DANIEL GALIANO

ANÁLISE DAS RELAÇÕES FÍSICAS E BIOLÓGICAS DO AMBIENTE COM UMA ESPÉCIE DE TUCO-TUCO – *CTENOMYS MINUTUS* (RODENTIA, CTENOMYIDAE)

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SUMÁRIO

RESUMO

Os roedores subterrâneos do gênero *Ctenomys*, popularmente conhecidos como tuco-tucos, são endêmicos ao sul da região Neotropical e apresentam a maior riqueza de espécies do mundo dentre os mamíferos subterrâneos, cerca de 60. Na região da planície costeira do sul do Brasil, a espécie *Ctenomys minutus* ocupa o maior gradiente latitudinal dentre os tuco-tucos da região. Esta espécie, objeto do presente estudo, foi muito estudada do ponto de vista evolutivo e molecular, mas negligenciada quanto a aspectos ecológicos básicos. Sendo assim, o objetivo deste estudo foi determinar e descrever as principais características ecológicas de *C. minutus*, bem como as relações da espécie com aspectos físicos e biológicos do ambiente. Este estudo visou à ampliação do conhecimento ecológico da espécie, no que se refere à ecologia espacial, seleção de habitat e implicações da presença deste roedor na planície costeira do Sul do Brasil, além de aspectos conservacionistas. Os dados obtidos ampliaram o conhecimento ecológico da espécie. Os modelos de distribuição gerados obtiveram sucesso ao identificar áreas com alta adequabilidade ambiental ao longo de sua distribuição. O modelo de máxima entropia (Maxent) indicou que existem áreas de alta adequabilidade ambiental ao longo da primeira linha de dunas e nos campos arenosos ao longo de toda a distribuição da espécie. As variáveis que determinam a distribuição potencial de *C. minutus* são os tipos de solo, tipos de vegetação e a altitude. Ao longo da planície costeira, observou-se um padrão de associação entre áreas com alta adequabilidade ambiental e maior variabilidade genética, o que indica que maiores valores de diversidade genética estão associados a estas áreas. Também se observou que a especulação imobiliária e fragmentação do ambiente de dunas ao norte da distribuição da espécie, e a silvicultura de *Pinus* sp. ao sul, são as principais ameaças

para a espécie. Nos locais habitados por *C. minutus*, a presença da espécie afeta a biomassa de plantas, cobertura de gramíneas, proporção de solo exposto, dureza do solo e as concentrações de nutrientes nos campos onde habita. A biomassa de plantas e a cobertura de gramíneas são significativamente inferiores nos locais habitados pelos indivíduos, assim como a proporção de solo exposto é maior. O solo se torna menos compacto nos perfis superficiais (10 e 20 cm), mas não em profundidades maiores (50 cm) nos mesmos locais onde os indivíduos estão presentes. Em relação à disponibilidade de nutrientes no solo, os locais habitados por *C. minutus* apresentam maior disponibilidade de P e K, assim como um pH significativamente menor. Todos estes efeitos observados são uma consequência das atividades de forrageio e escavação dos animais. Consequentemente, os locais onde os animais estão presentes sofrem um efeito significativo na dinâmica e composição da vegetação, assim como nas propriedades do solo, o que vai afetar diretamente processos ecossistêmicos, como decomposição e produtividade. Além disso, observou-se que a seleção de habitat da espécie é determinada principalmente com base em duas variáveis: umidade do solo e vegetação (disponibilidade de recursos). As características ambientais, tanto superficiais quanto subterrâneas, desempenharam um papel importante na ocorrência deste roedor subterrâneo. Acima da superfície do solo, um fator determinante é a oferta de recursos alimentares, que no caso do presente estudo foi caracterizado pela cobertura das espécies vegetais.

CAPÍTULO I

INTRODUÇÃO

Roedores subterrâneos

Os roedores são um grupo de mamíferos que tem despertado o interesse de vários pesquisadores, não apenas em virtude de sua abundância e da ampla classe de adaptações ecológicas, mas também por serem importantes componentes de quase todos os ecossistemas terrestres existentes (Delany 1974). A ordem Rodentia possui aproximadamente 2.227 espécies distribuídas em 34 famílias e 481 gêneros (Honeycutt 2009).

Este grupo de animais possui uma vasta classe de adaptações que permitem que eles explorem uma ampla variedade de ambientes, como terrestre, arborícola, aquático ou aéreo. Dentre os mamíferos terrestres, aqueles que em algum momento de sua vida utilizam túneis ou escavações abaixo da superfície do solo para realizar alguma atividade vital, seja ela reprodução, alimentação ou proteção, são denominados fossoriais e exibem adaptações morfológicas para a atividade de escavação (Nevo 1979; Lacey et al. 2000). Os grupos que, além de possuírem essas adaptações, conduzem a maioria de suas atividades vitais em galerias abaixo da superfície terrestre são denominados de subterrâneos. Estes roedores distribuem-se por todas as terras continentais, com exceção da Austrália e Antártica. Habitam, na sua maioria, campos, estepes, savanas ou desertos, e algumas espécies podem ocorrer em florestas ou mata arbustiva densa, porém não habitam solos saturados de água ou permanentemente congelados (Lacey et al. 2000).

Estes roedores são representados pelas famílias: Bathyergidae (África); Geomyidae (América do Norte); Muridae, dividida nas subfamílias Arvicolinae, Myospalacinae, Rhizomyinae e Spalacinae (Ásia, leste europeu e África) Octodontidae e Ctenomyidae (América do Sul; Lacey et al. 2000; Wilson e Reeder 2005) (Fig 1). As espécies possuem características marcadamente semelhantes em sua estrutura e tamanho, e apesar de possuírem métodos diferentes de escavação, constroem sistemas de túneis similares, sendo que diversos gêneros de roedores subterrâneos deram origem a um grande número de espécies e subespécies. Poucas dessas são simpátricas, o que indica primeiramente que em diferentes locais do mundo exista somente um nicho passível de exploração por mamíferos herbívoros subterrâneos (Pearson 1959).

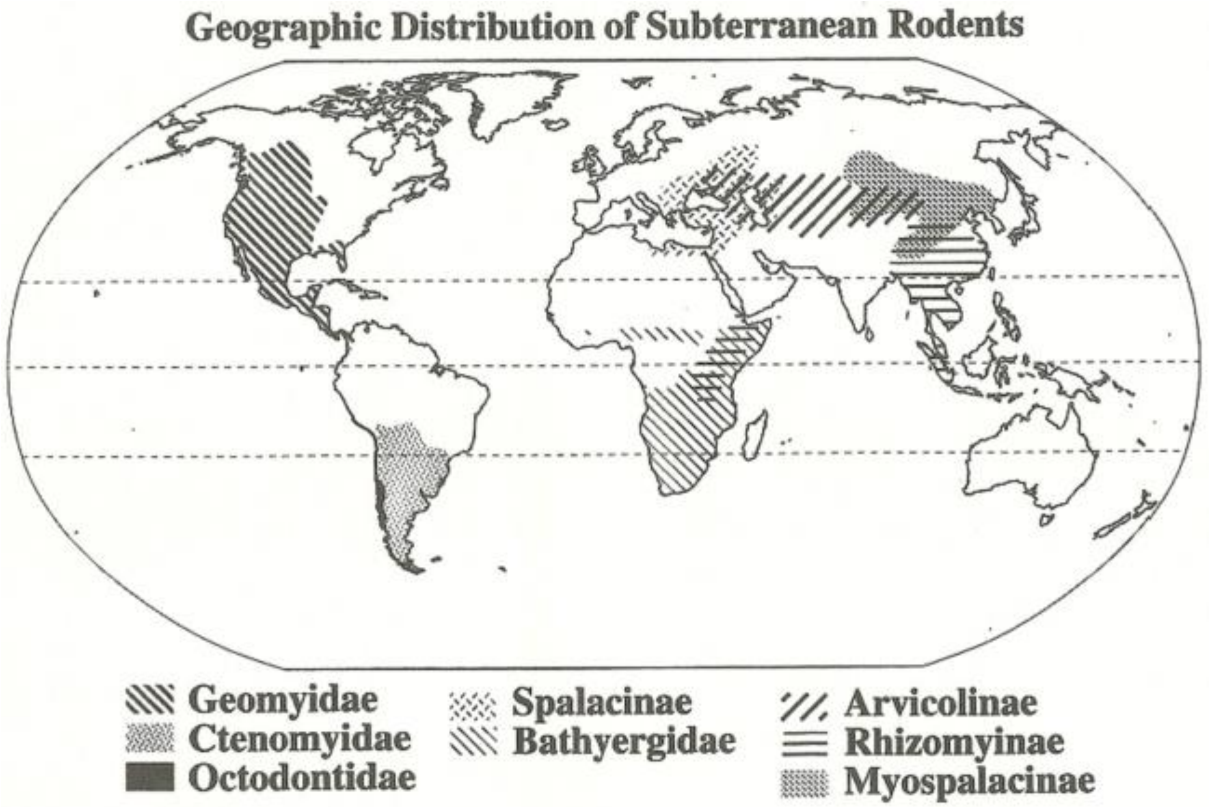

Fig. 1 Distribuição geográfica das famílias e subfamílias de roedores subterrâneos, de acordo com Lacey et al. (2000).

As semelhanças entre características corporais e hábitos de vida indicam uma evolução convergente na adaptação a este modo de vida. Estes animais possuem, de maneira geral, uma variação de peso corporal que vai de 50 a 1000 gramas, não importando qual a sua dieta ou origem evolutiva. Isto indica que para animais menores ou maiores, a construção de túneis é uma atividade muito dispendiosa do ponto de vista energético (Lacey et al. 2000;Luna e Antinuchi 2007 a,b). Segundo Reichmann e Smith (1990), o fato de que uma das características das tocas seja oferecer proteção, sugere que a predação sobre os animais fossoriais é pequena. Além disso, a pequena quantidade de indivíduos observados em uma ninhada indica uma baixa pressão de predação (Eisenberg 1981).

Na América do Sul os roedores subterrâneos estão representados pelas famílias Octodontidae, denominados popularmente de coruros, representados por uma única espécie (*Spalacopus cyanus* (Molina, 1782)), que ocorre na parte central do Chile; e Ctenomyidae (gênero *Ctenomys* Blainville, 1826), sendo este o gênero de roedores subterrâneos com a maior riqueza de espécies do mundo, cerca de 60 (Woods e Kilpatrick 2005; Parada et al. 2011). Os representantes do gênero *Ctenomys* são conhecidos popularmente como tuco-tucos (Lacey et al. 2000).

O gênero *Ctenomys* Blainville, 1826

A especiação dentro do gênero *Ctenomys* é considerada como uma das mais explosivas dentre os gêneros de mamíferos atuais (Cook e Lessa 1988; Lessa e Cook 1998). As espécies se encontram distribuídas na região Neotropical, sub-região Patagônica, desde a Terra do Fogo, na Argentina, até o sul da Bolívia e Peru, e em altitude desde o nível do mar até mais de 3700 metros, nos Andes peruanos (Pearson et al. 1968; Cook et al. 1990; Reig et al. 1990). A família Ctenomyidae é um clado de roedores caviomórfos com hábitos fossoriais e subterrâneos. Este grupo foi primeiramente reconhecido durante o final do período Mioceno, e sua história evolutiva demonstra uma disparidade gradativa e um aumento significativo na diversidade após o Plioceno, sendo que a maioria dos gêneros de mamíferos datam do final deste período. A diversificação do gênero *Ctenomys* foi ainda mais intensa durante o Pleistoceno (Reig et al. 1990; Verzi 2008), sendo que atualmente o gênero inclui aproximadamente 60 espécies com hábito subterrâneo (Woods e Kilpatrick 2005) e mais de 10 espécies extintas (Verzi et al. 2004), cuja definição sistemática está pendente devido à dificuldade de atribuir uma hierarquia taxonômica para a variabilidade interpopulacional (Lessa 2000). Além disso, *Ctenomys* é considerado um gênero com características morfológicas e adaptativas coesivas (Reig et al. 1990; Lessa e Cook 1998), sendo que os resultados encontrados em análises morfo-funcionais e alométricas de características crânio-mandibulares das espécies viventes, têm suportado esta noção de identidade morfológica (Lessa 1993; Mora et al. 2003; Schleich e Vassallo 2003; Verzi e Olivares 2006; Vassallo e Mora 2007). Porém estudos recentes utilizando métodos de morfometria geométrica (Fernandes 2008; Steiner 2009; Fornel 2010) contradizem esta visão, devido a grande variação intra e interpopulacional encontrada.

A maioria das espécies possui hábitos solitários e territorialistas, com apenas um indivíduo ocupando cada toca. No caso das poucas espécies sociais e semi-sociais (*C. sociabilis*, *C. peruanus* e *C. porteousi*) ou durante o período de cuidado parental, há o compartilhamento dos túneis por mais de um indivíduo (Lacey et al. 1998, 2000). Estes túneis são mantidos fechados pelos indivíduos ocupantes, o que proporciona não só proteção contra os predadores, mas também condições mais estáveis do que as do meio externo: menores flutuações de temperatura, alto grau de umidade relativa, concentrações de O_2 menos elevadas e de CO_2 mais elevadas (McNab 1966; Lacey et al. 2000; Perissinotti et al. 2009). A diversidade de ambientes em que ocorrem é reflexo da estabilidade conferida pelo tipo de vida subterrânea. Apesar disso, estudos mais detalhados de sua distribuição mostram que os tuco-tucos tendem a viver em solos arenosos ou, no mínimo, bem arejados (Contreras 1973; Lacey et al. 2000). Isto não é somente devido às restrições impostas pela dependência da atividade escavatória que realizam, mas também por restrições relacionadas com a manutenção do calor e o intercâmbio de gases com o meio externo (McNab 1966, 1979; Contreras e McNab 1990).

As adaptações morfológicas relacionadas ao hábito subterrâneo dos tuco-tucos são a redução da cauda e pavilhões auditivos, um maior desenvolvimento da musculatura (principalmente dos membros anteriores) e das unhas, e uma abertura bucal atrás dos incisivos os quais ficam expostos para fora da boca, mesmo em descanso. Os tuco-tucos são territoriais e apresentam baixa dispersão (Lacey et al. 2000). O gênero apresenta um caráter intrigante quanto a sua alta variabilidade cromossômica, com grande diversidade cariotípica, variando desde 2n=10 em *C.steinbachi* a 2n=70 em *C.pearsoni* (Reig e Kiblisky 1969; Kiblisky et al. 1977; Gallardo 1979; Freitas e Lessa 1984; Ortells et al. 1990; Massarini et al. 1991; Gimenez et al. 1997, 1999; Garcia et al. 2000; Mascheretti et al. 2000; Freitas 1990, 1994, 1997 e 2007). A estrutura populacional, com forte territorialidade individual, populações pequenas e semi-isoladas e baixa vagilidade dos adultos (Pearson 1959; Bush et al. 1989), parece favorecer a fixação de rearranjos cromossômicos, sendo esta uma das causas da alta frequência de espécies do gênero (Reig 1989).

