). Major sympatry was defined as the areas of major occurrence of both species (prevalence threshold value), while minor sympatry corresponded to the minor presence area of one or both species (sensitivity threshold value).

We used the program R version 2.6.0 (R Development Core Team 2007) in all model building procedures. The spdep package (ME function; Bivand et al. 2008) was used in extracting spatial eigenvectors; the dRedging package (dredge function; Burnham and Anderson 2002) was used in model selection; the DAAG package (CVbinary function; Maindonald and Braun 2008) was used in the predictive power assessment; kriging was developed with gstat package (krige function; Pebesma 2004). Maps were built in ArcView GIS version 3.2. (Environmental Systems Research Institute, Inc. 1999).

RESULTS

Lusitanian pine vole

The Lusitanian pine vole was found in 80 (53%) of the 150 UTM grid cells surveyed. After univariate analyses, multicollinearity checking, and model selection, the environmental set was reduced to three predictors (rain, frost, grass) and the soil set to five predictors (acid, acid^2, cambi, lito, solon). The two eigenvectors (vec150, vec148) were kept in the spatial set.

Multivariate models

The environmental model explained 54.1% of variation in *M. lusitanicus* data. The species is present in areas with higher rainfall and frost values and absence of grasslands as a landscape unit. The soil model explained 45.8% of variation, indicating that voles occurred preferentially in areas with higher cover of cambisols, lower cover of litosols, and presence of solonshaks. There was a unimodal response to soil pH, with high probabilities of species presence for minimum values, low probabilities at medium values of pH, but again arising for high pH levels. The Spatial model accounted for 66.6% of variation, and skows that the species tends to be present in the northern and central regions of the country (Tables 2, 3).

The full model presented 81.7% of explained variation. Most of this variability was due to the shared effects of all sets, which reached more than 50%. Most important shared effects were obtained in the joint models containing the spatial set (6.7% and 11.8%) and mostly in the full model (33.0%), indicating that more than 50% of variation is due to a spatial structure present in the environmental and/or soil variables used in our study. The spatial model alone presented a high pure effect value (15.0%) which corresponded to a spatial structure independent from environmental and soil variables used (Fig. 2).

Table 2. Coefficients and their significance of four models for *Microtus lusitanicus* data (environmental, soil, spatial, and full model).

Variables	**Partial models**		**Full model**	
	B	**p**	**B**	**p**
Environmental				
rain	5.819	0.000***	4.159	0.177.
frost	0.688	0.001**	1.223	0.002**
grass	-3.877	0.000***	-3.656	0.036*
constant	-38.717	0.000***		
Soil				
acid	-22.707	0.008**	0.612	0.973
acid^2	1.597	0.012*	-0.097	0.945
cambi	0.027	0.003**	0.004	0.872
lito	-0.120	0.080	-0.353	0.016*
solon	2.976	0.003**	2.510	0.399
constant	78.119	0.006**		
Spatial				
vec150	38.616	0.000***	29.356	0.007**
vec148	-31.837	0.000***	-22.629	0.004**
constant	-0.330	0.378	-29.306	0.634

Note: For variable definitions refer to Table 1. *** $p<0.001$; **$p<0.01$; *$p<0.05$.

Table 3. Summary of performance parameters of all models for *Microtus lusitanicus* data.

Models	**D^2**	**AIC**	**AIC_c**	**AUC**	**OCC**	**Sensitivity**	**Specificity**
ENV	0.541	103.1	103.4	0.928	86.0	82.3	90.0
SOIL	0.458	124.4	125.0	0.926	85.3	81.3	90.0
SPAT	0.666	75.3	75.5	0.970	91.3	95.0	87.1
ENV + SOIL	0.667	87.0	88.3	0.966	88.7	86.3	91.4
ENV + SPAT	0.758	62.1	62.7	0.982	94.7	93.8	95.7
SOIL + SPAT	0.726	72.8	73.8	0.974	95.3	95.0	95.7
ENV+SOIL+SPAT	0.817	60.0	61.9	0.988	95.3	93.8	97.1

Note: D^2, explained deviance; AIC, Akaike's information criterion; AIC_c, Akaike's information criterion corrected for small sample sizes; AUC, area under the receiver operating characteristics (ROC) curve; OCC, overall correct classification.

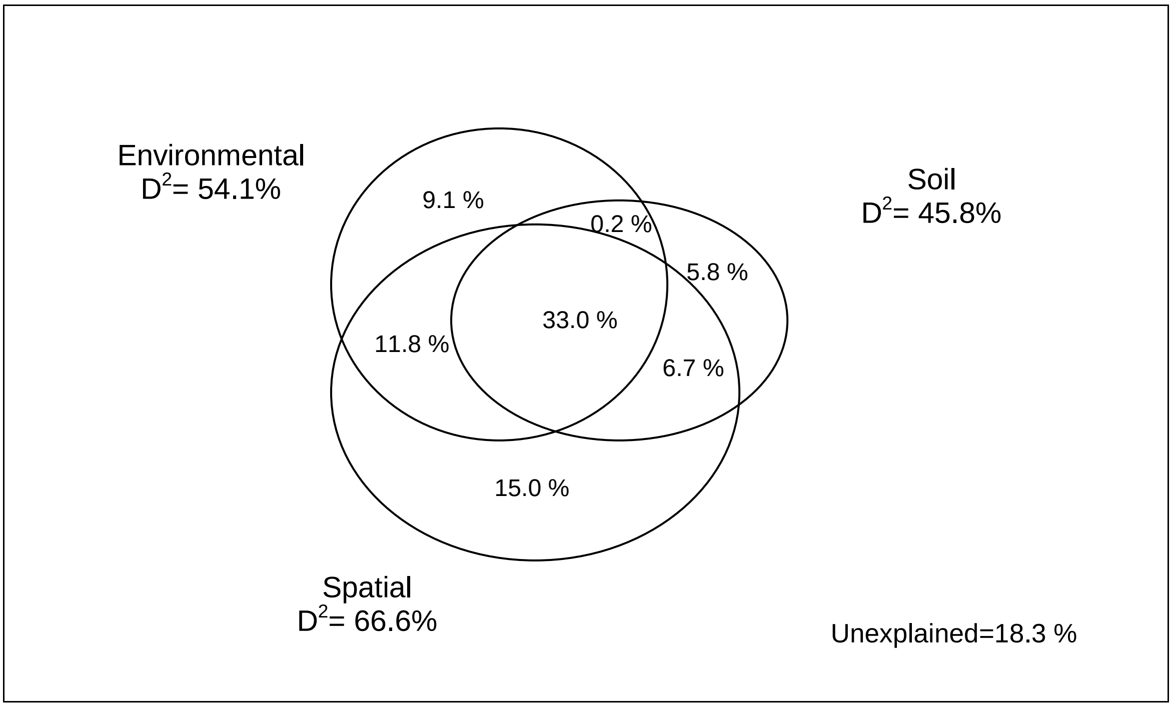

Fig. 2. Results of the variation partitioning for the Lusitanian pine vole (*M. lusitanicus*) data based on the three sets of independent variables: environmental, soil, and spatial sets. Unexplained is the percentage of unexplained variation. The size of circles and overlap are partially scaled according to the values of each component.

All seven models had very high discrimination ability (AUC > 0.90) and very good classification accuracy (OCC > 0.85). All models containing the spatial set presented the highest performance statistics, like AIC_c, AUC and percentage of correct classifications, although the full model performed slightly better than the others (Table 3).

The predictive power of the full model was assessed by the cross-validation procedure. The internal estimate of accuracy was 95.3% while the cross-validation estimate was 93.3%. This similarity in values indicates a good model performance outside the sampled areas.

Predicted maps

The predicted occurrences for the full and for each of the three individual model sets are presented in Figs. 3A-3D. The soil model failed to predict some known presence areas in the northeast and centre of the country, which the environmental and spatial models predicted as presences. Also, a false absence is predicted by the environmental model in the lowlands of central Portugal, while soil and spatial models predicted vole presence for that region. On the other hand, the environmental and soil models predicted occurrence areas scattered in the southern areas of the country, which are known as *M. duodecimcostatus* only areas. Although performance parameters of single model sets are all quite good, the three predictive maps show several differences in the predicted distribution patterns.

The full model presents the northern and central occurrence of *M. lusitanicus* with southwestern limits at Grândola (38º13’09’’N, -08º35’16’’E) and southeastern limits at Elvas (38º50’26’’N, -07º12’40’’E). The model also predicts occurrence areas for the southwest (Sines to Monchique) (Fig. 3D).

This model misclassified known sample presences in the centre of the country, and predicted presences for the localities of Elvas or Monchique, which are presently unknown.

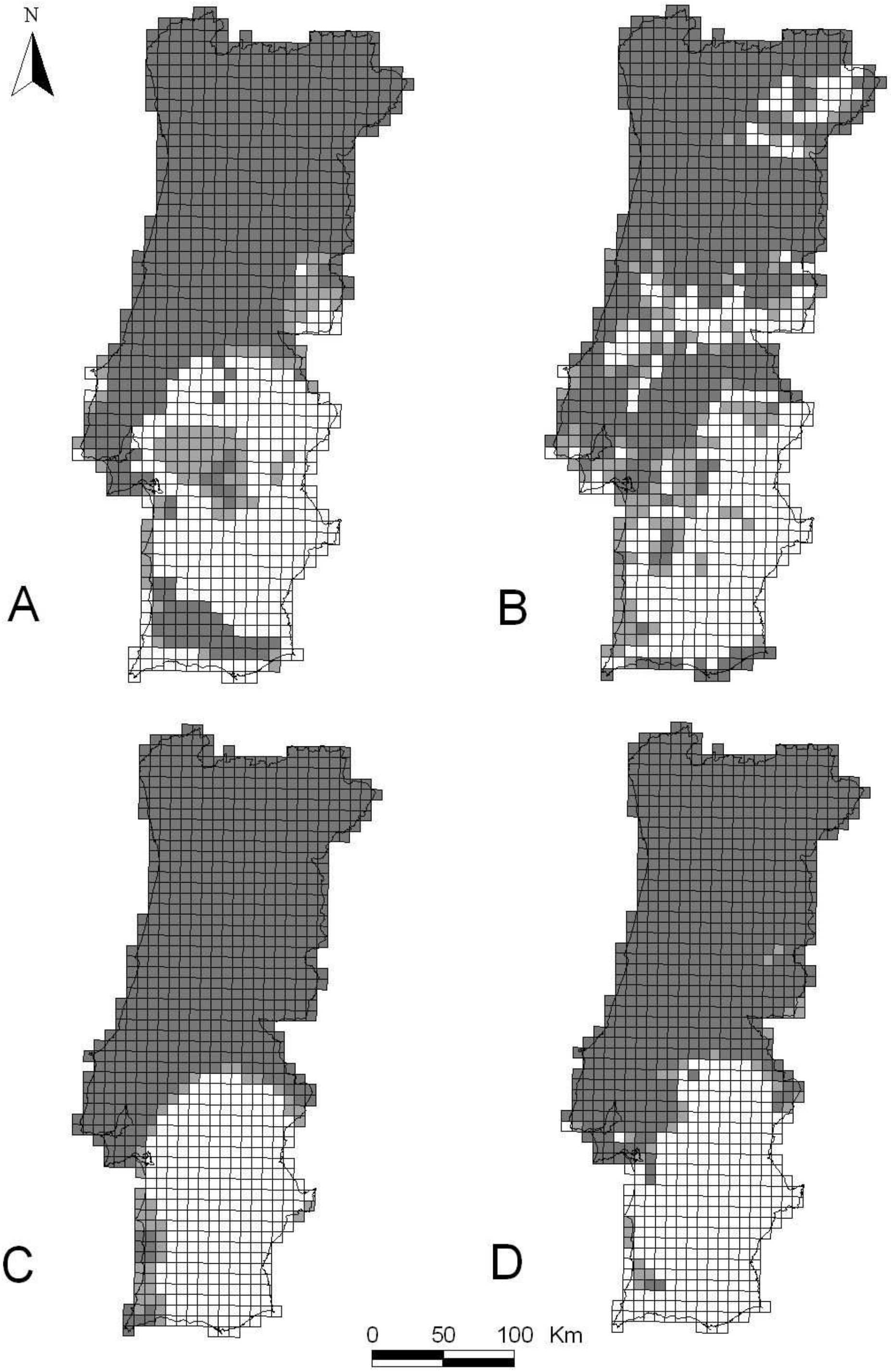

Fig. 3. Predicted occurrence of the Lusitanian pine vole (*Microtus lusitanicus*) in Portugal based on the four models presented: (A) environmental model, (B) soil model, (C) spatial model, and (D) full model. Light gray indicates a threshold of 0.30, while dark gray indicates a threshold of 0.53.

Mediterranean pine vole

The Mediterranean pine vole was found in 71 (43%) of the 166 UTM grid cells surveyed. After univariate regressions, multicollinearity checking, and model selection, the environmental set was simplified to three variables (rain, frost, grass) and the soil set to five variables (acid, acid^2, cambi, lito, luvi). The spatial set maintained the three eigenvectors (vec166, vec164, vec162).

Multivariate models

The environmental model explained only 34.4% of variation in *M. duodecimcostatus* occurrence. Highest probabilities of vole's presence can be found in areas with lower values of rain and frost and the presence of grasslands as a landscape unit. The soil model explained 40.6% of the variation, and the species is associated with higher abundance of litosols and luvisols, and lower abundance of cambisols. Soil pH showed a unimodal response, with low probabilities of species presence for lowest values, high probabilities at intermediate values of pH, and decreasing again for high pH levels. The spatial model accounted for 50.1% of the variation and indicated the occurrence of Mediterranean pine voles in the southern areas of Portugal (Tables 4, 5).

Table 4. Coefficients and their significance of four models for *Microtus duodecimcostatus* data (environmental, soil, spatial, and full model).

Variables	Partial models		Full model	
	B	**p**	**B**	**p**
Environmental				
rain	-4.430	0.000***	-4.373	0.022*
frost	-0.482	0.001**	-0.362	0.185
grass	2.309	0.000***	2.194	0.032*
constant	29.395	0.000***		
Soil				
acid	22.992	0.001**	16.869	0.041*
acid^2	-1.691	0.001**	-1.268	0.046*
cambi	-0.022	0.015*	-0.023	0.121
lito	0.123	0.064	0.010	0.928
luvi	0.086	0.165	-0.004	0.970
constant	-77.097	0.001**		
Spatial				
vec166	27.470	0.000***	9.582	0.086
vec164	22.363	0.000***	12.947	0.005**
vec162	21.930	0.000***	21.937	0.000***
constant	-0.782	0.003**	-26.627	0.324

Note: For variable definitions refer to Table 1. *** $p<0.001$; **$p<0.01$; *$p<0.05$.

Table 5. Summary of performance parameters of all models for *Microtus duodecimcostatus* data.

Models	D^2	AIC	AIC_c	AUC	OCC	Sensitivity	Specificity
ENV	0.344	156.7	157.0	0.850	75.9	83.1	70.5
SOIL	0.406	146.7	147.3	0.902	81.9	90.1	75.8
SPAT	0.501	121.1	121.4	0.924	89.8	90.1	89.5
ENV + SOIL	0.523	126.2	127.5	0.922	87.3	91.5	84.2
ENV + SPAT	0.626	98.8	99.6	0.953	91.6	95.8	88.4
SOIL + SPAT	0.625	103.1	104.4	0.956	91.6	94.4	89.5
ENV+SOIL+SPAT	0.682	96.1	98.4	0.964	93.4	95.8	91.6

Note: D^2, explained deviance; AIC, Akaike's information criterion; AIC_c, Akaike's information criterion corrected for small sample sizes; AUC, area under the receiver operating characteristics (ROC) curve; OCC, overall correct classification.

The full model showed 68.2% of the explained variation, and the models that included the spatial set had higher percentages of explained variation. The strongest pure effect was from the spatial model (15.9%), whereas environmental and soil models explained, respectively, 5.7% and 5.6% of the variation. More than 34% of the variance was explained by the shared effects of models that included the spatial set, indicating that pure spatial effects and spatially structured environmental and/or soil variables accounted for most of the variation in *M. duodecimcostatus* distribution (Fig. 4).

All seven models had high discrimination ability (AUC > 0.85) and good classification accuracy (OCC > 0.75). Only the environmental model presented lower performance statistics, while the joint models containing the spatial set had better performance (Table 5).

The cross-validation procedure confirmed that the model performed well and can be used to predict species presence outside the sampled area: the internal estimate of accuracy was 93.4% while the cross-validation estimate was 89.8%.

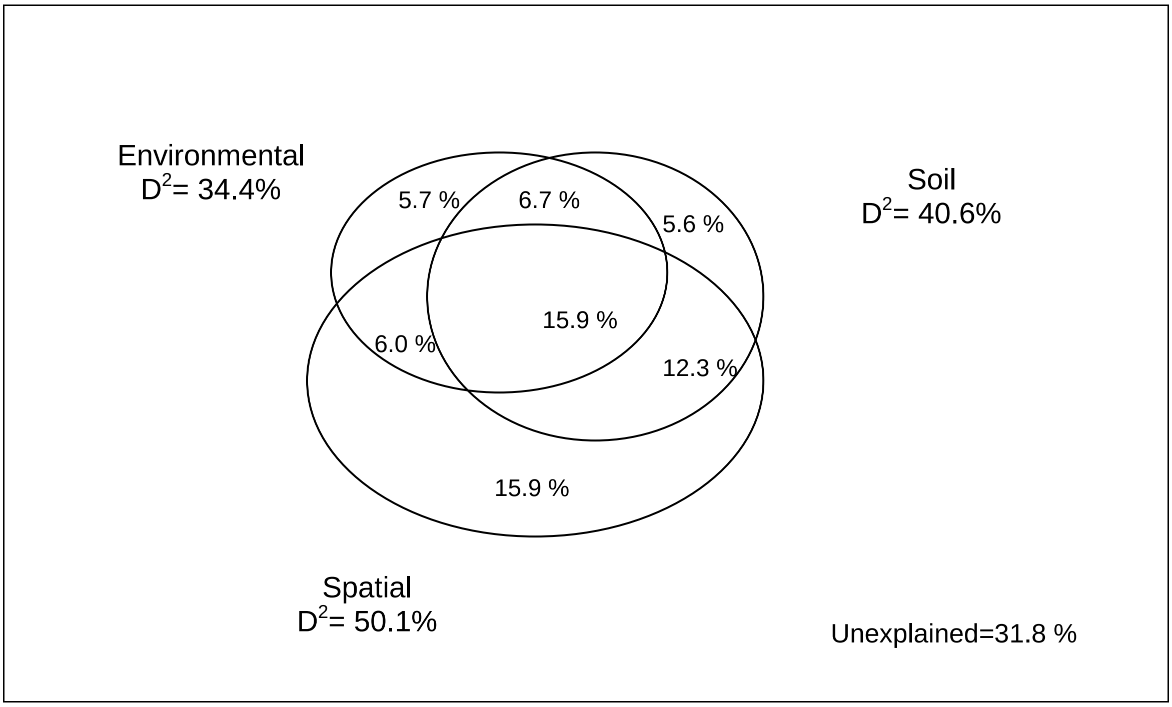

Fig. 4. Results of the variation partitioning for the Mediterranean pine vole (*Microtus duodecimcostatus*) data based on the three sets of independent variables: environmental, soil, and spatial sets. Unexplained is the percentage of unexplained variation. The size of circles and overlap are partially scaled according to the values of each component.

Predicted maps

The potential distribution maps produced by each model set and by the full model for *M. duodecimcostatus* are presented in Figs. 5A-D. Substantial differences can be observed between single models: the environmental model failed to predict known presences in areas of southern provinces. On the other hand, unknown presences are predicted to the west-central coast by the environmental and soil models, and to a large area in the centre and also in the northeastern region by the soil model.

The full model shows the potential distribution of *M. duodecimcostatus* in central and southern areas of the country. The northern limits are predicted to be Alcochete at west (38º46′55′′N, -08º56′44′′E) and Idanha-a-Nova district at east (39º52′48′′N, -07º09′32′′E). This model misclassified a few known sample presences only in the centre of the country, although presence areas are predicted for neighbouring cells. Absence cells are also predicted for the south (Monchique and Moura localities), and there is a discontinuity in the distribution area in a mountain region of the east-centre of the country (Fig. 5D).

Fig. 5. Predicted occurrence of the Mediterranean pine vole (*Microtus duodecimcostatus*) in Portugal based on the four models presented: (A) environmental model, (B) soil model, (C) spatial model, and (D) full model. Light gray indicates a threshold of 0.30, while dark gray indicates a threshold of 0.43.

Potential sympatric areas

Both species occur in potential sympatry in a fragmented belt in central Portugal, which runs from the southwest coast (Monchique to Setúbal: 37º20’16’’N to 38º46’55’’N, -08º29’44’’E to -08º56’44’’E) to the east Spanish border (Elvas to Idanha-a-Nova: 38º50’26’’N to 39º52’48’’N, -07º12’40’’E to -07º09’32’’E) (for details see Fig. 6). The general area is spatially discontinuous and has a maximum width of 80 km at the largest localities (west of the country). Distances between nearest sympatric areas (major or minor) varies between 20 and 70 km. Cells of potential sympatry represented 9.6 % of the potential distribution of *M. lusitanicus*, 18.1 % of that of *M. duodecimcostatus*, and 6.6 % of all studied area.

In the centre of the country there is a parapatric boundary (i.e. separated but contiguous distributions between pairs of taxa, abutting along common boundaries; sensu Bull 1991) surrounded by sympatric areas, both in the east and west. An isolated co-occurrence cell is predicted for the northwest coast and several joined absences are predicted for the centre and south of the country. From the pellet samples analysed, only four revealed both species presence. The predicted sympatric area failed to include three of these localities, although all of them were at 10 km of the predicted sympatric cell and one locality was in the parapatric border (Fig. 6).

Considering the explanatory variables integrating the models of both species, a mean value for these variables can be calculated from the full models that correspond to the range boundaries of both species (thus sympatry or parapatry). According to this, the mean contact area of the two species has 750-800 mm of annual rainfall, 40 days of frost per year, 40-50% cover of cambisols, 50-60% of litosols, soil pH of 5.5-6.0, and presence or absence of grasslands.

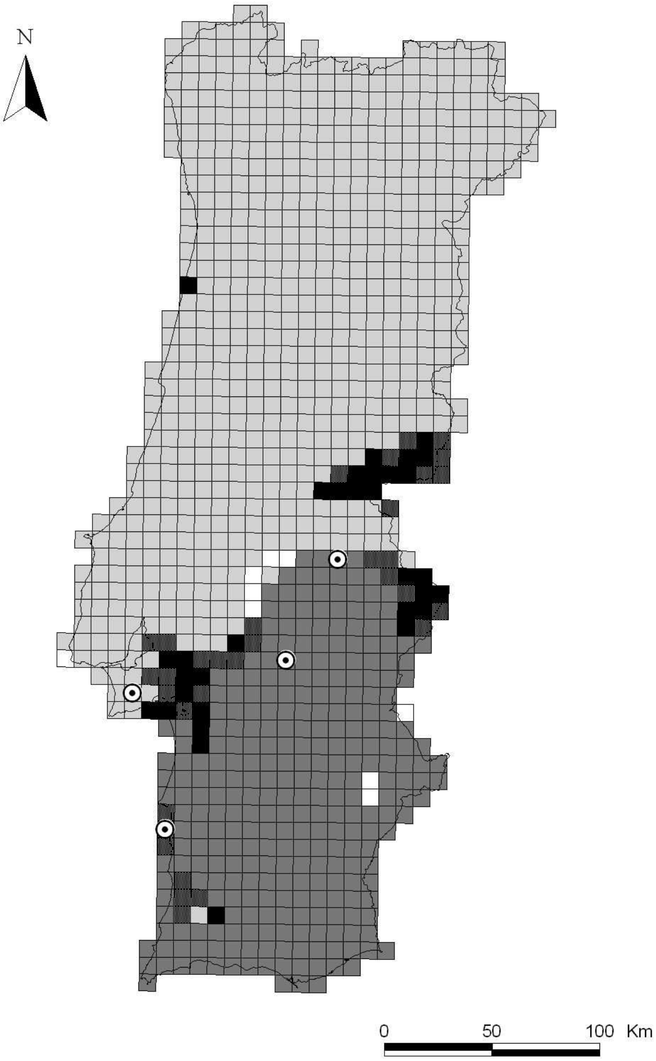

Fig. 6. Predicted occurrence of major sympatric areas for the two pine voles (black), minor sympatric areas (dark gray), allopatric areas for the Lusitanian pine vole (*Microtus lusitanicus*) (light gray), allopatric areas for the Mediterranean pine vole (*Microtus duodecimcostatus*) (medium gray), and absence areas for both species (white). The four localities where both species were sampled are indicated with a circle.

DISCUSSION

Factors influencing distribution

Results indicate that most of the variance in the large-scale distributions of *M. lusitanicus* and *M. duodecimcostatus* in Portugal is explained by spatial variables, alone or structured with environmental and soil effects. Considering only the pure fractions, the spatial variables still explained the major part of this variability in both species distributions, which was independent of the environmental and soil variables used in our study. This spatial structure present in the distribution of both species of pine voles may have originated from several sources, namely unmeasured explanatory variables spatially autocorrelated (possibly the most probable source of spatial autocorrelation at coarser scales; van Teeffelen and Ovaskainen 2007), but also environmental or geographical barriers, historical biogeography, long-distance movements, species interactions, metapopulation dynamics, or a combination of several of these factors (Bahn and McGill 2007; Dormann et al. 2007; Miller et al. 2007). Although we expected the presence of spatial effects, we have no detailed information on the ecology of each species that allow specification of which exact ecological processes are causing this spatial pattern. Future work needs to be done to identify the causal mechanisms that explain the autocorrelation in species' distributions, namely at coarse spatial scales (but see van Teeffelen and Ovaskainen 2007).

Contrasting with the usual high importance attributed to climate and topographical variables on large-scale distributions of species (Reutter et al. 2003; Anderson et al. 2002; Mira et al. 2008), recent studies referred also to the high prevalence of spatial effects in the distribution of bird species (Reino 2005; Bahn et al. 2006; Bahn and McGill 2007). Such differences in results can be explained by the increasing awareness of the importance of quantifying the spatial component in species distribution models, in addition to its simple inclusion in the models. Indeed, studies that do not account for spatial autocorrelation may falsely identify climate or topographical factors as most important factors influencing species distributions (Legendre 1993; Segurado et al. 2006; Dormann 2007).

It is widely known that different ecological processes may emerge at different scales, noticeably species distributions (Morris 1987; Delattre et al. 1996; Cushman and McGarigal 2002). Our results apply to the coarse scale that we used (10 km x 10 km). We should expect a higher importance of the environmental and soil components in studies conducted at finer resolutions that allow a more discontinuous vole occurrence pattern (with absence records within the general occurrence area of species), when compared to the continuous pattern presented in our study. Accordingly, Paradis (1995) suggest that *M. duodecimcostatus* shows a discontinuous distribution at local and landscape scales because of the heterogeneity of soil types at those finer scales.

Predicted distributions

The potential distribution maps confirmed that the Lusitanian pine vole occurs in the north and centre of the country, and revealed that the species is distributed outside landscape units of grasslands, in areas of high rainfall and frost, extreme values of soil pH (mostly acid soils), high abundance of cambisols, low abundance of litosols, and presence of solonshaks. In these areas the climate is colder and rainy with high abundance of mountainous areas, and dominance of fertile, well-drained and easy mobilized soils (cambisols; Ribeiro 1984; Varennes 2003). The combination of rain abundance and these soil characteristics offers good conditions for a burrowing rodent (like *M. lusitanicus*) in terms of energy expenditure in the tunnel construction and maintenance (Busch et al. 2000; Spinks et al. 2000; Romañach et al. 2005). On the other hand, the southern areas of the country are occupied by the Mediterranean pine vole, mainly in the grassland landscape unit, with low rainfall and frost, intermediate values of soil pH, higher abundance of litosols and luvisols, and lower abundance of cambisols. Here, the climate is more markedly Mediterranean with milder winters and dry and hot summers (Ribeiro 1984). Hard and shallow soils are common and fertility is generally low (Ribeiro 1984), which may require higher energy expenditure from voles in tunnel construction, especially during the dry season (Spinks et al. 2000; Romañach et al. 2005). These soil characteristics may possibly explain the larger body size, and the stronger fossorial habits of *M. duodecimcostatus* (reflected in morphological and behavioural characteristics), as observed by Mathias (1996). These macroecological differences (environmental and soil) experienced by the two species of pine voles have already been used in the interpretation of observed morphological and physiological differences between the species (Mathias 1990, 1996). Ecological conditions, however, are very similar in the sympatric area and present intermediate values for most studied variables (soil type, temperature, rainfall, etc.), when compared with the north and south of the country.

The presence of *M. lusitanicus* is not presently known for the most southern localities with predicted presence (and corresponding to predicted sympatric areas: Monchique and Elvas). The species presence in the extreme southwest (Monchique) is more plausible because it is supported by the three models (Figs. 3A, 3B, 3C). However, it may correspond to historical occurrences. If future fieldwork confirms present-day occurrence of the species in this region, it must concern isolated populations of high scientific and conservation value. The presence in the extreme southeast (Elvas) is only supported by the spatial model (Fig. 3C), and thus raises more doubts, as environmental and soil characteristics in this locality approach more the typical conditions of the *M. duodecimcostatus* geographical range.

The discontinuity in the predicted distribution of *M. duodecimcostatus* in its northern range may be explained by the influence of a mountain system (next to the border with Spain) which creates different climatic conditions in that region that is closer to the *M. lusitanicus* range characteristics.

Sympatry

The models selected areas predicted to support co-occurrence of both species. Our study shows, for the first time, that the sympatric area is fragmented into several geographical sections, contrasting with the previous thought of a continuous sympatric area, based on the work of Madureira (1981, 1984). In addition, the area estimated by this author encompasses the northern sympatric sections of the present work, but failed to include the southwestern section. Our study also suggests the existence of an extensive parapatric boundary between sympatric areas. Parapatry differs from sympatry by the extent of range overlap and may result from first meeting between taxa that have been isolated in geographical refugia, having spread until their ranges now meet (Bull 1991). It implies both hybridizing and non-hybridizing contact areas (Bull 1991). The present data are, however, insufficient to infer on the clear presence of a parapatric boundary, and thus on the existence of hybrids. This suggests that new questions need to be addressed within a finer scale framework, and our results show which regions of the country are best suited for these studies.

The pattern of small and separated sympatric areas interspaced by a parapatric area suggests a repulsed geographical distribution pattern between species. First evidence for interspecific competition has been derived from insights on sympatric species and contact areas (Miller 1964; Grant 1972; Bull 1991; Arif et al. 2007). An inverse spatial relationship and abrupt range limits in distribution areas presented by two sympatric species may indicate the existence of interspecific competition (Grant 1972; Case et al. 2005). There are, however, special properties that allow the coexistence of closely related species: mechanisms that guarantee reproductive isolation and competition avoidance with the other species that uses the same or similar environmental resources (Grant 1972; Mayr 1975). These mechanisms can be differences in diet, spatial and temporal patterns of activity, behaviour, or even microhabitat (Miller 1964; Grant 1972; Mayr 1975; Parren and Capen 1985). Although very little information exists to quantify some parameters for these pine voles, the results on habitat characteristics in an area of local sympatry in the Spanish Pyrenees provides evidence for the existence of habitat segregation at the microscale level between *M. lusitanicus* and *M. duodecimcostatus*, even though both species are herbivorous (Mathias 1999; Borghi et al. 1994). For *M. duodecimcostatus*, a more aggressive behaviour and larger body size was observed when compared with *M. lusitanicus* (Madureira 1984; Vinhas 1993), possibly enabling or maintaining reproductive isolation. Nevertheless, other causes may explain the observed patterns. Without evidence other than that of geographical distribution, it is impossible to confidently attribute any of these distribution patterns to a particular causal mechanism.

It would be very instructive to investigate how the two species may (or may not) interact, the mechanisms that maintain the limits of species distributions, and the ecological processes producing the observed spatial patterns in the distribution of both species. At the same time, this parapatry and sympatry pattern poses additional questions on what is the real pattern at finer scales, and questions whether hybridization occurs.

Implications of Barn Owls as collectors

The analysis of Barn Owl pellets is a widespread method for sampling small mammals over large areas (Millán de la Peña et al. 2003; Mira et al. 2008; Rodríguez and Peris 2007). It is often accepted that Barn Owls show no food preferences (Mikkola 1983), and that the abundance of small mammals in the diet is a reasonable estimate of true abundances in the hunting territory of the owls (Mikkola 1983; Agnelli and Marinis 1993; Avenant 2005). However, to obtain an unbiased sampling of the distribution of *M. lusitanicus* and *M. duodecimcostatus*, we have to assure that owls are equally distributed across the country (to avoid regions with lack of information or samples), and that birds hunt in all habitats (to ensure that there is no bias in habitat types sampled). Considering the first issue, Barn Owls occur across the entire country, albeit in the north its distribution is more fragmented because their preferred habitats are less abundant (Equipa Atlas 2008). The preferred habitats include open habitats and agricultural areas, but Barn Owls also occur in urban and suburban areas, open woodlands, and "montados" (Equipa Atlas 2008). The less preferred habitats are continuous areas of shrubland and of forestry plantations (Equipa Atlas 2008). The pine voles are also common in a similar range of (open) habitats, such as riversides, meadows, pastures, and agricultural areas (Mira and Mathias 2007; Cotilla and Palomo 2007). In addition, both vole species are common and abundant in their respective occurrence areas (Mira and Mathias 2007; Cotilla and Palomo 2007) and are within the range of body masses most captured by owls in Europe (range of body masses most captured: 10-30 g; *M. lusitanicus*: 14-19 g; *M. duodecimcostatus*: 19-32 g; Taylor 1994). Thus, although there are habitats undersampled by owls, they mostly correspond to habitats where vole presence is less frequently documented. According to this, the analysis of Barn Owl pellets appears to be a valid method to sample presence and absence of *M. lusitanicus* and *M. duodecimcostatus* in our study area.

Conclusions

The present work revealed how important spatial patterns can be in explaining species distribution and how critical it would be to ignore spatial autocorrelation, as it is often present in macroecological or biogeographical data sets (Dormann et al. 2007). In fact, a recent study highlighted that for large scale distribution modelling, the relationships between species' distribution and environment could be weaker than some investigators tipically claim, because both species and environment can be strongly dependent on space (Bahn and McGill 2007; but see Currie 2007).

Novel approaches were used to explore the nature of large scale distribution and interactions of two sister species of pine voles, and that stimulated new questions for the study of potential relationships between these species, which should be addressed in future studies. Specifically, our work made an important contribution to future fieldwork by suggesting environmental and soil conditions that are strongly associated with distribution limits and are important for field surveys. Furthermore, our work identified specific areas for investigation where the models predict sympatry and parapatry.

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Chapter 4

Regional scale distribution of pine voles

Paper II

The influence of local, landscape and spatial factors on the distribution of the Lusitanian and the Mediterranean pine voles in a Mediterranean landscape.

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The influence of local, landscape and spatial factors on the distribution of the Lusitanian and the Mediterranean pine voles in a Mediterranean landscape

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ABSTRACT

This study evaluated the relative contributions of local and landscape factors, and spatial structure on the regional distribution of the Lusitanian pine vole (*Microtus lusitanicus* (Gerbe, 1879)) and the Mediterranean pine vole (*Microtus duodecimcostatus* de Selys-Longchamps, 1839) over an area of 1613 km^2 on a Mediterranean landscape in central Portugal. The three sets of explanatory variables (local, landscape and spatial) were analysed independently with a generalized linear model, followed by a variance partitioning procedure. The variance in the distribution patterns of *M. lusitanicus* and *M. duodecimcostatus* was mostly explained by fine-scale environmental factors, but the spatial effects were also important, especially in the distribution of *M. duodecimcostatus*. The close proximity of verges, and the high abundance of humidity and coarse sand in the soil were the most influencing local-scale factors for the presence of *M. lusitanicus*, while at a larger scale the high abundance of linear habitats was the main landscape feature determining its occurrence. Regarding the presence of *M. duodecimcostatus*, the close proximity of verges, and the high tree canopy cover and herbaceous vegetation biomass were the most influencing local explanatory variables in its occurrence, while the absence of shrubs in the surrounding habitat was the main factor regulating the species presence at a landscape scale. The similarities that we found between the two species were mostly concerned with descriptors of cover, food and burrows, while the differences appeared as opposing spatial trends and coarse-scale descriptors.

Key-words: *Microtus duodecimcostatus*, *Microtus lusitanicus*, scale, spatial effects, variance partitioning

INTRODUCTION

Scale is a determinant aspect in species ecology because it influences the conclusions drawn by an observer and whether the results can be extrapolated to other times or locations (Legendre and Legendre, 1998; Turner et al., 2001, Cushman and McGarigal, 2002). Species habitat models have traditionally focused on fine spatial scales, but it is being increasingly recognized that habitat variation occurs across multiple scales, such as biogeographic, regional, and local (Chambers and Dickman, 2002; Suárez-Seoane and Baudry, 2002; Miller et al., 2004). In fact, many ecological processes, including species distributions, are driven by multiple factors acting at diverse organizational levels and across diverse spatial scales (Delattre et al., 1996; Cushman and McGarigal, 2002). Moreover, habitat patches are arranged hierarchically within landscapes, and the different species perceive and respond to these landscape features at different spatial scales (Kotliar and Wiens, 1990; Wiens, 1996; Farina, 2006). The spatial scale that a given species responds to is influenced by how habitat features are perceived and used by that species (Wiens, 1996; Suárez-Seoane and Baudry, 2002). These differences in scales of perception and habitat use by animals are largely influenced by physiological, morphological and behavioural characteristics, such as body size, ability to move, predators' avoidance and resource use (Farina, 2000).

On the other hand, the quantification of the spatial components can also help to correct for the presence of spatial autocorrelation and to clarify the importance of spatial trends and neighbourhood effects in species distribution processes (Legendre, 1993; Segurado et al., 2006).

A large number of endemic species of small mammals live in the Mediterranean Peninsulas (Bilton et al., 1998), including the Iberian, contributing to the recognised importance of the Mediterranean Basin as a biodiversity hotspot (Blondel and Aronson, 1999; Médail and Quézel, 1999). A large number of small mammal species play an important role in Mediterranean food webs as prey items for both terrestrial and avian predators (López-Gordo et al., 1976; Delibes et al., 1984; Veiga, 1986; Blondel and Aronson, 1999), but, despite this, there is still a lack of basic ecological data for some species, namely the microtines (Paradis and Guédon, 1993; Blondel and Aronson, 1999). The Lusitanian pine vole (*Microtus lusitanicus* (Gerbe, 1879)) and the Mediterranean pine vole (*Microtus duodecimcostatus* de Selys-Longchamps, 1839) are western European endemisms: the Lusitanian pine vole is distributed in the northwest of the Iberian Peninsula and in the French Pyrenees, and the Mediterranean pine vole occurs in the south-centre of Iberian Peninsula and in southern France (Cotilla and Palomo, 2007; Mira and Mathias, 2007). In Portugal the two species have a general allopatric occurrence pattern with a sympatric area occurring in the centre of the country (Madureira, 1984).

Both pine voles are referred to be common species with a preference for open areas such as meadows, pastures and agricultural areas, especially commercial orchards and irrigated crops where they can cause important damages (Vinhas, 1993; Mira and Mathias, 2007; Cotilla and Palomo, 2007). Although these general habitat preferences are broadly accepted, few studies have detailed and quantified these associations for either of the species in a region of potential sympatry. Borghi et al. (1994) conducted a small-scale habitat investigation in the Spanish Pyrenees where the two species coexisted. However, results report to a 1-ha area, at 2000 m of altitude, and extrapolations to wider areas or regions are difficult.

Some work has been conducted on the ecology and population dynamics of *M. duodecimcostatus,* mostly in agricultural areas (Guédon et al., 1992; Guédon and Pascal, 1993; Paradis, 1995; Mira, 1999). Equivalent studies are, however, rarer for *M. lusitanicus* (Rodríguez and Peris, 2007). Specifically, no attempt was made in modelling the habitat of the two species with multiple environmental variables, both at local and landscape scales.

It has been suggested that larger and more vagile rodents have a better perceptual range of the landscape (Mech and Zollner, 2002). This means that larger animals tend to observe landscape features over longer distances and, thus, will perceive the landscape at a larger spatial extent (Gehring and Swihart, 2003). The reverse should also be true and thus, smaller and less mobile rodents will perceive and respond to finer scales in the landscape. In addition, it has also been suggested that the distribution of mammals with larger home ranges and higher-distance movements are better explained by large-scale factors than by local-scale factors (Wiens, 1996; Gehring and Swihart, 2003). When compared with larger mammals, rodents should have higher costs for movements and lower perception of landscape structure (Wiens, 1996). Within rodents, microtines are known for being less vagile and having small home ranges when compared with non-microtines (McNab, 1963). The two pine voles here studied are both small sized (*M. lusitanicus*: 14-19 g and *M. duodecimcostatus*: 19-32 g; Cotilla and Palomo, 2007; Mira and Mathias, 2007) and both have fossorial habits (Giannoni et al., 1993), indicating a further lower vagility when compared with other surface-dwelling microtines.

Our main objective is to assess the relative contributions of local and landscape factors in the regional distribution of two pine voles across a

Mediterranean landscape in central Portugal, where the two species ranges are known to overlap. In addition, we also aim to: i) evaluate the role of the spatial factors on the distribution patterns of each species, and ii) identify the environmental factors (local and landscape) that most influence the distribution of *M. lusitanicus* and *M. duodecimcostatus* in central Portugal. According to the previous considerations on the perception scales of small fossorial microtines, we hypothesize that local factors should play a major role in the distribution of the two pine voles, when compared with the landscape ones.

MATERIAL AND METHODS

Study area

The study area is located in the northern Alentejo (UTM 0635030 W 4350608 N, Portalegre district, Portugal), over an area of 1613 km^2 (figure 1). It includes the highest mountain in southern Portugal (São Mamede mountain) and adjacent lowlands (Cancela d'Abreu et al., 2002). At the lowlands the landscape is dominated by large open fields mixed with patches of open oak forest (Cork, Holm, and/or Pyrenean oak trees with an agro-silvo-pastoral use, called "montado"; Pinto-Correia, 1993). Close to the villages these land uses are interspaced with polycultures. The mountain of São Mamede has contrasting characteristics when compared to surrounding lowlands. It presents a marked relief with 1025 m a.s.l. at the highest point and extensive areas above 600 m a.s.l.. The high geologic and microclimatic diversity of the mountains has conducted to a higher heterogeneity in the land uses. The agriculture is more pronounced in the valleys; perennial cultures are common in the plateaus; and forest plantations are dominant in the steep slopes (Cancela d'Abreu et al., 2002).

The climate is Mesomediterranean with a mean temperature of 7.4 ºC in January (3.1 – 11.7 ºC) and 24.7 ºC in July (16.8 – 33.3ºC), and an annual rainfall of 717 mm (Rivas-Martínez and Arregui, 1999; Ninyerola et al., 2005).

Vole sampling

From September 2006 to May 2007, 175 trapping plots were sampled across the study area. In each plot, 10 subterranean live-traps of multiple captures ("mole type") were set up for three consecutive nights, with apple as bait and hay for bedding. The selection of trapping plots was dependent on finding enough surface presence signs to allow the set up of all 10 traps (burrow openings and/or soil mounds, Santos et al. 2009a). Thus, the number and location of trapping plots was limited by finding vole presence signs. Even so, efforts were made to sample habitats in proportion to their availability. A typical monthly sampling (over a 12-day period) included 21 trapping plots arranged in three sets of seven simultaneous plots. Each plot was sampled only once (figure 1).

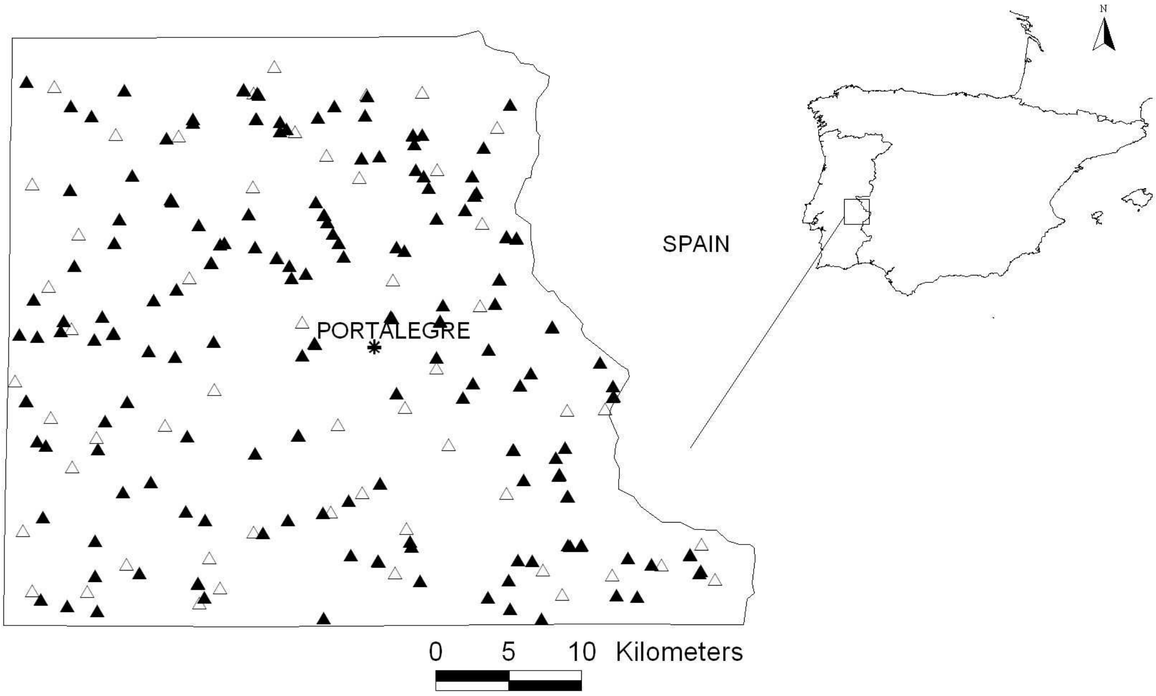

Figure 1. Distribution of sample sites across the study area (filled triangles: trapping plots (n=175); open triangles: absence plots (n=56)).

Captured voles were identified on the basis of body measurements: the hind feet length (HFL: *M. lusitanicus* < 16 mm > *M duodecimcostatus*), body length (BL: *M. lusitanicus* < 105 mm and *M duodecimcostatus* > 89 mm), and colour pattern (Madureira, 1982; 1984). Only adult animals were used in species identification, and juveniles and sub-adults captured in the same plot with an adult were assumed to be conspecifics. Because adult *M. lusitanicus* are morphologically similar to sub-adult *M. duodecimcostatus*, we used reproductive signs to help identification: voles with signs of sexual activity (scrotal testes in males, perforated vagina or lactating in females; McCravy and Rose, 1992) were considered to be adults. Plots with captured voles, assigned to one or the other species, were considered as presence plots in further analyses, while the plots with no pine vole captures were discarded. Fifty-six absence plots of 10 m radius were also defined throughout the study area (figure 1). The criterion for accepting an "absence plot" was the lack of any superficial presence signs of pine voles or moles (as there are reports of voles using mole tunnels; authors pers. observ.), after a careful search of 20 minutes. No trapping was undertaken at these plots.

Minimum distance between plots was set to 500 m to avoid pseudo-replication. This conducted to the elimination of nine presence plots: five of *M. lusitanicus* and four of *M. duodecimcostatus*.

Sets of explanatory variables

Three sets of explanatory variables were defined: local (LOC), landscape (LAND) and spatial (SPAT). The local and landscape sets are also referred in the text as Environmental variables. Initially, each set of explanatory variables was analysed independently with a generalized linear model (GLM) with binomial error structure (e.g. Guisan et al., 2002) to select the most parsimonious models to be used in further analyses. After that, models of each variable set were combined in joint (each pair of two sets) and full (all three sets) models, in order to perform the variance partitioning method (Borcard et al., 1992; Legendre, 1993; Cushman and McGarigal, 2002). The inclusion of a spatial component will correct for the existence of autocorrelation in the data and will also quantify its influence on species' distributions (Borcard et al., 1992; Legendre, 1993).

Environmental variables

Environmental data were collected at two scales of analysis: local and landscape. The local level was defined as the surface occupied by the traps (16 to 630 m^2, mean=62 m^2) at the presence plots, and the circular area of absence plots (314 m^2). The landscape level was defined as a 250 m radius surrounding the centre of the sampling plot (196 250 m^2). The local scale should correspond to high intensity of use within the home range of pine voles, as 62 m^2 is quite lower than the mean home range of *M. lusitanicus* (952 m^2; Santos et al., in press). The 250 m radius was selected as a reasonable approximation of the dispersal distance of voles, as it was also used in other similar studies (e.g. Delattre et al., 1999). We consider the value biologically relevant, as most estimates of dispersal distances for microtines range from 100 to 160 m (McShea and Madison 1992). Also, maximum observed distances between two points of a home range for *M. lusitanicus* was less than 230 m (Santos et al., in press).

At the local level we recorded 20 variables from six sub-groups: topography, local habitat, vegetation structure and composition, grazing, verges, and soil properties (table 1).

Altitude was measured with a hand-held GPS.

Most variables concerning vegetation structure and composition were surveyed in two to five 1x1 m quadrats by stratified random sampling (Kent and Coker, 1992). These variables included cover percentages of bare soil, herbaceous layer, woody vegetation (under 3 m height), grass species, composite species, and mean herbaceous vegetation height (obtained after ten random measures in each quadrat). Cover percentages of tree and shrub layers were obtained through visual estimation for the entire sampling plots (Bullock, 1996). The herbaceous vegetation was clipped in two to four 0.30 x 0.30 m sub-quadrats per plot (Pucheta et al., 1998). To obtain data on dry vegetation biomass, samples from sub-quadrats were oven dried in the laboratory, at 105 ºC, during 24 hours, and weighted afterwards at a 0.1 g precision (Kent and Coker, 1992). The inclusion of this sub-group of variables is justified as vegetation provides food, shelter and nesting sites for microtines (Rose and Birney, 1985; Lin and Batzli, 2001).

Grazing was defined as a presence/absence variable. Also, historical (or past) grazing was assessed through the measure of cover percentage of plant species with a prostrated growing that should be indicative of more long-term grazed sites (e.g. Lavorel et al., 1997; Sternberg et al., 2000) in 1x1 m quadrats (see details above). It is known the negative influence that high grazing pressure has on vegetation structure and herbaceous cover for small microtine species (Schmidt et al., 2005; Evans et al., 2006).

Distance to the nearest verge (field margins, hedgerows, roadsides, stone walls, ecotones) was registered from the sampling plot centre.

Because pine voles have fossorial habits, soil characteristics at 0.10 - 0.15 m depth were also described. This depth was chosen as it corresponds to highest densities of subterranean plant organs and covers also a high number of underground tunnels (Yeboah and Akyeampong, 2001). Soil penetration resistance was measured with a penetrometer (DICKEY-John Soil compaction tester) to a depth of 0.10 m. Ten readings were obtained in two orthogonal transects and the median value calculated for each plot (Martínez and Zinck, 2004). At the sub-quadrats with clipped vegetation, two soil samples were collected to assess soil humidity and texture values. The gravimetric method was used in humidity calculations, with oven at 105ºC during 48 h (Martínez and Zinck, 2004). The texture analyses were performed in the Soils Physics Laboratory of Phytotechny Department of University of Évora.

The number of quadrats and sub-quadrats sampled in each presence plot varied according to the area occupied by traps. A minimum of two quadrats and two sub-quadrats were sampled in smaller areas (< 40 m^2). In absence plots, five quadrats and four sub-quadrats were always sampled.

Table 1. Variable sets, correspondent explanatory variables, their original values, and transformations (a: *M. lusitanicus* data; b: *M. duodecimcostatus* data; log: natural logarithm; P(0.5): square root; P(0.33): cubic root; (0-1): binary).

Code	Description	Values	Transformation
Local set			
ALTI	Average elevation (m a.s.l.)	(167-820)	log
BARE	Cover percentage of bare soil	(1.5-67.0)	P(0.5) (a,b)
VEGET	Cover percentage of herbaceous vegetation	(33.0-99.0)	P(0.5) (a,b)
WOOD	Cover percentage of woody vegetation (under 3 m height)	(0-66.6)	P(0.5) (a) P(0.33) (b)
GRASS	Cover percentage of grasses	(4.0-80.0)	P(0.5)
COMP	Cover percentage of composites	(0.2-75.0)	P(0.5)
MHEIG	Mean herbaceous vegetation height (after 10 random measurements) (m)	(2.5-93.4)	log
TREE	Cover percentage of trees	(0-90)	P(0.5)
SHRUB	Cover percentage of shrubs	(0-75)	P(0.5)
BIOM	Dry vegetation biomass (g/m^2)	(57.2-803.9)	log
GRAZ	Presence of grazing signs (faeces, footprints, grazed grass, or observation of grazers)	(0-1)	
PROSTR	Cover percentage of plant species with a prostrated growing	(0-83)	P(0.5) (a) P(0.33) (b)
VERGED	Distance to the nearest verge (field margins, hedgerows, roadsides, stone walls, ecotones) (m)	(0-300)	P(0.33)
CMED	Median values of soil penetration resistance at 0.10 m depth (after 10 measurements in orthogonal transects) (psi)	(1-6)	
HUMID	Percentage of water in the soil at 0.10-0.15 m depth	(1.9-35.2)	P(0.5)
SAND2	Percentage of coarse sand (2-0.2 mm)	(5.7-71.5)	P(0.5)
SAND02	Percentage of fine sand (0.2-0.02 mm)	(13.3-51.7)	P(0.5)
SAND	Percentage of total sand (2-0.02 mm)	(27.2-91.4)	P(0.5)
LIME	Percentage of lime (0.02-0.002 mm)	(4.7-45.3)	P(0.5)
CLAY	Percentage of clay (< 0.002 mm)	(3.9-30.4)	P(0.5)
Landscape set			
URB	Percentage of urban areas	(0-13.7)	0-1
WAT	Percentage of water courses and dams	(0-7.1)	0-1
AGRI	Percentage of agricultural areas (crops, orchards, vines, etc.)	(0-85.6)	P(0.5)
OPEN	Percentage of open areas (pastures, meadows, fallow fields)	(0-97.0)	P(0.5)
LINH	Percentage of linear habitats (verges bordering roads, stonewalls and ecotones, and linear dense vegetation)	(0-13.4)	P(0.5)
SHRUB2	Percentage of shrubs formation	(0-83.0)	0-1
PLANT	Percentage of young oak forest plantation	(0-97)	0-1

Table 1. (continuation)

Code	Description	Values	Transformation
Landscape set			
MONT	Percentage of "montado" (cork, holm or Pyrenean oaks; Pinto-Correia 1993)	(0-100.0)	P(0.5)
FORE	Percentage of forest (natural, production or mixed)	(0-99.0)	0-1
NUMP	Number of patches	(1-49)	P(0.5)
MPS	Mean patch size (ha)	(0.4-19.5)	log+1
MEDPS	Median patch size (ha)	(0.2-19.5)	log+1
PSCOV	Variance coefficient of patch size (ha)	(0-190.2)	log+1 (a)
PSSD	Standard deviation of patch size (ha)	(0-9.6)	log+1
TE	Total edge of patches (m)	(1568.8-17153.1)	
ED	Edge density (m/ha)	(0.6-628.7)	log+1
MPE	Average amount of edge per patch (m)	(350.1-1568.8)	log+1
MSI	Mean shape index	(1.0-2.6)	
AWMSI	Area weighted mean shape index	(1.0-2.0)	
MPAR	Mean perimeter-area ratio	(80.3-21589.3)	log+1 (b)
MPFD	Mean patch fractal dimension	(1.2-1.5)	
AWMPFD	Area weighted mean patch fractal dimension	(1.2-1.4)	
SDI	Shannon's diversity index	(0-2.6)	
Spatial set			
AUTO_ML	Autologistic term for *M. lusitanicus* data	(0-1)	
AUTO_MD	Autologistic term for *M. duodecimcostatus* data	(0-1)	
X	X coordinates centred	(-1.615-2.211)	
Y	Y coordinates centred	(-1.727-2.188)	
XY	Multiplication of centred X and Y	(-3.498-2.471)	
X^2	Square of X coordinates centred	(0.000-4.888)	
Y^2	Square of Y coordinates centred	(0.000-4.786)	

Variables of landscape composition were established from aerial photographs (flights 2003 and 2005; IGeoE), corrected during field surveys. Land cover types were classified in a GIS with a working scale of 1:2000, and a minimum polygon size set to 190 m^2. Cover percentages of each land cover type were then calculated for each 250 m buffer. As most of these newly defined variables (n=38) presented zero-inflated data, we pooled them in eight broader categories: urban, water (small and large dams, rivers), agriculture (crops, orchards, vines), open (pastures, meadows, fallow fields), shrub, oak plantation, "montado" and forest (table 1). Moreover, we mapped linear habitats (verges bordering roads, stonewalls and ecotones, and linear dense vegetation) in a distinct category because they are considered potential dispersal corridors for voles (Bennett, 1990; Turner et al., 2001; Litvaitis et al., 2003; table 1).

Landscape metrics were calculated for the land cover types and thus, 14 additional variables were obtained, concerning size, edge, shape, and diversity metrics of the different land cover categories. Globally, the landscape set was described by 23 variables (table 1).

Spatial variables

The spatial group of variables was defined through an autologistic term and a second order polynomial of centred spatial coordinates, summing six variables (table 1). The autologistic term (Augustin et al., 1996; Dormann et al., 2007) is derived from the responses at neighbouring cells within 6 km of distance and corresponds to a weighted mean of the inverse of the square distance of the centre of each sample plot to each sampled neighbour. This autologistic term is intended to capture local or fine-scale spatial autocorrelation, whereas the polynomial of the geographic coordinates should capture more large-scale spatial variation (Legendre and Legendre, 1998; Méot et al., 1998; Miller et al., 2007).

Statistical modelling

Two data matrices were built, one for *M. lusitanicus* with 34 presence and 56 absence plots (n=90), and another for *M. duodecimcostatus* with 35 presence and 56 absence plots (n=91). For each species, we developed three single set models (LOC, LAND, and SPAT).