No Brasil, oito espécies de *Ctenomys* habitam as regiões Norte, Sul e Centro-Oeste. Nas regiões Norte e Centro-Oeste os registros de ctenomídeos são escassos, sendo que apenas três espécies foram coletadas nos estados de Rondônia e Mato Grosso (*C. bicolor* Ribeiro, 1914, *C. naterreri* Wagner, 1848, e *C. rondoni* Ribeiro, 1914), porém ainda existem poucos estudos com estas espécies, sendo que ainda persistem dúvidas taxonômicas básicas (Bidau e Avila-Pires 2009). As cinco espécies restantes (*C. torquatus* Lichtenstein, 1830; *C. flamarioni* Travi, 1981; *C. minutus* Nehring, 1887; *C. lami* Freitas, 2001; e *C. ibicuiensis* Freitas, Fernandes, Fornel & Roratto, 2012) ocorrem no Sul do Brasil, particularmente nos estados do Rio Grande do Sul e Santa Catarina. Em Santa Catarina é registrado apenas a espécie *C. minutus* (Freitas 1995). No Rio Grande do Sul, cinco espécies apresentam distribuição conhecida, sendo três endêmicas (*C. flamarioni*, *C. lami* e *C. ibicuiensis*). As outras duas espécies (*C. minutus* e *C. torquatus*) apresentam parte de sua distribuição no estado de Santa Catarina e no Uruguai, respectivamente (Freitas e Lessa 1984; Freitas 1995, 1997) (Fig. 2).

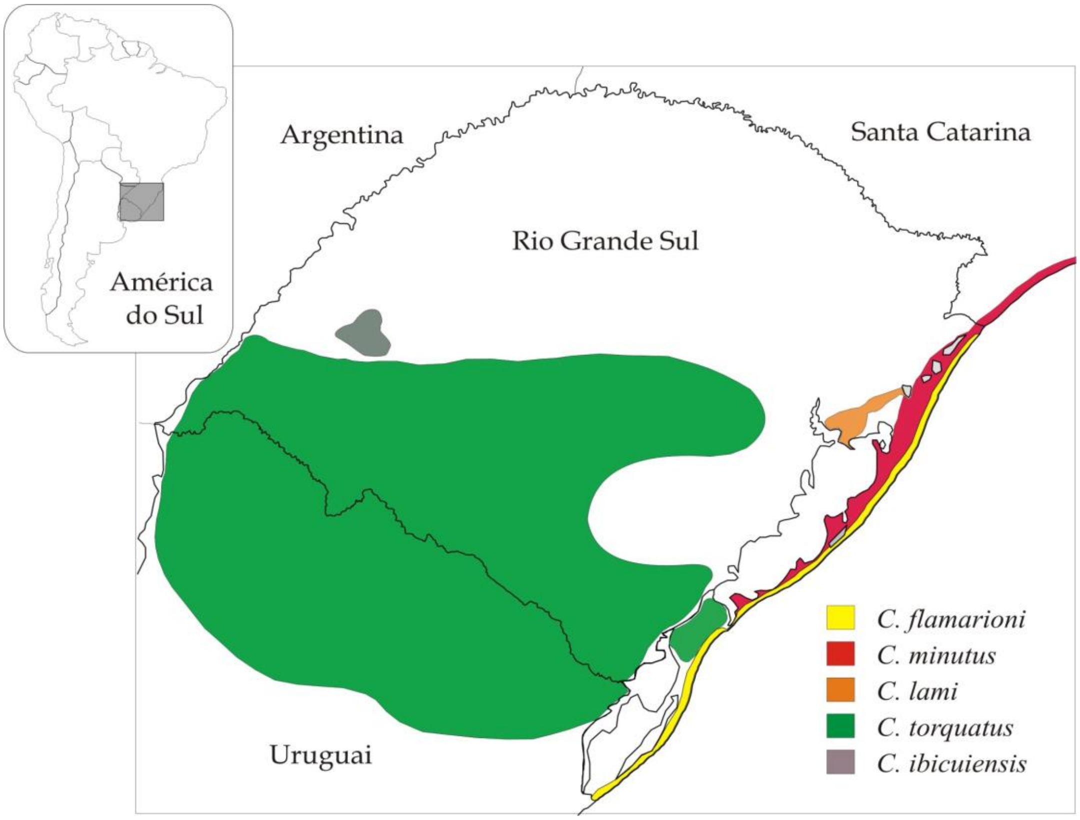

Fig. 2 Distribuição das espécies do gênero *Ctenomys* no estado do Rio Grande do Sul. Fonte: Tatiane Noviski.

Ctenomys minutus Nehring, 1887

Ctenomys minutus é uma espécie endêmica da planície costeira do Sul do Brasil, e pertence filogeneticamente ao grupo *torquatus* no gênero *Ctenomys* (Freitas 1995; Parada et al. 2011) (Fig. 3). Os indivíduos apresentam coloração castanha claro com tons da cor areia na parte inferior e uma coloração castanho-médio/escura. Os indivíduos jovens com dois ou três meses de idade apresentam coloração mais clara que a maioria dos adultos. Em geral, as colônias desta espécie são localizadas em campos arenosos e secos, ocupando preferencialmente as partes mais elevadas do campo, tais como taipas e taludes de estrada. No entanto, as regiões onde as colônias se localizam estão frequentemente associadas a corpos d’água (Fonseca 2003).

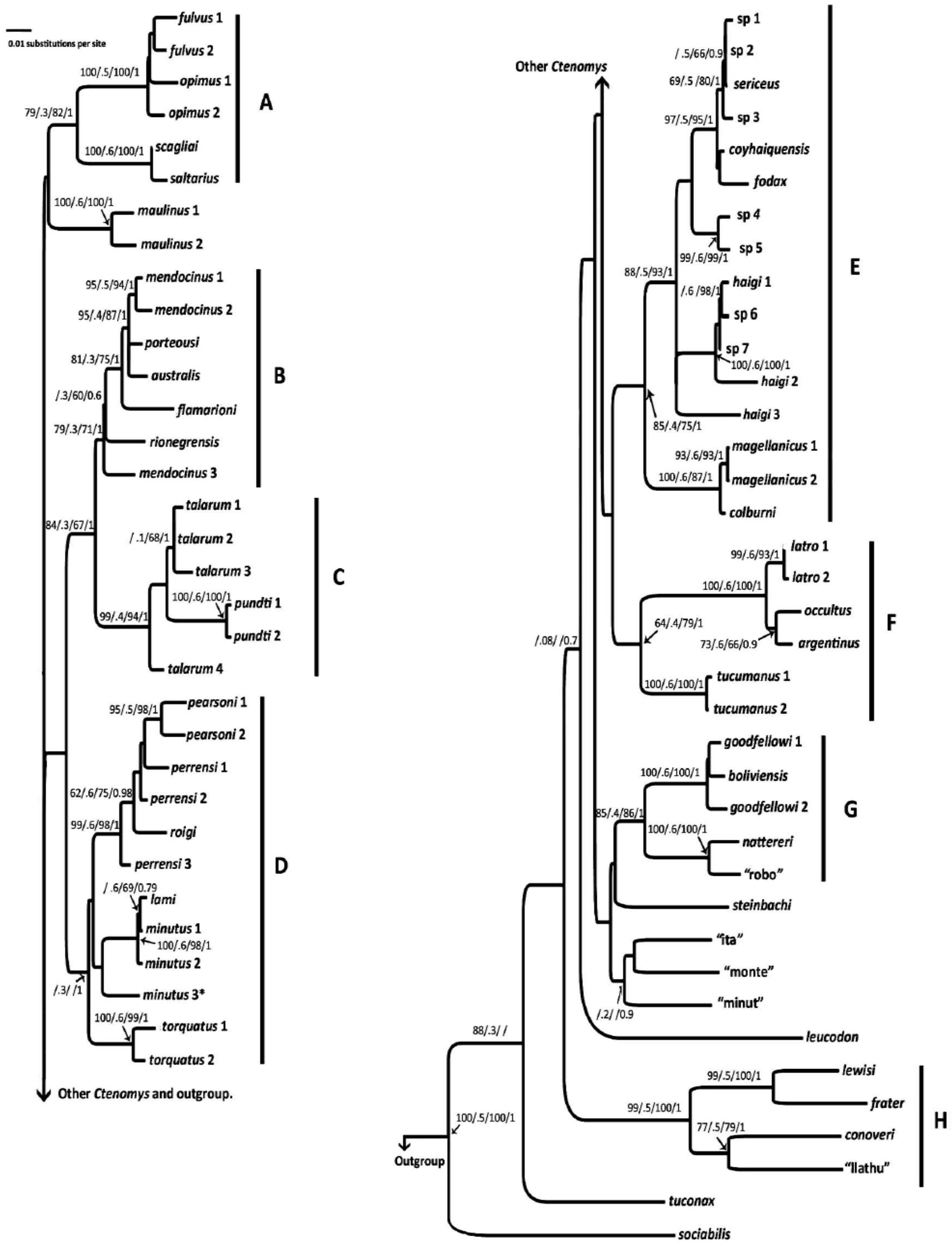

Fig. 3 Árvore filogenética obtida de Parada et al. (2011), com base em sequencias de citocromo-b. As letras designam os grupos de espécies: (A) *optimus*; (B) *mendocinus*; (C) *talarum*; (D) *torquatus*; (E) *magellanicus*; (F) *tucumanus*; (G) *boliviensis*; e (H) *frater*.

Ctenomys minutus (Fig. 4) ocupa o maior gradiente latitudinal dentre os tuco-tucos no Sul do Brasil, sendo que sua distribuição desde a praia de Jaguaruna no estado de Santa Catarina, até o município de São José do Norte no estado do Rio Grande do Sul (Freygang et al. 2004). Essa espécie ocupa no norte de sua distribuição a primeira linha de dunas da beira da praia, até as proximidades de Capão da Canoa (RS), e a partir de Capão da Canoa em direção ao sul a espécie passa a ocupar o segundo e terceiro sistemas de barreiras-lagunas, correspondente aos campos arenosos da segunda linha de dunas, interiorizando-se na costa até as proximidades das margens da Lagoa dos Patos (Freitas 1995; Kubiak 2013).

As populações de *C. minutus* parecem ser compostas em sua maioria de indivíduos adultos, com uma pequena participação de sub-adultos e jovens, o que sugere uma alta territorialidade dos adultos que pode forçar os jovens à dispersão (Gastal 1994; Fonseca 2003). Segundo os mesmos autores, a razão sexual encontrada entre as diferentes classes etárias sofre variação, sendo que essa alteração pode ser atribuída a algum fator que favorece as fêmeas, como a maior predação de machos sub-adultos durante a dispersão (Gastal 1994; Marinho 1997; Fonseca 2003).

A espécie possui hábito social tipicamente solitário, compartilhando os sistemas de galerias somente para a cópula e o cuidado das crias, que é realizado pelas fêmeas (Fonseca 2003). Suas tocas são constituídas por um túnel principal e túneis secundários laterais, sendo as aberturas igualmente distribuídas em todas as direções (Gastal 1994). Apresenta reprodução em época preferencial de acasalamentos nos meses de inverno (início) e de nascimentos a partir do final do inverno e início da primavera, podendo, eventualmente acontecer ao longo de todo o ano. Possui um tamanho de ninhada de um ou dois filhotes, sendo que a idade estimada dos indivíduos mais velhos é de dois anos e seis meses, com alguns podendo chegar até três anos (Marinho 1997; Fonseca 2003).

Fig. 4 Exemplar de *Ctenomys minutus*, município de Osório, Rio Grande do Sul, Brasil. Fonte: Daniel Galiano.

Além disso, uma região de hibridação interespecífica foi relatada entre as espécies *C. minutus* e *C. lami* na porção oeste da Lagoa dos Barros (El Jundi 2003; Gava e Freitas 2003). Gava e Freitas (2003) sugerem que esta zona híbrida seria produto de contato secundário entre as espécies, uma vez que no passado, uma extensa área úmida, a oeste da Lagoa dos Barros, isolava geograficamente as populações. Uma análise do ponto de vista ecológico e populacional nesta possível região de hibridação se torna necessário para uma melhor compreensão deste processo e suas consequências para as espécies. É fundamental inclusive, determinar a exata extensão da região de contato.

Planície costeira do Sul do Brasil: área de ocorrência de *Ctenomys minutus*

Planícies costeiras são habitats bastante instáveis e fortemente submetidos às influências dos oceanos (Dillenburg e Hesp 2009). A planície costeira do Sul do Brasil compreende uma área em torno de 35.000 km^2 de terras baixas, que se estendem na direção geral nordeste-sudoeste, ao longo de mais de 600 km. Aproximadamente a metade desta superfície encontra-se ocupada por um complexo sistema lagunar (Ceco 1984; Schwarzbold e Schafer 1984; Villwock 1984). Os limites naturais são formados, a leste, pela linha de costa do Oceano Atlântico e a oeste pelos terrenos mais altos e mais antigos. Os cursos do Rio Mampituba, no extremo norte, e do Arroio Chuí, no extremo sul, definem os limites políticos do litoral Sul-Riograndense, que se estende como região geomorfológica desde o sul de Santa Catarina até o leste do Uruguai, aproximadamente entre 28°30'S e 34°30'W. Os morros basálticos de Torres constituem a única interrupção rochosa ao longo de toda linha de costa, que se caracteriza principalmente pela linearidade das praias arenosas (Waechter 1990).

Nos últimos 12.000 anos esta área sofreu importantes transformações quanto a sua fisionomia (Vilwock et al. 1986). Estes autores sugeriram que o desenvolvimento da Planície Costeira do Rio Grande do Sul no Pleistoceno e início do Holoceno originou quatro barreiras. A primeira, que se localiza entre Porto Alegre e a Lagoa dos Barros com cerca de 250 km de extensão, é denominada de Coxilha das Lombas, que surgiu no início do Pleistoceno, resultado do primeiro evento transgressional-regressional, através de depósitos marinhos e eólicos. A segunda e terceira barreiras (formadas aproximadamente há 325 e 125 mil anos, respectivamente) deram origem à denominada “Barreira Múltipla Complexa”, correspondente à segunda linha de dunas, e foram responsáveis pela separação das Lagoas dos Patos e Mirim. A quarta e última barreira formou-se por volta do início do Holoceno e teria atingido seu máximo transgressivo há cerca de cinco mil anos, elevando o nível do mar de dois a quatro metros acima do atual (Vilwock et al. 1986; Tomazelli et al. 2000; Tomazelli e Villwock 2005).

Embora a planície costeira caracterize-se por uma relativa uniformidade altitudinal, pequenas variações topográficas podem determinar diferentes processos pedogênicos e, consequentemente, um mosaico de tipos edáficos peculiares. O regime de umidade no solo constitui-se em um dos principais fatores de diferenciação taxonômica vegetal, tanto para os tipos menos desenvolvidos, como para os tipos mais desenvolvidos (Waechter 1990). A distribuição do tipo de solos relaciona-se também com o material de origem e a idade dos diferentes depósitos geomórficos (Schneider et al. 1987). O revestimento vegetacional da planície costeira, muitas vezes referido simplesmente como vegetação pioneira, litorânea ou de restinga, compõe-se, na realidade, de um imenso mosaico de comunidades florística e estruturalmente diferenciadas (Klein 1967; Waechter 1985). As comunidades encontram-se, em diversas oportunidades, interrompidas por áreas ocupadas por lagunas ou lagoas costeiras ou por dunas móveis completamente desprovidas de plantas vasculares (Waechter 1990).

A fauna e flora presentes nas regiões de planícies costeiras são adaptadas a hostilidade destes ambientes. Atualmente a planície costeira do Sul do Brasil apresenta um terreno bastante irregular, formado por um mosaico de barreiras geográficas naturais ao fluxo gênico entre diferentes populações das espécies do gênero *Ctenomys* que habitam a região (Freitas 2007; Lopes e Freitas 2012; Lopes et al. 2013).