In each case, due to the large number of environmental variables we started our variable selection with a univariate screening of all environmental variables. Only variables that achieved significant univariate models ($P<0.05$; Bussche et al., 2008) were considered for further model development. Logarithmic, square root and cubic root transformations were performed on several explanatory variables to achieve normality. Zero-inflated variables were transformed into binary classes (table 1). There was no need of a previous univariate screening of the spatial variables due to their reduced number. To avoid multicollinearity, we performed pairwise Pearson correlations among all pairs of continuous independent variables (environmental and spatial) and, for pairs with r > |0.7|, we excluded the variable with lower univariate model performance from further analyses (Tabachnick and Fidell, 2001).

When building multivariate models we used the information-theoretical model comparison approach (ITMC; Burnham and Anderson, 2002) for variable selection because when a large number of explanatory variables are available, the traditional stepwise method performs poorly and results are often unstable (Whittingham et al., 2006). Models with all possible combinations of remaining variables (after univariate screening) were developed for each variable set and compared with the Akaike's information criterion corrected for small samples (AIC_c; Burnham and Anderson, 2002). Models with $\Delta AIC_{c,\ i} < 4$ (ΔAIC_c = difference between each model AIC_c and $AIC_{c,\ min}$) are considered to have substantial support as candidate models (Burnham and Anderson, 2002). If no single model was clearly superior to others in the set $\Delta AIC_{c,\ i} < 4$, a model averaging approach was performed which bases inference on the entire set of models (Burnham and Anderson, 2002).

The variance partitioning procedure implies building a full model with all variables selected in each variable set, besides the development of joint models. However, to achieve the rule of a minimum of 10 cases for each explanatory variable included in the model (Harrell et al., 1996), only variables with more than 90% of importance value within the best models ($\Delta AIC_c < 4$) of each variable set were considered for variation partitioning calculations. Before these calculations, interactions and quadratic terms were tested on best models (within each set) and retained if a decrease in the AIC_c was verified.

Besides the three single set models (LOC, LAND and SPAT), another three joint models (LOC + LAND, LOC + SPAT, LAND + SPAT), and one full model (LOC + LAND + SPAT) were estimated. Seven components of explained variation were obtained through the partitioning method (Borcard et al., 1992): a) pure effects of LOC; b) pure effects of LAND; c) pure effects of SPAT; d) shared effects of LOC + LAND; e) shared effects of LOC + SPAT; f) shared effects of LAND + SPAT; and g) shared effects of LOC + LAND + SPAT (Borcard et al., 1992; Borcard and Legendre, 1994).

The explained deviance (D^2) was used as a measure of variance explained by each logistic model (Guisan and Zimmermann, 2000).

Model goodness-of-fit was evaluated by explained deviance (Guisan and Zimmermann, 2000) and validation graphs. Model discrimination accuracy was assessed with the Area Under the receiver operating characteristics Curve (AUC), which is a threshold-independent criterion for model discrimination (Fielding and

Bell, 1997). We also calculated the overall correct classification rate (OCC) and the proportion of presences (sensitivity) and absences (specificity) correctly classified, using the prevalence (0.38 for both species) as the threshold value (Jiménez-Valverde and Lobo, 2007). Model performance procedures were evaluated independently for the three simple models, for the three joint models, and for the full model, for each species.

To evaluate the degree of non-independence in error terms, plots of residuals were examined for spatial patterns and the global Moran's I of residuals was tested for significance (Dormann et al., 2007). This procedure was done only in single set and full models.

We used R software 2.6.0 (R Development Core Team, 2007) in all model building procedures. The dRedging package (dredge function; Burnham and Anderson, 2002) was used in the ITMC approach, and the autocovariate terms were calculated with spdep package (version 0.4-21, Bivand et al., 2008). Landscape metrics were calculated with Patch Analyst and Spatial Analyst extensions for Arcview 3.2. (ESRI, 1999).

RESULTS

Distribution patterns

We captured a total of 217 individual voles: 105 *M. lusitanicus* and 112 *M. duodecimcostatus*. Out of 175 trapping plots, 55.4% (n=97) failed to catch any voles, despite existing presence signs. Considering only the presence plots used for data analyses (n=69), *M. lusitanicus* were captured in 19.4% of trapping plots (n=34) and another 20.0% of trapping plots (n=35) corresponded to *M. duodecimcostatus* presence. Local abundances of either species were low as most of presence plots had one or two captured individuals only (58.8% of presence plots of *M. lusitanicus* and another 57.1% of presence plots of *M. duodecimcostatus*). Plots with six or more individuals were also registered, although less frequently (5.9% of presence plots of *M. lusitanicus* and 11.4% of presence plots of *M. duodecimcostatus*), never exceeding eleven (*M. lusitanicus*) and nine (*M. duodecimcostatus*) individuals per plot.

The locations of presence and absence of each species are shown in figures 2 and 3 *M. lusitanicus* occurs mainly in the north and *M. duodecimcostatus* in the south of the study area.

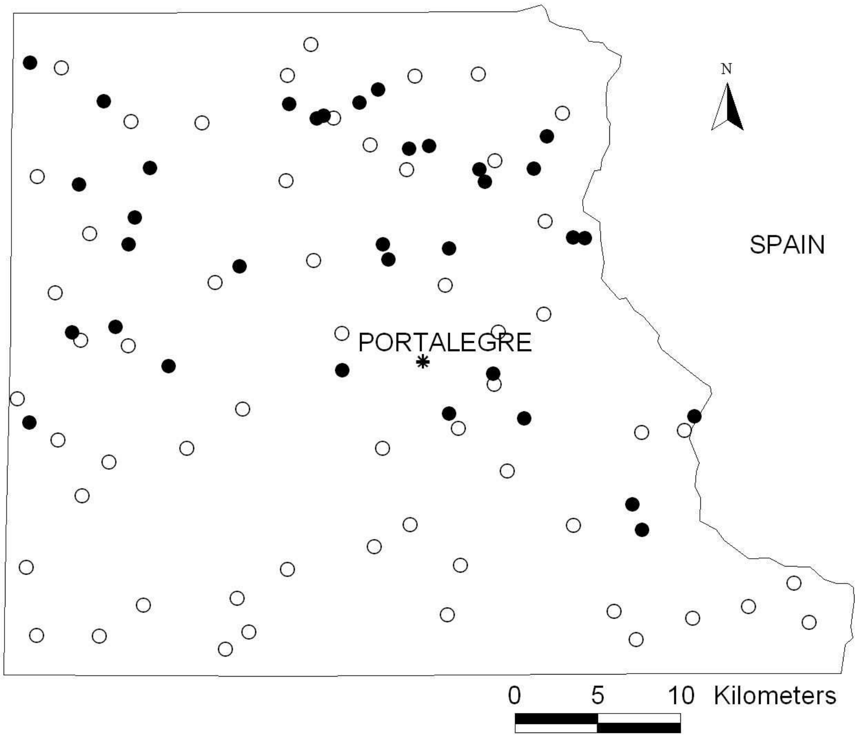

Figure 2. Distribution of presence (n=34) and absence sites (n=56) of Lusitanian pine vole (*Microtus lusitanicus*) across the study area (open circles: absence sites; filled circles: presence sites).

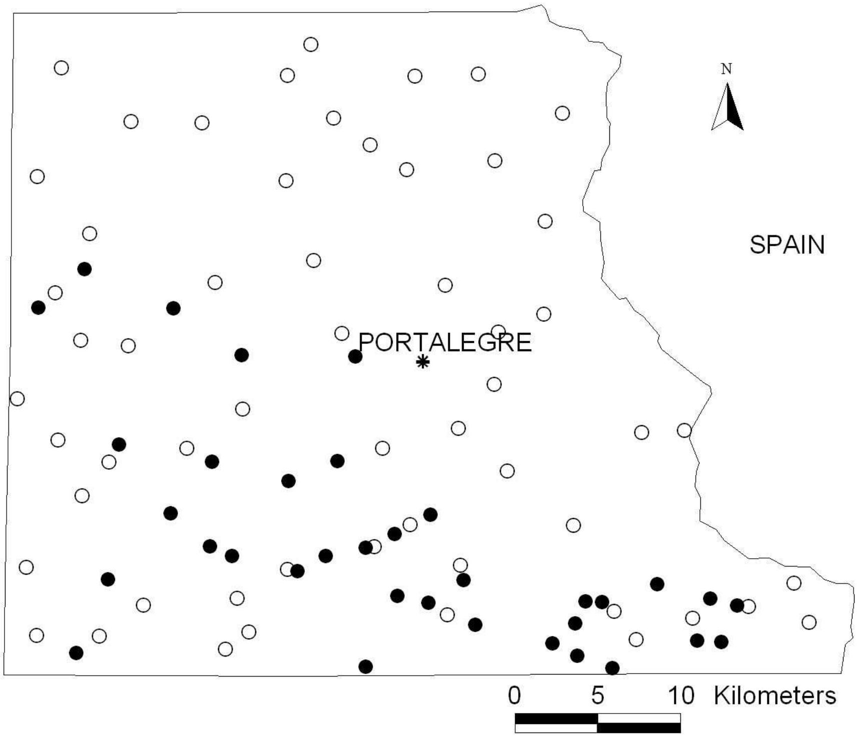

Figure 3. Distribution of presence (n=35) and absence sites (n=56) of Mediterranean pine vole (*Microtus duodecimcostatus*) across the study area (open circles: absence sites; filled circles: presence sites).

Microtus lusitanicus

Seven variables reached the highest importance values (>0.90) within the candidate models, thus receiving more support. Distance to verges, soil humidity and coarse sand content in the soil were selected in the local set; abundance of linear habitats in the landscape set; and the autologistic term, Y and Y^2 coordinates in the spatial set. The best single models of each variable set (local, landscape and spatial) and the full model from the variation partitioning procedure are summarized in table 2.

The local set model explained 56.5% of variation in *M. lusitanicus* data. The species is present in areas with high proximity of verges (< 50 m) and high content of soil humidity (> 20%) and coarse sand in the soil (> 20%). The landscape set model explained only 12.6% of variation, indicating that voles occurred more often in areas with high abundance of linear habitats (> 10%). The spatial set model accounted for 28.7% of variation, revealing the negative associations of the autologistic term and Y^2 and positive associations of Y with the species presence (tables 2 and 3). The full model presented 83.5% of explained variation. Most of this variability was due to the pure effect of local set that reached 44.1%, while the pure effect of spatial set still accounted for 18.8%. The landscape set performed poorly in explaining variance. Most shared effects were not meaningful (< 3%), although part of the variance captured by the local set is also captured by the spatial set (8%) (figure 4).

Table 2. Partial models (local, landscape and spatial) and full model showing the coefficients (B) and their significance (p) for the Lusitanian pine vole (*M. lusitanicus*); for variables' transformations see table 1.

Variables	**Partial models**		**Full model**	
	B	**p**	**B**	**p**
Local				
VERGED	-1.741	0.000	-2.821	0.009
HUMID	1.408	0.003	1.732	0.028
SAND2	0.609	0.049	0.138	0.803
constant	-5.969	0.035		
Landscape				
LINH	1.412	0.001	4.084	0.033
constant	-3.493	0.000		
Spatial				
AUTO_ML	-4.034	0.001	-11.527	0.014
Y	2.052	0.000	2.833	0.017
Y^2	-1.580	0.002	-3.882	0.022
constant	2.392	0.003	-2.707	0.642

Table 3. Summary of performance parameters of all models for the Lusitanian pine vole (*M. lusitanicus*) (D^2: explained deviance, AIC: Akaike's information criterion, AIC_c: Akaike's information criterion corrected for small samples, AUC: area under the ROC curve, OCC: overall correct classification, sensiv.: sensivity, specif.: specificity).

Models	**D^2**	**AIC**	**AIC_c**	**AUC**	**OCC**	**sensiv.**	**specif.**
LOC	0.565	59.9	60.4	0.945	91.1	94.1	89.3
LAND	0.126	108.3	108.4	0.738	66.7	67.6	66.1
SPAT	0.287	93.1	93.6	0.869	78.9	85.3	75.0
LOC + LAND	0.647	52.1	52.8	0.961	91.1	91.2	91.1
LOC + SPAT	0.749	44.0	45.4	0.979	94.4	94.1	94.1
LAND + SPAT	0.394	82.3	83.0	0.892	80.0	85.3	76.8
LOC+LAND+SPAT	0.835	35.7	37.5	0.991	94.4	94.1	94.6

(OCC cut value 0.38)

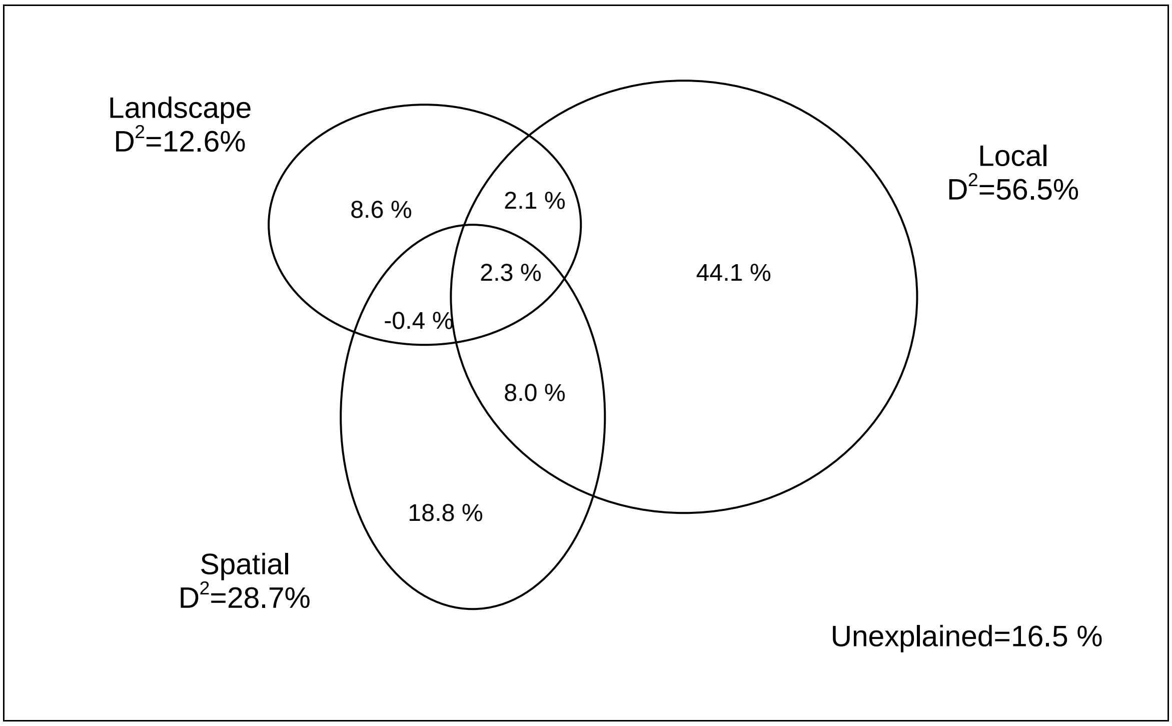

Figure 4. Results of the variation partitioning for the Lusitanian pine vole (*M. lusitanicus*) data according to the three sets of independent variables: local, landscape and spatial sets (Unexplained: percentage of unexplained variation); the size of circles and overlap are partially scaled according to the values of each component.

All seven models had excellent discrimination ability (AUC > 0.85), except for the single landscape model which presented a lower but still considered acceptable ability (0.738; table 3). The map of residuals of the three single models and the full model (not shown) exhibited no clear spatial pattern, indicating the absence of spatial autocorrelation in the models' residuals. The corresponding results on the Moran's I randomization test also conducted to the same observation, as no significant values of autocorrelation were detected (local set:

Moran's I stat= -0.204, p = 0.581; landscape set: Moran's I stat= 1.32, p = 0.093; spatial set: Moran's I stat= -0.078, p = 0.531; Full set: Moran's I stat= 0.037, p = 0.485).

Considering the two scale approaches, the local one performed much better than the landscape scale in explaining the species distribution pattern. However, the spatial component also explains a meaningful proportion of variance.

Microtus duodecimcostatus

Ten variables received high support by the candidate models with importance values higher than 0.90. Distance to verges, cover of tree canopy, and herbaceous vegetation biomass were selected in the local set; presence of shrubs in the landscape set; and all six spatial variables in the spatial set. Table 4 shows the best single models of each variable set (local, landscape and spatial) and the full model for this species.

The local set model explained 40.4% of variation in *M. duodecimcostatus* occurrence. Highest probabilities of vole's presence can be found in sites closer to verges (< 50 m) and with high cover of trees (> 50%) and herbaceous vegetation biomass (> 300 g/m^2). The landscape set model explained only 16.6% of variation and associates the species presence with absence of shrub landscape units. The spatial set model accounted for 41.4% of the variation and highlights a negative association between the presence of the Mediterranean pine vole and all spatial variables (tables 4 and 5).

The full model showed 68.6% of explained variation, and the models that included both the local and spatial sets had percentages of explained variation of 62.7% (joint model) and 68.6% (full model).

The strongest pure effects resulted from the local and the spatial models (18.1% and 17.1% respectively), whereas the landscape model only reached 5.9% of pure effect. The greatest amount of shared variance was explained by the combined influence of local and spatial sets (16.8%). The remaining shared fractions were lower than 5% (figure 5).

Table 4. Partial models (local, landscape and spatial) and full model showing the coefficients (B) and their significance (p) for the Mediterranean pine vole (*M. duodecimcostatus*); for variables' transformations see table 1.

Variables	Partial models		Full model	
	B	p	B	p
Local				
TREE	0.05710	0.000	0.040	0.079
VERGED	-0.92736	0.000	-0.810	0.021
BIOM	2.09835	0.004	2.969	0.006
constant	-11.07739	0.006		
Landscape				
SHRUB2	-3.16535	0.003	-4.441	0.024
constant	0.02985	0.903		
Spatial				
AUTO_MD	-5.2155	0.001	-5.805	0.020
X	-1.8071	0.007	-1.332	0.218
Y	-4.3044	0.000	-4.684	0.013
XY	-3.8984	0.001	-4.850	0.012
X^2	-1.7799	0.001	-1.5280	0.079
Y^2	-1.9373	0.035	-2.402	0.095
constant	2.4646	0.011	-12.282	0.034

Table 5. Summary of performance parameters of all models for the Mediterranean pine vole (*M. duodecimcostatus*) (D^2: explained deviance, AIC: Akaike's information criterion, AIC_c: Akaike's information criterion corrected for small samples, AUC: area under the ROC curve, OCC: overall correct classification, sensiv.: sensivity, specif.: specificity).

Models	D^2	AIC	AIC_c	AUC	OCC	sensiv.	specif.
LOC	0.404	80.3	80.8	0.898	80.2	82.9	78.6
LAND	0.166	105.2	105.3	0.691	62.6	97.1	41.1
SPAT	0.414	85.1	86.5	0.894	82.4	91.4	76.8
LOC + LAND	0.505	70.0	70.7	0.926	81.3	82.9	80.4
LOC + SPAT	0.627	65.2	67.9	0.958	86.8	88.6	85.7
LAND + SPAT	0.515	74.8	76.6	0.926	83.5	88.6	80.4
LOC+LAND+SPAT	0.686	60.0	63.3	0.969	90.1	94.3	87.5

(OCC cut value 0.38)

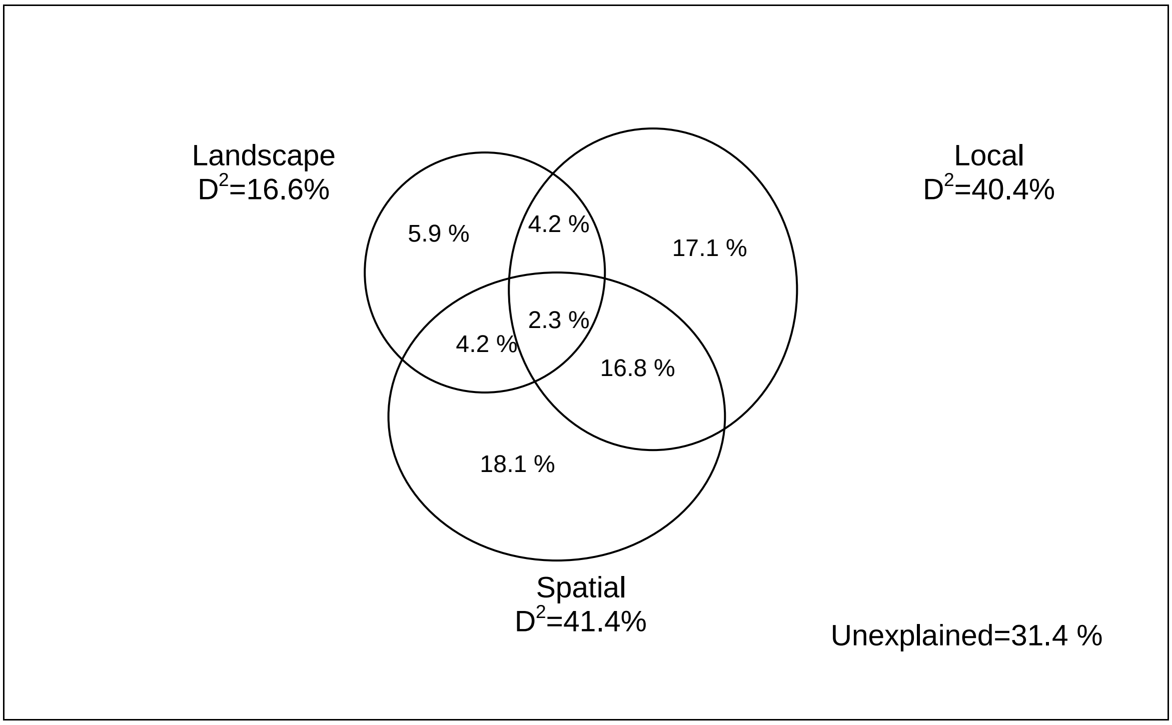

Figure 5. Results of the variation partitioning for the Mediterranean pine vole (*M. duodecimcostatus*) data according to the three sets of independent variables: local, landscape and spatial sets (Unexplained: percentage of unexplained variation); the size of circles and overlap are partially scaled according to the values of each component.

All seven models had excellent discrimination ability (AUC > 0.89), excepting for the single landscape model which presents a poor discrimination ability (0.691; table 5). The map of residuals of the three single models and the full model (not shown) presented no clear spatial pattern, although local and full models presented some points with higher residuals (of both signs) at southern areas. Still, the general pattern of maps indicated that the spatial autocorrelation in the models' residuals was not of concern. The corresponding results on the Moran's I randomization test confirmed our interpretation of maps, as no significant values of autocorrelation were detected (local set: Moran's I stat= -0.073, p = 0.529; landscape set: Moran's I stat= 1.295, p = 0.098; spatial set: Moran's I stat= -0.293, p = 0.615; Full set: Moran's I stat= -0.093, p = 0.537).

From the two studied scale approaches, the local scale presented a higher performance when compared to the landscape scale in explaining the species distribution pattern. The weight of spatial component was very similar to the local component, but considering the contribution of the two environmental scales together (local and landscape), the environmental group showed a better performance.

DISCUSSION

In the present study we were able to discriminate, for the first time, the relative contributions of local and landscape scales on the distribution of the two species of pine voles occurring in central Portugal. As predicted, the variation in the distribution patterns of both species is mostly explained by fine-scale environmental factors, being this effect particularly strong in the Lusitanian pine vole. An important spatial component was also observed for both species. This result was expected if we take into account the strong spatial segregation registered in the distribution of each species (see figures 2 and 3).

Distribution patterns

Although both species are considered regionally common (Mira, 1995), our results did not clearly support it. Because the distribution limits of both species meet in our study area, lower abundances are expected, a frequent characteristic in range margins of most species (Hengeveld, 1990; Case and Taper, 2000).The low success of captures however, may not be attributed solely to the low abundance of populations, but also to other constraints such as trap avoidance, or circumstantial absence of animals at the time of trapping. Even accepting that this could happen occasionally, the occupied sites, for both species, were spatially very localized and discontinuous, thus supporting the idea that the two pine voles may not be very common in the study area.

The analysis of the distribution maps of both species suggests the existence of a narrow sympatry area located west from Portalegre city, which is in general agreement with previous large-scale spatial predictions (Santos et al., 2009b).

Scale effects

As already mentioned, the distributions of the Lusitanian and Mediterranean pine voles in central Portugal are determined mostly by local factors, although sometimes with an important spatial structure. Landscape features play a minor role in this process.

Our results concerning scale are supported by the work of Gehring and Swihart (2003) who claimed that distribution of mammals of smaller size should be better explained by local-scale factors than by large-scale factors. In addition, selection of burrow sites, cover from predators and food resources, and other specific habitat characteristics within the home range of small mammals are primarily determined by microhabitat features (Bussche et al., 2008), thus explaining further the importance of local-scale factors. Moreover, as *M. lusitanicus* is smaller than *M. duodecimcostatus*, we should expect that local-scale factors would have a stronger influence on the distribution of *M. lusitanicus*. Our results support this view. The local-scale factors explain about 5 times more the variation in the occurrence of *M. lusitanicus*, while in the *M. duodecimcostatus* local-scale factors are nearly 3 times more important than the landscape-scale factors. This suggests that *M. duodecimcostatus* may perceive the landscape at a slightly larger spatial extent.

Similar results were obtained with *M. pennsylvanicus* and *Myodes (C.) gapperi*, where distribution of both individuals and populations depended more of fine-scale than larger-scale factors (Dooley and Bowers, 1996; Orrock et al., 2000). However, Rodríguez and Peris (2007) obtained different results and claimed that the abundance of *M. lusitanicus* in the region of Zamora (Spain) was best explained by models at the broadest regional scale, although referring to abundance data. This diversity of results may be explained by the different parameters used (presence/absence vs. abundance) and by differences in the definition of "local" and "landscape" scales, making comparisons between studies difficult. According to Warren et al. (2005), local-scale factors are more likely to be more important than landscape composition in habitat selection by specialist species. Our target species are patchily distributed in the study area and local abundances are rather low, suggesting that both voles have a specialized ecological niche. In addition, the dependency of the subterranean biotope by both species (Giannoni et al., 1993) must impose physiological and spatial limitations to populations (see Reichman and Smith, 1990) that conduct to specialization of the ecological niche, thus supporting the importance of local-scale effects.

Spatial effects

The variation partitioning evidenced that spatial variables were also important in explaining the pine voles occurrence, especially in the case of *M. duodecimcostatus*. The variation partitioning procedure allowed the quantification of part of spatially structured ecological processes, revealed by the shared fraction between the spatial set and each of the environmental sets. The strong pure spatial effects observed in the distribution of the two pine voles (ca. 18% each) may be caused by unstudied explanatory variables spatially structured themselves, and/or by non-measurable factors, such as historical events spatially structured (i.e. past disturbances that are still reflected on its present spatial structure, biotic processes (such as dispersal, competition or metapopulation dynamics; Legendre and Legendre, 1998). In fact, low distance dispersal can cause spatial autocorrelation if vole populations are subject to extinction-recolonization dynamics, as has been proposed for *M. duodecimcostatus* (Paradis, 1995). Similar data on the population dynamics of *M. lusitanicus* is lacking for comparative purposes. Although this pure fraction of the spatial component can have origin from diverse processes, it is not possible to infer, from our data, the exact ecological processes that are causing this spatial pattern (but see Van Teeffelen and Ovaskainen, 2007).

The autologistic terms revealed a negative association with both species presence (negative autocorrelation). This indicates that the neighbours of a presence site are mostly absence sites, and vice-versa. Although there are many absence sites surrounded by absence sites, the general pattern within the regional range of species is composed of presence sites intermixed with absence sites (figure 2 and 3). While the geographical terms explained the general north and south regional trends exhibited by the species, the autologistic term evidenced the local patchy pattern of presence sites.

Local scale characteristics

Both species occur preferentially at sites with verges. *M. lusitanicus* is present in soils with higher humidity and sand content, while *M. duodecimcostatus* occurs in sites with higher tree cover and herbaceous biomass.

Edge habitats, like verges, are less disturbed than most open fields, maintaining high plant cover and density throughout the year, thereby providing good habitat conditions (food resources and shelter) for small mammals (Desy and Batzli, 1989; Hodara and Busch, 2006 and references therein). Verges offer cover from aerial predators (diurnal and nocturnal) and allow short superficial movements by voles. The positive selection of verges by *M. lusitanicus* has already been reported in a Mediterranean polyculture (Santos et al., in press), and also for other microtines as *M. cabrerae* (Santos et al., 2007) and *M. ochrogaster* (Getz, 1985).

The high moisture content and coarse texture of the soil should be important for all burrowing rodents, as it allows a cost-effective excavation of tunnels and burrows (Reichman and Smith, 1990; Romañach et al., 2005b). The high moisture content is especially advantageous in arid or Mediterranean environments where evaporative water loss may be significant above ground (Reichman and Smith, 1990; Andreu, 1995). Several studies indicate that most burrowing activities of small mammals take place following first rains that increase the friability of the soil (*M. ochrogaster* in Rose and Birney, 1985; *Thomomys bottae* in Romañach et al., 2005a). The soil texture also strongly influences the distribution of *M. pinetorum* (Rose and Birney, 1985), while the burrow system area of pocket gophers (*Thomomys* spp) decreased with increasing soil clay content (i.e. increasing expense of digging) (Romañach et al., 2005b).

Our results concerning the vegetation characteristics associated to the presence of *M. duodecimcostatus* are supported by the work of Mira and Mathias (1994) who found a strong association of the species with presence of small trees and dense herbaceous vegetation cover. The importance of trees could be explained because its canopy and root systems promotes the maintenance of moisture in the soil (Joffre and Rambal, 1993) and provide safety against human interventions (e.g. soil ploughing) and terrestrial predator access (as digging by foxes and wild boars; King, 1985; Borghi et al., 1994). The importance of herbaceous vegetation is well known for microtine species as both protection and food (e.g. Getz, 1985; Lin and Batzli, 2001). For fossorial voles, the cover provided by high values of plant biomass has an additional buffering effect, maintaining adequate temperatures and humidity values inside burrows and nests (Getz, 1985). Moreover, lower survival rates of *M. duodecimcostatus* are reported in areas with poor cover of vegetation (Paradis, 1995).

In a reduced area of sympatry in the Spanish Pyrenees, it was shown that while *M. lusitanicus* occupied areas of shallow soils and medium to sparse plant cover, *M. duodecimcostatus* was present in deeper soils and dense plant cover (Borghi et al., 1994), partially supporting our data.

Although it is tentative to conclude that one species is mostly influenced by soil conditions and the other by vegetation structure, all factors are strongly interrelated: soils with an intermediate texture have a superior capacity for water retention, more nutrients, easier working ability and are capable of providing water for growing vegetation during the dry season (Varennes, 2003). At the same time, vegetation structure (tree cover and herbaceous biomass) is related to microclimate conditions. Sparse vegetation causes more extreme temperature and moisture conditions at the surface and at the underground burrows (Getz, 1985), a situation particularly restrictive during the Mediterranean dry season and most evident in *M. duodecimcostatus* occurrence areas. The ecological interpretation of the variables here selected (soil conditions, vegetation structure, abundance of herbaceous plants) suggests that both species seem to be responding to cover, food and burrowing within their habitat.

Landscape scale characteristics

At a landscape scale, *M. lusitanicus* is associated to higher availability of linear habitats, and *M. duodecimcostatus* to the absence of shrub landscape units.

As already stated, the variation explained by landscape factors in our study tended to be lower than that attributed to local or spatial factors. This does not mean that landscape factors are not important, but rather suggests that landscape factors alone will not suffice as indicators of suitable occurrence areas for the pine voles.

The associations between landscape and species distributions could reflect important differences. *Microtus lusitanicus* occurs often in more heterogeneous and diverse landscapes with abundance of potential corridors, and where the mountain of São Mamede is included. On the other side, *M. duodecimcostatus* occurs in the southern lowlands, in the simplified Mediterranean landscapes like the "montado" where extensive grazing prevents shrub encroachment (Pinto-Correia, 1993). Also, *M. lusitanicus* is much less fossorial, when compared to *M. duodecimcostatus* (Mathias, 1996). This may suggest that the stronger association of *M. lusitanicus* with areas with higher abundance of potential corridors and presence of shrublands could be related to a greater dependency of protection cover from predators, due to a higher vulnerability caused by surface movements.

Concluding remarks

The distributions of the Lusitanian and the Mediterranean pine voles were explained mostly by fine-scale factors within their habitat, although spatial factors were also relevant. The similarities that we found between the species were mostly related to cover, food and burrows, while the differences appeared as opposing spatial trends and to coarse-scale descriptors reflecting landscape heterogeneity. Considering that *M. lusitanicus* and *M. duodecimcostatus* are sister taxa (Jaarola et al., 2004), a possible evolutionary explanation of their habitat niche similarity may be their close phylogenetic relationship.

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Chapter 5

Local coexistence and niche differences between pine voles

Paper III

Local coexistence and niche differences between the Lusitanian and Mediterranean pine voles (*Microtus lusitanicus* and *M. duodecimcostatus*).

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Local coexistence and niche differences between the Lusitanian and Mediterranean pine voles (*Microtus lusitanicus* and *M. duodecimcostatus*)

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ABSTRACT

In the present study we analysed the coexistence pattern of the Lusitanian pine vole (*Microtus lusitanicus*) and the Mediterranean pine vole (*Microtus duodecimcostatus*) in a potential area of sympatry in a Mediterranean landscape (Portugal). We also determined the relative contribution of local, landscape and spatial factors explaining the differences in the distribution patterns of the two species in the region. Using a kriging interpolation method, we obtained a map of sympatric and allopatric areas of species occurrence. The estimated sympatry area corresponded to a northwest-southeast belt representing 11.3% of the study area. Habitat niche differences were assessed with binomial GLMs followed by a variance partitioning. At a local scale, the most important factors distinguishing the presence of *M. lusitanicus* from *M. duodecimcostatus* were the higher altitude, higher cover of shrubs, lower clay content in the soil, and lower cover of tree canopy for the *M. lusitanicus* presence sites. At a larger scale, the presence of forest landscape units and the low abundance of "montado" units were the most influencing landscape factors in the identification of *M. lusitanicus* occurrence sites when compared to *M. duodecimcostatus*. Our results suggested that local coexistence of *M. lusitanicus* and *M. duodecimcostatus* in the field was a rare event. The differences in distribution patterns of the two species of pine voles were mostly explained by fine-scale environmental factors and by shared spatial effects. The use of novel approaches, such as kriging, may be interesting for other scientists aiming to build species distribution maps as, in many cases, kriging would be far more appropriate for their data, although less widely known amongst ecologists.

Key-words: *Microtus lusitanicus*, *Microtus duodecimcostatus*, kriging, sympatry, niche differences

INTRODUCTION

Closely related species often occupy similar ecological niches. However, one of the most important premises of the niche theory predicts that ecologically identical species cannot coexist for long periods of time due to competitive and stochastic factors (Hutchinson 1957, 1959). Yet, some authors argue that species coexistence is the general rather than the exceptional case in ecological systems (e.g. Conley 1976). A stable coexistence among members of the same guild in local communities may be possible if some degree of morphological, ecological, or behavioural diversity is shown (Chesson 2000b; Douglass 1976; Hutchinson 1959; Kronfeld-Schor and Dayan 2003; Spaeth 2009).

Interspecific competition is an important process affecting the distribution and abundance of animals at scales ranging from a single microhabitat to the entire mapped geographical distribution of a species (Connor and Bowers 1987; Krebs 1994). The reason for this influence is that competition leads species to diverge in their occupancy of space and use of resources (Krebs 1994). Indeed, one of the consequences of interspecific competition between closely related species can be the presence of spatial segregation (Amarasekare 2003; Chesson 2000a; Connor and Bowers 1987), although the observation of such segregation is not, per se, indicative of competition (Connor and Bowers 1987).

There are several documented cases of spatial segregation and parapatry among small mammals, as in pocket gophers (genera *Geomys*, *Cratogeomys*, and *Thomomys*; Miller 1964), in African rodents (*Mastomys coucha* and *M. natalensis*; Venturi et al. 2004), and as a general rule in strictly subterranean rodents (Nevo 1979). However, for some microtine species, many examples can be found that report varying scales of coexistence and sympatric populations (e.g. Douglass 1976; Randall 1978; Spaeth 2009; Whitney 1976).

The Lusitanian and the Mediterranean pine voles (*Microtus lusitanicus* (Gerbe, 1879) and *Microtus duodecimcostatus* de Selys-Longchamps, 1839) are western European endemisms with similar and marked burrowing behaviour (Cotilla and Palomo 2007; Giannoni et al. 1993; Mira and Mathias 2007). *Microtus lusitanicus* is distributed in the northwest of the Iberian Peninsula and in the French Pyrenees, and *M. duodecimcostatus* occurs in the south-centre of Iberian Peninsula and in southern France (Cotilla and Palomo 2007; Mira and Mathias 2007). In Portugal the two species have a general allopatric occurrence pattern with a predicted area of sympatry in the centre of the country (Madureira 1984). Although the general geographic ranges in Portugal are known for the voles, these are based solely on dot maps (e.g. documentation of scattered locations plotted in a map) which lack precise information in several areas and particularly in the predicted area of sympatry. Specifically for this area, there is no detailed information that reports or quantifies the coexistence of the two species at finer scales. In addition, the two voles are sister species, derived from a common and unique ancestor (Jaarola et al. 2004). Although detailed and quantitative studies are scarce, both species occupy preferentially open habitats, natural and agricultural (Cotilla and Palomo 2007; Mira and Mathias 2007), and both species feeds on roots, tubers, fruits and also aerial parts of plants (Cotilla and Palomo 2007; Mira and Mathias 2007; Vinhas 1993). In this sense, it would be valuable to obtain more information on niche characteristics, at local and landscape scales that differentiate the distribution of the two species within an area of potential sympatry.

Both species can become important agricultural pests (Mira and Mathias 1994; Vinhas 1993), but in the predicted area of sympatry it is often uncertain which species may be responsible for the reported damages, also due to their morphological similarity (Madureira 1982, 1984) and the need of specific trapping techniques for the survey of these voles (Guédon et al. 1992). Besides the usefulness for agencies of pest control, the clarification of species distribution patterns is also crucial to differentiate situations of allopatry, parapatry or sympatry (Bull 1991) and to provide insights on biogeography and evolutionary issues of closely related species (Arif et al. 2007; Raxworthy et al*.* 2007), as ecological niches can be more easily compared when projected in a geographic space. Moreover, the examination of the degree of coexistence between two species can reveal clues on important ecological processes, such as interspecific competition (Grant 1972). Thus, the above pine voles represent good candidate species for the investigation on these issues.

Here we analyse the coexistence pattern and habitat niche differences of two pine voles, *Microtus lusitanicus* and *M. duodecimcostatus*, in a Mediterranean landscape that includes occurrence areas for both species. In particular, we aimed to determine: i) the presence or the extent of a regional sympatric area for the two pine voles; and ii) the relative contribution of local, landscape and spatial factors explaining the differences in the distribution patterns of the two species.

METHODS

Study area

The study area is located in the northern Alentejo (UTM 0635030 W 4350608 N, Portalegre district, Portugal), covering an area of 1613 km^2. The study area includes a landscape of mountain characteristics (São Mamede mountain) and a landscape of lowlands (Cancela d'Abreu et al. 2002). At the lowlands the landscape is dominated by open fields with low fragmentation, scattered oak trees with varying densities, called "montado" (Pinto-Correia 1993), sometimes interspaced with polycultures close to the villages (Cancela d'Abreu et al. 2002). The mountain of São Mamede has contrasting characteristics when compared to surrounding lowlands. It presents a marked relief with 1025 m a.s.l. at the highest point and extensive areas above 600 m a.s.l.. The high geologic and microclimatic diversity of the mountains result in a higher heterogeneity in land uses (Cancela d'Abreu et al. 2002).

The climate is Mesomediterranean with a mean temperature of 7.4 ºC in January (3.1 – 11.7 ºC) and 24.7 ºC in July (16.8 – 33.3ºC), and an annual rainfall of 717 mm (Ninyerola et al. 2005; Rivas-Martínez and Arregui 1999).

Vole sampling

From September 2006 to May 2007, 175 trapping plots were sampled across the study area. In each plot, 10 subterranean live-traps of multiple captures ("mole type") were set up in underground tunnels for three consecutive nights. The traps were provided with apple as bait and hay for bedding. The location and selection of trapping plots was dependent on finding enough surface presence signs to allow the set up of all 10 traps (burrow openings and (or) soil mounds; Santos et al. 2009a). Efforts were made to sample habitats in proportion to their availability. Each plot was sampled only once.

Captured voles were identified on the basis of body measurements: the hind feet length (HFL: *M. lusitanicus* < 16 mm > *M duodecimcostatus*), body length (BL: *M. lusitanicus* < 105 mm and *M duodecimcostatus* > 89 mm), and colour pattern (Madureira 1982, 1984). Plots with captured voles, assigned to one or the other species, were considered as presence plots in further analyses, while the plots with no pine vole captures were discarded. Fifty-six absence plots of 10-m radius were additionally defined throughout the study area. The criterion for accepting an "absence plot" was the lack of any superficial presence signs of pine voles or moles (as there are reports of voles using mole tunnels; authors pers. obs.), after a careful search of 20 minutes. No trapping was undertaken at these plots.

Environmental variables

The environmental data were collected at two scales of analysis: local and landscape. The local level was defined as the surface occupied by the traps (16 to 630 m^2, mean=62 m^2; 0.0062 ha) at the presence plots, while the landscape level was defined as a 250 m radius surrounding the centre of the sampling plot (196 250 m^2). The local scale should correspond to high intensity of use within the home range of pine voles, as 62 m^2 is much lower than the mean home range of *M. lusitanicus* (952 m^2; Santos et al. in press). The 250 m radius was selected as a reasonable approximation of the dispersal distance of voles, as considered in other similar studies (Delattre et al. 1999).

At the local level we recorded 20 variables belonging to six categories: topography, local habitat, vegetation structure and composition, grazing, verges, and soil properties (appendix A). Most variables concerning vegetation structure and composition were surveyed in two to five 1x1 m quadrats (as the size of the trapping plots varied) by stratified random sampling (Kent and Coker 1992). These variables included cover percentages of bare soil, herbaceous layer, woody vegetation (under 3 m height), grass species, composite species, and mean herbaceous vegetation height (obtained after ten random measures in each quadrat). Cover percentages of tree and shrub layers were obtained through visual estimation for the entire sampling plots (Bullock 1996). The herbaceous vegetation was clipped in two to four 0.30 x 0.30 m sub-quadrats per plot (Pucheta et al. 1998). To obtain data on dry vegetation biomass, samples from sub-quadrats were oven dried in the laboratory, at 105 ºC, during 24 hours, and weighted afterwards at a 0.1 g precision (Kent and Coker 1992). The inclusion of this sub-group of variables is justified as vegetation provides food, shelter and nesting sites for microtines (Lin and Batzli 2001; Rose and Birney 1985).

Grazing was described as a binary variable. Also, historical (or past) grazing was assessed through the measure of cover percentage of plant species with a prostrated growing that should be indicative of more long-term grazed sites (Lavorel et al. 1997; Sternberg et al. 2000) in 1x1 m quadrats (see details above). The negative influence of high grazing pressure on vegetation structure and herbaceous cover for small microtine species has been well documented (Evans et al. 2006; Schmidt et al. 2005).

Distance to the nearest verge (field margins, hedgerows, roadsides, stone walls, ecotones) was measured from the sampling plot centre.

Because pine voles have fossorial habits, soil characteristics at 0.10 - 0.15 m depth were also described. This depth was chosen as it corresponds to the highest densities of subterranean plant organs and covers also a high number of underground tunnels (Yeboah and Akyeampong 2001). Soil penetration resistance was measured with a penetrometer (DICKEY-John Soil compaction tester) to a depth of 0.10 m. Ten readings were obtained in two orthogonal transects and the median value calculated for each plot (Martínez and Zinck 2004). At the sub-quadrats with clipped vegetation, two soil samples were collected to assess humidity and texture values. The gravimetric method was used in humidity calculations, with oven at 105ºC during 48 h (Martínez and Zinck 2004). The texture analyses were performed by a technical laboratory (Soils Physics Laboratory of Phytotechny Department of University of Évora).

Variables of landscape composition were established from aerial photographs (flights 2003 and 2005; IGeoE) and confirmed during field surveys. Land cover types were classified in a GIS (Arcview 3.2.; ESRI 1999) with a working scale of 1:2000, and a minimum polygon size set to 190 m^2. Cover percentages of each land cover type were then calculated for each 250-m buffer (appendix A). We mapped linear habitats (verges bordering roads, stonewalls and ecotones, and linear dense vegetation) in a distinct category because they are considered potential dispersal habitats for voles (Bennett 1990; Litvaitis et al. 2003; appendix A).

Landscape metrics were calculated for the nine land cover types and thus, 14 additional variables were obtained for each buffer concerning size, edge, shape, and diversity metrics of the different land cover categories. Globally, the landscape set was described by 23 variables (appendix A).

Spatial variables

The spatial group of variables was defined through an autologistic term, derived from the responses at neighbouring cells within 7 km of distance and corresponds to a weighted mean of the inverse of the square distance of the centre of each sample plot to each sampled neighbour (Augustin et al. 1996; Dormann et al. 2007); and a second order polynomial of centred spatial coordinates, summing six variables (appendix A).

Data analyses

Interpolation maps of species occurrence

As our environmental explanatory variables were not available as spatially continuous digital layers, prediction of species occurrence patterns as maps with the usual Generalised Linear Models (GLM) or presence-only models (e.g. ENFA, GARP, MAXENT) was unfeasible. The kriging method (Mathereon 1969, 1970) is an interpolation technique widely used in mining, soil, engeneering and climate working groups (Jerosch et al. 2006; Largueche 2006), but seldom in ecology, where regression distribution models (as GLMs) are more widespread. Nevertheless, this technique is becoming more popular in ecology (Monestiez et al. 2006; Rossi et al. 1992; Stelzenmüller et al. 2004; Tröltzsch et al. 2009). Kriging assumes that the distance between sample points reflects a spatial correlation that can be used to explain and model the spatial variability of a variable in the surface. It fits a function to all points within a specified radius, in order to determine the output value for each location. Indicator kriging (Solow 1986) is a kriging analysis performed on a binary-transformed sample population taking no assumptions concerning the distribution of the modeled variable(s) (Marioni 2003).

Two spatial data matrices were built, one for *M. lusitanicus* with 34 presence and 56 absence plots (n=90), and another for *M. duodecimcostatus* with 35 presence and 56 absence plots (n=91). Each species was first analysed independently from each other to obtain individual probability maps of occurrence.

A semi-variogram was calculated using the binary variable (species presence/absence) and then we visually fitted theoretical semi-variogram models that were applied for interpolation at unsampled locations to predict the probability of a species' occurrence. No directional effects were observed for four directional semi-variograms, and so, isotropic autocorrelation of the data was assumed (Bivand et al. 2008a). A cell size of 1 km^2 was chosen for the output maps.

In order to validate map estimations of potential species distributions, we performed a leave-one-out cross-validation of the models (Bivand et al. 2008a; Trötlzsch et al. 2009). A good representation of the data by the variogram model can be assumed if standardized residuals (z-scores) have a mean and variance values close to 0 and 1 respectively (Bivand et al. 2008a).

The output maps of indicator kriging represent the spatial distribution for each species as a continuous probability surface of values ranging from zero to one. To obtain occurrence maps, these probabilities must be converted into presence and absence information. The potential occurrence areas were obtained with two threshold values: major presence areas were defined with the standard threshold (0.5), while minor presence areas were defined with a lower value that maximised correct prediction of presence cells (0.29 for *M. lusitanicus* and 0.30 for *M. duodecimcostatus*). The chosen threshold values should prevent an underestimation of the regional extent of the species potential distributions, but overestimate the local extent. The definition of two threshold values intends to highlight a gradient in species boundaries, as distribution limits are rarely lines or sharp borders (Fortin et al. 2005).

The potential distribution maps of both species were overlaid to obtain areas of potential sympatry with the predicted presence of both species (Anderson and Martínez-Meyer 2004; Martínez-Freiria et al. 2008). Major sympatry was defined as the areas of major occurrence of both species (standard threshold), while minor sympatry corresponded to the minor presence area of one or both species (sensitivity threshold).

Environmental analyses

For the environmental analyses, we built a presence-only data matrix, concerning the 34 and 35 presence plots of *M. lusitanicus* and *M. duodecimcostatus*, respectively. This data matrix combined the species presence and all previously described environmental variables (local, landscape scales) plus spatial variables. *Microtus duodecimcostatus* was coded as "0" and *M. lusitanicus* was coded as "1". The analyses performed here allowed us to investigate the ecological differences between species and to discuss the spatial coexistence between them. These ecological niche differences were analysed with a multivariate GLM with binomial error structure (Guisan et al. 2002) for each variable set, followed by a variation partitioning procedure (Borcard et al. 1992; Legendre 1993).

Three sets of explanatory variables were defined: local (LOC), landscape (LAND) and spatial (SPAT). This classification will allow the study of ecological factors involved within each scale, followed by the understanding of their combined effect and relative influence on the niche differentiation of each species. Moreover, the definition of a spatial component will correct the possible presence of autocorrelation in the data and also quantify its influence on species' distributions (Borcard et al. 1992; Legendre 1993).

We started our variable selection with a univariate screening of all variables, and considered only those environmental variables for further model development that achieved significant univariate models ($P<0.05$; Bussche et al. 2008). Logarithmic, square root and cubic root transformations were performed on several explanatory variables to achieve normality. Zero-inflated variables were transformed into binary classes (appendix A). No previous univariate screening of the spatial variables was undertaken due to their reduced number. We performed pairwise Pearson correlations among all continuous independent variables (environmental and spatial) and, if $| r |> 0.7$, we excluded the one with lower univariate model performance, thus avoiding multicollinearity problems (Tabachnick and Fidell 2001).

We used the information-theoretical model comparison approach (ITMC; Burnham and Anderson 2002) for selection of variables in multivariate models (Johnson and Omland 2004). We developed models with all possible combinations of remaining variables (after univariate screening) for each variable set and compared with the Akaike's information criterion corrected for small samples (AIC_c;

Burnham and Anderson 2002). Models with $\Delta AIC_{c,\ i} < 4$ (ΔAIC_c = difference between each model AIC_c and $AIC_{c,\ min}$) are considered to have substantial support as candidate models (Burnham and Anderson 2002). If no single model is clearly superior to the others in the set of $\Delta AIC_{c,\ i} < 4$, a model averaging approach was performed which bases inference on the entire set of candidate models (Burnham and Anderson 2002).

We performed a variance partitioning procedure to specify how much of the variation of the final model was explained by the pure effect of each set of explanatory variables, which proportion was derived from their interaction, and how these sets interact together in explaining the variance in pine voles' distributions (Borcard et al. 1992; Legendre 1993; Legendre and Legendre 1998). As this procedure implies building a full model with all variable sets, we had to establish a maximum number of variables, according to the rule of a minimum of 10 cases for each explanatory variable included in the model (Harrell et al. 1996). To achieve this, only variables with more than 90% of importance value within the best models ($\Delta AIC_c < 4$) of each variable set were considered for variation partitioning calculations. Interactions and quadratic terms were tested on best models (within each set) before variance calculations and retained if a decrease in the AIC_c was verified.

Additionally to the three single set models (LOC, LAND and SPAT), another three joint models (LOC + LAND, LOC + SPAT, LAND + SPAT) and one full model (LOC + LAND + SPAT) were estimated with the previous variables of the three single sets. The explained deviance (D^2) was used as a measure of variance explained by each logistic model (Guisan and Zimmermann 2000).

Model goodness-of-fit was evaluated by explained deviance (Guisan and Zimmermann 2000) and validation graphs. The accuracy in model discrimination was assessed with the Area Under the receiver operating characteristics Curve (AUC). We also calculated the overall correct classification rate (OCC) and the proportion of presences (sensitivity) and absences (specificity) correctly classified, using the prevalence (0.49 for both species) as the threshold value (Jiménez-Valverde and Lobo 2007). Model performance procedures were evaluated independently for the three single models (one variable set), for the three joint models (two sets) and for the full model.

To evaluate the degree of non-independence in error terms, plots of residuals were examined for spatial patterns and the global Moran's I of residuals was tested for significance (Dormann et al. 2007). This procedure was done only in single set and full models.

All statistical analyses were performed with the free software R (version 2.6.0 R Development Core Team 2007): the autocovariate term was calculated with spdep package (version 0.4-21, Bivand et al. 2008b), GLMs were selected with dRredging package (version 0.11.1, Burnham and Anderson 2002), kriging was developed with gstat package (version 0.9-47, Pebesma 2004). Landscape metrics were calculated with Patch Analyst and Spatial Analyst extensions for Arcview 3.2. (ESRI 1999), and kriged maps were transposed and edited in Arcview 3.2. (ESRI 1999).

RESULTS

Kriging and sympatry maps

The local co-occurrence of both species in the field was not confirmed for neither of the 69 presence sites here analysed.

Figure 1 shows the kriged maps of the probability of *M. lusitanicus* occurrence in the study area (Fig. 1A) and the respective variance map (Fig. 1B), showing an increasing presence probability at the northern areas and an increasing uncertainty of predictions at the limits of the study area. The cross-validation produced z-scores with a mean of -0.003 and variance of 2.105, suggesting that the variogram model is adequate to the general data.

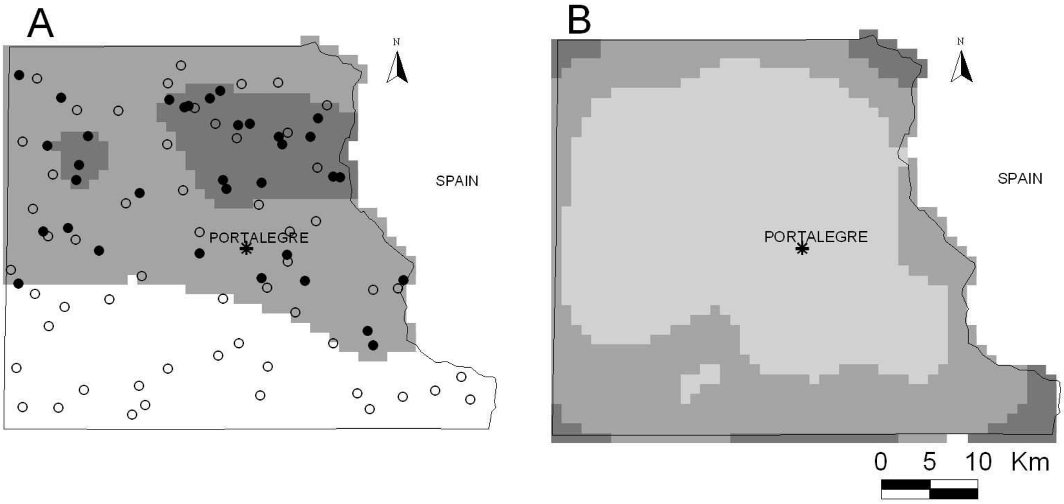

Figure 1. Kriging results for *M. lusitanicus* data; A: kriged probability of *M. lusitanicus* presence (dark gray: major presence areas with prob > 0.50, light gray: minor presence areas with 0.29 < prob < 0.50, white: absence areas with prob < 0.29; filled circles: observed presences, open circles: observed absences); B: kriging variance of estimated probabilities (light gray: 0.22-0.23, intermediate gray: 0.23-0.24, dark gray: 0.24-0.26).

The probabilities of presence of *M. duodecimcostatus* in the study area are plotted in figure 2 (Fig. 2A) along with respective error variances (Fig. 2B). Occurrence sites concentrate on most southern areas, while major prediction errors are located at the limits of the study area. The z-scores of the cross-validation presented a mean value of -0.006 and a variance of 1.915, indicating that the variogram model describes the data reasonable well.

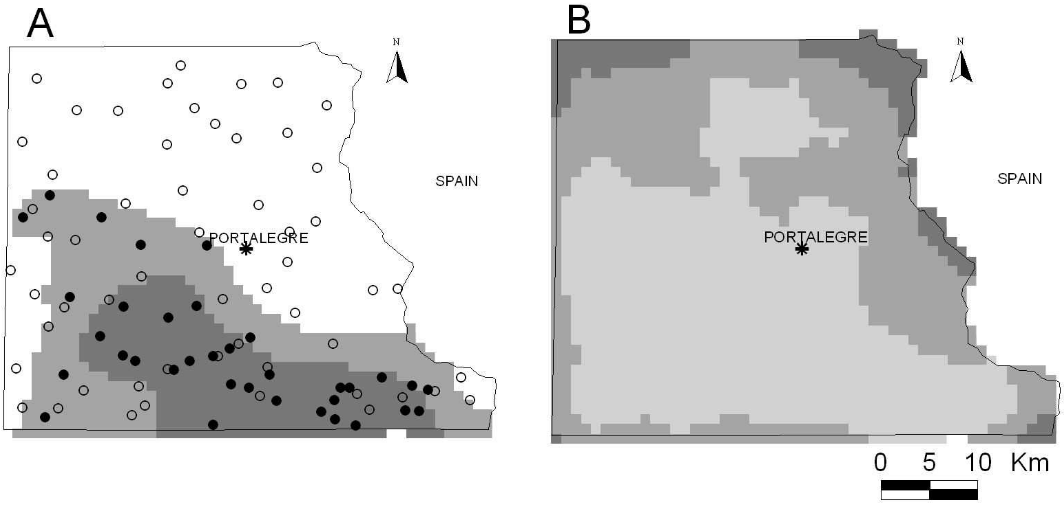

Figure 2. Kriging results for *M. duodecimcostatus* data; A: kriged probability of *M. lusitanicus* presence (dark gray: major presence areas with prob > 0.50, light gray: minor presence areas with 0.30 < prob < 0.50, white: absence areas with prob < 0.30; filled circles: observed presences, open circles: observed absences); B: kriging variance of estimated probabilities (light gray: 0.10-0.20, intermediate gray: 0.20-0.22, dark gray: 0.22-0.26).

The area of sympatry obtained from the overlay of both kriged maps is plotted in figure 3, with a calculated surface area of 183 km^2 (11.3 % of the regional study area): 178 km^2 of minor sympatry and only 5 km^2 of major sympatry. This northeast-southwest belt is divided in two regions of different size, having a maximum width of 10 km in the west and a width of 3 km in the east. Between these two regions a contact zone (or a parapatric boundary; Bull 1991) separating allopatric regions appears to exist: *M. lusitanicus* at the north and northeast, and *M. duodecimcostatus* at the south and southwest. The estimated sympatric area includes six observed presences of *M. lusitanicus* and five of *M. duodecimcostatus*.