Relações de *Ctenomys* com o ambiente: nicho ecológico, uso do habitat e efeitos no ambiente

Nicho é um conceito amplamente utilizado em ecologia e recebeu várias definições ao longo do tempo. A quantificação de nichos (largura e sobreposição) é frequentemente utilizada em estudos que abordam partilha de recursos (Gliwick 1987; Ganzhorn 1989), competição (Colwell e Futuyma 1971; Hulbert 1978; Llewellyn e Jenkins 1987), coexistência de espécies (M'Closkey 1978) e estrutura de comunidades (Deuser e Shugart 1979; Findley e Black 1983; Marti et al. 1993).

O nicho ecológico inclui não apenas o espaço físico ocupado por um organismo, como também o seu papel funcional na comunidade e a sua posição em gradientes ambientais de temperatura, umidade, pH, solo e outras condições de existência (Odum 1983). As definições de nicho ecológico seguem dois enfoques: autoecológico e sinecológico. O primeiro conceito de nicho ecológico foi proposto por Grinnel (1924) e apresenta um enfoque autoecológico, enfatizando as relações entre uma espécie e seu ambiente. Nicho seria "o intervalo de valores de fatores ambientais que são necessários e suficientes para permitir que uma espécie desenvolva sua história de vida" (James et al. 1984), ou seja, a distribuição que esta espécie poderia potencialmente atingir sem interações com outras espécies (Vandermeer 1972). O segundo conceito considera as interações com outras espécies (enfoque sinecológico): "Local do animal no ambiente abiótico, suas relações com os alimentos e inimigos" (Vandermeer 1972).

Hutchinson (1957) sintetizou estas duas linhas de definições de nicho, apresentando o nicho como um hipervolume com *n* dimensões e estabelecendo a diferença entre nicho fundamental e nicho observado. Nicho fundamental é um conceito pré-interativo (autoecológico), sendo o conjunto de condições ambientais que são favoráveis à sobrevivência da espécie em questão. Nicho observado é um conceito pós-interativo (sinecológico), sendo à parte do nicho ecológico que permaneceu ocupada pela espécie após terem ocorrido interações com as outras espécies (Vandermeer 1972).

Entre os mamíferos, a separação de nicho ocorre mediante diferenças na dieta, na distribuição espacial e no uso do tempo (Schoener 1974; M'Closkey e Fieldwick, 1975; Brown et al. 1994). Kotler e Brown (1988) apresentam estas diferenças como mecanismos de coexistência de espécies, diferenciando seleção de habitat (uso de recursos similares em locais ou tempos diferentes) de partilha de recursos (uso de recursos diferentes no mesmo local e tempo). Além disso, a distribuição espacial de uma espécie envolve duas dimensões: vertical e horizontal. A distribuição horizontal pode ser analisada em diversas escalas, desde a escala biogeográfica até a de microhabitat (Morris 1987; Connor e Bowers 1996). De modo análogo, a segregação ecológica por diferenças na dieta é resultado das diferenças nos conteúdos energético e nutricional, na abundância e na distribuição espacial dos recursos, tamanho corporal, morfologia e fisiologia das espécies (Kotler e Brown 1988).

O nicho subterrâneo difere dos habitats de superfície devido aos indivíduos que o ocupam utilizarem uma terceira dimensão: a profundidade. Esta dimensão em conjunção com o solo dos túneis escavados gera uma gama distinta de fatores de seleção que criam e conferem oportunidades de resposta adaptativa às condições ambientais distintas (Lacey et al. 2000). Geralmente assume-se que o nicho subterrâneo é relativamente invariável (Nevo 1979), apesar da diversidade geográfica e de habitats de superfície em que os roedores subterrâneos ocorrem. Este pressuposto é bastante reforçado pelas similaridades morfológicas entre os *taxa* subterrâneos, que refletem peculiaridades impostas pela vida subterrânea (McNab 1979; Lacey et al. 2000). Apesar destas suposições, a variação regional do clima, solo e vegetação, podem ser importantes fatores que geram diferenças adaptativas entre as populações ou espécies. Como resultado, taxa convergentes podem apresentar diferentes picos adaptativos em cada localidade, que refletem a variabilidade do ambiente local. Além do mais, devido às adaptações em cada ambiente envolverem um grau de comprometimento distinto com o hábito subterrâneo, diferenças ecológicas nas populações destas espécies podem refletir efeitos combinados dos processos seletivos da superfície e subterrâneos (Lacey et al. 2000).

De maneira geral, os sistemas de túneis dos tuco-tucos são caracterizados pela ausência de luz, excesso de umidade, baixa variação na temperatura e baixas concentrações de oxigênio. Devido à luz ser absorvida pelas paredes, até mesmo túneis abertos se tornam escuros a uma pequena distância da entrada. Todos os túneis apresentam altos valores de umidade relativa, e em túneis fechados os valores podem ser próximos de 100% (Kennerly 1964). O fluxo de ar é geralmente reduzido e relativo ao ambiente superficial, o que causa uma baixa ventilação e elevados níveis de CO_2 (Darden 1972; Arieli 1979).

Os túneis de *Ctenomys* atingem em média uma profundidade que varia de 0.31m a 0.44m, sendo esta alterada pela presença de rochas, raízes e pela altura do lençol freático (Altuna et al. 1992). Segundo Altuna (1983), não existe variação da profundidade das tocas conforme a estação do ano, mas existem diferenças de comprimento entre o túnel principal e suas ramificações para a espécie *C. pearsoni*. Também foi verificado por Comparatore (1990), que a textura e a dureza do solo provavelmente não são fatores limitantes na dispersão e ocupação de novas regiões dentro da área de distribuição da espécie *C. talarum.* Segundo os autores, a distribuição agrupada da espécie pode estar correlacionada com fatores ambientais, tais como a presença de vegetação rasteira e a umidade do solo. Ainda, indicam que o comportamento dos animais é um fator determinante na distribuição da espécie.

Além disso, a presença de tuco-tucos influencia diretamente na composição florística da região e na modificação das condições do solo, através do revolvimento e aeração do mesmo (Zenuto e Busch 1995; Rosi et al. 2000; Del Valle et al. 2001; Lara et al. 2007), ao mesmo tempo em que disponibiliza uma maior biomassa de presa para alguns predadores que possuem condições de capturá-los (Pia et al. 2003).

Os resultados encontrados por Lara et al. (2007) para uma comunidade de plantas e para a concentração de nutrientes no solo no Deserto de Puna do Sul, Argentina, sugerem que a espécie *C. mendocinus* pode ser essencial neste local, sendo capaz de orientar a dinâmica da comunidade de plantas estudadas. Os autores ainda sugerem que a herbivoria de *Ctenomys* afetou a cobertura vegetal, a estrutura da comunidade, a diversidade e a mortalidade das plantas e a composição florística do local estudado. O efeito na diversidade de plantas pode ser altamente dependente das densidades das populações locais de *Ctenomys*, bem como das condições iniciais da estrutura e da composição da comunidade de plantas (Campos et al. 2001). A dieta dos tuco-tucos é inteiramente herbívora, na forma de raízes, sementes e grama, que podem ser puxados para dentro da galeria. Os tuco-tucos alimentam-se ainda de partes de plantas que se encontram na superfície, abrindo orifícios ao longo dos túneis e consumindo a vegetação adjacente (Barlow 1969; Travi 1983; Albanese et al. 2010).

A espécie *C. minutus*, objeto do presente estudo, ainda é pouco explorada quanto as suas características ecológicas. Desta forma, estudos que visem compreender características do nicho ocupado pela espécie, bem como o padrão de seleção de habitat e consequências da presença deste roedor no ambiente, são necessários para uma melhor compreensão das relações da espécie com o ambiente em que habita, bem como capazes de contribuir para o esclarecimento de questões relativas a sua distribuição espacial e aspectos conservacionistas.

Modelagem de nicho ecológico e Maxent

Os modelos de distribuição de espécies (MDEs), também chamados de modelos de nicho ecológico ou modelos de envelope bioclimático, são ferramentas úteis para complementar a informação sobre a distribuição geográfica das espécies ao longo do tempo (Peterson et al. 2011). Atualmente, constituem um dos campos de pesquisa mais ativos na ecologia (Zimmermann et al. 2010) e têm sido aplicados em estudos com diversos interesses, como determinação de distribuições potenciais, avaliação do potencial de invasão de espécies exóticas, genética da paisagem, estudos populacionais, estudos conservacionistas (Diniz-Filho et al. 2008; Phillips e Dudik 2008; Diniz-Filho et al. 2009; Václavík e Meentemeyer 2009; Bernardo-Silva et al. 2012), entre outros. O uso dos MDEs se tornou um procedimento comum para determinar a amplitude da distribuição geográfica das espécies. Uma lista de aplicações atuais para esses métodos nunca será completa, principalmente porque seu uso está ainda em crescimento, com inovações que permitem novas abordagens (De Marco Júnior e Siqueira 2009).

A teoria do nicho ecológico é o principal fundamento para o desenvolvimento de MDEs (Peterson 2011) considerando que indivíduos de uma mesma espécie respondem a um conjunto de recursos e condições que definem sua distribuição (Grinnell 1917; Hutchinson 1957). De modo geral, os MDEs podem ser considerados como o ajuste a uma função entre os pontos de ocorrência de uma espécie e um conjunto multivariado de dados ambientais (Phillips et al. 2006). Os métodos utilizados são baseados em soluções matemáticas mais simples (Distância Euclidiana, BIOCLIM) até algoritmos derivados de inteligência artificial (Maxent, GARP, Redes Neurais).

Atualmente, um dos métodos de modelagem mais aplicados é o Maxent (Máxima Entropia), que se enquadra na lista dos modelos mais complexos. É uma técnica de aprendizagem automática (*machine-learning*) que estima a distribuição de probabilidades mais próxima à distribuição uniforme sob a restrição de que os valores esperados para cada variável ambiental estejam de acordo com os valores empíricos observados nos pontos de ocorrência (Phillips et al. 2006). As principais vantagens da técnica são: i) necessita apenas de dados de presença; ii) o produto final gerado (distribuição potencial) é contínuo, indicando uma adequabilidade relativa dentro do intervalo de 0 a 100; iii) possibilita o uso de variáveis categóricas; iv) apresenta uma definição matemática concisa e facilmente interpretável dentro dos conceitos clássicos de análise de probabilidades (Phillips et al. 2006). Além disso, o desempenho desta técnica tem se demostrado favorável em relação às demais abordagens (Elith et al. 2006; Wisz et al. 2008).

Diversos trabalhos têm demostrado à possibilidade de integrar os resultados dos MDEs com outras ferramentas ou áreas do conhecimento, como por exemplo, dados moleculares (Jakob et al. 2007; Knowles et al. 2007; Diniz-Filho et al. 2009). Embora estas abordagens possam apresentar algumas limitações, os resultados oriundos da integração destas ferramentas têm se demostrado muito promissores, sendo que esta abordagem está sendo utilizada em muitos trabalhos desenvolvidos atualmente. No entanto, é importante que as escolhas feitas durante o processo de modelagem sejam pautadas no objetivo do estudo e no tipo e qualidade dos dados obtidos.

OBJETIVOS

Objetivo geral

Determinar e descrever as principais características ecológicas de *Ctenomys minutus*, bem como as relações da espécie com aspectos físicos e biológicos do ambiente. Este estudo visa à ampliação do conhecimento ecológico da espécie, no que se refere à ecologia espacial, seleção de habitat e implicações da presença deste roedor na planície costeira do Sul do Brasil, além de aspectos conservacionistas.

Objetivos específicos

Capítulo II - Artigo 1

- Conduzir a primeira análise de distribuição espacial das espécies *C. minutus* e *C. lami* ao longo de toda a sua distribuição geográfica;
- Determinar quais fatores ecológicos podem estar limitando a distribuição destas espécies;
- Desenvolver um quadro integrado para avaliar padrões de diversidade genética dentre os potenciais pools genéticos;
- Discutir questões conservacionistas à luz desta abordagem concatenada.

Capítulo III - Artigo 2

- Determinar os efeitos do roedor subterrâneo *C. minutus* nos campos arenosos da planície costeira do Sul do Brasil, por meio da comparação da cobertura vegetal, biomassa de plantas, dureza do solo e concentração de nutrientes no solo, em áreas com a presença e ausência deste roedor.

Capítulo IV - Artigo 3

- Analisar a relação entre *C. minutus* com o ambiente em que habita ao longo de sua distribuição, por meio da comparação de características ambientais entre áreas presentes ao longo de sua distribuição, com o intuito de determinar o porquê a espécie seleciona determinadas áreas em detrimento de outras.

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CAPÍTULO II

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(Anexo I)

Genetic pool information reflects highly suitable areas: the case of two parapatric endangered species of tuco-tucos (Rodentia: Ctenomyidae)

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Abstract

Conservation of small mammals requires knowledge of the genetically and ecologically meaningful spatial scales at which species respond to habitat modifications. Conservation strategies can be improved through the use of ecological niche models and genetic data to classify areas of high environmental suitability. In this study, we applied a Maxent model integrated with genetic information (nucleotide diversity, haplotype diversity and Fu's *Fs* neutrality tests) to evaluate potential genetic pool populations with highly suitable areas for two parapatric endangered species of tuco-tucos (*Ctenomys minutus* and *C. lami*). Our results demonstrated that both species were largely influenced by vegetation and soil variables at a landscape scale and inhabit a highly specific niche. *Ctenomys minutus* was also influenced by the variable altitude; the species was associated with low altitudes (sea level). Our model of genetic data associated with environmental suitability indicate that the genetic pool data were associated with highly suitable areas for *C. minutus*. This pattern was not evident for *C. lami*, but this outcome could be a consequence of the restricted range of the species. The preservation of species requires not only detailed knowledge of their natural history and genetic structure but also information on the availability of suitable areas where species can survive, and such knowledge can aid significantly in conservation planning. This finding reinforces the use of these two techniques for planning conservation actions.

Introduction

Subterranean rodents of the genus *Ctenomys* inhabit the southern part of the Neotropical region from the extreme south up to southern Peru, including the entire

Patagonian region and showing a wide latitudinal variation. The range of the genus extends from sea level to 4,000 m in the Andean region [1]. These small subterranean herbivores are among the most geographically variable mammals and are also the most speciose of all subterranean rodent groups [2]. Presently, the genus includes approximately 60 species [2]. South American tuco-tucos (*Ctenomys*) have attracted special interest in terms of speciation and evolution by virtue of their patchy distributions, low vagility, territoriality, and extensive karyotypic variation [1].

In the coastal plain of southern Brazil, the species with the widest geographic distribution is *Ctenomys minutus*, which inhabits sandy fields and dunes and has a range extending from Jaguaruna Beach in the state of Santa Catarina to the town of São José do Norte in the state of Rio Grande do Sul. *Ctenomys lami* is an endemic species inhabiting a sandy region named 'Coxilha das Lombas' along a narrow line of old dunes that extends from north of Guaiba Lake to the northwestern sandbanks of Barros Lake [3,4]. A hybrid zone between *C. minutus* and *C. lami* is also present. It is probable that this zone was formed due to habitat alterations. A wide humid zone once separated both species and represented a barrier [5]. *Ctenomys lami* is cited as vulnerable in the Red List of the International Union for Conservation of Nature [6], and *C. minutus* is listed as data deficient. However, given the current state of information about tuco-tucos in Brazil, Fernandes et al. [7] have affirmed that the vulnerability of these subterranean rodents is greater than presently supposed and that any conservation effort should be based on consistent and detailed studies of habitat occupation.

Ecological niche models (ENMs), based on the use of multiple strategies in conservation plans, are an important tool for determining the distribution of threatened species for conservation purposes [8]. In particular cases of restricted distribution or sparse populations of rare or endangered species, the use of ENMs may be necessary to identify a set of areas for protection [9]. Functionally, ENMs could be considered to furnish a specification of the relationship between the points of occurrence of a species and a set of multivariate environmental data [10]. The need to base strategies of ENM development on niche theory facilitates the interpretation and discussion of the resulting models relative to conservation issues. Conservation of small mammals requires knowledge of the genetically and ecologically meaningful spatial scales at which species respond to habitat modification. Understanding this information is important because small mammals represent a major assemblage of species occupying the most varied environments [11]. Large-scale, presence-only models are vital to complement the information obtained by local studies. They reveal otherwise-overlooked ecological requirements by identifying the environmental parameters that influence species distributions on a broad geographical scale [12].

Ctenomys minutus and *C. lami* inhabit the coastal plain of southern Brazil. Several studies have addressed the degradation of this coastal environment, identifying both natural and human-induced factors contributing to long- and short-term change [13,14]. Anthropogenic changes, such as urbanization in active dune areas [14,15], have modified the natural landscape over the years. As a result of human activity, native habitats have become increasingly fragmented or destroyed, changing the patterns of gene flow between populations and modifying the levels of genetic diversity. In our particular case, conservation strategies can be improved with the use of ENMs and genetic data to classify areas of high environmental suitability and to investigate how genetic characteristics diverge in different areas.

In this study, we applied ENMs integrated with genetic information to evaluate potential genetic pool populations with highly suitable potential areas. We developed maximum-entropy (Maxent, Phillips et al. [10]) presence-only distribution models for *C. lami* and *C. minutus* based on the following goals:

1. To conduct the first geographical distribution analysis for both species over their entire distributional range;
2. To determine which ecological factors may be limiting the distributions of the species;
3. To develop an integrated framework to evaluate geographic patterns of genetic diversity within potential genetic pools;
4. To discuss conservation issues in the light of this concatenated approach.

Methods

Study area

The study was conducted on the southern coast of Brazil. The environment consists of sand dunes and relict ‘restinga’ forests [16]. The landscape of the coastal plain of southern Brazil is characterized by lakes, lagoons, rivers, and dunes that represent natural geographical barriers for different populations of *Ctenomys* [4,17] (Figure 1).

Species records

Current knowledge about the distributions of *C. minutus* and *C. lami* is based on records from the collection of the Laboratório de Citogenética e Evolução of the Universidade Federal do Rio Grande do Sul and collected by researchers [3-5,18-21]. All of the coordinates were recorded using a GPS at the exact point of collection or observation. We considered a total of 74 records for *C. minutus* (n=45) and *C. lami* (n=29), each representing an established population of these tuco-tucos, to generate the ENMs (Figure S1).

Environmental data

We used 19 Worldclim bioclimatic variables obtained through interpolated data from derived rainfall and temperature, with a resolution of 2.5 arc-minutes (five kilometers); one altitude variable [22], available at http://www.worldclim.org/download; and two categorical variables for soil and vegetation composition [23] available at http://mapas.mma.gov.br/i3geo/datadownload.htm. We included those two categorical variables due to their recognized ecological importance to the study species and genus [24-27]. The vegetation and soil maps were converted to rasters at the same level of resolution as the bioclimatic variables using ArcMap v. 10.0. We generated a matrix with the values of each climatic variable for the entire study area. We performed a Principal Components Analysis (PCA) on this matrix to identify correlations between variables, selecting the axes that explained ≥95% of the correlation structure. From this result, we selected variables with the highest absolute coefficient in each axis. This procedure yielded seven variables for *C. minutus* (mean temperature of coldest quarter, precipitation seasonality, mean temperature of warmest quarter, mean diurnal range (mean of monthly (max temp - min temp)), soil, vegetation, and altitude) and five variables for *C. lami* (maximum temperature of warmest month, minimum temperature of coldest month, mean temperature of warmest quarter, soil, and vegetation). To avoid information loss, we included all variables represented in the PCAs from both species to generate models for the two species. The models were constructed with a total of nine variables because equal variables were selected for the two species.

Modeling

The models were constructed with Maxent version 3.3.3k [10], available at (http://www.cs.princeton.edu/~schapire/maxent/). Maxent estimates the ecological niche of a species by determining the distribution of maximum entropy (ME), subject to the constraint that the expected value of each environmental variable under this estimated distribution matches its empirical average. The technique was designed as a machine learning algorithm [10,28,29]. The default parameters in Maxent were used to construct the models: automatic features election, regularization multiplier at unity, maximum iterations 500 and convergence threshold 10^{-5}. We produced maps of the potential distribution of the species using the logistic output format [29]. This format is an attempt to ensure the closest possible approach to an estimate of the probability that the species is present given the environment [28]. We chose Maxent because it requires only presence data, because it can process categorical data and model interactions, and because it has performed favorably when compared with alternative approaches [30,31]. The models were validated by calculating the area under the curve (AUC) from a receiver operating characteristic curve (ROC). The relative importance of the variables was assessed with Maxent’s built-in Jackknife functionality.

Genetic data

Our basic data consisted of mitochondrial DNA (mtDNA) sequences corresponding to the cytochrome oxidase I gene and the hypervariable control region.

The data were obtained from Lopes et al. [20] for *C. minutus* and Lopes and Freitas [19] for *C. lami*, where a detailed description of the genetic data can be obtained. For *C. minutus*, a total of 30 localities were sampled across the entire distribution of the species, and seven principal clades were highlighted in the Bayesian phylogenetic tree. We focused on these seven main genetic haplogroups described by Lopes et al. [20] (North 1, North 2, Coast, Barros Lake, Mostardas, Tavares, and South; see Lopes et al. [20] for details). For *C. lami*, 28 locations were sampled, and the data are presented by the authors based on the four karyotypic blocks proposed for the species (Block A, B, C and D; see Freitas [4], Lopes and Freitas [19]). We chose to present our results for this concatenated approach (genetic data and modeling) based on the haplogroups for *C. minutus* and karyotipic blocks for *C. lami* since we did not directly compare these two different units, but discuss the results independently for each species. We believe that the use of this approach may not overestimate the interpretation of the results.

Briefly, the geographical genetic structure of *C. minutus* was characterized by examining 340 individuals over the entire distributional range and using information from cytochrome oxidase I gene and control region sequences. For *C. lami*, a total of 178 specimens were sampled, using information from the same mitochondrial DNA markers. Our analyses were based primarily on three different genetic parameters obtained from genetic data estimated by these authors [19, 20]. The first parameter, nucleotide diversity (π), is defined by the equation

$$\pi = \sum_{ij} x_i x_j \pi_{ij} \text{ ,}$$

where π_{ij} is the proportion of nucleotide differences between the ***ith*** and ***jth*** types of DNA sequences and x_i and x_j are the respective frequencies of these sequences. Nucleotide diversity is a measure of genetic variation. The second parameter, haplotype diversity (H_d), is a measure of the uniqueness of a particular haplotype in a given population. H_d is defined by the equation

$$Hd = \frac{N}{N-1}\left(\ 1-\sum i\,\chi^{2} i\ \right),$$

where x_i is the (relative) haplotype frequency of each haplotype in the sample and N is the sample size. Haplotype diversity is given for each sample. The third parameter, Fu's *Fs*, is the probability of observing a random sample with a number of alleles equal to or smaller than the observed value given the observed level of diversity and the assumption that all of the alleles are selectively neutral. If we call this probability $\hat{S}$, then

$$Fs = \ln\left(\frac{\hat{S}}{1-\hat{S}}\right),$$

so that a negative value of *Fs* would be expected from a recent population expansion or from genetic hitchhiking. A positive value of *Fs* is evidence for a deficiency of alleles, as would be expected from a recent population bottleneck or from overdominant selection [32]. To display the genetic information on a map, we established a geographic coordinate associated with each haplogroup for *C. minutus* and with each karyotypic block for *C. lami*.

Results

The models

The models constructed with Maxent produced distributional predictions for each species. The map with the widest distributional area was generated for *C. minutus*, whereas *C. lami* presented a very narrow distribution probability (Figure 2).

According to the ME model, the occurrence of *C. lami* was most strongly associated with soil and vegetation. The variable with the highest gain when used in isolation was vegetation. For this reason, vegetation appears to be the single variable that furnishes the most useful information. The variable that decreased the gain most markedly when omitted was soil. For this reason, soil appears to furnish the greatest amount of information not present in the other variables. For *C. minutus*, occurrence was strongly associated with altitude, vegetation, and soil. The variable with highest gain when used in isolation was vegetation, and the variable that decreased the gain most markedly was also vegetation (Figure 3).

Both species were strongly influenced by vegetation and soil variables. Model performance, defined as the area under the curve, was highly discriminative for both species (AUC values for *C. lami* = 0.997 and *C. minutus* = 0.993), indicating that they inhabit a highly specific landscape niche.

Genetic pool data

The three local genetic parameters estimated showed no apparent patterns for *C. lami*. The suitability values calculated between the blocks were the same (high), and block C showed the highest value of π; however, the same pattern was not followed by the H_d values, which were higher in blocks B and D. The *Fs* values were not significant for any of the analyzed blocks (Figure 4).

For *Ctenomys minutus*, areas of high suitability were in accordance with the seven principal genetic haplogroups found for the species (see Lopes et al. [20] for details). For π values, the northern portion of the distribution presented higher values (except for North 1). H_d presented the same trend, with the center of the distribution showing greater diversity. For the *Fs* index, only the Barros Lake haplogroup showed a significant result (-5.30), as would be expected from a recent population expansion or from genetic hitchhiking (Figure 4).

Discussion

Maximum entropy models and environmental variables

This is the first study to use ENM methods to test parameters that influence the occurrence of the genus *Ctenomys* and one of the first to use these techniques on subterranean mammals. The models presented here were successful in identifying regions that are suitable for the species. Both models showed few commission errors, such as the areas of medium environmental suitability on the east side of the Coxilha das Lombas region for *C. lami* and the areas of low suitability in the countryside along Patos Lagoon for *C. minutus*. In addition, model performance was highly discriminative for both species. Although we identified these commission errors, we must consider that is very difficult to incorporate the dispersal abilities of the species and geographical accessibility to the model (landscape configuration) at present or through history; however, this factor is necessary for the actual presence of species [33]. In our particular case, geographical accessibility is a very important issue because the tuco-tucos are characterized by limited individual mobility and a patchy distribution of local populations [25,34]. *Ctenomys lami* appears to be restricted to the region of Coxilha das Lombas and showed a potential distribution at low to medium levels of occurrence beyond the limits of this region. The species was shown to be associated with local vegetation and soil type and to be geographically limited by the presence of wetlands and geographical inaccessibility, factors that characterize this region.

The distribution of *Ctenomys minutus* was restricted to the coastal plain of southern Brazil, including the region's first line of dunes and extending to sandy fields. This species shows a sympatric zone with another tuco-tuco species (*C. flamarioni*) in a range of approximately 15 km [35]. In an area extending to the northern portion of this region, *C. minutus* inhabits the first line of dunes. Below the sympatric zone, *C. minutus* occupies only the regions of sandy fields, although the ME model indicates that it can potentially occur up to the line of dunes. The reason for this apparent discrepancy is that near the southern limits of this sympatric zone, the first line of dunes is already occupied by *C. flamarioni* up to the southern boundary of Rio Grande do Sul state. This finding is, most likely, related to the historical patterns of occupation by these species. *Ctenomys minutus* shows a pattern of north-south occupation extending from Santa Catarina to Rio Grande do Sul state [20], and *C. flamarioni* shows a pattern of occupancy from south to north [36,37]. This transition between the sand fields and dune line represents an environmental discontinuity along the range of *C. minutus*. According to Fornel et al. [38], who have found significant differences in skull morphology between specimens of *C. minutus* that inhabit sand fields and those that inhabit dunes, environmental characteristics such as soil hardness produce morphological adaptations in tuco-tucos. These authors found some variation among different populations belonging to different habitats in cranial shape, and these intraspecific phenotypic differences appear to arise as a combination of selection and drift acting as diversifying forces. However, according to our model of environmental suitability, this discontinuity may not inhibit free dispersal by specimens from different habitats. Therefore, these different environments may also be associated with distinct levels of environmental suitability, although we could not measure this difference in our models.

Although they are morphologically similar, both species studied here occupy distinct areas of the coastal plain. According to the ME models, *C. minutus* appears to show higher environmental plasticity than *C. lami*. The models proposed for these two species showed a low overlap of areas of high suitability. This characteristic appears to be defined primarily by the presence of the Coxilha das Lombas region and the geographical inaccessibility of this region. A hybrid zone between *C. lami* and *C. minutus* is present in the northern portion of this coxilha. This zone has been the subject of previous study. According to Gava and Freitas [5], it was formed by modifications of the environment because a humid zone that separated these species was drained for rice cultivation. Environmentally, our results suggest a medium probability of occurrence for both species in the region where they hybridize.

Moreover, edaphic conditions can be very important for the definition of a species' fundamental niche [39,40]. The occurrence of *C. lami* and *C. minutus* was strongly influenced by soil and vegetation. *C. minutus* was also influenced by altitude, as it was associated with low altitudes (sea level). According to Kubiak [35], *C. minutus* is associated with areas with higher vegetation and biomass, which strengthens the evidence for the importance of this variable to the species. Given that tuco-tucos are exclusively subterranean, the use of variables that are directly linked to the species niche demonstrated to be extremely important in building the models, regardless of soil and vegetation variables, might have suffered from their degree of generalization. It is recommended that the choice of predictors in ENMs should consider the ecological relevancy of the predictors to the target species [41]. Such considerations are extremely important to improve conservation actions.

Genetic information and conservation actions

Our analyses of the genetic data associated with the ME model for *C. lami* showed that there are differences in the levels of genetic diversity (π and H_d) between populations, although the suitability is currently the same for all of the blocks. Lopes and Freitas [19] found that the genetic structure associated separately with each of the four different karyotypic blocks was inconsistent for mtDNA and microsatellite data. Environmentally, we also found no differences among these four karyotypic blocks. Thus, the presence of chromosomal rearrangements or distinct karyotypes between individuals noted by Freitas [4] does not appear to be limited by the environment.

Lopes et al. [20] recovered a pattern of genetic structure of the sampling sites subdivided into seven main haplogroups for *C. minutus*. In the current study, these haplogroups, represented by three genetic parameters, were associated with areas of high environmental suitability. This pattern may result from the occurrence of higher genetic diversity in the areas of higher suitability for the species. According to Lopes et al. [20], a relatively ancient genetic structure was present in the northern area of the geographical distribution, whereas the southern sampling sites may exhibit a founder effect of more recent occurrence. However, areas with high suitability showed different values of π and H_d, thus other extrinsic (historical or contemporary) or intrinsic (behavioral) factors could be affecting the pattern of genetic diversity and structure.

The only value of the *Fs* index that was statistically significant for *C. minutus* was from the Barros Lake haplogroup, which was associated with a highly suitable area. As this pattern was not found for *C. lami* or for the other haplogroups of *C. minutus*, it is not possible to support our hypothesis that highly suitable areas favor population expansion or genetic hitchhiking, as indicated by a significant negative value of the *Fs* index.