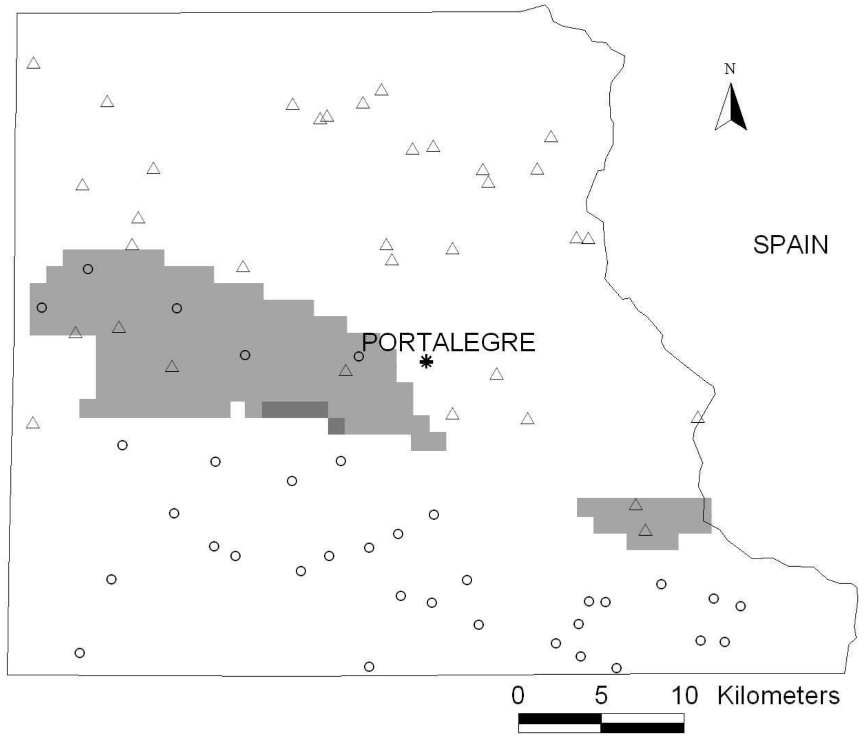

Figure 3. Predicted occurrence of minor sympatric areas (gray) and major sympatric areas (black); open triangles: *M. lusitanicus* presence sites, open circles: *M. duodecimcostatus* presence sites, asterisk: the district main city Portalegre for reference.

Ecological differences between species

Seven variables reached the highest importance values (>0.90), thus receiving more support by the candidate models. Altitude, cover of shrubs and trees, and clay content in the soil were selected in the Local set; presence of forest and abundance of "montado" units in the Landscape set; and Y coordinate in the Spatial set. In table 1 the best single models of each variable set (Local, Landscape and Spatial) and the full model are presented.

There was a weak spatial pattern in the map of residuals of the three single models and the full model (not shown) revealing mostly low residuals, but with positive residuals in the north and negative residuals in the south. The results on the Moran's I randomization test revealed no significant values of autocorrelation for the Landscape set (Moran's I stat= 1.187, p = 0.118), the Spatial set (Moran's I stat= 0.106, p = 0.458) and the Full set (Moran's I stat= 0.009, p = 0.497). Only the Local set revealed a nearly significant spatial autocorrelation in the residuals (Moran's I stat= 1.648, p = 0.050). Even so, the Moran's I results for the Full model indicate that the spatial autocorrelation in the models' residuals should not be a problem in the interpretation of results.

Table 1. Partial models (Local, Landscape and Spatial) and full model showing the coefficients (B) and their significance (p) for both species data; for variables' transformations see appendix A.

Variables	Partial models		Full model	
	B	**p**	**B**	**p**
Local				
ALT	8.243	0.000	8.627	0.174
SHRUB	0.086	0.001	0.196	0.080
TREE	-0.048	0.005	-0.129	0.075
CLAY	-3.416	0.020	5.269	0.242
constant	-39.242	0.002		
Landscape				
FORE	3.141	0.004	-1.166	0.632
MONT	-0.038	0.003	-0.108	0.120
constant	0.781	0.138		
Spatial				
Y	2.903	0.000	7.027	0.049
constant	0.082	0.840	-60.249	0.093

All seven models had outstanding discrimination ability (AUC > 0.93) excepting for the single Landscape model set (0.868), although the discrimination was still high. All joint models containing the Local set presented the best performance statistics (AUC > 0.97 and correct classifications >94%), with maximum classification accuracy for the full model (table 2).

Table 2. Summary of performance parameters of all models for both species data (D^2: explained deviance, AIC: Akaike's information criterion, AIC_c: Akaike's information criterion corrected for small samples, AUC: area under the ROC curve, OCC: overall correct classification, sensiv.: sensivity, specif.: specificity).

Models	D^2	AIC	AIC_c	AUC	OCC [a]	sensiv.	specif.
LOC	0.548	53.3	54.0	0.934	85.5	91.2	80.0
LAND	0.341	69.1	69.4	0.868	73.9	70.6	77.1
SPAT	0.566	45.5	45.6	0.949	84.1	82.4	85.7
LOC + LAND	0.732	39.6	41.0	0.979	94.2	97.1	91.4
LOC + SPAT	0.780	33.1	34.1	0.986	94.2	97.1	91.4
LAND + SPAT	0.617	44.6	45.1	0.961	88.4	88.2	88.6
LOC+LAND+SPAT	0.835	31.8	33.6	0.990	95.6	97.1	94.3

[a] (OCC cut value 0.49)

The strongest pure effects resulted from the Local model (21.8%), whereas the Landscape model presented a minimum value of pure effect (5.5%). The greatest amount of shared variance was explained by the three fractions containing the spatial component (12.9 - 17.3%). The remaining shared fraction (Local + Landscape) was extremely low (figure 4).

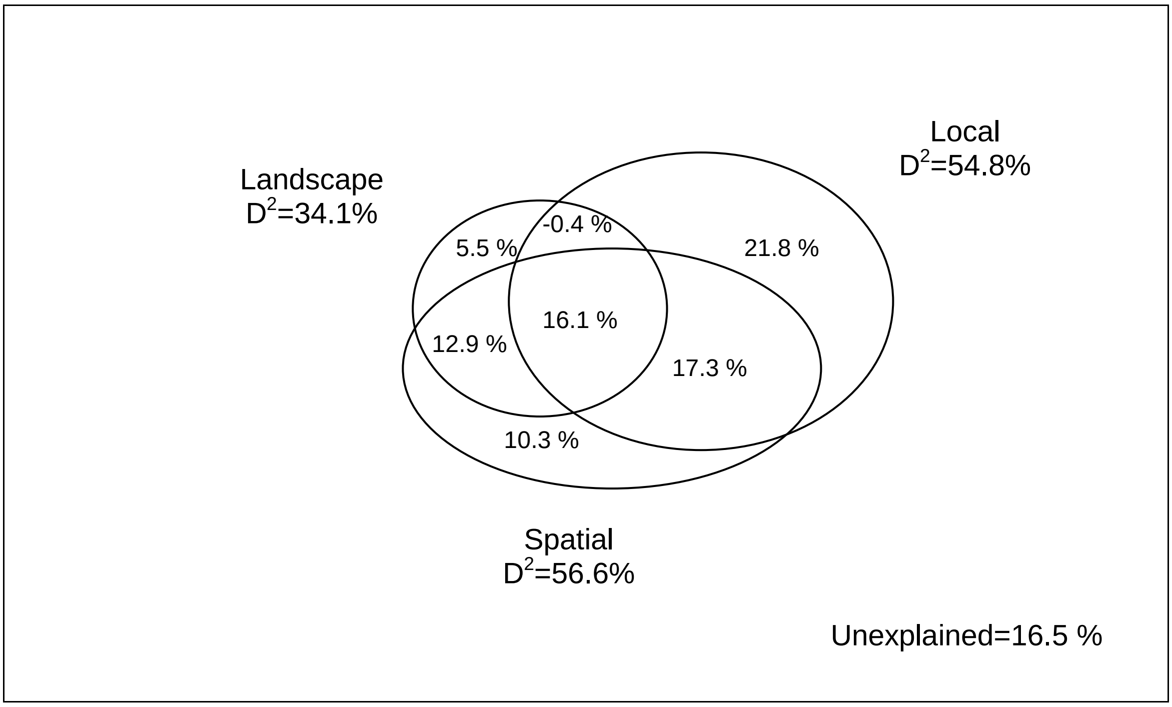

Figure 4. Results of the variation partitioning for both species data according to the three sets of independent variables: Local, Landscape and Spatial sets (Unexplained: percentage of unexplained variation); the size of circles and overlap are partially scaled according to the values of each component.

At the local scale, the most important factors distinguishing the presence of *M. lusitanicus* from *M. duodecimcostatus* were the higher altitude, higher cover of shrubs, lower clay content in the soil, and lower cover of tree canopy for the *M. lusitanicus* presence sites. The presence of forest landscape units and the low abundance of "montado" units were the most influencing landscape factors in the identification of *M. lusitanicus* occurrence sites when compared to *M. duodecimcostatus*. Considering the two scale approaches, the local one presented a higher performance when compared to the landscape scale in explaining the two species variability in distribution patterns. The (pure) importance of the spatial component was much lower than the local component, and considering the sum of the two environmental scales (Local and Landscape), a better performance was evidenced by the environmental set.

In short, the differences in distribution patterns of the two species of pine voles are mostly explained by fine-scale environmental factors and by shared spatial effects.

DISCUSSION

Regional occurrence pattern and coexistence

Local coexistence of *M. lusitanicus* and *M. duodecimcostatus* in the field, although possible, is not a common event, as our estimated area of sympatry was small and the simultaneous co-occurrence of the two species was not confirmed in the field. Previous results indicated that the two species have low abundance in the region and occurrence sites are localized and spatially discontinuous (Santos et al. submitted). These characteristics and average dispersal distances of 100-160 m for some *Microtus* species (*M. pennsylvanicus* and *M. arvalis*; McShea and Madison 1992) may create conditions that shorten the possibilities for local co-occurrence to become common and widespread in the region. In addition, ecological niche differences of species can better explain the reduced size of sympatric areas (see discussion below).

Iberian localities with simultaneous presence of both pine voles are commonly cited in literature (e.g. Brunet-Lecomte et al. 1987; Madureira 1981; Rodríguez and Peris 2007; Veiga 1978), and have contributed to the general idea of a sympatric belt through the Iberian Peninsula (Cotilla and Palomo 2007). However, as these works are based on Barn owl (*Tyto alba*) diet, the documented co-occurrence records correspond to the scale of hunting areas of owls (ca. 4 km^2 during the breeding season, and 16-25 km^2 outside the breeding season; Shawyer 1998). In spite of that, we found one study reporting coexistence of the two species in a 1-ha area, in the Spanish Pyrenees (Borghi et al. 1994).

Our results suggested the existence of a parapatric boundary between sympatric areas, differing from these by the extent of range overlap (Bull 1991). This variation in the prediction of overlap extent (from sympatry to parapatry) could be explained by a sharp environmental cline (measured or not by us) or by the difficulty in obtaining presence samples of either species for neighbouring areas in the region of parapatry. However, the kriging error was minimal for this area, validating the existence of a contact zone between the two species, which lead us to exclude the second hypothesis.

Environmental and spatial effects

Although there was a spatial component explaining the species segregation in a north and south pattern, the microhabitat characteristics were the most important in discriminating each species ecological niche.

As *M. lusitanicus* is less fossorial (Mathias 1996; Vinhas 1993), it should be more dependent on cover protection for occasional surface movements and activity.

The high cover of shrubs should be more efficient in providing protection from predators than tree cover, for instance, as owls can use trees as hunting perches (Shawyer 1998). In the case of *M. duodecimcostatus*, the more fossorial species that lives in drier environments, the close proximity of trees (and their roots) ensures both protection from digging predators (foxes and wild boars; King 1985; Borghi et al. 1994), and higher humidity and lower temperatures in the burrows conditions during the dry season, when compared to the nearby areas outside tree canopy (Joffre and Rambal 1993).

This less fossorial behaviour of *M. lusitanicus* may also influence other characteristics in habitat selection, such as higher elevations that offer better microclimatic conditions during critical seasons (soil moisture and temperature, and green food during the dry season), and soils with low clay content to allow less energy expenditure in burrow construction. As the range of this species includes major areas of Temperate bioclimates (Rivas-Martínez et al. 2002), it is expected that, at finer scales, it prefers fresher and more humid sites, which tend to occur more frequently at higher altitudes within a mediterranean environment. Indeed, previous results indicated that in this study area the presence of *M. lusitanicus* was positively associated to more humid soils (Santos et al. submitted). These differences in niche patterns could also be related to the physiological traits of the two species as a response to environmental variability. Recent physiological results indicated that the resting metabolic rate of *M. duodecimcostatus* is lower than the rate of *M. lusitanicus* (Monarca R., unpublished data), which could be a strategy for saving water and reducing thermal stress inside the burrows (Armitage et al. 1990; McNab 1966).

In a local area of sympatry in the Spanish Pyrenees, it was shown that soil depth and plant cover were the habitat factors discriminating the different sites occupied by the two species: while *M. lusitanicus* occupied areas of shallow soils and medium to sparse plant cover, *M. duodecimcostatus* was present in deeper soils and dense plant cover (Borghi et al. 1994). These results report to a 1-ha area, in a mountain environment at 2000 m of altitude (Borghi et al. 1994), and so comparisons with our data are difficult due to the different scales and environments. Regional differences may exist however, in the way species select habitat features across their ranges and in different ecosystems, reflecting local adaptations to different biotic pressures, such as other competitors or predators presence. This may explain different results for the same species, but for different regions of the species ranges.

Niche differences between *M. lusitanicus* and *M. duodecimcostatus* were better explained by fine-scale factors when compared to landscape ones. This higher importance of local factors was also obtained in a previous research for the presence/absence patterns of each species (Santos et al. submitted). Both results can be explained by the small distances in perception of the landscape by these small mammals that conduct to a higher importance of finer scales in explaining general patterns and differences in species distributions (see Gehring and Swihart 2003). This reinforces the importance of microhabitat characteristics in providing not only cover, food and burrowing conditions for the presence of each species, but also in providing enough heterogeneity in those characteristics that allow for niche differences between species, and the avoidance of interspecific competition in sympatric areas and contact zones. Indeed, heterogeneous habitats may promote species coexistence, by providing enough (intermixed) habitats that allow the co-occurrence of different species and by reducing their competition, as has been suggested for *M. montanus* and *M. pennsylvanicus* (Douglass 1976) and several other North American *Microtus* species (Getz 1985; Rose and Birney 1985).

Spatial effects, alone and shared with environmental sets, were also very important in explaining the segregation of the two species. Shared spatial effects may arise because several local and landscape factors are spatially structured themselves and originate distribution patterns also spatially structured (Fortin and Dale 2005; Legendre and Legendre 1998). On the other hand, the pure spatial component may be due to unstudied explanatory variables also spatially structured, and to contagious biotic processes such as growth, dispersal, predation, resource dynamics, social interactions, or metapopulation dynamics that may produce spatial structuring of communities (Bahn and McGill 2007; Fortin and Dale 2005; Legendre and Legendre 1998). Although several processes may contribute to this pure fraction of the spatial component, it is not possible to determine which ecological processes are causing this spatial pattern (but see Van Teeffelen and Ovaskainen 2007).

The landscape component contributes to the segregation of the two species mostly through a combined effect between landscape and spatial variables. This means that forest patches are more abundant in the north part of the study area, and "montado" habitats dominate in the south. Even so, there is ca. 5% of variation attributed to forest and "montado" that is independent of a geographic trend, and thus contribute to niche segregation in sympatric areas with presence of both landscape units.

Interspecific competition

The ecological niche differences found between species should allow, per se, local coexistence of the two pine voles. However, the area available for this coexistence is rather limited and confirmation with field data was not possible. Thus, we believe that local coexistence in the field is not common and a spatial segregation between species is evident in some areas, possibly as a consequence of interspecific competition.

Dominance and aggression behaviours of one species towards another indicate that interference competition may be a common behavioural pattern among sympatric small mammal species (Eccard and Ylönen 2003). In small mammals, it is frequent that larger species emerge as superior competitors in a community (Grant 1972; but see Koivisto et al. 2007). In our example, *M. duodecimcostatus* is the larger species and is referred to as more aggressive than *M. lusitanicus* (Vinhas 1993), possibly being the dominant species. Moreover, in a previous national-scale work it was found that *M. duodecimcostatus* avoids regions with high abundance of cambisols and prefers the abundance of luvisols, while *M. lusitanicus* occurs in regions with opposite characteristics (Santos et al. 2009b). When we overlay the referred national soil map with our regional presences of the two species, we find that *M. lusitanicus* presences occurred only once outside the cambisol class, whereas *M. duodecimcostatus* occurred outside the luvisol class in six sites (figure not shown). Although the resolution of this soil classes may be too coarse for a regional approach, this indicates that *M. duodecimcostatus* is able to occupy less favourable (or less common) soil conditions, while *M. lusitanicus* may not. However, from the analysis of the sympatry map here presented, neither species consistently excludes the other, as both species show equivalent number of presence sites within the estimated sympatry area. More intensive trapping within the regional sympatry area could clarify this apparent contradiction.

Our work shows for the first time predicted areas of co-occurrence of *M. lusitanicus* and *M. duodecimcostatus* at a regional scale, using Kriging methods. This approach may be interesting for other scientists aiming to build species distribution maps as, in many cases, kriging would be far more appropriate for their data, although less widely known amongst ecologists. We also demonstrate that regional niche differences between *M. lusitanicus* and *M. duodecimcostatus* are better explained by local scale factors by using a multiscale approach. However, a more detailed and quantitative study of the diet niches of each species are needed, as well as laboratory trials or enclosures experiments (removal and exclusion) for testing behaviour responses and habitat choices when both species are present. Although this lack of information may have limited some of our conclusions, it stimulates future researches on this topic.

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Appendix A. Variable sets, correspondent explanatory variables, their original values, and transformations (log: natural logarithm; P(0.5): square root; P(0.33): cubic root; 0-1: binary).

Code	Description	Values	Transformation
Local set			
ALTI	Average elevation (m a.s.l.)	(167-820)	log
BARE	Cover percentage of bare soil	(1.5-67.0)	P(0.33)
VEGET	Cover percentage of herbaceous vegetation	(33.0-99.0)	P(0.33)
WOOD	Cover percentage of woody vegetation (under 3 m height)	(0-66.6)	P(0.33)
GRASS	Cover percentage of grasses	(4.0-80.0)	
COMP	Cover percentage of composites	(0.2-75.0)	P(0.5)
MHEIG	Mean herbaceous vegetation height (after 10 random measurements) (m)	(2.5-93.4)	log
TREE	Cover percentage of trees	(0-90)	P(0.5)
SHRUB	Cover percentage of shrubs	(0-75)	
BIOM	Dry vegetation biomass (g)	(57.2-803.9)	log
GRAZ	Presence of grazing signs (faeces, footprints, grazed grass, or observation of grazers)	(0-1)	
PROSTR	Cover percentage of plant species with a prostrated growing	(0-83)	P(0.33)
VERGED	Distance to the nearest verge (field margins, hedgerows, roadsides, stone walls, ecotones) (m)	(0-300)	0-1
CMED	Median values of soil penetration resistance at 0.10 m depth (after 10 measurements in orthogonal transects) (psi)	(1-6)	
HUMID	Percentage of water in the soil at 0.10-0.15 m depth	(1.9-35.2)	P(0.33)
SAND2	Percentage of coarse sand (2-0.2 mm)	(5.7-71.5)	log
SAND02	Percentage of fine sand (0.2-0.02 mm)	(13.3-51.7)	
SAND	Percentage of total sand (2-0.02 mm)	(27.2-91.4)	
LIME	Percentage of lime (0.02-0.002 mm)	(4.7-45.3)	
CLAY	Percentage of clay (< 0.002 mm)	(3.9-30.4)	log
Landscape set			
URB	Percentage of urban areas	(0-13.7)	0-1
WAT	Percentage of water courses and dams	(0-7.1)	0-1
AGRI	Percentage of agricultural areas (crops, orchards, vines, etc.)	(0-85.6)	P(0.33)
OPEN	Percentage of open areas (pastures, meadows, fallow fields)	(0-97.0)	P(0.33)
LINH	Percentage of linear habitats (verges bordering roads, stonewalls and ecotones, and linear dense vegetation)	(0-13.4)	P(0.5)
SHRUB2	Percentage of shrubs formation	(0-83.0)	0-1
PLANT	Percentage of young oak forest plantation	(0-97)	0-1

Appendix A. (continued)

Code	Description	Values	Transformation
Landscape set			
MONT	Percentage of "montado" (cork, holm or Pyrenean oaks)	(0-100.0)	
FORE	Percentage of forest (natural, production or mixed)	(0-99.0)	0-1
NUMP	Number of patches	(1-49)	log+1
MPS	Mean patch size (ha)	(0.4-19.5)	log+1
MEDPS	Median patch size (ha)	(0.2-19.5)	log+1
PSCOV	Variance coefficient of patch size (ha)	(0-190.2)	log+1
PSSD	Standard deviation of patch size (ha)	(0-9.6)	log+1
TE	Total edge of patches (m)	(1568.8-17153.1)	log+1
ED	Edge density (m/ha)	(0.6-628.7)	log+1
MPE	Average amount of edge per patch (m)	(350.1-1568.8)	log+1
MSI	Mean shape index	(1.0-2.6)	
AWMSI	Area weighted mean shape index	(1.0-2.0)	
MPAR	Mean perimeter-area ratio	(80.3-21589.3)	
MPFD	Mean patch fractal dimension	(1.2-1.5)	
AWMPFD	Area weighted mean patch fractal dimension	(1.2-1.4)	
SDI	Shannon's diversity index	(0-2.6)	
Spatial set			
AUTO_LD	Autologistic term	(0-1)	
X	X coordinates centred	(-1.615-2.211)	
Y	Y coordinates centred	(-1.727-2.188)	
XY	Multiplication of centred X and Y	(-3.498-2.471)	
X^2	Square of X coordinates centred	(0.000-4.888)	
Y^2	Square of Y coordinates centred	(0.000-4.786)	

Chapter 6

Spatial and temporal ecology of the Lusitanian pine vole

Paper IV

Spatial and temporal ecology of the Lusitanian pine vole (*Microtus lusitanicus*) in a Mediterranean polyculture.

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Spatial and temporal ecology of the Lusitanian pine vole (*Microtus lusitanicus*) in a Mediterranean polyculture

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ABSTRACT

In this study we report the first data on the spatial ecology of the Lusitanian pine vole (*Microtus lusitanicus*). Data report to the breeding season and to a traditional Mediterranean agricultural landscape in Central Portugal, using radio-telemetry methods. We documented large home range areas with values of 1042 m^2 for males and 862 m^2 for females (MCP method; 95% kernel method with values of 229 m^2 and 159 m^2 for males and females, respectively). Although no significant differences between sexes or reproductive status were found, longer daily movements were observed in reproductively inactive males. Pair bonding and home range overlap was observed between males and females, as well as between females and sub-adults. Voles showed no distinct preference for day or night for activity periods and movements. However, this result was dependent on interactions between sex, reproductive status and time of day. Voles revealed habitat preference for both spatial scales of analysis: they selected verges, vines and olives, within the study area, and used more verges within their home ranges, when compared to the other habitat types. The use of space by *Microtus lusitanicus,* in comparison with other microtines, suggests the occurrence of spatial associations between males and females in monogamous pairs. The importance of verges and linear habitats within an agricultural context is apparent, once they provide food and shelter from predators and human interventions.

Key-words: Activity pattern; habitat; home range; *Microtus lusitanicus*; radio-tracking; social behaviour.

INTRODUCTION

The Mediterranean Peninsulas have favoured the formation of a large number of endemic species of small mammals (Bilton et al., 1998). This has contributed to the importance of the Mediterranean Basin as a biodiversity hotspot (Blondel and Aronson, 1999; Médail and Quézel, 1999). Despite this, very little is known about the spatial ecology of some of these species, namely microtines

(Paradis and Guédon, 1993; Blondel and Aronson, 1999). A specific example of a pragmatic case of data deficiency is the Lusitanian pine vole (*Microtus lusitanicus* (Gerbe, 1879)). This small herbivore is a Southwestern European endemism with fossorial habits and a probable absence of cycling populations (Giannoni et al., 1993; Mira and Mathias, 2007; IUCN, 2007). The voles live in open habitats such as meadows and pastures, but can also be found in agricultural areas such as commercial orchards and irrigated crops, where it is known to cause important damages (Madureira, 1984; Vinhas, 1993; Mira and Mathias, 2007).

The intensification of commercial orchards and changes in the irrigation techniques that started in the 1970's have resulted in a transformation of the pine voles' habitat. This has resulted in higher densities of voles and increased amounts of damage since then (Madureira, 1984; Santini, 1988; Guédon et al., 1992; Vinhas 1993). The potential of *M. lusitanicus* to cause damage stimulated research on their taxonomy, morphology, reproduction and abundance. However, the majority of this focus has been in large and homogeneous agricultural habitats with high densities of voles (Madureira, 1982; 1984). Knowledge on the ecology of this species in natural habitats is still scarce (Mira and Mathias, 2007; Rodríguez and Peris, 2007). Lack of information on *M. lusitanicus* may be due to its small body size and fossorial habits, which makes the vole difficult to study in a natural environment.

In central Portugal, where our study took place, traditional Mediterranean agriculture is characterized by small-scale mosaics of vineyards, olive groves and crops fields that have existed in the region for centuries (Guédon et al., 1992; Blondel and Aronson, 1999; Farina, 2006). Although pine voles can be abundant in these habitats, there are no reports of these animals causing noticeable damage (Guédon et al., 1992). This type of ecosystem has characteristics that make studying *M. lusitanicus* very attractive. These areas have high fine-scale heterogeneity (vertical and horizontal diversity of habitats) with an abundance of hedgerows and verges. Although these ecosystem types cannot be classified as natural areas, they diverge very much from the commercial orchards and crops where most available information on the species exists. In addition, local results from this study can be applied to other populations in equivalent landscape units, since this landscape unit (Corine land cover unit "complex cultivation patterns"; IGeoE, 2003) is very common across the species distribution range in Portugal (Madureira, 1984; Cancela d'Abreu et al., 2002).

Many animals restrict their movements to specific areas (Burt, 1943; Kernohan et al., 2001; Yletyinen and Norrdahl, 2008). This has several important consequences on the spatial patterns of distribution and abundance of animals (McNab, 1963; Zwicker, 1989; Macdonald et al., 1997). In microtines, spacing and social behaviour are important to understand the population regulation mechanisms (Taitt and Krebs, 1985; Ostfeld, 1990; Ishibashi et al., 1998; Moorhouse and Macdonald, 2008). It has been argued that spacing and social behaviours influence population regulation through changes in the proportion of reproducing individuals (Madison, 1980; Taitt and Krebs, 1985), in aggressiveness, or in restrictions of dispersing animals (Madison and McShea, 1987). Commonly, sex and reproductive status of voles are key factors influencing those space use patterns (Ostfeld, 1990). Microtines have highly variable social systems, ranging from solitary species with almost no pair bonding and no paternal care (e.g. *Microtus pennsylvanicus;* Madison, 1980; Madison and McShea, 1987), to species with strong pair bonding and paternal care (e.g. *Microtus pinetorum;* FitzGerald and Madison, 1983). An indication of the type of social system present in *M. lusitanicus* would allow a better understanding of its population dynamics and regulation mechanisms.

On the other hand, patterns of animal movements can provide useful information on dispersal, activity area, and site selection for reproduction, and may provide a solid basis for the implementation of agricultural practices in areas facing risk of damage by this vole species (Buckle et al., 1997).

Resource selection by animals occurs on several spatial scales (Batzli and Lesieutre, 1991; Dooley and Bowers, 1996; Orrock et al., 2000). The selection of a home range by an individual within a landscape and the selection of different habitat units within a home range or territory are important criteria. Information on which resources are preferred or avoided improves our understanding of ecological and physiological needs of a species.

Quantitative assessments of home range, movements, social behaviour, activity rhythms, or habitat selection by *M. lusitanicus* remain to be elucidated. In the present study, we report data on these issues using radio-telemetry carried out during the breeding season in a Mediterranean agricultural landscape in Central Portugal.

The main goals of our study were to characterize the basic patterns of space use by *M. lusitanicus* and answer to the following questions: *i*) How do sex and reproductive factors influence home range size and movements of *M. lusitanicus*? *ii*) What type of social system is present? *iii*) What is the daily activity pattern of the species, and what is the periodicity of short-term activity rhythms? and *iv*) What are the habitat preferences at different spatial scales?