The preservation of species requires not only detailed knowledge of their natural history and genetic structure but also information on the availability of suitable areas where species can survive; such knowledge can aid significantly in conservation planning. Both species analyzed here have suffered intense pressure from the fragmentation and reduction of their habitats. This process results in the death or isolation of individuals because these species are extremely niche-specific. The principal threats to *C. lami* are the progressive urbanization and human settlement in its territory, associated, for example, with agricultural activities [7,19]. In the northern portion of the coast, *C. minutus* is threatened by land speculation associated with urban constructions along the coast and on the shores of the lagoon and by the fragmentation of the dune habitat. In the south, silviculture involving *Pinus* sp. directly affects the populations of the species ([7], authors' personal observations). A study by Bernardo-Silva et al. [42] focused on the conservation of two endangered species of red-bellied toads, and the authors suggested five important areas for species conservation based on a hotspot analysis. These areas included a significant number of threatened species, including *C. minutus*. Our results underscore that the areas proposed by those authors should be considered for reserve planning because they cover three highly suitable areas within the range of *C. minutus* (see Bernardo-Silva et al. [42]). Although their results did not focus on underground rodents, these hotspot areas are of great interest for *C. minutus* because considering conservation of habitats as a priority, the protection of sandy field areas with high suitability for underground tunneling is an interesting solution for protecting the species. Additionally, *C. minutus* has only two protected areas within its entire distribution (Parque de Itapeva and Parque da Guarita). These areas are in neighboring locations in the northeastern portion of the distribution. However, none of the southern populations are located in protected areas. Two state conservation units (Parque de Itapuã and Refugio de Vida Silvestre Banhado dos Pachecos) are located within the range of *C. lami*, but only parts of these areas overlap the distribution of the species. Over the past year, extensive field work has been conducted to search for *C. lami*, but few populations have been found outside these two units (authors' personal observations).

Our models of genetic data associated with environmental suitability indicated that genetic pool data were associated with highly suitable areas for *C. minutus*. This result supports the use of these two techniques to plan conservation actions. This pattern was not evident for *C. lami*, perhaps due to the restricted range of the species. Despite the current wide use of molecular markers to estimate genetic variation at different geographical scales and originating from different evolutionary processes [43,44], it is still difficult to match these data with ecological parameters at broad scales. Moreover, despite many studies testing the relationship between genetic diversity and geographical populations, it is necessary to test alternative mechanisms, such as fragmentation and human effects on species distributions, by integrating genetic and demographic data in an ecological context.

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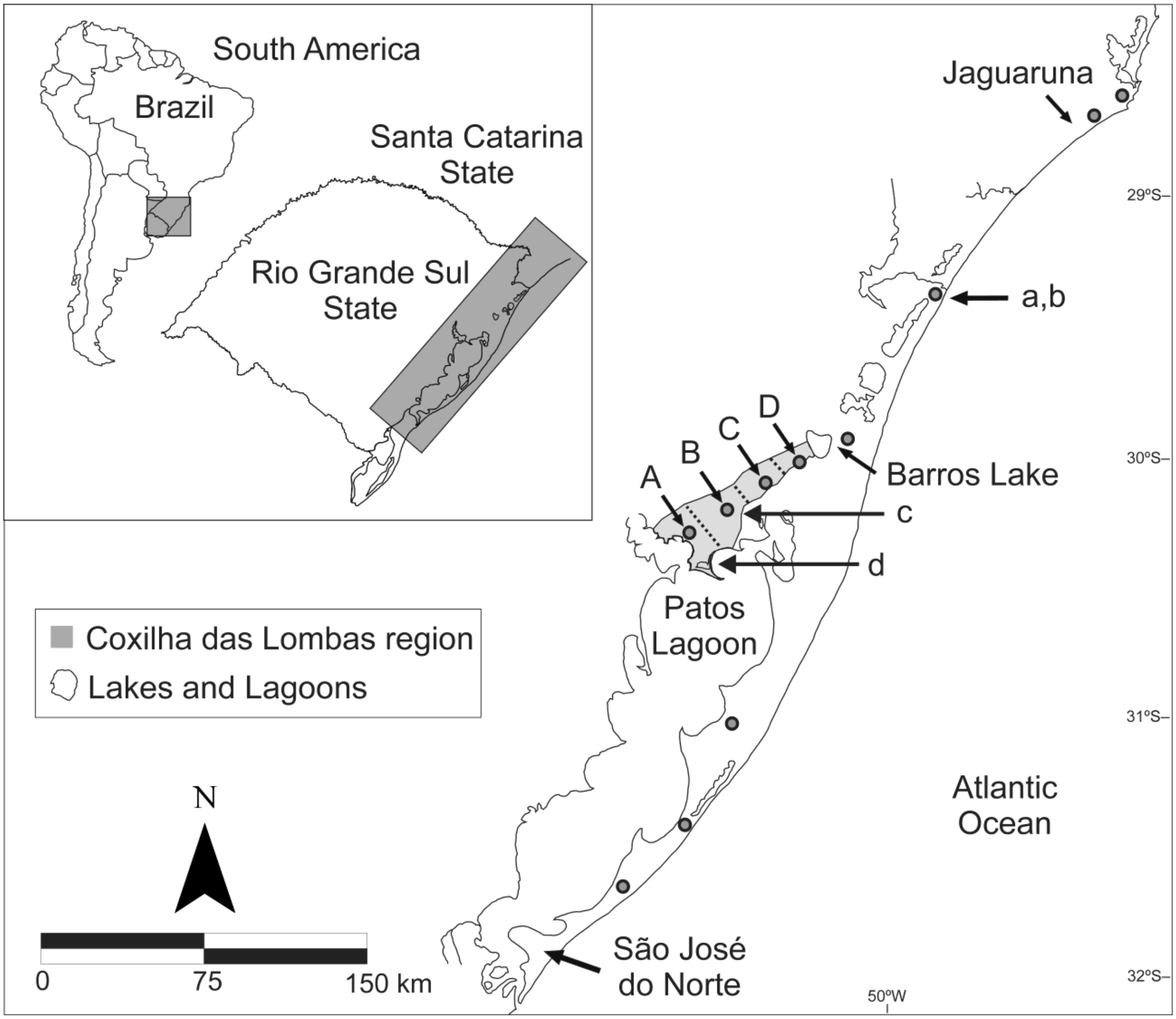

Figure 1. Coastal plain of southern Brazil with geographic details. The most important lakes and rivers are shown. Points (●) represent geographical regions for which genetic data are available from Lopes and Freitas (2012) and Lopes et al. (2013). In the region of Coxilha das Lombas are displayed the four karyotypic blocks described by Freitas (2007) (A, B, C and D). Conservation areas within the distribution of each species are also displayed (a, b: Parque de Itapeva and Parque da Guarita; c: Parque de Itapuã; d: Refugio de Vida Silvestre Banhado dos Pachecos).

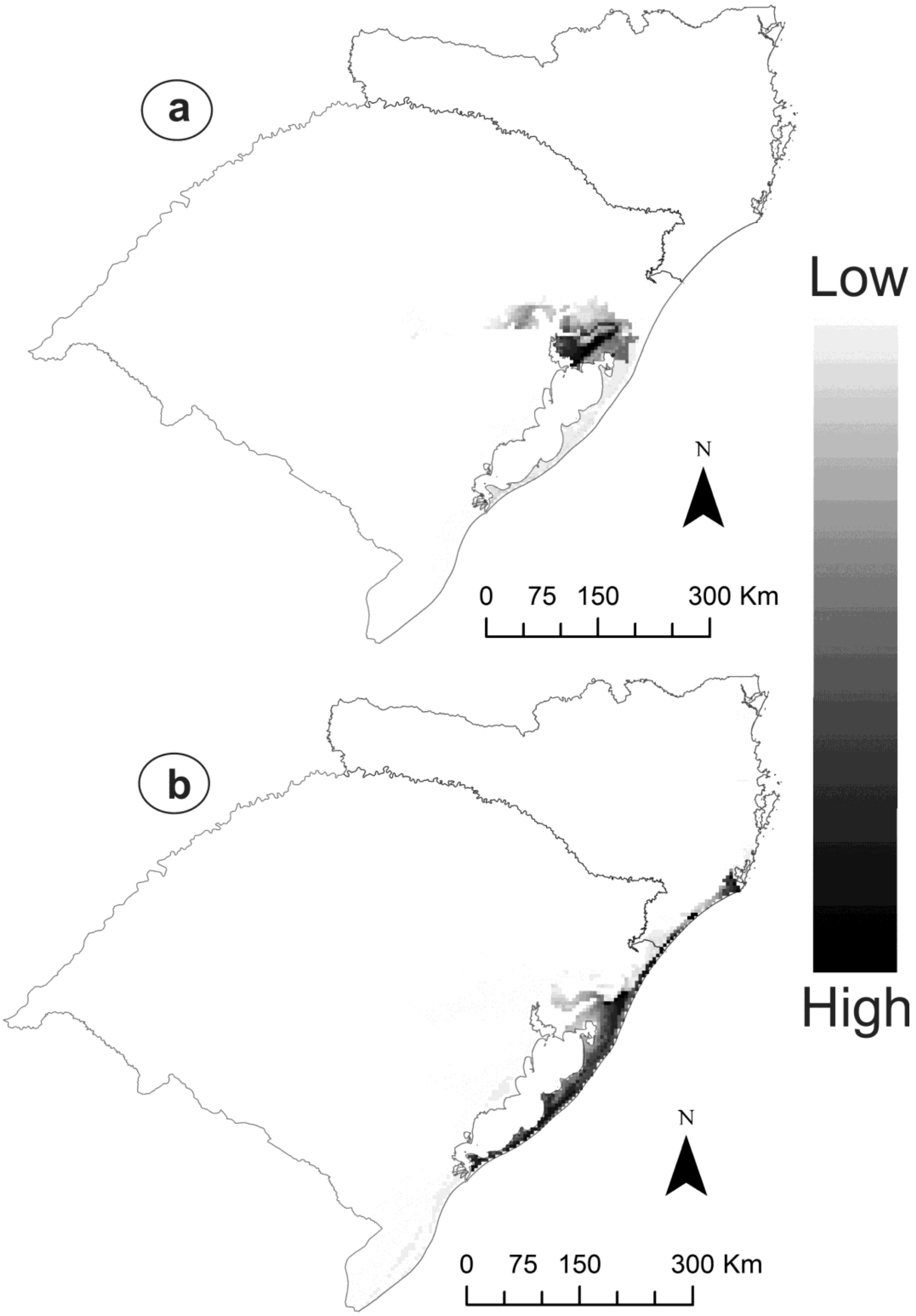

Figure 2. Suitable areas for (a) *Ctenomys lami* and (b) *Ctenomys minutus*, according to Maxent (maximum entropy) model in southern Brazilian coastal plain. Darker regions indicate greater ecological suitability.

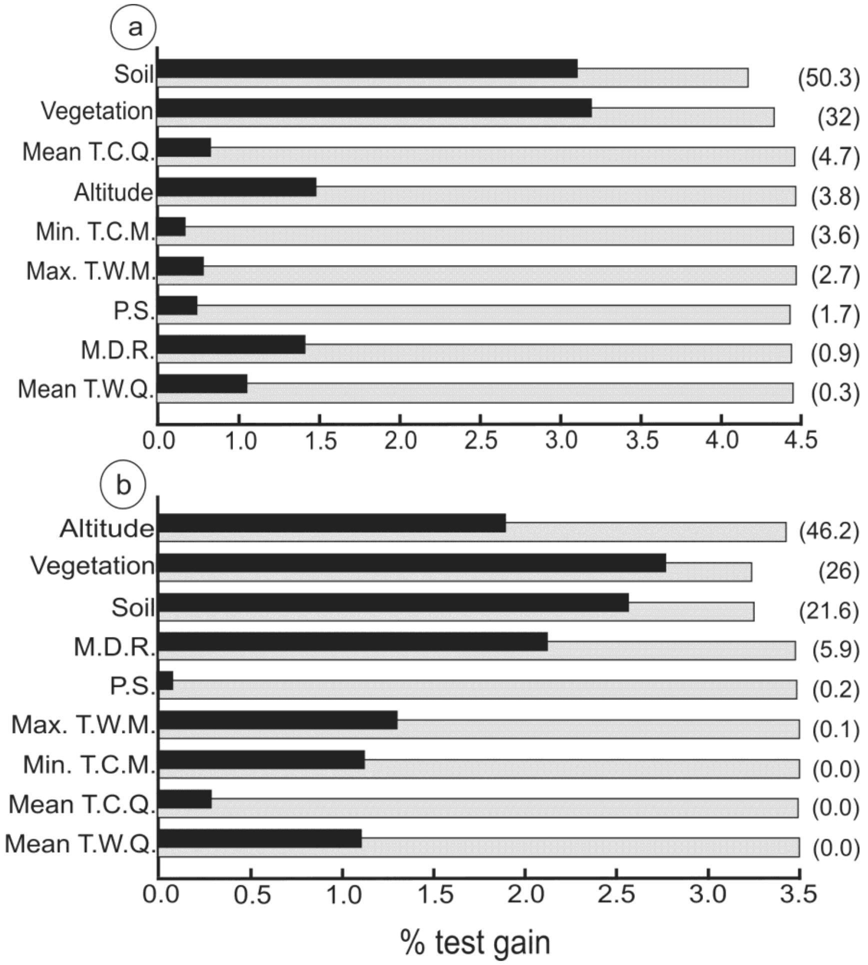

Figure 3. Jackknife analyses of the importance of environmental variables in maximum entropy modeling of *C. lami* (a) and *C. minutus* (b) occurrence. A heuristic estimate of the relative contribution of each variable to the global model is given in parentheses, with variables listed in descending order of importance. Grey bars show the performance of the global model (known as test gain) without each variable, and black bars show the influence of each variable in isolation (derived from a univariate model only). The variables are the following: Mean T.C.Q.: mean temperature of coldest quarter; Min. T.C.M.: minimum temperature of coldest month; Max. T.W.M.: maximum temperature of warmest month; P.S.: precipitation seasonality; M.D.R.: mean diurnal range (mean of monthly (max temp - min temp)); Mean T.W.Q.: mean temperature of warmest quarter.