METHODS

Study area

The study area is 3 ha and is located in the District of Tomar, Central Portugal (-08º18’29’’ N, 39º37’47’’ W). The landscape is characterized by generally low elevation values (between 150 and 300 m a.s.l.). On a regional scale, the mosaics of olive groves, vineyards and polycultures are common and are generally concentrated around small villages. These small-scale mosaics, are still managed traditionally and extensively, and are often surrounded by intensive production or mixed forests (Cancela d’Abreu et al., 2002). In the study area, the fruit trees and crop species that dominate are: the olive tree (*Olea europaea*) and the vine (*Vitis vinifera*). However, there are other common fruit trees (*Pyrus communis*, *Malus domestica*, *Cydonia oblonga*, *Prunus* spp., *Citrus* spp., *Ficus carica*). The herbaceous vegetation is mainly composed by ruderal and nitrophilous species, typical of agro-ecosystems (e.g. *Calendula arvensis*, *Hordeum murinum*, *Bromus* spp., *Avena barbata*, *Coleostephus myconis*, and other species).

The climate is Mediterranean with mild winters (8.8 ºC average daily temperature in January) and hot summers (23.5 ºC average daily temperature in July). Annual precipitation averages 986 mm, and is concentrated in autumn and spring months (see Ninyerola et al., 2005).

Trapping

In order to obtain animals for the radio-tracking, trapping sessions were conducted once every month from January to May 2008 over a period of four days, setting up 70 traps. The voles were caught in live-traps of multiple captures (“mole-type”) modified by the addition of a nest box, with apple as bait and hay for bedding, that were set up in the underground tunnels.

The captured animals were sexed, measured, weighted and checked for reproductive condition (active males: scrotal testes; inactive males: abdominal testes; active females: perforated vagina or lactating; inactive females: non-perforated vagina and non-lactating; McCravy and Rose, 1992). Information on body masses of different maturation stages is not available for this species. The criterion used for the body mass intervals of adults was the minimum weight observed in animals with visible signs of reproductive activity. Because the lightest animal reproductively active was 16.4 g, we set the limit for 15 g. The remaining animals (sub-adults and juveniles) were all less than 15 g. The discrimination between sub-adults and juveniles was more arbitrary and was based on strongly reduced body size and weight: animals under 10 g were considered juveniles, 10 to

15 g were considered sub-adults. We expected that, in the study area, the breeding season of *M. lusitanicus* should occur from November to May, with maximum activity in February and March. This was based on data from a similar species, the Mediterranean pine vole *M. duodecimcostatus*, for Central Portugal (Mira, 1999).

Surgical procedures

The radio transmitters (nine BD-2H, ten BD-2NTH, one BD-2THX, Holohil Systems Ltd., Ontario, Canada) were implanted in aseptic conditions in the laboratory. General anaesthesia was achieved with a single intramuscular injection of 0.1 ml, and was a combination of Ketamine 75 mg/kg (Imalgene 1000 ®) and Medetomidine 1 mg/kg (Domitor ®; Cruz et al., 1998). An incision of 12 mm was made in the ventro-lateral side of the animal and the transmitter was inserted into the abdominal cavity. Three to four interrupted sutures (Surgicryl, synthetic absorbable suture, USP 2/0 EP3) were used to close the muscle and peritoneum as a single layer. This was followed by a similar number of sutures in the skin. Surgical procedures followed guidelines from Madison et al. (1985) and were conducted under the supervision of a veterinarian.

The transmitters weighed 0.73 to 0.95 g, depending on the model, and represented on average 5.2 % (range: 4.0 – 7.5 %) of the voles' body mass. The expected battery life varied between 10-12 days (BD-2NTH and BD-2THX), and 28 days (BD-2H).

Telemetry

Twenty animals were implanted with radio transmitters (eight adult males, ten adult females, and two sub-adults: male and female). One vole was preyed by a domestic cat and we lost radio contact with three others before achieving enough data collection. The remaining 16 voles comprised seven adult males, eight adult females and one sub-adult male.

An average of 3.2 voles (range: 2 - 5) was radio-tracked simultaneously each month. From January to May 2008, the tagged animals were tracked on foot using the "homing-in" method (White and Garrot, 1990) with a hand-held 2-element Yagi antenna and a TR-5 receiver (Telonics, Mesa, Arizona, USA). The position of animals was determined with an accuracy of 0.25 - 1 m^2. All actions that would potentially disturb the voles were avoided (Madison et al., 1985).

Since we were also interested in the activity patterns of the animals, we decided to use clustered sampling (Garton et al., 2001). As most of the batteries lasted only a few days, the tracking method chosen was discontinuous tracking with short intervals of 30 min. (Collins and Barret, 1997; Hansteen et al., 1997). Though longer time intervals would yield more data independence, there would be too few locational fixes (Harris et al., 1990; Hansteen et al., 1997; Rooney et al., 1998). Hence tracking was done in 4-h bouts, comprising eight locational fixes and separated 16 to 24 h from the next bout, to sample all the 24 h period (8-12 h, 12-16 h, 16-20 h, 20-24 h, 0-4 h, 4-8 h). Frequently, occasional fixes were also taken between bout periods.

We registered individual locations by plotting each fix by hand, with an estimated error of ± 2 m, on a digital map of the study area. Habitat classes were mapped for the entire study area based on field work combined with orthophoto map interpretation (2004 flight, resolution of 0.5 m, IGeoE). Even so, habitat classes were also registered in the field and added to each individual location: verge, vineyard, olive grove, orchard, vegetable crops, pasture, urban and other. Verges included herbaceous field margins, hedgerows and edges of small agricultural fields, while the class "other" corresponded to set-asides and uncultivated areas. All information was imported for analysis into ArcView 3.2 (ESRI, 1999).

On average, animals were tracked for 9.1 days (range: 5 – 14 days, n= 16) with 11.4 fixes per animal each day (range: 9.8 – 12.5, n=16). An incremental area plot of the number of fixes versus the cumulative minimum convex polygon area (Kernohan et al., 2001) revealed that an asymptote in the area estimation is reached at 79.8 fixes (range: 39 – 143). This value would exclude one animal with 62 fixes, a sampling effort that corresponds to more than 70% of the average home range of the remaining animals and well above minimum sample size recommended by Kernohan et al. (2001). For this reason, we decided to include the above animal in home range calculations.

Data analysis

Home ranges and movements

The minimum convex polygon (MCP; Mohr, 1947; Harris et al., 1990) was used to define the outer boundary of the home ranges since it yields more precise contours when several potential barriers that may restrict movements (e.g. roads, stonewalls, houses) are present, as compared to utilization distribution methods (Kernohan et al., 2001). Because this method is widely used in rodent researches (Madison, 1985) and permits easy comparisons between studies (Harris et al., 1990), we used the MCP method for comparisons of home range values. Despite this, the fixed kernel estimator (95% and 50% probabilities; Worton, 1989) was also calculated for each animal to compare the results of both estimators and obtain data on core areas (Harris et al., 1990; Kernohan et al., 2001). The least squares cross validation (LSCV) was used to calculate the smoothing factor, *h* (Worton, 1989; Seaman et al., 1999; Kernohan et al., 2001). To validate our choice of *h*, we first calculated kernel home ranges with varying values of LSCV *h*. Higher values of LSCV *h* reduced area fragmentation, but increased bias (low precision of contours at home range limits). For this reason, smoothing factors were calculated for each animal, and then averaged (*h* = 3 m) to allow easier comparisons between animals (Bontadina et al., 2002). We used all fixes for home range estimation, as moderately autocorrelated observations do not invalidate the use of MCP or kernel estimators (Rooney et al., 1998; De Solla et al., 1999; Kernohan et al., 2001). Mean daily speed (mean number of meters travelled per day) was also calculated to summarize the movement information of each animal.

Logarithmic transformations (MCP and 95% kernel) and square root transformation (mean daily speed) were performed on the dependent variables to achieve normality and were then tested for the effects of sex and reproductive status with linear models. These variables were selected because they are known to influence space use patterns of most microtines (Ostfeld, 1990; Collins and Barrett, 1997). The assumptions of normality and variance homogeneity were verified after inspection of residual plots against fitted values and predictors (Zuur et al., 2007).

Social behaviour

Despite our small sample size, we used the information from simultaneous radio-tracking of the individuals, as it can provide insight to the degree of interaction within and between the sexes, revealing dominating social patterns (FitzGerald and Madison, 1983; Wolff, 1985). We calculated the percentage of MCP home range overlap between adjacent individuals, radio-tracked simultaneously in the same month, to quantify the spacing pattern. Individuals that had at least 20% of home range overlap were assumed to have a social bond (Lambin and Krebs, 1991). The overlap of core areas (50% kernel) was determined qualitatively (overlap/no overlap). Moreover, capture results should reveal some basic information on the local population structure (adults/non-adults and males/females ratios) and multiple captures should reveal information on the type of social groupings (FitzGerald and Madison, 1983).

Activity patterns

We used the activity index (mean change of position of each animal for each 4h bout) to measure activity patterns, and bout mean distance (square root of mean distance travelled in each bout) to measure movement intensity of voles. Then, we tested these parameters for the effects of sex, reproductive status, and time of day (day and night). We applied linear mixed-effects models, with individuals as a random factor to correct the correlation between observations within the same animal (Faraway, 2006). The bout period and month variables were not included in the models because they did not show any pattern with dependent variables during exploratory analyses and caused numerical instability when included in the models (Zuur et al., 2007).

Habitat selection

Habitats were divided into eight classes, according to the field measurements and orthophoto confirmation (see telemetry methods). The MCP home range estimator was used to define the outer limits of each individual's movements and the outer limits of the study area (Harris et al., 1990; Aebischer et al., 1993).

Our analysis focused on two levels of habitat selection (Johnson, 1980): i) a second-order selection of home ranges within the study area, and ii) a third-order selection of habitats within the home range used by voles. Habitat preference was evaluated using compositional analysis (Aebischer et al., 1993), accounting for randomisation as recommended by Aebischer et al. (1993). This non-parametric technique uses the individual as the sampling unit and takes into account the serial correlation between radio fixes within each individual. Zero values in the matrix of used habitats were replaced by 0.01%, while zero values in the matrix of available habitats induced the calculation of the "weighted mean lambda" (Aebischer et al., 1993).

Home-range and distance calculations were made using the ArcView 3.2 (ESRI, 1999) extension: Animal Movement Program 2.0 (Hooge and Eichenlaub, 1999). We used R software 2.6.0 (R Development Core Team, 2007) in model building procedures (nlme package, lme function; Pinheiro et al., 2007), and for compositional analysis (package adehabitat, Calenge, 2006). All mean values presented are reported with standard errors and followed by the range.

RESULTS

Home ranges and global movements

A total of 16 individuals (eight males and eight females) provided enough fixes to obtain reliable estimates of home range size (n ≥ 62 fixes).

Average home range values obtained with the MCP method were 1042.9 ± 430.9 m^2 for males (range: 5.5 – 3063.0 m^2) and 861.9 ± 409.0 m^2 for females (range: 16.5 – 2866.5 m^2), with a mean overall value of 952.4 ± 287.9 m^2 (range: 5.5 – 3063.0 m^2). Home range size did not differ based on sex, or reproductive status. No interaction effects were found (all $p > 0.05$, table 1).

The 95% kernel estimator presented average home range values of 228.8 ± 35.7 m^2 for males (range: 116.8 – 394.7 m^2), 158.7 ± 20.2 m^2 for females (range: 90.9 – 237.5 m^2), and 193.8 ± 21.8 m^2 for the two groups pooled (range: 90.9 – 394.7 m^2). Again, no significant effects were detected for sex, reproductive status or their interaction ($p > 0.05$, table 1). Core areas (50% kernel) averaged 42.0 ± 3.3 m^2 for overall data (range: 32.5 – 66.2 m^2). The distance between two most remote points of a home range varied from 10.6 to 225.8 m (mean ± SE: 65.3 ± 14.1m).

Table 1. Summary results of the linear models for the effects of sex, reproductive status and their interaction on home ranges and daily speed of *Microtus lusitanicus* (MCP: home range with the minimum convex polygon, 95 kernel: home range with the 95% kernel method, Daily speed: mean daily speed, Adj R^2: adjusted R^2, n= 16 animals).

	Factors	Adj R^2	F test	p-value
MCP		0.035		
	sex		0.007	0.933
	reprod		2.385	0.148
	sex * reprod		1.156	0.303
95 Kernel		0.141		
	sex		2.674	0.128
	reprod		2.482	0.141
	sex * reprod		0.314	0.586
Daily speed		0.230		
	sex		0.082	0.779
	reprod		0.276	0.609
	sex * reprod		7.112	0.021 *

The kernel estimator produced a fragmentation of home ranges of most individuals (10 out of 16 monitored individuals), leading to smaller area estimates when compared to MCP areas (not significant; Wilcoxon signed ranks test, V = 100, p = 0.105). Each individual had a mean of 2.2 ± 0.3 95% kernel areas (range: 1-4) and 1.2 ± 0.1 50% core areas (range: 1 – 2).

Mean daily speed was 72.8 ± 12.9 m/day (range: 3.9 – 206.5 m/day), and the variation of these values between individuals could not be explained by any effects of sex or reproductive status as main factors (both $p > 0.05$, table 1). Despite this, there was a significant effect of the interaction effect ($p < 0.05$, table 1): i.e. reproductively inactive males made higher daily distances when compared to reproductively active males and inactive females.

Social behaviour

Our trapping results revealed a globally low abundance of voles with mean captures of 4.5 ± 0.8 voles/ha each month (range: 4.2 – 6.3 voles/ha). Age structure favoured adults (58.5%, n = 24) over sub-adults and juveniles (41.5%, n = 17), but we captured sub-adults every month and juveniles in March and May. Overall sex-ratio (male:female) was 0.67M:1F (considering adult and sub-adult animals, n = 35), and was not significantly different from 1:1 (one-sample proportion test with continuity correction: $\chi^2 = 1.029$, df = 1, p = 0.311).

The spatial disposition of home ranges is presented in figure 1. The degree of territoriality shown by males and females during the studied period was not very clear. However, no large intrasexual overlap is observed in males, or in females; and the extension of intrasexual overlap is similar for both sexes. For three male-female pairs that were radio-tracked simultaneously (in January, March and April), all had more than 50% of overlap of the MCP areas for both sexes. Male home ranges had a mean overlap with the home ranges of females equalling 75.5% (range: 61.3 – 100%), while for the females the overlap averaged 58.7% (range: 53.6 – 61.3%). Simultaneous nest cohabitation (and core areas overlap) was registered in two vole pairs, but not for the male-female pair from March which showed non-overlapping core areas. Another pair of voles evidenced sharing of the nest site: the February female with 2866.5 m^2 MCP and the March male with 5.5 m^2 MCP. Although they were not radio-tracked in the same month, both voles were captured in the same place in March, close to the nest site. Nevertheless, the animals were not included in the above overlap calculations because the female changed her condition to reproductively active and may have reduced her home range area.

Also, results from trapping revealed 32.4% of multiple captures in traps (11 out of 34 total captures). Of these, most common situations were male-female (3 out of 11), female-sub-adult (3 out of 11) and sub-adult groups (3 out of 11).

Captures of female-female occurred only once and male-male captures did not occur.

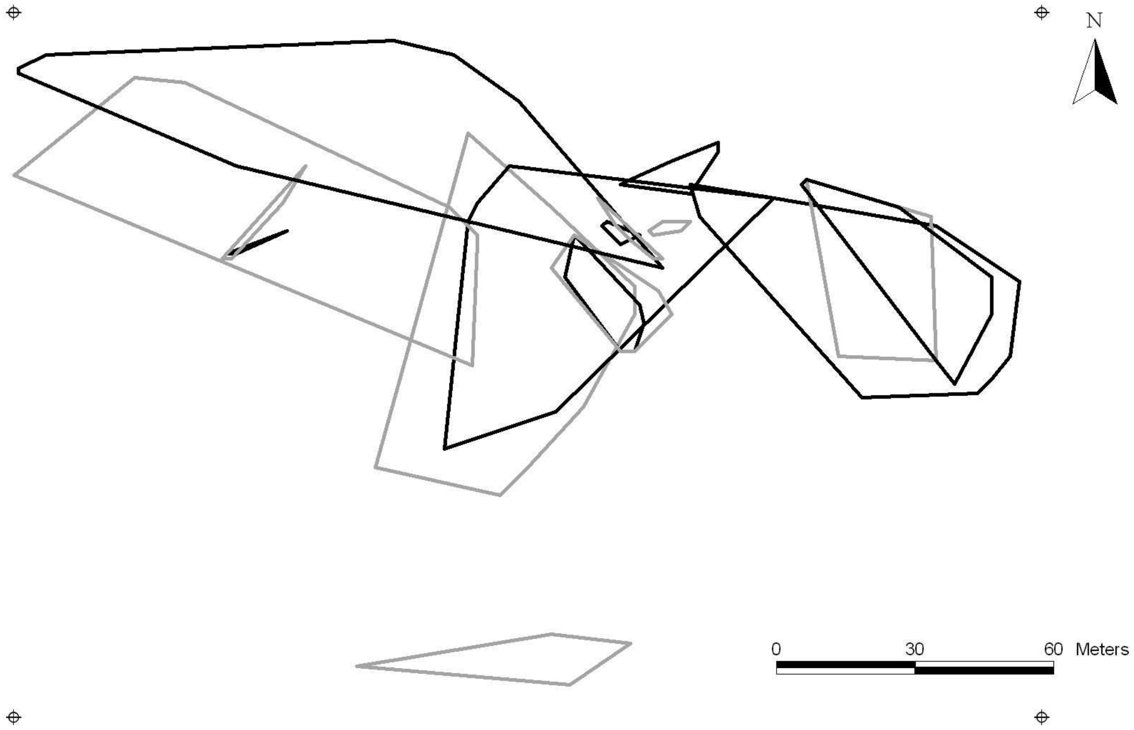

Figure 1. Estimated MCP home ranges of males (black lines) and females (grey lines) of *Microtus lusitanicus* present in the study area from January to May 2008.

Activity patterns

The most frequent activity pattern, within the 4-h bout, was 1 h of activity plus 3 h of inactivity (39% of bouts), although a balanced pattern of 2-h was also frequent (23.8%). Bouts with no registration of activity comprised 25.6% and a 4-h activity was rare (2.3%).

The tracked voles showed an average of 2.2 ± 1.6 change of position during each 4-h bout (range: 0 – 6.2). Variation of activity could not be explained by sex, reproductive status, or time of day as main factors (all $p > 0.05$, table 2); however, the interaction between sex and time of day was significant ($p < 0.05$, table 2), indicating that males were more active during the daylight, while females were less active, with similar activity values between sexes for the night period.

Table 2. Summary of linear mixed models for the fixed effects of sex, reproductive status, time of day and their interaction on activity index and mean distance made by *Microtus lusitanicus* (animal as random factor; Intercept SD: Estimated standard deviation for animals, Res SD: estimated residual standard deviation of the model; n=171 observations, 16 animals).

	Factors	Intercept SD	Res SD	F test	p-value
Activity index		0.564	1.513		
	sex			0.576	0.466
	reprod			0.003	0.954
	time_day			0.057	0.811
	sex * reprod			0.127	0.728
	sex * time_day			5.975	0.016 *
	reprod * time_day			1.656	0.200
	sex * reprod * time_day			1.741	0.189
Mean distance		0.823	1.634		
	sex			0.153	0.702
	reprod			0.094	0.765
	time_day			2.329	0.129
	sex * reprod			5.461	0.038 *
	sex * time_day			5.989	0.016 *
	reprod * time_day			4.322	0.039 *
	sex * reprod * time_day			0.196	0.659

Considering the movements per 4-h sampling bout (n = 171), voles travelled mean distances of 9.5 ± 1.0 m per bout (range: 0 – 73.3 m). Total distances made during each bout averaged 35.9 ± 3.4 m (range: 0 - 279.5 m), and maximum values between two consecutive fixes (30 min) were in average 16.7 ± 1.5 m (range: 0 – 76.0 m).

The variation in mean distance travelled could not be attributed to effects of sex, reproductive status or time of day as main factors (all p > 0.05, table 2). However, there were significant effects for all three two-way interactions terms (all p < 0.05, table 2): reproductively inactive males moved larger distances when compared to active males and inactive females (interaction sex - reproductive status); females travelled larger distances during the night when compared to distances travelled by females during the day (interaction sex - time of day); and reproductively active animals wandered larger distances during the night period than during the day period (interaction reproductive status - time of day).

Habitat selection

The verges and vines were the most frequently used habitats with a mean 49.3 ± 10.9% of fixes (range= 0-100 %) and 29.7 ± 10.9% of fixes by vole (range= 0-100 %), respectively. Less used units were orchards (9.8 ± 5.8% of fixes; range=0-90.4%), olive groves (5.2 ± 2.5% of fixes; range= 0-28.4%), and crops (5.0 ± 3.3% of fixes; range=0-48.0%). The least frequently used habitat units were urban (0.6 ± 0.6% of fixes; range=0-9.6%), and pasture (0.3 ± 0.3% of fixes; range=0-4.8%). No fixes were registered for the unit "other".

Compositional analysis revealed that percentages of habitat composition of individual home ranges were significantly different from the study area (Wilk's λ= 0.040, randomisation $p < 0.001$). A ranking matrix ordered the habitat types from most to least selected, as follows: verge > vine > olive > orchard > other > pasture > crops > urban. Table 3 (section a) shows that verge and vine habitats were selected significantly more often than other, pasture, crops and urban habitats. There was no detectable difference between the three most selected habitats (verge, vine and olive), and also between the two least selected (crops and urban). The three habitat types with lower rankings were absent from the home ranges in 50% of the voles and were dropped from the third-order selection (Aebischer et al., 1993).

Habitat use within the home range differed significantly from random (Wilk's λ = 0.021, randomisation $p < 0.05$). The ranking matrix (table 3, section b) ordered the habitats as follows: verge > orchard > olive > vine > other, with no significant differences between the use of verge and orchard, although the first one was significantly selected over the last three habitats (olive, vine and other).

Table 3. Simplified ranking matrices for *Microtus lusitanicus* based on a) comparison of proportions of habitat types within the home range with proportions of total types available within the study area; and b) comparison of percent use (fixes) within the home range with available habitat types within each individual home range. Lines are ordered from the most to least selected habitat type, with indication of rank number. The signs show whether the habitat type on each row was more (+) or less (-) selected than the corresponding colomn of the matrix. A triple sign (+++ or ---) means a significant (P<0.05) difference between the two habitat types. One sign (+ or -) indicates a non-significant difference.

a) Home range vs. Study area

	Habitat								
Habitat	Verge	Vine	Olive	Orchard	Other	Pasture	Crops	Urban	Rank
Verge	0	+	+	+++	+++	+++	+++	+++	1
Vine	-	0	+	+	+++	+++	+++	+++	2
Olive	-	-	0	+	+	+	+++	+++	3
Orchard	---	-	-	0	+	+	+	+++	4
Other	---	---	-	-	0	+	+	+	5
Pasture	---	---	-	-	-	0	+	+++	6
Crops	---	---	---	-	-	-	0	+	7
Urban	---	---	---	---	-	---	-	0	8

b) Percent use vs Home range

	Habitat					
Habitat	Verge	Orchard	Olive	Vine	Other	Rank
Verge	0	+	+++	+++	+++	1
Orchard	-	0	+	+	+++	2
Olive	---	-	0	+	+++	3
Vine	---	-	-	0	+	4
Other	---	---	---	-	0	5

DISCUSSION

Home range

The large discrepancy between the two methods for home range estimation (MCP: 952 m^2; kernel: 194 m^2) was also reported for *M. arvalis* for an agricultural habitat (Briner et al., 2005). This highlights the importance of examining more than one home range estimator. In our study, we found that the MCP method obtained more reliable results for *M. lusitanicus* data when compared to the kernel estimator. One flaw of the kernel estimator in this study was that it produced several fragmented areas, especially in those animals that made longer movements.

The space use patterns of *M. lusitanicus* seem to be distinct from most vole species because these animals had larger home range values and differences did not exist between the sexes (see Madison, 1985; Gliwicz, 1997; Jacob and Hempel, 2003; Yletyinen and Norrdahl, 2008). Although we would expect to find similarities among fossorial voles, other studies report home range values of 50 m^2 (family groups of *M. duodecimcostatus*; Guédon, 1992), 445 and 298 m^2 (male and female of *M. savii*; Salvioni, 1988), 342 and 229 m^2 (male and female of *M. multiplex*; Salvioni, 1988), or 44.7 and 41.7 m^2 (male and female of *M. pinetorum*; FitzGerald and Madison, 1983). All four species present smaller home range values than *M.*

lusitanicus, albeit the last two species showed similar home range areas between sexes, according to the respective authors.

Even though some studies have also been carried out in agricultural habitats (Guédon, 1992; FitzGerald and Madison, 1983), data from this study were gathered in a traditional agricultural system of small patches used for self-production. This kind of management could be responsible for distinct resource availability to voles and habitat quality, influencing home range size (McNab, 1963; Mares et al., 1982; Collins and Barret, 1997; Fisher, 2000). A negative association between home range size and habitat quality has also been recorded in *M. pennsylvanicus* and other mammalian herbivores (Collins and Barret, 1997; Fisher, 2000). Similarly, higher home ranges in *M. lusitanicus* may be related to higher habitat heterogeneity of the fine-grained mosaics with the inclusion of patches of low quality and low intensity of use, but frequently crossed in foraging activities, as also stated by Miller (1964) for pocket gophers (Geomyidae).

Moreover, in our study area, some of *M. lusitanicus* home ranges overlapped areas also used by moles (*Talpa occidentalis*). Although we do not have radio-tracking data of moles, we captured two animals in sites that were within home ranges of radio-tracked voles. We also know that *M. lusitanicus* and *T. occidentalis* must share tunnels or burrows because in other study areas we have caught both species in the same trap. It is thus possible that voles used mole tunnels (occupied or abandoned) for underground movements, as also described for *Microtus multiplex*, *M. savii* (Salvioni, 1988) and *Arvicola terrestris* (Delattre et al., 2006). Thus, the availability of an underground pathway and a probably over-dispersed spatial distribution of resources could explain the larger home ranges and high daily distances travelled by *M. lusitanicus*.

Our results show longer daily movements for reproductively inactive males and shorter movements for active males and inactive females. If reproductive active males display some paternal care of young (see discussion below), we will expect shorter daily movements, perhaps more localized around the nest or in mate guarding behaviours, as observed in *M. ochrogaster* (MacGuire et al., 1990). Mironov (1990) defends that smaller movements made by some males of *Myodes (C.) glareolus* were related to the restricted activity by the lactating females, while larger movements were explained by the search for nearby estrus females. Behavioural interactions between dominant and subordinate males can also influence movements, as suggested for *M. (C.) glareolus* (Sikorski & Wójcik, 1990), assuming that reproductive active animals dominate over inactive ones. However, until now, our knowledge about *M. lusitanicus* is not enough to advance any of the above explanations.

Social behaviour

Our data indicate a balanced sex ratio, a spatial association of males and females (pair bonding and high overlap of space use for some pairs) and similar home range values for both sexes (see above). As multiple captures of male-male and female-female were rare, we suggest the existence of intrasexual territoriality for both sexes (Madison, 1980), but not intersexual territoriality. The combination of the characteristics above indicates that some males and females are spatially associated in monogamous pairs, suggesting the possible presence of a monogamous mating system (Salvioni, 1988; Dewsbury, 1990, Lambin and Krebs, 1991), which is reinforced by the absence of sexual dimorphism in size in this species (Madureira, 1982; Heske and Ostfeld, 1990). Although a balanced sex ratio is not exclusive of monogamous species (Madison, 1980), multiple captures or nest co-habitation of males and females do not occur in promiscuous systems (Madison, 1980; Wolff, 1985).

A monogamous mating system was reported for *M. duodecimcostatus* (Paradis and Guédon, 1993), *M. multiplex* (Salvioni, 1988), *M. ochrogaster* (Thomas and Birney, 1979; Getz and Hofmann, 1986), and *M. pinetorum* (Dewsbury, 1990). Though it is tempting to associate the burrowing habits of all these species (Salvioni, 1988; Dewsbury, 1990; Giannoni et al., 1993; Mankin and Getz, 1994) with monogamous pair formations, other mating systems may occur in these and other burrowing or fossorial species (e.g. Getz and Hofmann, 1986; Salvioni, 1988).

Because the total and monthly number of radio-tracked voles was low, suggestions regarding territoriality and spatial associations must be interpreted with caution and need to be examined through future studies. Our results open the possibility for the presence of a monogamous mating system and in the least a monogamous pair association.

Activity patterns

The analysis of the periods of activity within the 4 h bouts showed an agreement with the triphasic pattern of activity observed for *M. lusitanicus* during the daytime period (Madureira, 1984). The activity pattern also agreed with the typical microtine ultradian rhythms of 2 to 4 h, mainly associated with feeding and digestion rhythms (Madison, 1985; Zynel and Wunder, 2002). According to the metabolic demands of a small herbivore microtine, the duration of bouts of activity of *M. lusitanicus* (mean weight of 18 g) should be close to 2 h (Zynel and Wunder, 2002), which roughly agrees with our data.

All our individuals in this study were active day and night, although there were some interaction effects: activity was higher during the day for males than for females; reproductively active voles and females travelled larger distances during night periods than during day periods; and inactive males travelled higher distances than active males and inactive females (interaction of sex and reproductive status).

The temporal discrepancy observed in diurnal activity of both sexes may be related to alternate parental behaviour (Lonstein and de Vries, 1999) or sex-specific behaviours such as territory patrolling by males (Schradin, 2006). Also, the higher distances made by reproductively inactive males could be explained by mate searching behaviours (Madison, 1985). When studying activity patterns of *M. breweri*, Zwicker (1990) also found a temporal partitioning between sexes, and explained it as a way of sharing food resources and contributing to greater social stability. Our results can be caused by one or all of the above situations.