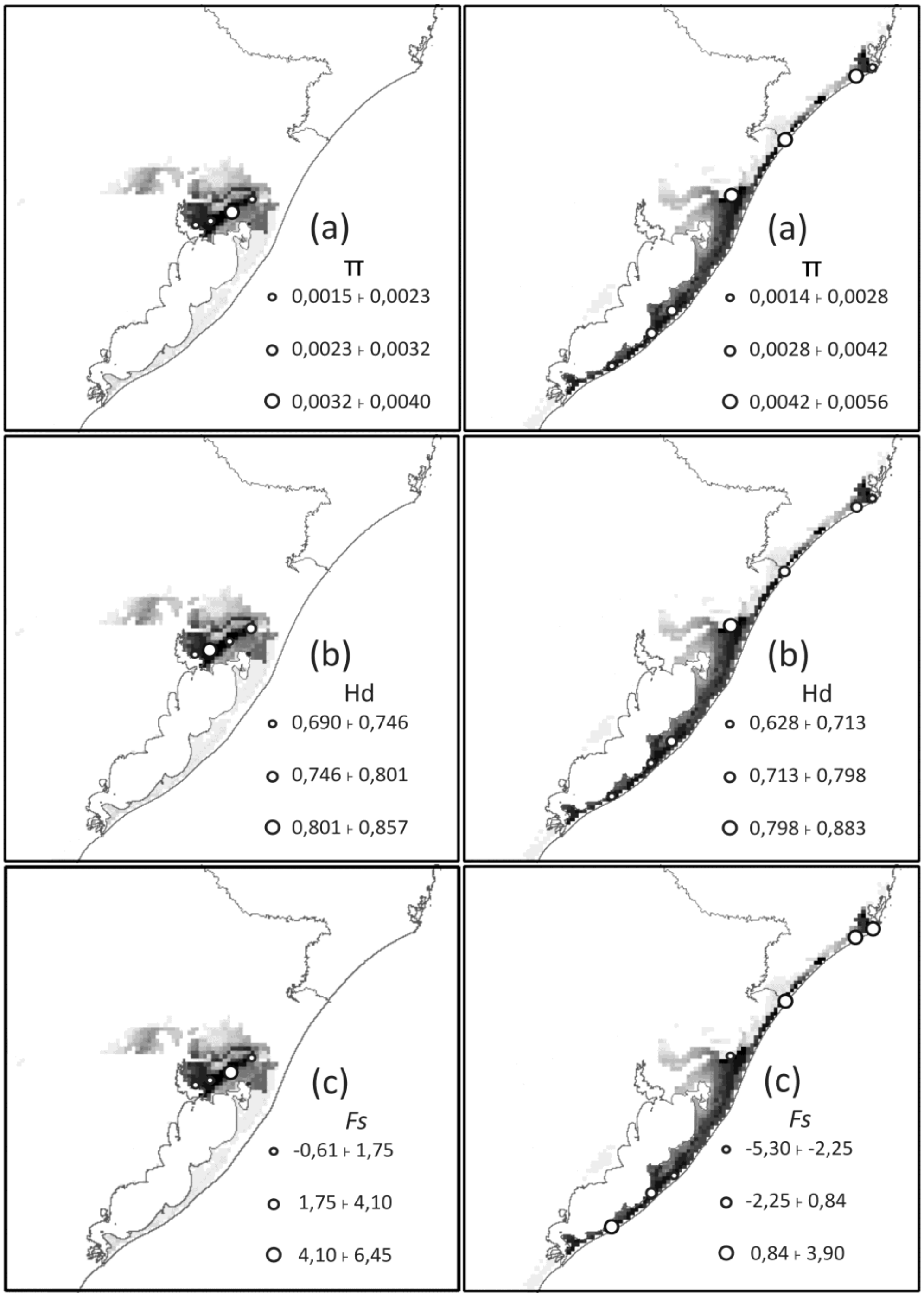

Figure 4. Geographical patterns of genetic parameters estimated within phylogeographic groups (*C. minutus*) and karyotype blocks (*C. lami*). Nucleotide diversity, *Π* (a); Haplotype diversity, *Hd* (b); Fu´s neutrality parameter, *Fs* (c), overlapping the potential distribution of *C. lami* (left column) and *C. minutus* (right column). Darker regions indicate greater ecological suitability.

Supporting Information

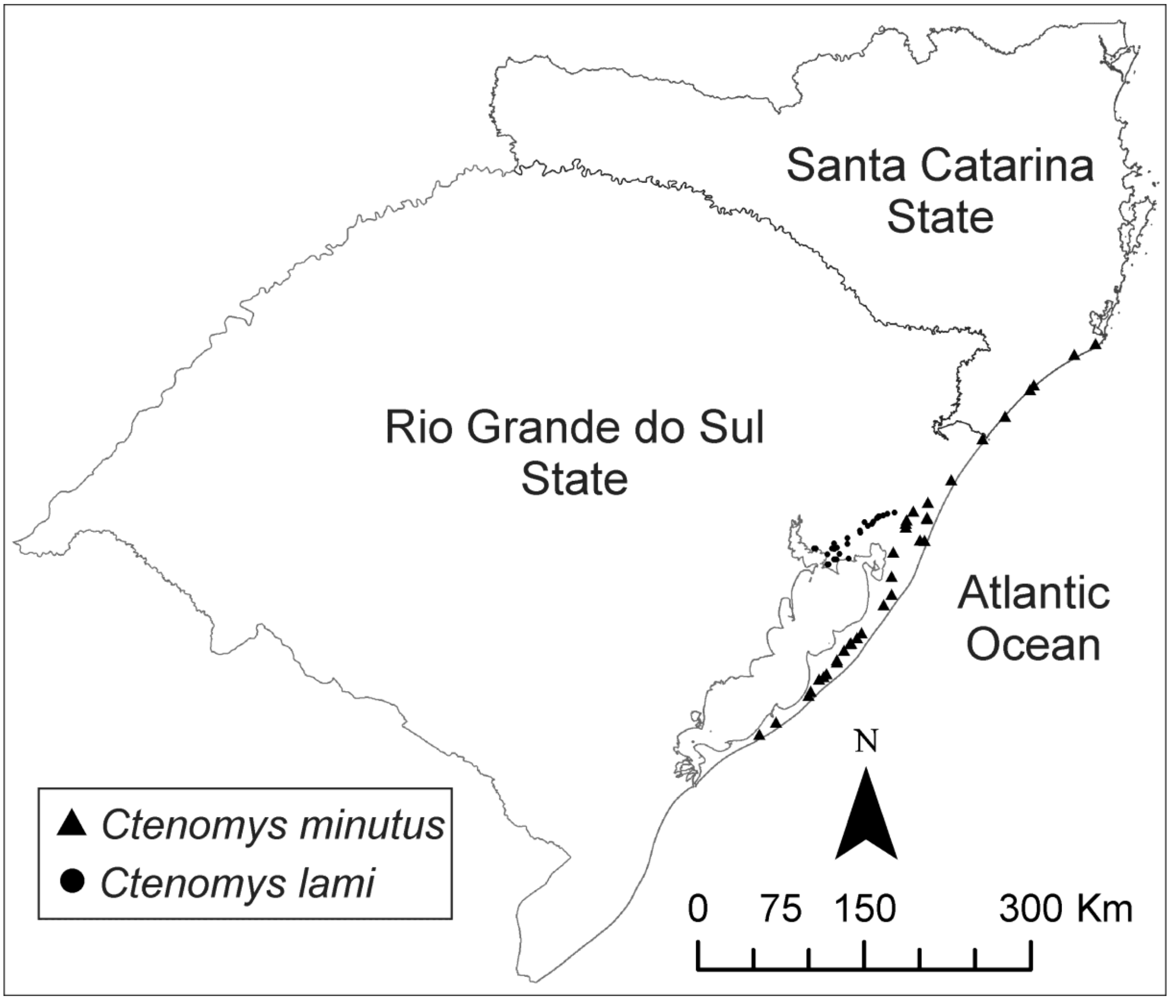

Figure S1. Input points given to the software (Maxent).

CAPÍTULO III

Manuscrito publicado no periódico Acta Theriologica

(Anexo II)

Effects of rodents on plant cover, soil hardness and soil nutrient content: a case study on tuco-tucos (*Ctenomys minutus*)

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Abstract

We analyzed the effects of tuco-tucos (*Ctenomys minutus,* Ctenomyidae) on plant cover, plant biomass, soil hardness, soil pH and variables related to nutrient disposition (P, K, Mg and Ca), using data from three areas in the South Brazilian coastal plain. In each area, samples were taken from sites with and without *C. minutus* and results are presented in a concatenate way. Our results show that the presence of *C. minutus* modifies total plant biomass, grass cover, bare soil, soil hardness, soil pH and nutrient content. Soils horizons at the depths of 10 and 20 cm are significantly softer in sites with *C. minutus* and phosphorus and potassium had higher concentrations. The content of magnesium and calcium were not affected. Soil pH was significantly lower where tuco-tucos occurred. Altogether, our results show that these animals may have a significant effect on vegetation composition and dynamics as well as on soil properties.

Keywords: Animal interactions; Coastal grasslands; Subterranean mammals; Southern Brazil.

Introduction

Tuco-tucos (Ctenomyidae) belong to a highly specialized mammalian family of subterranean rodents endemic to South-America, with approximately 60 species (Woods and Kilpatrick 2005). This family is characterized by a conspicuous ecological and morphological diversity (Busch et al. 2000; Lacey et al. 2000; Mora et al. 2003), and species occur across extensive areas (Lacey et al. 2000). They can, for instance, be found in deserts or semi-deserts in the Andean region, as well as in open areas in subtropical forests (Reig et al. 1990). *Ctenomys minutus* Nehring, 1887 is a medium-sized herbivorous rodent restricted to the southern Brazilian coastal plain. This species inhabits sandy grasslands and dunes, and it occupies the longest latitudinal gradient among tuco-tucos in southern Brazil (Freitas 1995; Freygang et al. 2004).

Subterranean rodents excavate and inhabit extensive burrow systems, and changes in plant diversity, abundance, and community composition are typical consequences of the many activities of these animals (Andersen 1987; Contreras and Gutierrez 1991; Huntly and Reichman 1994; Malizia et al. 2000; Campos et al. 2001; Reichman and Seabloom 2002; Kerley et al. 2004; Lara et al. 2007; Hagenah and Bennett 2013). They also may alter soil conditions (Schauer 1987; Cox and Roig 1986; Borghi et al. 1990; Malizia et al. 2000; Lara et al. 2007; Šklíba et al. 2009; Hagenah and Bennett 2013). These effects of subterranean rodents on vegetation and soil can arise from burrow dynamics, diet selection, or foraging behavior (Huntly and Reichman 1994). Because of the great impact they have on entire ecosystems, including on water and air in soil, decomposition processes of plant material, nutrient cycling and composition of local biota (Hole 1981), subterranean rodents are regarded as ecosystem engineers (Cameron 2000; Reichman and Seabloom 2002; Reichman 2007). Because of the magnitude of their effects, their actions can constitute a major factor in soil and vegetation dynamics.

Several studies have focused on the effects of *Ctenomys* on vegetation (Malizia et al. 2000; Campos et al. 2001; Tort et al. 2004; Lara et al. 2007) and soil conditions (Malizia et al. 2000; Lara et al., 2007). In most of these studies, *Ctenomys* alters vegetation and nutrient content of soil, although there is some variation in magnitude of these effects among studies. Like other subterranean rodents, *Ctenomys* spends most of its life in burrows that provide safety and foraging opportunities (Lacey et al. 2000). While direct observation of the species in the field is impossible, the investigation of the influence of *Ctenomys* on vegetation and soil conditions may provide valuable information about the magnitude of the effects of these animals in the ecosystem they inhabit. In this context, the aim of our study was to analyze the effects of a subterranean rodent (*Ctenomys minutus*) on its environment by comparing vegetation cover, plant biomass, soil hardness, and nutrient concentration in the soil between areas with and without this tuco-tuco.

Material and methods

Study area

The study was conducted between May and July 2013 in the coastal plain of the state of Rio Grande do Sul, southern Brazil. We sampled three areas where *C. minutus* occurred, situated in the municipalities of Torres (29º23'S, 49º45'W), Capão da Canoa (29º40'S, 50º01'W) and Osório (29º57'S, 50º13'W). Climate at these sites is mild mesothermal, wet, with no dry season. Average annual temperature ranges between 16 and 20 ºC and annual rainfall varies between 1,000 and 1,500 mm (Nimer 1977; Vieira and Rangel 1988). The region is a mosaic of lakes, lagoons, sand dunes, and sand fields, and its geomorphology is continuously influenced by the fluctuations of the Atlantic Ocean (Tomazelli et al. 2000).

The vegetation in the region consists of mosaics of dune vegetation, coastal grasslands, and 'restinga' forests (Hesp et al. 2009). Our study sites were on grasslands under cattle grazing with rather low stocking rates, as typical in the region. Feces produced by cattle covered less than 3% of the area (patches with *C. minutus*: 2.02±2.03%, and areas without *C. minutus*: 1.86±1.96%; t=0.85, without significant differences between areas). Grasslands in the region are dominated by C4 grasses and are rich in plant species, with high importance especially of species from the Poaceae, Asteraceae, Cyperaceae and Fabaceae (see Overbeck et al. 2007 for a review on South Brazilian grassland vegetation). The most dominant species at the study sites was the prostrate grass *Axonopus* aff. *affinis* Chase. The vegetation also contains some small grassland shrubs (e.g. *Baccharis* sp., C*roton lanatus* lam.). Total vegetation cover is approximately 50% to 80%, and proportion of bare sandy substrate is high.

Sampling methodology and statistical analysis

To evaluate the influence of *C. minutus* on vegetation and soil, we sampled ten burrow sites of active individuals at each of the three study sites (‘presence points’). We sampled the burrow systems found in each area which presented the activity of tuco-tucos, and the first 10 sites in which animals were captured were considered the presence points. In addition, ten randomly allocated points without presence of tuco-tucos were established in the same grasslands where animals were captured (‘control points’). Control points were a minimum of 80 meters from presence points containing *C. minutus*. No obvious differences in vegetation or topography were apparent between points of presence and absence of tuco-tucos, other than the presence or absence of burrows of *Ctenomys*. In total, we had 60 sample points (20 at each study site). We used live traps (Oneida Victor No. 0 traps protected with rubber strips) to capture the animals in burrows systems. Each trap was introduced into the entrance of a burrow, checked every 10 minutes, and animals were released at their point of capture. Trapping was conducted under the IBAMA (Brazilian Institute for the Environment and for Renewable Natural Resources) permit number 14690-1.

At each of the presence and control points, vegetation structure, plant biomass, and soil hardness were sampled in four sampling units (SU) situated at a distance of 2 m in the four cardinal directions. To evaluate vegetation structure, we used a 1-m^2 quadrat to measure percent cover of grasses, herbs, and bare ground. The side of the quadrat was placed 2 m away from each presence/control point. Plant biomass was measured in these same quadrats with a vertical quadrat sampler with a side length of 16 cm and a side depth of 22 cm (one sample per SU). We removed all above- and below-ground vegetation from inside this quadrat sampler. After removing sand, plant samples were oven-dried to a constant weight at 80 ºC, and then dry weight was recorded to determine total biomass. We used an impact soil penetrometer (Model IAA / Planalsucar, Stolf 1991) to measure soil hardness. At each presence and control point, we took four measurements (one per SU), recording the number of strokes necessary to penetrate to three soil depths: 10 cm, 20 cm and 50 cm (results are expressed in kg/cm^2). For analysis of vegetation variables and soil hardness, we computed the mean of the four sampling units and then used the mean in the analysis (60 data points; 10 from presence points, 10 from control points, for each of 3 study sites). For plant biomass, the weights of the four samples per unit were summed for the subsequent analysis.

We also collected soil samples to evaluate the influence of *C. minutus* on soil pH, phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg). Soil samples were collected from the top 10 cm of soil at each of the 60 points. Phosphorus and potassium were determined by Mehlich-1 method (expressed in g/m^3) and calcium and magnesium were extracted with potassium chloride (KCl, 1mol/L) (expressed in g/m^3) (Mehlich 1953; Embrapa 1997).

Differences between areas with and without tuco-tucos were analyzed regarding variables of vegetation structure, mean dry biomass (g/sample), soil hardness, and soil nutrient concentration. Each area (Torres, Capão da Canoa e Osório) was considered a block in all analyses. Considering that not all variables had normal distribution, we used a permutational ANOVA analysis (PERMANOVA) to compare variables between presence and control sites (Underwood 1998; Zar 1999). The tests were performed with matrices based on Euclidean distances, and we ran 9999 permutations. All analyses were conducted using the vegan package in R (R Development Core Team 2012; Oksanen et al. 2012).

Results

Total plant biomass and grass cover were significantly lower at sites where tuco-tucos were present, but herb cover did not differ between sites with and without tuco-tucos. There also was significantly more bare ground at sites with tuco-tucos (Table 1).

Soil hardness was significantly lower at 10 cm (F = 6.13, P = 0.0016) and 20 cm (F = 2.08, P = 0.049) in areas containing tuco-tucos. There was no significant difference in soil hardness at 50 cm between sites with and without tuco-tucos (F = 0.52, P = 0.375) (Fig. 1).

Areas with tuco-tucos had significantly higher concentrations of phosphorus and potassium than areas without tuco-tucos, but there was no significant difference in calcium and magnesium between sites with and without tuco-tucos. Soil pH was significantly lower where tuco-tucos occurred (Table 1).