Habitat selection

Microtus lusitanicus revealed habitat preference at both scales of analysis: they selected verges, vines and olives within the study area, and verges within their home ranges. This general preference for the verges may be due to the high cover and density of herbs throughout the year, abundance of root systems, and occasional presence of hedgerows (vine rows) and stonewalls and a different surface relief pattern that often presents different soil moisture conditions from the surrounding matrix. All of these characteristics make the verges a more complex and diverse habitat, providing better food resources and improved shelter from predators and human interventions. Tunnels under the verges are more protected from agricultural machines and soil ploughing. This makes verges safer places that enable normal movements (above- or underground) within the home range and permit dispersal movements. The high levels of use of linear habitats have also been reported for other microtines, such as *M. ochrogaster* (Getz, 1985), *M. cabrerae* (Santos et al., 2007), and *M. duodecimcostatus* (Mira A, pers. obs.).

In our study area, vines and olive grows also provided satisfactory conditions for home range establishment with regards to vegetation cover and intensity of agricultural interventions. In fact, these units typically underwent pruning and herbicide application once per year. Other advantages of these units are the infrequent disturbances in the subterranean tunnels and the physical protection provided by the shrub-like vine plants and old olive trees and their root systems. Contrarily, the crops and urban units had the most intense human interventions (including soil ploughing) and vegetation cover was generally low and sparse at this time of year.

The habitat characteristics suggest that *M. lusitanicus* prefer habitat units with high herbaceous cover. This has also been described for other microtine species (Dooley and Bowers, 1996; Lin and Batzli, 2001; Pusenius and Ostfeld, 2002; Jacob and Hempel, 2003; Yletyinen and Norrdahl, 2008).

Although *M. lusitanicus* is fossorial, it is a regular prey of the Barn owl *Tyto alba* (e.g. Mira and Mathias, 2007; Rodríguez and Peris, 2007) and presence signs at the surface (fragments of grass clippings and faeces) are commonly found during the peak of the reproductive season (authors pers. obs.). However, the relative importance of the surface activity of this vole compared to underground activity remains an open question. From our data, 71.4% of fixes could not be determined as reflecting above or underground activity due to the high vegetation cover. For the remaining fixes, 28.4% were underground positions whereas only 0.2% corresponded to aboveground activity (n = 2029 fixes). We suspect that surface movements are not infrequent and occur under abundant vegetation cover, but this could not be definitively determined in our study. Occasional surface activity by *M. pinetorum* was also suggested as an explanation for smaller movements after an experiment with vegetation cover reduction (Madison et al., 1981). It is worthwhile to compare the results of habitat preferences between typically subterranean rodents and fossorial microtines. A study on the distribution of mole-rats *Cryptomys zechi* revealed the negative influence of mechanized ploughing of soil, which restricted the presence of animals to the field margins. This study also showed that vegetation cover was not related to movement patterns (Yeboah and Akyeampong, 2001). In this context we can say that pine voles occupy an intermediate ecological position between "above ground" microtines and strictly subterranean rodents.

Final remarks

This study is the first attempt to obtain data on the spatial ecology of *M. lusitanicus*. Novel information concerning space use, social behaviour, activity patterns and habitat selection of this European endemism was attained from this field study. A more detailed study of the issues focused on in this study and a higher sample size are clearly needed in order to validate some of the results obtained and the suggestions made. A larger number of simultaneously radio-tracked voles are necessary to allow stronger inferences on issues like space use and social behaviour. Despite the limitations, we are confident that our data provides an important step in understanding the ecology of a species whose population trends are unknown (IUCN, 2007).

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Chapter 7

Using presence signs to discriminate between similar species

Paper V

Using presence signs to discriminate between similar species.

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Using presence signs to discriminate between similar species

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ABSTRACT

The Lusitanian and the Mediterranean pine voles (*Microtus lusitanicus* (Gerbe, 1879) and *Microtus duodecimcostatus* de Selys-Longchamps, 1839) are fossorial sister species and have an allopatric pattern of distribution in Portugal, which includes a potential sympatry area in the centre of the country. The present study aimed to determine the validity of using presence signs in the field for discrimination of the two species in an area of sympatry (Northern Alentejo) and the characteristics that achieve the best classification accuracy. A total of 175 trapping plots were sampled across the study area. Prior to the set up of traps, 10 presence signs were randomly selected for measurements of four variables: proportion of soil mounds, mean diameter of mounds, proportion of burrow openings and mean diameter of burrow openings. On the basis of a classification tree analysis, results showed that presence signs can be used to discriminate plots inhabited by one or the other species in the studied sympatry area. The characteristic that most accurately enables species identification is the proportion of burrow openings: for every 10 presence signs found in a plot, if more than 8 have an opening, then it is inhabited by *M. lusitanicus* (i.e. mostly burrow openings with few or no mounds present); if 8 or fewer have an opening, *M.duodecimcostatus* is present (i.e. mostly mounds with few or no burrow openings).

Key-words: *Microtus duodecimcostatus*, *Microtus lusitanicus*, presence signs, species identification.

INTRODUCTION

The Lusitanian and the Mediterranean pine voles (*Microtus lusitanicus* (Gerbe, 1879) and *Microtus duodecimcostatus* de Selys-Longchamps, 1839) are fossorial sister species (Jaarola *et al*. 2004) with similar and marked burrowing behaviour and a restricted European distribution (Giannoni *et al*. 1993; Cotilla & Palomo 2002; Mira & Mathias 2002). In Portugal, these species have a mainly allopatric distribution pattern, with a narrow area of potential sympatry in the centre of the country (Madureira 1984; Santos SM, in press). Both species can become agricultural pests (Vinhas 1993; Mira & Mathias 1994), although no cyclic populations are known for either species (Cotilla & Palomo 2002; Mira & Mathias 2002). Other similarities between the species include occurrence in the same type of habitat: open areas such as meadows, pastures and agricultural areas (Mathias 1999). In the area of sympatry, it is often uncertain which species is responsible for the reported damages, because of their morphological similarity (Madureira 1982, 1984) and the need for specific trapping techniques for the survey of these voles (Guédon *et al*. 1992; Mira 1999). Some morphological features of skulls and teeth have been used with a relevant degree of accuracy in species discrimination (Madureira 1982; Brunet-Lecomte *et al*. 1987; Mathias 1996), but frequently require the killing of animals.

Although there is empirical field knowledge suggesting the association of soil mounds with the presence of *M. duodecimcostatus* and burrow openings with *M. lusitanicus* (Vinhas 1993), a validation of this method as an identification tool is lacking. Moreover, there are also reports of soil mounds being presence signs of *M. lusitanicus* (Borghi & Giannoni 1997; Mira & Mathias 2002) which raises doubts in terms of species identification.

The advantages of using presence signs as a sampling method are numerous, from easier sampling to being a more cost effective and non-invasive method (Giraudoux *et al*. 1995; Sutherland 1996; Battersby & Greenwood 2004). This has stimulated works aimed at validating the use of presence signs as indicators of relative abundance of small mammals (Liro 1974; Mankin-Rogalska *et al*. 1986; Giraudoux *et al*. 1995; Fichet-Calvet *et al*. 1999; Van Horne *et al*. 1999). Consequently, the use of presence signs is now widespread among small mammal ecological studies, namely, for our study species (Borgui & Giannoni 1997; Mira & Mathias 1994), for *Microtus cabrerae* Thomas, 1906 (Santos *et al*. 2005; Pita *et al*. 2006), *Microtus arvalis* (Pallas, 1778) (Delattre *et al*. 1996, 1999), *Arvicola sapidus* Miller, 1908 (Fedriani *et al*. 2002), *Arvicola terrestris* (Linnaeus, 1758) (Giraudoux *et al*. 1997; Duhamel *et al*. 2000; Fichet-Calvet *et al*. 2000; Delattre *et al*. 2006), *Psammomys obesus* Ctretzschmar, 1828 (Fichet-Calvet *et al*. 1999), *Talpa europaea* Cabrera, 1907 (Delattre *et al*. 2006), *Cryptomys zechi* (Matschie, 1900) (Yeboah & Akyeampong 2001) and other species (Giraudoux *et al*. 1998) We defend the importance and urgency of developing and validating methods, such as using presence signs only, that allow a rapid assessment of species identity in the field, avoiding the logistical constraints of trapping.

Two questions are addressed in the present study: (i) Can presence signs be used accurately to discriminate the presence of the two species of voles in an area of sympatry?; and (ii) if so, which characteristics achieve the best classification accuracy?

MATERIALS AND METHODS

Study area

The study area is located in the Northern Alentejo (Portugal), in the District of Portalegre, and covers an area of 1613 km^2. The climate is Mesomediterrananean, with a mean temperature of 7.4°C in January (3.1–11.7ºC) and 24.7°C in July months (16.8–33.3°C), and an annual rainfall of 717 mm (Rivas-Martínez & Arregui 1999; Ninyerola *et al*. 2005). The most important phytosociologic units are woods of cork oak, *Sanguisorbo agrimoniodis–Quercetum suberis* Rivas Goday 1959, of holm oak, *Pyro bourgaenae–Quercetum rotundifoliae* Rivas-Martínez 1987, and of Pyrenean oak *Arbuto unedonis–Quercetum pyrenaicae* Rivas-Martínez 1987, most of the times transformed in open woodlands, designated as "montados" (Costa *et al*. 1998). The study area includes allopatric and sympatric areas of species occurrence (Santos SM, in press).

Data collection

From September 2006 to May 2007, 175 trapping plots were sampled across the study area. In each plot, 10 subterranean live-traps (mole type) were set up for three consecutive nights, and selection of plots was dependent on finding enough surface presence signs to allow the set up of all traps. We considered as a "presence sign" either a soil mound, a burrow opening or a mound with a burrow opening (as in Giraudoux *et al*. 1995; Fichet-Calvet *et al*. 1999; Reichman and Seablomm 2002; Werner *et al*. 2005). Prior to the set up of traps, 10 presence signs were randomly selected for measurement in each plot. Four variables were calculated for each plot by averaging the measurements taken in the 10 presence signs: *P_MOUND* (proportion of soil mounds: number of soil mounds/10 presence signs), *DIAM_M* (mean diameter of mounds, when present), *P_OPEN* (proportion of openings to subterranean burrows: number of presence signs with burrow opening/10 presence signs) and *DIAM_O* (mean diameter of burrow openings, when present).

The presence of either of the species was recorded in 69 trapping plots: 32 with *M. lusitanicus* and 37 plots with *M. duodecimcostatus.* Voles were identified on the basis of the hind feet length (*M. lusitanicus* < 16 mm > *M duodecimcostatus*; Madureira 1982, 1984). Animals presenting border values (16 mm) were identified through measurements of body length (*M. lusitanicus* < 105 mm and *M* *duodecimcostatus* > 89 mm) and color pattern of fur (Madureira 1982, 1984). Only adult animals were used in species identification, and juveniles and subadults captured in the same plot with an adult were assumed as conspecifics. Only plots with captured voles assigned (either species) were considered in further analyses, whereas plots with no captures or "only-mole" captures were discarded.

The present study is part of a habitat selection project, conducted on a landscape scale and covering a high diversity of natural and semi-natural habitats (olive orchards, pastures, meadows, fallow areas, verges, hedgerows, oak plantations and oak "montados").

Data analysis

The Mann–Whitney *U*-test (Zar 1999) was used for preliminary data analysis of the four explanatory variables to compare presence signs between the two vole species. A classification tree (Breiman *et al*. 1984) was used to discriminate plots inhabited by one or the other species with the available variables. Classification trees (Breiman *et al*. 1984) consist of repeated partitions of the data using a rule based on a single explanatory variable at each time. At each partition, the data are split into two mutually exclusive groups, each of them as homogeneous as possible (Segurado & Araújo 2004; Zuur *et al*. 2007). The main difficulty with tree models is determining the optimal tree size: a full tree with many splitting rules is likely to overfit the data, whereas a tree with one or two splits might provide a poor fit. The process of determining the best tree size is called "tree pruning" (Zuur *et al*. 2007).

This type of model deals better with nonlinearity and interactions between explanatory variables than regression, Generalised Linear Models, Generalised Additive Models or Discriminant Models (Zuur *et al*. 2007). In addition, they produce a "probability" of class membership based on the proportion of observations of each class (each species in the present study) at any terminal node of the tree (Miller & Franklin 2002).

We used the statistical software SPSS for Windows version 16.0 (SPSS, 2007) for model building. Classification and Regression Trees was the growing method chosen. This method maximizes the within-node homogeneity. The tree pruning option was selected to avoid overfitting. Validation was performed using a cross-validation procedure (Zuur *et al*. 2007).

RESULTS

All four variables revealed significant differences between the two species (Table 1): *M. lusitanicus* occurrence was associated with plots characterized by a lower proportion of mounds and a higher proportion of burrow openings, both of smaller dimensions when compared to presence signs of *M. duodecimcostatus*.

Table 1. Summary values (mean and standard deviations, median, minimum and maximum values) and results of the Mann–Whitney *U*-test of four explanatory variables concerning presence signs of *Microtus lusitanicus* (ML) and *Microtus duodecimcostatus* (MD); *P_MOUND*, proportion of mounds; *DIAM_M*, mean diameter of mounds; *P_OPEN*, proportion of openings; *DIAM_O*, mean diameter of burrow openings.

		P_MOUND	*DIAM_M*	*P_OPEN*	*DIAM_O*
ML	Mean ± standard deviation	0.2 ± 0.2	10.6 ± 5.0	0.9 ± 0.1	2.6 ± 0.2
	Median	0.1	9.8	1.0	2.7
	Minimum – maximum	0.0 – 0.8	4.3 – 20.7	0.5 – 1.0	2.1 – 3.1
MD	Mean ± standard deviation	0.7 ± 0.3	14.4 ± 2.5	0.4 ± 0.4	3.0 ± 0.4
	Median	1.0	14.7	0.2	3.0
	Minimum – maximum	0.0 – 1.0	6.0 – 19.7	0.0 – 1.0	2.5 – 4.0
	Mann–Whitney *U*-test	*U* = 134.5 *P* = 0.000	*U* = 169.5 *P* = 0.006	*U* = 121.5	*U* = 193.0 *P* = 0.000

The classification tree accurately discriminated the two vole species, and correctly classified 85.5% of plots: 87.5% plots of *M. lusitanicus* and 83.8% plots of *M. duodecimcostatus*. The species identity predicted by the model failed for 14.5% (standard error (SE) = 0.042) of cases and the correspondent value for cross-validation was 18.8% (SE = 0.047).

Only one variable (*P_OPEN*) was retained by the model after the pruning procedure. The plots inhabited by *M. lusitanicus* are described by proportions of burrow openings higher than 0.8 (for every 10 presence signs found, more than 8 have an opening). *M. duodecimcostatus* plots are described by proportions of openings equal to or lower than 0.8 (for every 10 presence signs found, 8 or fewer have an opening; figure 1).

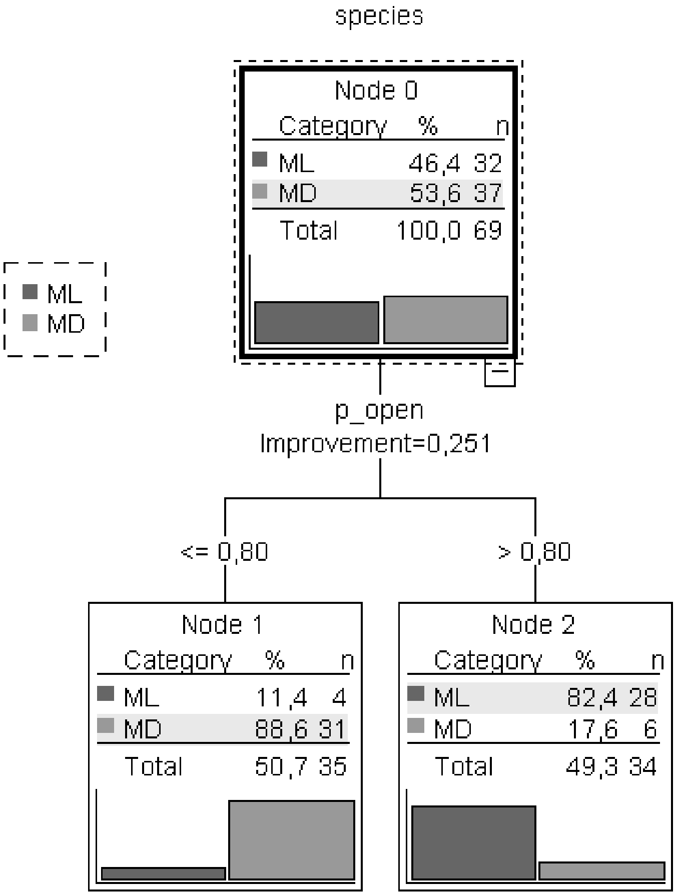

Figure 1. Classification tree diagram for pine voles data showing the results for the two groups based on one explanatory variable (proportion of burrow openings); the left branch corresponds to the *Microtus duodecimcostatus* group and the right branch to the *Microtus lusitanicus* group (MD, *M. Duodecimcostatus*; ML, *M. Lusitanicus*; %, percentage of correct classification of plots; *n*, number of plots; p_open, proportion of burrow openings).

DISCUSSION

Results revealed that presence signs can be satisfactorily used to discriminate between the two species of pine voles in the studied area of sympatry, at least in the period between autumn and spring. During these months, activity by the animals is most intense and soils are easier to work with (Mira, personal observation). The characteristic that better discriminates the presence of the two species in the field is the proportion of burrow openings: plots with more than 0.8 correspond to *M. lusitanicus* presence, whereas plots up to 0.8 correspond to *M. duodecimcostatus*. Our results are in agreement with existing field observations that relate the presence of *M. lusitanicus* to mostly burrow openings with just a few mounds, and the presence of *M. duodecimcostatus* to mostly mounds with few or no burrow openings (Vinhas 1993). Explanations for this difference in presence signs between similar species might lay in the differentiated vertical use of space by the two species. *M. duodecimcostatus* is considered to exhibit more fossorial behaviour than *M. lusitanicus* (Mathias 1996), which explains the higher volume of soil removed when digging the tunnels (therefore, higher abundance of mound signs) and lower surface activity (therefore, lower abundance of burrow openings).

In some plots we registered cases of similar presence signs for both species, and so our results confirmed that *M. lusitanicus* also produces soil mounds, although less frequently and always of smaller size than its sister species. However, several plots where we captured *M. duodecimcostatus* have a high number of burrow openings adjacent to typical soil mounds (*n* = 6 plots). Therefore, our estimated classification error was not as low as we would have expected. Reasons for this might include soil differences that affected behaviour and burrow construction by the animals, as also suggested by Mankin-Rogalska *et al*. (1986) for size differences found in burrow systems of *M. arvalis*.

The aim of the present work was to provide a tool to distinguish between the presence of two species of pine voles. Although presence–absence information has limited value for agricultural managers, presence data collected in sample transects or plots can be transformed in a frequency index and used as an indirect abundance estimative (Delattre *et al*. 1996; Duhamel *et al*. 2000). It would be interesting to further develop this method and to build models to estimate abundance indices that could be applied in large areas, as is done by Giraudoux *et al*. (1995) and Fichet-Calvet *et al*. (1999). The inclusion of study areas with high abundance of animals in such research would be desirable, as our work did not include agricultural areas with high densities of voles.

A certain caution is needed in applying these results: sampling should be undertaken in potential sympatry areas (centre of Portugal and the north–centre of Spain; Cotilla & Palomo 2002; Mira & Mathias 2002), during winter–spring (due to higher humidity in the soils and increased vole activity) and using fresh signs only, to minimize prediction error. Also, because there is a time lag between the production of the presence signs by animals and our observation, sampling based on presence signs might not correspond to current presence (as opposed to trapping methods).

Most of our land is continuously affected by human activities (e.g. agriculture, urbanization and pollution) and so, species experience changes in their habitat quality and fragmentation (Saunders *et al*. 1991; Battersby & Greenwood 2004). Information on changes in the distribution and abundance of these species over time is thus required to determine the need for changing conservation measures, policies, and management controls. The Mediterranean ecosystems have a long history of human presence (Blondel & Aronson 1999). Although they are rich in endemic species of small mammals (Bilton *et al*. 1998; Baquero & Tellería 2001), for many of these species, information on population trends is lacking, while for many others, basic research studies (including those focusing on conservation), status assessments or population monitoring are needed (IUCN 2007). Despite sometimes being considered agricultural pests (Cotilla & Palomo 2002; Mira & Mathias 2002), pine voles are a key element of the Mediterranean food web, as the main prey of mammalian and avian predators (Lopez-Gordo *et al*. 1976; Delibes *et al*. 1984; Veiga 1986). The present study provides an additional tool for easy monitoring of the occurrence and distribution of pine voles in Portugal. The proposed method allows cost effective sampling of populations and the use of non-specialized technicians in species surveys. Major applications of results presented here include presence sampling surveys for species habitat models (based on "logistic regression" type models), large scale biodiversity assessments and environmental impact assessments.

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Chapter 8

General discussion

8. GENERAL DISCUSSION

Information on species distribution ranges and on the interactions between species and their environment is crucial not only for management actions in both pest control and community conservation, but also to get insights on the selective forces that have driven speciation and biogeographic patterns (Delattre *et al.* 1996, Bustamante 1997, Osborne *et al.* 2001, Silva *et al.* 2002, Peterson and Robins 2003, Venturi *et al.* 2004, Raxworthy *et al.* 2007, Mira *et al.* 2008). When focusing on coexisting species and parapatric or sympatric patterns, additional insights on interspecific competition and evolutionary processes should appear (Raxworthy *et al.* 2007, Kozak *et al*. 2008). As large scale environmental factors may be responsible for allopatric patterns (geographically separated) or parapatric patterns (geographically abutting) in sister species, they could be determinant in understanding the role of ecology in speciation processes (Kozak *et al.* 2008). Accordingly, these environmental factors can influence the evolution of many fundamental biological traits, such as diet items, timing of reproduction, number of offspring and body size (Kozak *et al.* 2008).

As the first detailed ecological study of two closely related pine voles in Portugal, the present research answers a number of questions related to the ecological determinants of the distribution of Lusitanian and Mediterranean pine voles at several scales. Results found provide very relevant and diverse ecological data on the Lusitanian pine vole, an Iberian endemism with a relatively restricted geographic distribution and a poorly known ecology. Using owl pellets (Chapter 3), trapping (Chapters 4, 5, 7), and telemetry data (Chapter 6) locations where vole species are occurring are identified and related with environmental or biological information.

Some of the modelling approaches used are relatively novel, and offer powerful tools for investigating the nature of interactions of closely-related species, providing species distribution maps at two spatial scales, and environmental or habitat associations at three scales, with different extents and resolutions. The integration of this information helps to focus future field efforts by: (1) suggesting the nature of the interaction (e.g. particular environmental variables that are strongly associated with range boundaries and should be important for field studies; Chapters 3, 4, 5), (2) indicating particular geographic areas for research (e.g., sympatric zones or isolated contact areas where the models predicts sympatry; Chapters 3, 5), and (3) suggesting alternative methods for species identification in the field, based on presence signs (Chapter 7). Specifically for the Lusitanian pine vole, this study provides the first sound contribution on species ecology, with information on spatial and social organization, activity patterns and habitat selection (Chapter 6).

8.1. Summary of the main findings

This study was started by investigating the relative effects of environmental, soil and spatial characteristics on the large-scale distribution of two species of pine voles in Portugal, and aiming to obtain predicted occurrence maps for each species, with emphasis on the identification of sympatric areas (**Chapter 3**). This larger scale study confirmed the clear spatial separation into a northern and a southern species distribution, with a small central sympatric belt. It also showed, for the first time, that this belt is spatially disjunct and of reduced extension, and includes a parapatric boundary, suggesting that, at finer scales, the contact zones are narrow. The obtained models suggested presence areas of Lusitanian pine vole, unknown until now (Monchique and Elvas). Further results indicated that at a national context, the Lusitanian pine vole occurs outside grassland dominated landscapes, in areas of higher rainfall, frost and cambisols, with mostly acid soils, lower abundance of litosols, and presence of solonshaks. In contrast, the Mediterranean pine vole is distributed in grassland dominated areas within intermediate values of soil pH, dominated by lito- and luvisols and lower values of rainfall, frost and cambisols. However, most of the variance in the large-scale distributions of the Lusitanian and Mediterranean pine voles is explained by spatial variables, alone or structured with the environmental and soil effects.

Moving to a smaller scale, one would expect a higher importance of environmental (or soil) components and, thus attention was focused on a smaller area within the predicted area of sympatry. The main goal was to determine the relative contributions of environmental factors and spatial structure, in order to explain the regional distribution of each of the two pine voles in central Portugal, at two scales of spatial resolution: local and landscape (**Chapter 4**). As expected, the local-scale environmental factors explained more variation in the distribution patterns of both species than did landscape-scale or spatial factors, being this effect particularly evident in the Lusitanian pine vole. An important spatial component was also observed in the regional distribution of each species, although less relevant when compared to the one in the national scale. Locally, both species occurred preferentially at sites with verges. However, the Lusitanian pine vole occupied soils with higher humidity and sand content, while the Mediterranean pine vole occurred mostly in sites with high tree cover and herbaceous biomass. At a landscape context, the Lusitanian pine vole is associated to higher availability of linear habitats, and the Mediterranean pine vole to the absence of shrub landscape units. Main similarities between the two species in environmental associations concern the cover, food and burrow conditions.

A further goal was to determine if the two species coexist in the same patch in a sympatric regional area, and to find out how the two species differ from each other concerning habitat niche characteristics. These aims were evaluated using again the local and landscape spatial scales (**Chapter 5**). This research revealed a small co-occurrence area and a parapatric boundary for central Portugal, thus confirming and detailing the previous pattern obtained at a larger scale. At a local scale, the most important environmental factors separating presence sites of the Lusitanian from the ones of the Mediterranean pine vole were higher altitude, higher cover of shrubs, lower clay content in the soil, and lower cover of tree canopy in the presence sites of Lusitanian pine vole. The presence of forest landscape units and the low abundance of "montado" units were the most influencing landscape factors in correctly identifying occurrence sites of Lusitanian pine vole when compared to the southern species.

Niche differences between the two pine voles are better explained by local-scale factors, when compared to landscape ones. Nevertheless, the spatial effects are also very important in explaining the segregation of both species. The local coexistence of both species in the field is possible, but should not be a common situation.

Further reducing the scale extent, the final step of this study focused on the spatial and temporal ecology of the Lusitanian pine vole, the least studied of the two focal species. The aims were to evaluate the effects of sex and reproductive status on the space use (home range and movements), the dominant social system, the daily activity pattern, and habitat preferences at two spatial scales in a Mediterranean polyculture of central Portugal (**Chapter 6**). Large home ranges were reported: 1042 m^2 for males and 862 m^2 for females, with no significant effect of sex or reproductive status. However, longer daily movements were observed for reproductively inactive males. Social affinities were observed between males and females, as well as between females and sub-adults through the observation of pair bonding and home range overlap. Voles showed no distinct preference for day or night in their activity periods and movements. However, this result was dependent on interactions between sex, reproductive status or time of day. The Lusitanian pine vole revealed habitat preference at both scales of analysis: they positively selected verges, vineyards and olive groves within the entire study area (in opposition to set-asides, pastures, crops and urban habitats), and verges were selected positively within their home ranges (when compared to olive groves, vineyards and set-asides).

Aiming to explore alternative tools in species identification, the validity of using presence signs in the field for the discrimination of the two species were evaluated in an area of sympatry and the characteristics of presence signs that achieve the best classification accuracy were selected (**Chapter 7**). Results showed that presence signs can be used with high confidence to differentiate the two species of pine voles in the studied sympatric area. Best discrimination accuracy is achieved with the proportion of burrow openings within a sample of 10 presence signs: plots with more than 0.8 correspond to the presence of Lusitanian pine vole (i.e. mostly burrow openings with few or no mounds present), whereas plots up to 0.8 correspond to Mediterranean pine vole (i.e. mostly mounds with few or no burrow openings). The use of this method allows cost effective sampling of populations and the use of non-specialized technicians in species surveys.

8.2. The influence of scale

8.2.1. Ecological factors

Considering the ecological associations at the largest scale (Chapter 3), the distribution of vole species was related to climate variables, landscape units, and soil types. Together, these characteristics reflect the macro-ecological conditions responsible for the segregation of a "northern species" and a "southern species". In the north, the rainy and colder areas inhabited by the Lusitanian pine vole, along with acidic and fertile soils, tend to offer better conditions for a burrowing rodent with an important surface activity (Winking 1976, Mathias 1996). On the contrary, the warmer and drier southern areas, with less acidic and fertile soils, present harder conditions for living and burrow construction, and may be related to the stronger fossorial behaviour and robust morphology of the Mediterranean pine vole (Winking 1976, Madureira and Ramalhinho 1981, Mathias 1996).

The ecological associations at a landscape scale (Chapter 4) reflect the importance of habitat connectivity and heterogeneity for the Lusitanian pine vole, and of open and shrub free areas, often related with extensive grazing, for the Mediterranean pine vole.