Discussion

Our results indicate that tuco-tucos exert significant effects on vegetation and soil conditions. The influence of *C. minutus* on plant biomass (a 37% reduction) is within the range of other herbivorous subterranean rodents (plant biomass reduced by 25 to 50%; Reichman and Smith 1985). This reduction in plant biomass also was similar to that measured for other species of *Ctenomys* (*C. mendocinus*: 44% reduction of biomass on a plant community in the southern Puna Desert, Lara et al. 2007; *C. talarum*: 31% reduction in biomass in grasslands of Buenos Aires Province, Malizia et al. 2000). We also observed a decrease in grass cover on sites with tuco-tucos. The reduction in grass cover and plant biomass at sites with *C. minutus* is likely a result of foraging activity of individuals, since this species feeds mostly on grasses (C.M. Lopes, pers. comm.), which are the dominant species group in the vegetation at our study sites. This reduction also can be a direct result from activity related to burrow construction. Albanese et al. (2010) also found that grasses were the dominant life form consumed by C. *mendocinus* (79%), and grass leaves were the most representative item among consumed plant parts (89.5%). Decreased densities of grasses in areas with *Ctenomys* also were reported by Campos et al. (2001) and Lara et al. (2007). This pattern suggests an effect of *Ctenomys* foraging on the abundance of grasses, which may then, in turn, have further effects on community dynamics, e.g. by relaxing competition and effects on different plant types, thus changing, for example the ration of species with sexual and vegetative propagation (see also Lara et al. 2007).

There was significantly more bare ground at areas where tuco-tucos were present (61% more bare ground). Bare ground is created by both burrowing and foraging activities of tuco-tucos. However, the direct effect of the creation of burrow systems and the resultant deposition of soil on the surface that can bury existing vegetation is easily visible and likely much more important in quantitative terms.

Values from the soil penetrometer provide a clear indication of the effect of tuco-tucos on soil hardness at soil depths of 10 and 20 cm. According to Gastal (1994) the average depth of the burrow systems built by *C. minimus* is 25 cm and the maximum depth is 29.1 cm. This range of burrow depths is consistent with our finding of a significant effect of tuco-tucos on soil hardness at depths of 10 cm and 20 cm, but not at 50 cm. Sampling areas situated two meters from the burrow opening could present some undiscovered burrows that were nearby sampling units, thus affecting soil hardness. During burrowing, the animals redistribute soil among different horizons which contributes to aeration, irrigation, and fertilization of soils (Reichman and Smith 1990). The latter can be observed from higher P and K concentrations just below the soil surface, but not concerning Ca and Mg. According to Malizia et al. (2000), the formation of new mounds by tuco-tucos (*C. talarum*) increased the levels of N, P, Na, K, and Mg, but higher levels of Ca and pH were found in undisturbed areas. Lara et al. (2007) also found that the disturbing activity of *C. mendocinus* increased nutrient concentration (N, K, P) in bare soil compared to bare soil in undisturbed areas. Even though effects on different nutrients vary between studies, it is well known that tuco-tucos and other subterranean mammals (pocket gophers, Geomyidae) modify the distribution of nutrients (Abaturov 1972; Mielke 1977; Grant and Mc Bryer 1981; Hole 1981; Spencer et al. 1985; Inouye et al. 1987; Koide et al. 1987; Reichman and Seabloom 2002). In our case, this was shown even for patches within the same grassland area. Thus, we can assume that sites close to *C. minutus* burrows are nutrient hotspots (for P and K), resulting from the animals' activities moving, mixing or bringing soil to the surface from lower levels and the incorporation of nutrients into the soil. In contrast, the more acid pH found on sites with *C. minutus* may be a consequence of the dead vegetal material resulting from foraging activity.

Altogether, our results show that *C. minutus* affects plant biomass, grass cover, the proportion of bare soil, soil hardness, concentrations of P and K, and pH in the grasslands under study. In addition, we demonstrated that this tuco-tuco affects soil hardness and vegetation characteristics in an area greater than just above the burrowing mount (two meters), probably because of the tunneling and feeding of individuals. Still, if we measured these characteristics above the opening or over the tunnel itself, this difference could be greater. These animals may thus have a significant effect on vegetation composition and dynamics as well as on soil properties. While this obviously will affect ecosystem processes such as decomposition or productivity, the consequence of all the effects that tuco-tucos have on ecosystems are difficult to predict. Studies with pocket gophers have revealed that the extensive excavations and their associated impacts generate a dynamic mosaic of nutrients and soil conditions that promote diversity and maintains disturbance-dependent components of plant communities (Reichman and Seabloom 2002). The possible importance of herbivory by small rodents on plant composition and ecosystem properties in coastal grassland communities in South Brazilian has been neglected and detailed effects of *C. minutus* still have to be investigated.

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Table 1 Mean and standard deviation of vegetation variables (plant biomass, grass cover, herb cover and bare soil) and soil variables (pH, and nutrient concentrations: phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg)). The results of permutational ANOVA (PERMANOVA) tests for differences between areas with and without tuco-tucos (*Ctenomys minutus*) are displayed.

	Without Tuco-tucos (n = 30)	With Tuco-tucos (n = 30)	F	*p*-level
	Mean ± SD	Mean ± SD		
Vegetation				
Plant Biomass (g)	146.64 ± 75.16	91.21 ± 59.13	5.88	0.0014
Grasses (%)	56.88 ± 18.51	35.14 ± 19.01	12.84	0.0001
Herbs (%)	8.01 ± 7.01	5.98 ± 4.47	0.95	0.2192
Bare Soil (%)	18.01 ± 18.44	47.39 ± 24.52	19.38	0.0001
Soil				
pH	5.62 ± 0.95	5.28 ± 0.38	6.18	0.0013
P (g/m^3)	4.32 ± 2.08	5.84 ± 4.09	2.43	0.0315
K (g/m^3)	17.30 ± 10.58	22.95 ± 9.28	3.75	0.0033
Ca (g/m^3)	146.66 ± 383.06	106.66 ± 77.07	0.14	0.9701
Mg (g/m^3)	33.61 ± 33.26	44.95 ± 26.34	1.08	0.1801

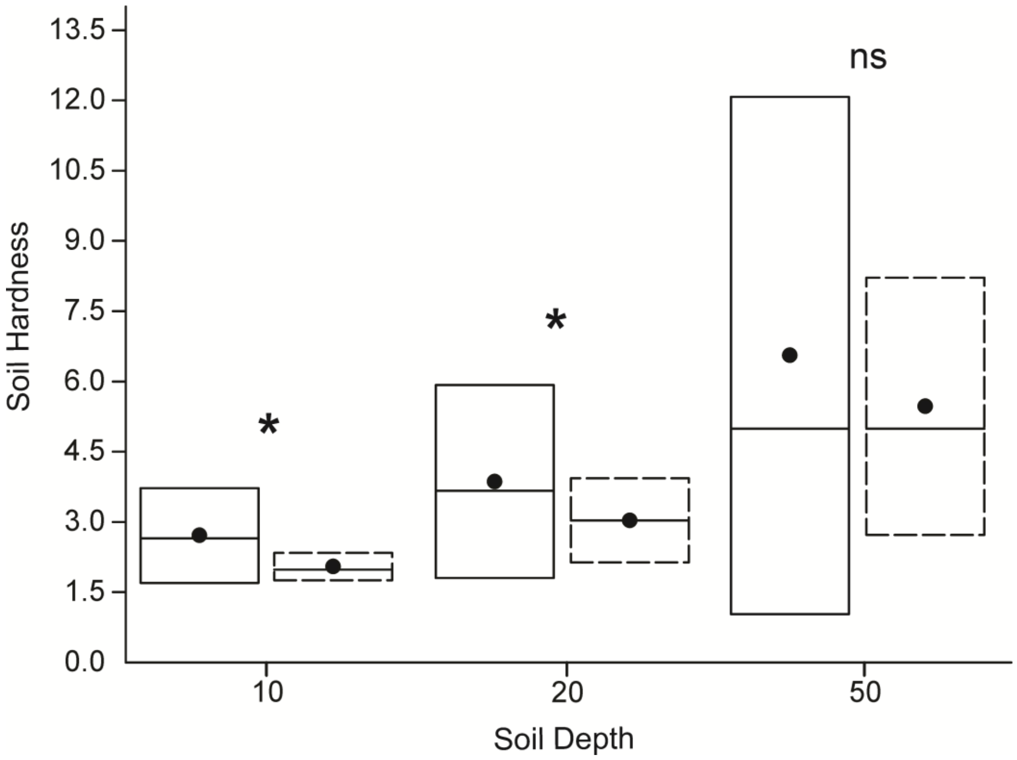

Fig. 1 Soil hardness (kg/cm^2) at three different depths (cm) from sites without (solid boxes) and with (dashed boxes) individuals of *Ctenomys minutus*. Top and lower horizontal lines of boxes represent standard deviations. Black points and the center horizontal lines represent the mean and the median, respectively. The results of the statistical tests are displayed in the text. Asterisk indicates a significant difference and "ns" non-significant.

CAPÍTULO IV

Manuscrito em preparação

Wet soils affect habitat selection of a solitary subterranean rodent in the Neotropical region

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Abstract

Subterranean rodents are characterized by limited individual mobility and a patchy distribution of local populations. Therefore, certain habitat patches where the species is considered to be absent may have been patches of unoccupied but suitable habitat or a patch in which some habitat features make impossible for individuals to inhabit. In the coastal plain of southern Brazil the species *Ctenomys minutus* inhabits sandy fields and dunes of the region, and its distribution is strongly influenced by altitude, soil and vegetation. The aim of our study was to analyze the relationship between a subterranean rodent and its environment through a comparison of environmental features between habitats present in the distribution of the species. Our results showed that the patch occupancy of *C. minutus* is clearly determined by the soil moisture and vegetation. Although no obvious differences in vegetation and topography were apparent between sampled areas, these two habitat features seems to define the presence of populations in the areas they inhabit. The species *C. minutus* had its habitat selection guided by soil moisture and by vegetation (food supply), but probably there might be other habitat features that regulate the habitat choice of this mammal when choosing for an area. It is evident that the environment and the habitat discontinuities in the coastal plain of southern Brazil (habitat structure is rather heterogeneous and provides numerous effective potential barriers to dispersal) are responsible to maintain the individuals in their natal areas. In contrast, the intraspecific competition within each population might consequently increase, as well as inbreeding.

Keywords: coastal grassland, *Ctenomys minutus*, habitat availability, habitat use, soil properties.

Introduction

The distribution of critical resources, including suitable habitats and food supply, is thought to be an important factor favoring small mammal species, including subterranean rodents (Jarvis et al., 1998; Tammone et al., 2012; Galiano et al., 2014). When resources such as suitable habitats or food supply are patchily distributed, habitat use typically does not occur at random, since animals preferentially occupy patches where resources are available (Garshelis, 2000). To understand habitat selection, it is critical to analyze the different factors that shape the habitat of a given species.

Subterranean rodents play an important role in ecosystem dynamics, as they possess the ability to directly or indirectly modify surface, nutrient dynamics of soils, and the composition and abundance of vegetation (Malizia et al., 2000; Campos et al., 2001; Reichman and Seabloom, 2002; Lara et al., 2007; Hagenah and Bennett, 2013; Galiano et al., 2014). At the same time as these species modify their environment, some habitat features, e.g. soil and vegetation characteristics, influence their habitat choice. Most subterranean rodent species live in deep, soft and well-drained soils (Cameron et al., 1988; Heth, 1989).

The most specious of all subterranean mammals are the South American tuco-tucos (*Ctenomys*), comprising approximately 60 species (Woods and Kilpatrick, 2005; Parada et al., 2011) that are distributed from southern Peru to Tierra del Fuego in Argentina, and from the Andes to southeastern Brazil (Cook et al., 1990; Pearson et al., 1968; Reig et al., 1990). As in other subterranean rodents, tuco-tucos show disjunts areas of distribution in favorable habitats (Mirol et al., 2010). These rodents are characterized by limited individual mobility and a patchy distribution of local populations, and generally defend multipurpose territories in which breeding and foraging take place

(Lacey et al., 2000). Habitat patches where the species is absent may either be suitable, but currently unoccupied, or be characterized by some habitat features that make it impossible for individuals of the species to inhabit it.

In the coastal plain of southern Brazil, the species with the widest geographic distribution is *Ctenomys minutus* Nehring, 1887, which inhabits grasslands on sandy soils and dunes of the region. According to Galiano et al. (2014) the habitat of sandy grasslands found along the distribution area of this species have distinct levels of environmental suitability, and the distribution patterns of *C. minutus* are strongly influenced by altitude, soil and vegetation. The authors also sustain that highly suitable areas are found along the entire distribution of the species associated with these environmental characteristics. Understanding these habitat requirements has implications for numerous aspects on the process of a species' habitat selection, especially in solitary species, like *C. minutus* that show a territorial behaviour in complex landscapes that can be described as more or less continuous habitats, irregularly interrupted by discontinuities (Galiano et al., 2014).

In this context, the aim of our study was to analyze the relationship between a subterranean rodent (*C. minutus*) and its environment through a comparison of environmental features between habitats present in the distribution of the species. The evaluation of habitat features of different sandy grasslands can give important insights into habitat selection processes of the species, and thus contribute to understanding of ecological and biodiversity patterns of the entire region.

Material and methods

Study area

The study was conducted between October 2012 and February 2013 in the coastal plain of southern Brazil. We sampled 13 sites along the entire distribution of *C. minutus*: four sites at the north limit of the species distribution, at Santa Catarina state (SC1: 28º49'S, 49º15'W; SC2: 28º55'S, 49º22'W; SC3: 28º59'S, 49º35'W; SC4: 29º18'S, 49º44'W); five sites at the central region, at the north littoral of Rio Grande do Sul state (LN1: 29º251S, 49º49'W; LN2: 29º57'S, 50º13'W; LN3: 30º08'S, 50º14'W; LN4: 30º15'S, 50º28'W; LN5: 30º27'S, 50º29'W); and four sites at the south limits, at the medium littoral of Rio Grande do Sul state (LM1: 31º06'S, 50º55'W; LM2: 31º17'S, 51º06'W; LM3: 31º32'S, 51º19'W; LM4: 31º38'S, 51º25'W) (Figure 1). Climate is mild mesothermal, wet, with no dry season. Average annual temperature ranges between 16 and 20 ºC. The annual rainfall varies between 1.000 and 1.500 mm (Nimer, 1977; Vieira and Rangel, 1988). The region is a mosaic of several lakes, lagoons, dunes and grasslands, and its geomorphology is continuously influenced by the fluctuations of the Atlantic Ocean (Tomazelli et al., 2000). The vegetation in the region consists of mosaics of sand dunes, coastal grasslands and 'restinga' forests (Hesp et al., 2009). Although the coastal plain is characterized by relative altitudinal uniformity, small topographic variations can determine different pedogenic processes, and consequently a peculiar mosaic of soil types are found (Waechter, 1990).

Sampling methodology

To evaluate which habitat features regulate the presence of *C. minutus* at the sites they inhabit, we sampled 13 sites (100 ×100m) along the distribution of this species. We categorized these sites into three different abundance classes according to the estimated number of individuals present in each area: (a) Absence of individuals (n=4); (b) ≤ 20 individuals (n=5); and (c) > 20 individuals (n=4). Abundances were estimated by counting the number of fresh mounds at each site. Groups of mounds with distances below 10 meters were considered to be of the same individual.