At a local scale the importance of verges for both voles (Chapter 4) and for space use by the Lusitanian pine vole (Chapter 6) highlights the several advantages of these structures, where vegetation is usually abundant: protection from predators and harsh meteorological conditions, low soil machinery intervention, no grazing influence, and higher food supply. Soil humidity and higher sand content are mostly related to the presence of the Lusitanian pine vole, while greater tree canopy and plant biomass influence the occurrence of the Mediterranean pine vole. On the other hand, segregation of species (Chapter 5) is explained by higher altitudes, abundance of shrubs, scarcity of tree canopies, and lower soil clay content in the Lusitanian pine vole occurrence areas when compared to the ones of the Mediterranean pine vole. The combination of these results seems to be linked with differences in behaviour and physiological needs. Accordingly, the lowest energy expense in soil digging (high humidity and sand, and low clay contents) and summer coolest localities (with green food throughout the season) are found in sites where the Lusitanian pine vole occurs, this species being least fossorial and less acclimated to the typical Mediterranean conditions (Winking 1976, Brunet-Lecomte 1991). In contrast, the stronger fossorial behaviour and the need to cope with dry soil and poorer vegetation conditions, with high energy expenditure in burrow construction, should explain why the Mediterranean pine vole inhabiting the lowland southern areas with clayey soils, must find sites to live with high tree cover and abundant herbaceous plants, as they provide more suitable microclimatic conditions within a Mediterranean environment (Joffre and Rambal 1993). The presence of trees and their roots also offers protection from digging predators and soil machinery interventions. The buffering function of trees and herbaceous vegetation against hard climate conditions (Getz 1985, Joffre and Rambal 1993) is also recognized as additional advantages for the Mediterranean pine vole. The ecological interpretation of the environmental variables associated with the regional distribution of each vole (soil conditions, vegetation structure, and abundance of herbaceous plants) suggests that both species may be responding to cover, food and burrowing needs within their habitat. On the other side, niche differences between species (topography, soil conditions and vegetation structure) seem to be related with dissimilar fossorial degrees and morphological adaptations (such as proodonty of the upper incisors, and stronger musculature of the head and neck; Madureira 1982b, Mathias 1990).

At the smallest scale (spatial ecology of Lusitanian pine vole; Chapter 6), the animals selected different habitat units according to the resolution scale. While establishing the home ranges within the study area (larger scale), the animals preferred verges, vineyards and olive groves, and avoided set-asides, pastures, crops and urban habitats. Within the established home ranges (smaller scale), animals preferred the verges, while avoiding olive groves, vineyards and set-asides.

8.2.2. Spatial autocorrelation

The spatial variables were among the most important predictors of site occupancy by voles (Chapter 3 and 4). Therefore, distribution data were characterized by strong spatial autocorrelation even after accounting for several significant environmental effects. At the coarsest scale (Chapter 3) the spatial effects were the most important, but at a finer scale (Chapters 4 and 5) these effects, although important, played a secondary role when explaining the species distributions and segregation. This difference of the importance of spatial autocorrelation in the two scales was not unexpected. While, at a national scale, the pattern of occurrence records of each species is almost continuous (absence records concentrated in half of the study area, and not within the presence records area; Chapter 3, Figure 1); at a regional scale, the occurrence records are intermixed with absence sites (absence records were found across the entire study area; Chapter 4, Figures 2 and 3).

Spatial autocorrelation in the distribution of species may result from several factors, going from unmeasured environmental variables with spatial structure, historical and biogeographic restrictions, to diverse biotic processes (Bahn and McGill 2007, Dormann *et al.* 2007, Miller *et al.* 2007). At coarser scales (e.g. 10 km x 10 km cells) spatial autocorrelation may arise because large scale datasets are usually characterized by low variation between sampling units, high variation within units, and high predictability (Farina 2006). Moreover, at these scales, climate factors and unmeasured environmental variables play a major role in explaining species distributions patterns, showing frequently high spatial autocorrelation (Lennon 2000, Rangel *et al.* 2006, van Teeffelen and Ovaskainen 2007). At finer scales (such as the used 62 or 196 250 m^2-plots), the variation between sampling units is commonly high, and predictability is low (Farina 2006). Here, as the climate is more homogeneous, we should expect the influence of biotic processes, such as reproduction and death, species interactions, dispersal, metapopulation dynamics, or vegetation patterns that may also cause spatial autocorrelation in species distributions (Legendre 1993, Legendre and Legendre, 1998). Dispersal movements and synchronous population fluctuations are among potential contributors to this spatial autocorrelation (Mackinnon *et al.* 2001, Knapp *et al.* 2003). Dispersal of individuals may result in spatial autocorrelation in species distribution if, for instance, the presence of a colony (or family) of pine voles in one site increases the chance that some individuals will settle in nearby sites. If these vole populations are subject to extinction-recolonization events, such as in metapopulation dynamics (Pulliam and Danielson 1991, Dias 1996), the dispersal of individuals could also cause spatial autocorrelation. Synchronous population fluctuations could also be responsible for spatial autocorrelation, as populations in close proximity are more likely of experiencing similar environmental pressure (e.g. rainfall, soil type, land uses) than will populations separated by long distances, as has been suggested for field voles (Mackinnon *et al.* 2001). This may result in a positive correlation in population dynamics (Knapp *et al.* 2003). On the other hand, most of the success of herbivores in finding food depends on the spatial arrangement and density of vegetation, and animals must make scale-dependent choices in habitat use and/or foraging (Batzli and Lesieutre 1991, Caughley and Sinclair 1994, Turner *et al.* 2001). Food resources for a microtine may vary spatially in abundance and quality across a landscape as a function of plant species composition, moisture, soil fertility, and topography (Turner *et al.* 2001, Tzialla *et al.* 2006, Carr *et al.* 2009). Therefore, the spatial patterns present in the abundance or distribution of vegetation should also influence the spatial distribution of consumers.

The techniques used in this thesis allowed the explicit incorporation of spatial autocorrelation into the modelling procedures, but did not allow the identification of the ecological processes that have generated it. Spatially autocorrelated data were modeled with autoregressive models like the autologistic regression (Augustin *et al.* 1996, Keitt *et al.* 2002) or spatial eigenvector mapping (Dray *et al.* 2006, Griffith and Peres-Neto 2006). While such models have been successful in capturing spatial patterns (Segurado *et al.* 2006, Dormann *et al.* 2007, Miller *et al.* 2007), they do not provide clear information about the underlying processes that gave origin to the observed patterns. These causal mechanisms that explain the spatial autocorrelation observed in voles distribution data should be further investigated, in coarse and finer scales, particularly to distinguish the effects of contagious population processes (or endogenous processes) from the effects of environmental factors with spatial structure (or exogenous processes). However, this is not an easy task, as different processes may create similar statistical patterns and, most of the times, it is not possible to identify the mechanism causing the aggregation of species from distributional data alone (van Teeffelen and Ovaskainen 2007).

It is clear that multiple factors may influence the distribution of pine voles in Portugal. Some of these factors gain importance at specific spatial scales and others can only be discussed using more than one scale approach. Thus, conclusions about the relative effects of factors on the species distributions are largely dependent on the scale at which the data are analysed. These findings could have major implications for management and conservation decisions, which, in an effort to maximize efficiency and minimize cost, are often based on a single scale approach. Therefore, the scale is an essential component in the study of species ecology because it influences the conclusions that can be drawn and limits the results validity when extrapolations to other geographical areas are made (Kotliar and Wiens 1990, Suárez-Seoane and Baudry 2002).

8.3. Sympatry maps and scale

Two maps presenting an area of sympatry for both pine voles occurrence, at two different scales, were obtained: one for the entire country (Chapter 3) and another allowing closer examination of a section of the predicted national parapatric area (Chapter 5). The prediction results for this small regional area are largely coincident. At the larger scale, the national map indicates the presence of a narrow sympatric belt fragmented into four separate areas and interspaced with parapatric boundaries (Table 1). The area chosen for conducting the smaller scale study includes predicted allopatric, sympatric and parapatric localities from the previous national study (Figure 8). According to this larger scale study, the northern half of the regional study area is predicted as Lusitanian pine vole in allopatry and parapatry, the southwestern half of the area is predicted as Mediterranean pine vole in allopatry and parapatry, and the southeastern half of the study area is categorized as minor and major sympatric localities. The second map produced at this finer scale (regional map) also predicts a very narrow sympatric area, consisting in two sub-areas (Table 1). The general area of the regional map is quite coincident with the pattern observed in the national map. The larger sub-area of sympatry (west sub-area) has 10 km width and overlaps the parapatric boundary of the national map. This suggests that national parapatric areas may correspond to narrow sympatry belts at a finer scale. The smaller sub-area of sympatry (east sub-area) has 3 km width and overlaps also sympatry areas predicted in the national approach. However, there are some differences in predictions when the two maps are overlaid. First, the regional map shows a parapatric boundary that lies within an area predicted to be (minor) sympatry in the national map; second, the regional map indicates an allopatric area for the Mediterranean pine vole in the southeastern localities, while the larger scale map shows (minor and major) sympatry for those areas. In the first case, the observed spatial discrepancy may be explained by scale differences between the two studies, or by a difficulty in sampling the presence of each species in that area, conducting to a possible model failure when predicting a discontinuous regional area of sympatry (Chapter 5). In the second case, one of the four national sympatric areas starts in the southeastern extreme of the small scale study area (Arronches) and extends further south to Elvas. In fact, it is suggested in the discussion of the national study (Chapter 3) that the prediction of Lusitanian pine vole presence (and thus sympatry) in Elvas municipality corresponds to model misspecification and thus, its occurrence is questioned. The smaller scale study reinforces this suggestion by predicting the presence of only one species for part of that area: the Mediterranean pine vole. In addition, the voles captured during fieldwork in the surroundings of that area were all clearly identified as the southern species (12 capture plots), further supporting the suggestions made.

On one hand, the finer scale approach can provide very detailed and precise maps that the larger scale approach often overlooks. On the other hand, these larger scale maps: (1) provide predictions for large areas avoiding the prohibitive costs of trapping in extensive regions; (2) give predictions for unknown areas of species occurrence (e.g. the potential presence of Lusitanian pine vole in Monchique); and (3) complement and improve previously sampled areas that rendered few or too sparsely data.

The results of the finer scale study (map, field captures and niche differences) point out that, although possible, the local coexistence of the two voles should be a rare event. This conclusion reinforces the credibility of a regional sympatry area separated by a parapatric boundary and thus, the spatial discrepancy of predictions between the two maps may be better explained by a scale difference and a lower precision of the national map.

The figures 9 and 10 provide brief examples on each species habitat characteristics, in national allopatric areas and within the regional study area.

Table 1. List of municipalities and districts of Portugal included in the sympatric and parapatric areas predicted in the national and regional maps.

National sympatry	
Monchique sub-area	parts of municipalities of Monchique, Aljezur, Odemira and Sines
Setúbal sub-area	parts of municipalities of Grândola, Alcácer do Sal, Setúbal, Alcochete, Benavente, Montijo, Montemor-o-Novo, all municipality of Palmela, Vendas Novas, and a small portion of Coruche municipality
Elvas sub-area	parts of municipalities of Elvas and Portalegre, and all municipalities of Campo Maior and Arronches
Idanha-a-Nova sub-area	parts of municipalities of Nisa, Vila Velha de Ródão, Castelo Branco and Idanha-a-Nova
National parapatry	parts of municipalities of Ponte de Sôr, Alter do Chão and Crato, more specifically to the line crossing the cities of Ponte de Sôr, Crato and Portalegre

Regional sympatry	
West sub-area	parts of district of Vale do Peso, Monte da Pedra, Aldeia da Mata, Crato, Fortios, the entire Flor da Rosa, and very small part of Urra
East sub-area	parts of district of Alegrete, and very small part of Mosteiros
Regional parapatry	parts of districts of Urra and Alegrete

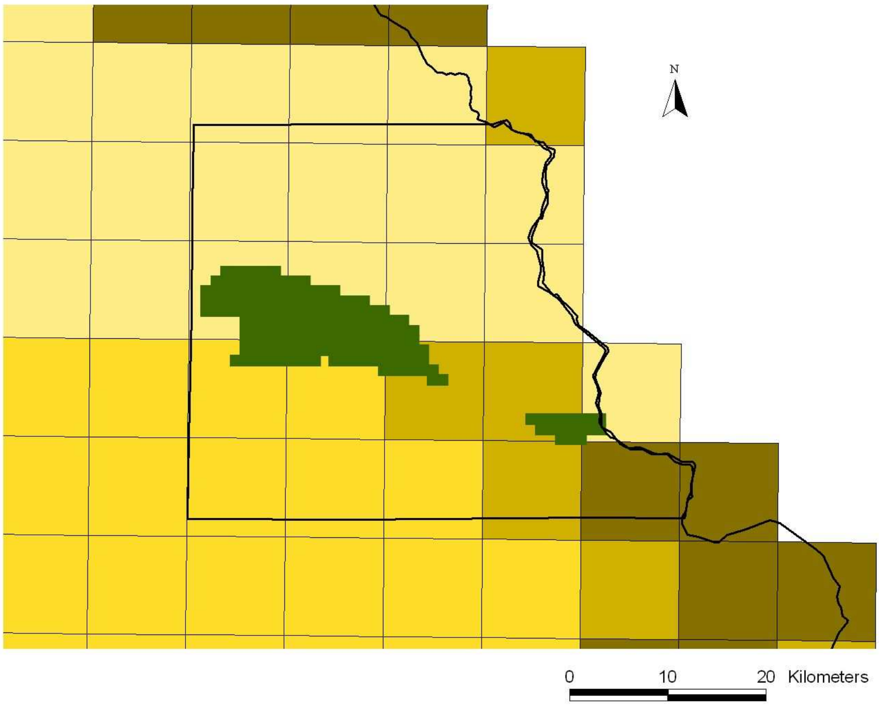

Figure 8. Overlap of the national and regional maps showing sympatric areas for the two pine voles in Alto Alentejo region (see Chapter 3 - figure 6 and Chapter 5 - figure 3); the larger size cells (10x10 km) correspond to the national map predictions (dark brown: major sympatric areas; light brown: minor sympatric areas; dark yellow: allopatric areas for the Mediterranean pine vole; light yellow: allopatric areas for the Lusitanian pine vole); the smaller size cells (1 x 1 km) correspond to the regional map predictions (for simplicity, both major and minor sympatric areas are represented by the same green color).

8.4. Unknown predicted presence localities and distribution patterns

The national prediction maps for the occurrence of the Lusitanian pine vole (Chapter 3) extend the limits of species distribution far south than previous estimates (Madureira 1981, 1984, Mira and Mathias 2007), namely in Elvas and Monchique regions, where the species has not been confirmed until now. Although the first case has already been discussed, additional comments are still relevant. For Elvas, only the national spatial model predicted species presence in the region. Because no obvious barriers exist to prevent colonization of this southern area from known localities (e.g. São Mamede mountain and surroundings), historical restrictions as an explanation for current absence seems unlikely. Hence, the lack of records there may be due to other unstudied factors or a too coarse resolution of environmental and soil variables that originated the prediction of a false presence. Moreover, the referred region holds unsuitable habitat according to the results of the finer scale models (mostly grasslands and "montado") and presents markedly xeric conditions (IGeoE 2003, Instituto do Ambiente 2007), unsuitable for a species with more mesic requirements.

The presence of Lusitanian pine vole predicted for Monchique (and a very narrow belt for the southwestern coast) is supported by the three models (Environmental, Soil and Spatial), thus receiving more credibility. In this region (Monchique) the rainfall is higher than 800 mm/year, the cambisols are locally dominant, reaching lower pH values when compared to the surrounding region, and landscape is dominated by forest (pine and eucalyptus plantations and dense oak woods), while "montados" are scarce (IGeoE 2003, Instituto do Ambiente 2007). Despite these favorable ecological conditions for the Lusitanian pine vole, a possible occurrence in this region must correspond to a population isolate, as the distance from the main range seems too long to allow frequent (long-distance) dispersal events between the two areas. In addition, the prediction area for the southwestern coastline may be too narrow to function as a dispersal corridor between Monchique and the main distribution range. Hence, the lack of presence records in the region must correspond either to historical presence followed by local extinction, or actual presence of the species but failure in obtaining data samples from this area. This second explanation is the most likely. During the first year of this thesis, several fieldtrips were conducted throughout the country in order to increase our experience with the methods and presence signs, and to capture animals for an ongoing project (SAPIENS project POCI/BIA-BDE/57053/2004). One of the trapping sites was located on the top of the mountain of Monchique, in grazed grassland with dispersed shrubs at 900 m of elevation. Although none of the three individuals captured had visible signs of sexual maturity, their measurements did not exceeded 85 mm of body length and 14 mm of hind feet length, values that correspond to the Lusitanian pine vole (Madureira, 1982b; 1984). At that time, we considered to be possible the presence of the species also because the presence signs at the site were characteristic of this species (i.e. mostly burrow openings). However, the absence of clearly reproductive animals, made us be cautious about the identification of those individuals. Very recently, the analysis of genetic data of the animals captured during these countrywide fieldtrips (and during the fieldwork of this thesis) confirmed our suspicions of a localized occurrence of Lusitanian pine vole in the mountain of Monchique, surrounded by occurrence areas of Mediterranean pine vole in the lowlands (C. Bastos-Silveira, unpublished data). Other population isolates are known for this region, such as the Schreiber's green lizard (*Lacerta schreiberi*) which has a northern distribution in Portugal and occurs in population isolates in the mountains of Monchique and São Mamede (Brito *et al.* 1999). A more detailed fieldwork in this mountain and surroundings is required to address the area occupied by Lusitanian pine vole, local coexistence with Mediterranean pine vole, and genetic differentiation between the two species and populations.

The national maps of predicted presence of Mediterranean pine vole provide novel information as its distribution area is not continuous across its range as previously assumed (Madureira 1981, 1984), but is spatially disjunct between the mountain of São Mamede and Castelo Branco district. A first possible explanation for this discontinuity could be the presence of the mountain and, second, the Tagus river, which could act as geographical barriers for the movements of animals between the areas. The mountain hypothesis does not seem very plausible, as the mountain is northwest-southeast orientated and occupies only a small fraction of the "disjunct" area. However, although the mountain may not act as an effective geographical barrier, its presence should influence climatic conditions in adjacent areas, which lead to a combination of environmental and soil conditions more typical of regions with Lusitanian pine vole presence. Additionally, the large size and torrent of the Tagus river in this region should pose serious threats to animals crossing between São Mamede and Castelo Branco, although the Mediterranean pine vole is described as a good swimmer (Giannoni *et al.* 1994).

8.5. Species coexistence and competition

The results presented in this thesis open a door to future tests on geographic predictions of competitive exclusion and competitive release. The two congeneric voles are rarely syntopic and when their ranges meet (at national and regional scales), the overlap area is narrow. Moreover, the ecological associations and niche differences found should allow species segregation into distinctive microhabitats, thus meeting several of the assumptions required to perform such tests (Anderson *et al.* 2002, Amarasekare 2003).

It should be noted that only two records of Lusitanian pine vole were detected within the national sympatric area, while eight records of Mediterranean pine vole were obtained for the same area (Chapter 3). In contrast, six presence sites of Lusitanian pine vole and five sites of Mediterranean pine vole were found in the regional sympatric area (Chapter 5). While the first case seems to suggest the possibility of the southern species may be competitively excluding Lusitanian pine vole, the regional approach seems to indicate that none of the species consistently excludes the other (Anderson *et al.* 2002). However, Amarasekare (2003) stated that, considering both fine and large scales, a pattern of species regional exclusion and a national coexistence is the more likely situation in a spatially heterogeneous environment. In spite of the low sample size, the results obtained in this thesis do not seem to support this view. More intensive trapping within the regional sympatry area could clarify this apparent contradiction. In either case, more detailed field studies on microhabitat use, behaviour and diet, using removal experiments and performed in the sympatric or parapatric areas are necessary to address the issue of competition (exploitative or interference) between the two voles.

The reduced estimated sympatric area, at both national and regional scales, along with the low abundance of both species in Alto Alentejo region further suggests a low probability of contact between species. The modelling techniques used in this thesis proved to be powerful tools to identify multi-scale environmental factors sustaining the location of potential contact zones among congeneric and sympatric species, such as the pine voles here studied. In fact, research on this species-pair has received recently financial support to continue, allowing future deeper knowledge on the evolutionary history of these species.

8.6. The mating system in the Lusitanian pine vole

Facultative or obligate monogamy in the Lusitanian pine vole could explain several of the results obtained in this thesis (Chapter 6). It could explain the balanced sex-ratio registered in the field, the pair bonding and high overlap in space use between males and females (Madison 1980, Wolff 1985), the similar home range values between sexes (Madison 1980, Salvioni 1988), and the shorter daily movements for reproductively active males (MacGuire *et al.* 1990). Also, the results on social behaviour support previous suggestions that the Lusitanian pine vole individuals live in small groups of one couple and offspring (Mira and Mathias 2007) as it was observed nest sharing between males and females, and between one female and one subadult. However, the sample size used for this study was limited and conclusions regarding the presence of a monogamous mating system need to be supported by further studies with a larger number of simultaneously radio-tracked voles. Despite these limitations, this study provided novel information on space use, social behaviour, activity patterns, and habitat selection of the Lusitanian pine vole, and it raised the possibility for further investigations on the occurrence of a monogamous mating system in this species.

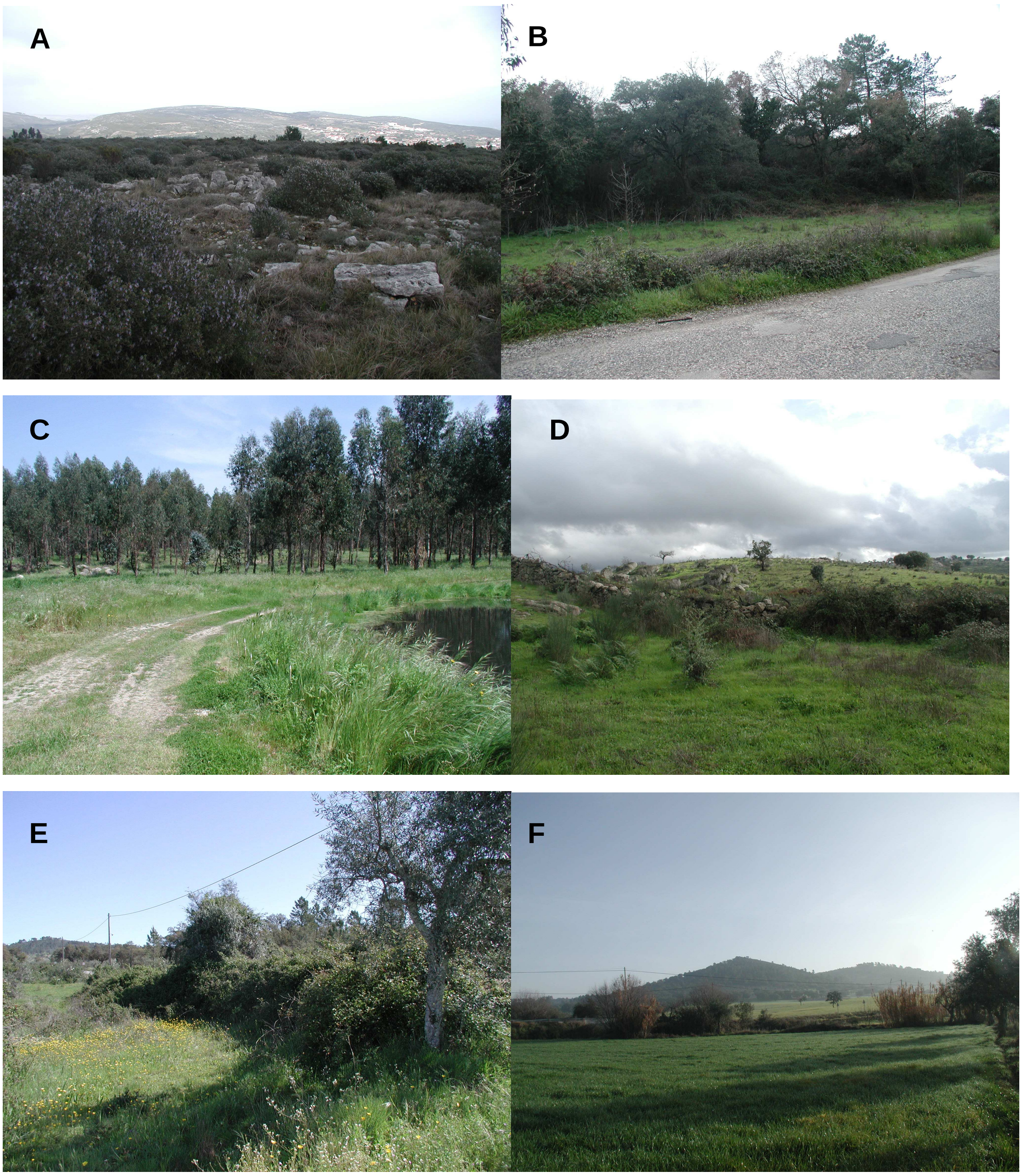

Figure 9. Examples of the habitat of Lusitanian pine vole (*Microtus lusitanicus*) in national allopatric areas (photos A and B: Alcobaça municipality), regional allopatric areas (photos C: Vale do Peso district, photo D: Santiago Maior district), and regional sympatric areas (photo E and F: Alegrete district).

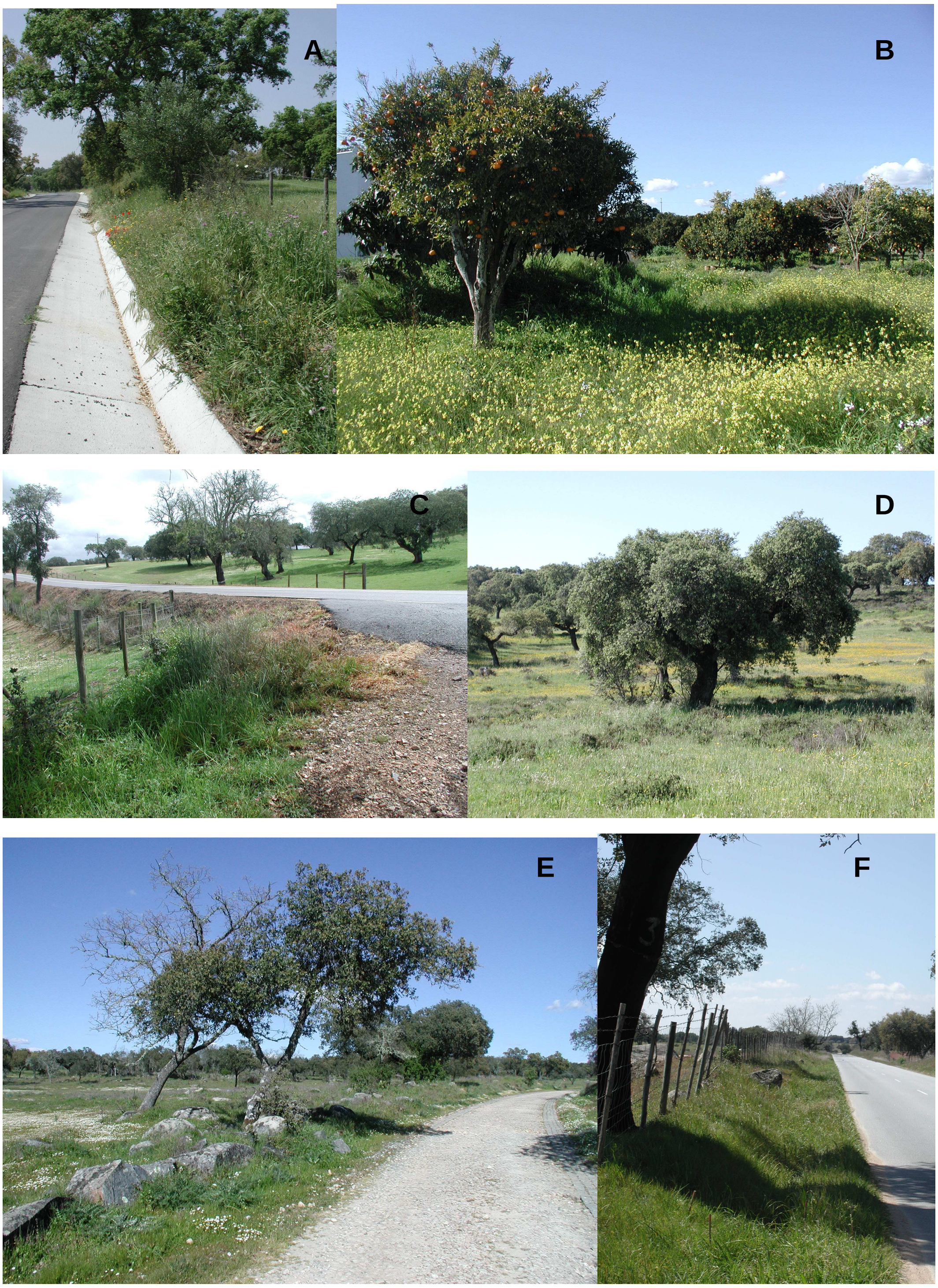

Figure 10. Examples of the habitat of Mediterranean pine vole (*Microtus duodecimcostatus*) in national allopatric areas (photo A: Vendas Novas municipality; photo B: Portimão municipality), regional allopatric areas (photo C: Urra district; photo D: Mosteiros district), and regional sympatric areas (photo E: Vale do Peso district; photo F: Crato district).

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