We sampled three characteristics of the vegetation layer (i) VEG: vegetation species composition; (ii) VH: average vegetation height; and (iii) BS: proportion of bare soil; and four edaphic variables (iv) HARD: soil hardness; (v) GRAN: soil granulometry; (vi) SM: soil moisture; and (vii) SOIL: soil chemical composition (Tables A.1 and A.2). For vegetation, sampling was performed during southern hemisphere spring and summer, when a large percentage of the plants are flowering. Species composition data was obtained in 15 plots of one square meter per site, distributed randomly. In each plot, all vascular plant species were identified and had their cover estimated by Londo’s decimal scale (Londo, 1976). Plant samples were collected for posterior taxonomic identification whenever necessary. Classification of species into families followed the APG III (2009); nomenclature of species follows Boyle et al., (2013). To compose the variable VEG, we considered only the 18 plant species which presented more than one per cent of relative cover among all sampled areas.

For characterization of soil hardness (HARD), we used an impact soil penetrometer, Model IAA / Planalsucar (Stolf, 1991). At each site we made 30 measurements (10 m apart) along three 100 m transects, recording the number of strokes necessary to penetrate into a 50 cm soil depth.

Furthermore, twenty soil samples were collected randomly at each site at 0-20 cm depth, and then homogenized in a plastic recipient where a sample representative of the entire site was taken. From this sample, the variables SM, SOIL and GRAN were determined. To compose the variable SOIL, we considered the soil pH, organic matter and concentrations of the nutrients phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg). Phosphorus and potassium were determined by Mehlich-1 method and calcium and magnesium were extracted with potassium chloride (KCl, 1mol/L), and organic matter by wet digestion (%) (Mehlich, 1953; Embrapa, 1997). To characterize the variable GRAN, a representative 100 g subsample from each soil sample was vibrated through a series of nine sieves for a period of five minutes. Individual sieve contents were weighed and the relative proportions of the sample were calculated, thereby providing the particle size distribution of each sample. We used Shannon's heterogeneity index (H), which accounts for both abundance and evenness of the particle sizes as a measure representing the soil granulometry of each site (Smith and Smith, 2003; Jackson et al., 2008). A low Shannon's index value indicates that the majority of soil particles are of a similar size.

Data analysis

For statistical analysis, we summarized the variables VEG and SOIL of each area using a Principal Components Analysis (PCA) for each variable (Legendre and Legendre, 1998). In case of SOIL, the variables were on different scales and therefore we standardized them by their ranges. For both variables, the first axis of the PCA’s (PC1) was used as a variable in the subsequent analysis. We chose to exclude the PC2 and other axis because their explanations were less relevant. To define a gradient of the 13 sampled areas based on environmental conditions, we performed a PCA analysis based on a matrix using these seven habitat variables: VEG, VH, BS, HARD, GRAN, SM and SOIL. We also employed a Multivariate Analysis of Variance based on distances (db-Manova) to test the differences between areas with (n=4) and without the presence of individuals (n=9), not considering abundance classes (Warton and Hudson, 2004). The test assesses whether squared distances between-groups (i.e. from the group centroids to the overall centroid) are larger than squared distances within-groups (i.e. from the observations to group centroids). In our case, groups were constituted by two types of areas (absence and presence of individuals). The tests were performed with matrices based on Euclidean distances, and we ran 9999 permutations.

We also tested the association between the abundance of individuals and specific plant species. We performed linear regressions between the abundance classes of the animals and the relative cover of each plant species. All analyses were conducted in the R program for statistical computing (R Development Core Team, 2012) with the vegan package (Oksanen et al., 2014).

Results

Results of the PCA for habitat characterization and description showed that the two first axes explain 91% of the total variability. Soil moisture loaded positively on PC1 comprising 78% of the variability. Vegetation loaded positively on PC2, which explained 13% of the variance. In Table 1, correlation coefficients for each of the first two PC vectors.

The significance of these differences in environmental variables to discrimination among the areas with and without the presence of *C. minutus* is evident from the bivariate PC plots (Figure 2). One group is formed by areas without the presence of individuals, and it is characterized by the highest soil moisture. A second group is formed by areas with the presence of *C. minutus*, independently of the abundance of individuals, having lower soil moisture. Therefore a sharp delimitation of areas selected by *C. minutus* majorly driven by soil moisture seems to exist. Soil chemical and physical content (hardness and granulometry) seem not to influence individuals in coastal grasslands when choosing for areas. The db-Manova supported the main finding of a difference between areas selected or not by *C. minutus* pointed out in the PCA analysis (db- Manova: F = 24.79, p = 0.001).

The results of the regressions showed that from all the 18 plant species analyzed, only *Centella asiatica* and *Kyllinga odorata* were negatively correlated with the abundance of this tuco-tuco (F=9.28; p=0.01 ; F=5.82; p=0.03, respectively). The main plant species present along the distribution of *C.minutus* are shown in Table 2.

Discussion

The feedback of organisms and their environment is of great relevance to the understanding of habitat selection among mammals. Our results showed that the patch occupancy of *C. minutus* is clearly determined by the soil moisture. Although no obvious differences in vegetation and topography were apparent between sampled areas, these two habitat features seems to define the presence of populations in the areas they inhabit, while other variables measured here might be important for the species related to other ecological requirements.

Although we did not measured dispersion of individuals, it is very widespread that ctenomids present a limited mobility and ability of dispersion (Lacey et al., 2000). Obviously, a fundamental role of locomotory constraints is important in determining habitat selection by this species, as also suggested for other small mammals (Holbrook, 1979). In turn, the scale at which an individual can perceive habitat heterogeneity depends on its ability to move in the environment (Boulinier et al., 2008), which in the case of *C. minutus* presumably is low. Thus, it is possible that juveniles looking for territories or even individuals that disperse occasionally, could find difficulty in occupying and establishing in new areas far away from their natal group, because the grasslands present in the coastal plain of southern Brazil present several habitat discontinuities for *C. minutus* (such as swamps, flooded areas and other typical intergrasslands habitats) which may have a significant impact on the establishment of individuals. Therefore, individuals may benefit by being in their natal group. This is reflected by the low genetic diversity found between populations of *C. minutus*, which is maintained by the effects of low levels of gene flow hamper the gene exchange between the subpopulations (Lopes et al., 2013).

Moreover, group living is believed to occur when ecological features make it unlikely that individuals dispersing from their natal area can establish themselves elsewhere in the environment (Emlen, 1982; Lacey and Wieczorek, 2003; Solomon, 2003). Numerous factors may lower the probability of successfully establishing residence in new habitats for solitary animals (Lacey and Wieczorek, 2003). Also, the spatial distribution of suitable settlement sites and the nature of the intervening habitat may significantly affect dispersal success (Lacey and Wieczorek, 2003; Solomon, 2003). According to Galiano et al. (2014), several high suitable areas are present along the distribution of *C. minutus*, but these areas are geographically limited by the presence of wetlands and geographical inaccessibility. As a result, the areas that we found absence of individuals could be suitable areas for this species to inhabit, because they have similar ecological conditions and food resources as the populated fields, with exception of higher soil moisture. Moreover, our results suggest that both above and belowground habitat features play important roles in the occurrence of fossorial rodents. Above ground use could be driven by food supply or protection provided by vegetation, in our case vegetation cover (Busch et al., 2000; Albanese et al., 2010).

Regarding the vegetation cover, we found that habitat patches which present high cover of *Centella asiatica* and *Kyllinga odorata*, two plant species negatively correlated with the abundance of *C. minutus*, might be areas that individuals would avoid to inhabit or colonize. However, this avoidance is probably not related to the presence of these plants themselves. In the case of *C. asiatica*, the negative correlation is probably related to the fact that the species is abundant in recently disturbed areas (Mathias et al., 1972). The species *K. odorata* belongs to the family Cyperaceae, which is quite common and represented by several species in the coastal plain (Boldrini, 2009), and whose species are known to establishing well in humid areas (Simpson et al., 2011), which according to our data is the determinant environmental condition for the absence of *C. minutus*. However, according to Lopes et al. (2015), plants of this family are not a part of the diet of *C. minutus*, and consequently habitat patches with high abundance of this plant might be not preferable by the species. *Ctenomys minutus* consume most frequently plants of the families Poaceae and Asteraceae (Lopes et al. 2015), which are the most abundant vegetation families in our study sites (72%), and also very abundant in the southern Brazilian coastal plain, figuring among the families with the highest species richness in the region (Palma and Jarenkow, 2008; Boldrini, 2009; Filho et al., 2013).

In conclusion, the species *C. minutus* had its habitat selection guided by soil moisture and by vegetation (food supply or protection against predators), but probably there might be other habitat features that regulate the habitat choice of this mammal when choosing for an area. It is evident that the environment and the habitat discontinuities in the coastal plain of southern Brazil (habitat structure is rather heterogeneous and provides numerous effective potential barriers to dispersal) is responsible to maintain the individuals in their natal areas, which is reflected by the low success of migrators (Lopes et al., 2013). In contrast, the intraspecific competition within each population might consequently increase, as well as inbreeding. Additionally, we must consider that habitat selection results from multiple choices made by individuals during their activities including foraging, escape from predators, mate searching, and refuge use (Holbrook, 1979; Garshelis, 2000). In an ideal scenario, studies with estimates of all these activities to accurately determine patterns of habitat selection should be conducted.

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Table 1. Correlation coefficients for environmental variables and the first two principal components (PC) axes. VEG: vegetation species composition; BS: proportion of bare soil; VH: vegetation height average; SOIL: soil composition; HARD: soil hardness; SM: soil moisture; GRAN: soil granulometry.

Variable	PC 1	PC 2
VEG	0.210	0.940
BS	-0.088	0.030
VH	0.038	0.254
SOIL	0.023	-0.073
HARD	0.075	-0.025
SM	0.969	-0.207
GRAN	-0.009	0.011

Table 2. Relative cover of plant species present along the distribution of *C. minutus*. The results of linear regressions tests for correlations between abundance of individuals and each plant species are displayed.

Family	Species	Relative cover (%)	R^2	F	*p* level
Poaceae	*Axonopus* aff. *affinis* Chase	31.38	0.09	1.17	0.301
Poaceae	*Paspalum notatum* Fluegge	10.21	0.01	0.08	0.781
Poaceae	*Andropogon lateralis* Nees	7.76	0.19	2.61	0.135
Poaceae	*Paspalum pumilum* Nees	3.64	0.10	1.24	0.290
Poaceae	*Paspalum arenarium* Schrad.	3.20	0.22	3.01	0.110
Poaceae	*Paspalum lepton* Schult.	3.00	0.07	0.82	0.386
Poaceae	*Panicum brizanthum* Hochst. ex A. Rich.	2.74	0.02	0.20	0.661
Apiaceae	*Centella asiatica* (L.) Urb.	2.27	0.46	9.29	0.011
Poaceae	*Eragrostis cataclasta* Nicora	2.06	0.02	0.27	0.615
Poaceae	*Ischaemum minus* J. Presl	1.91	0.01	0.09	0.770
Poaceae	*Dichanthelium sabulorum* (Lam.) Gould & C.A. Clark	1.61	0.22	3.18	0.102
Fabaceae	*Desmodium incanum* DC.	1.21	0.09	1.06	0.325
Poaceae	*Stenotaphrum secundatum* (Walter) Kuntze	1.20	0.00	0.01	0.979
Euphorbiaceae	*Croton lanatus* Lam.	1.19	0.18	2.47	0.144
Cyperaceae	*Kyllinga odorata* Vahl	1.13	0.35	5.82	0.034
Poaceae	*Sorghastrum setosum* (Griseb.) Hitchc.	1.10	0.23	3.32	0.096
Cyperaceae	*Rhynchospora tenuis* Link	1.09	0.12	1.55	0.239
Asteraceae	*Vernonanthura nudiflora* (Less.) H.Rob.	1.08	0.18	2.44	0.147

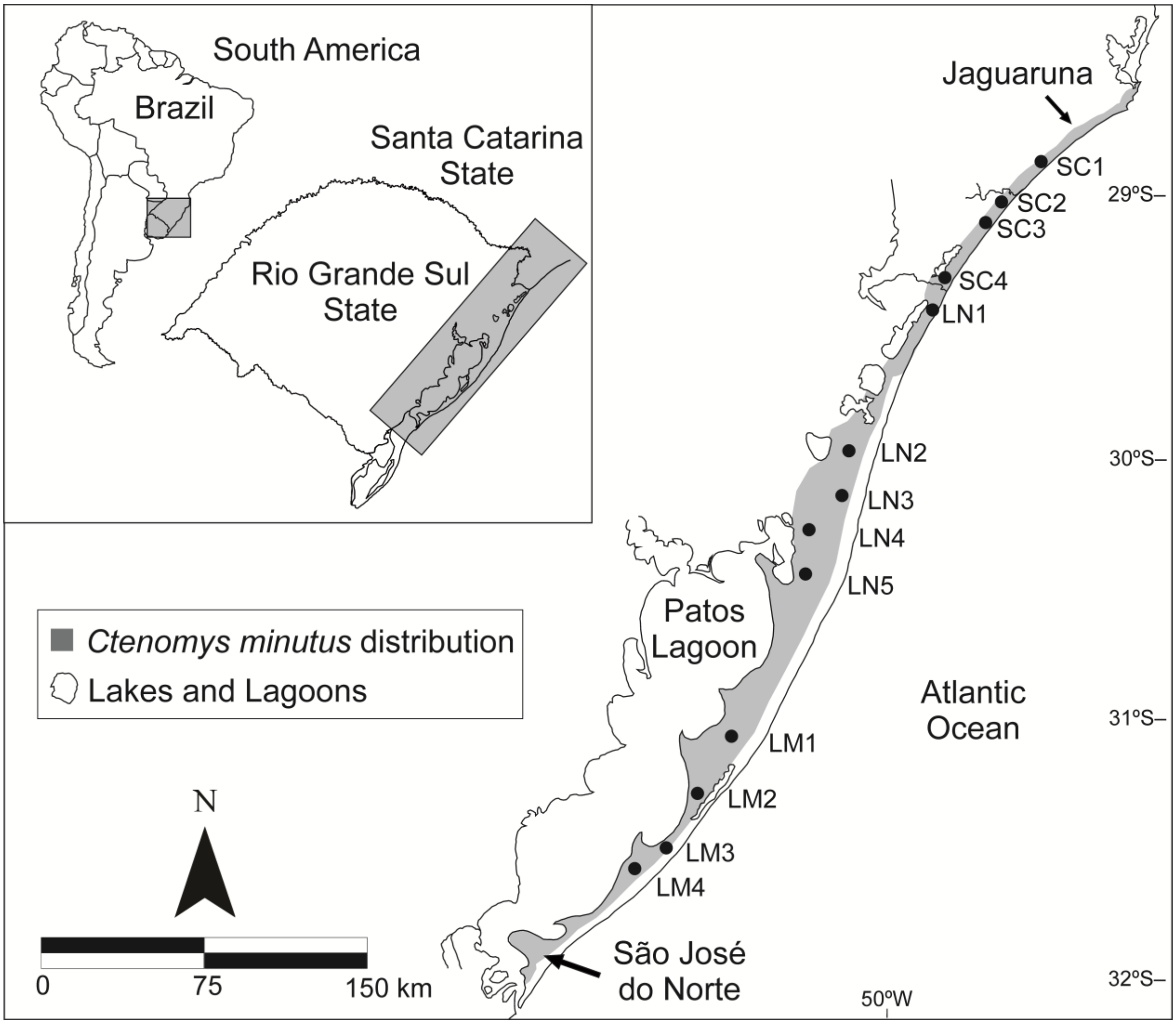

Figure 1: Coastal plain of southern Brazil with geographic details. Points (●) represent the sampled areas. SC, Santa Cataria state areas; LN, north littoral of Rio Grande do Sul state areas; LM, medium littoral of Rio Grande do Sul state areas.

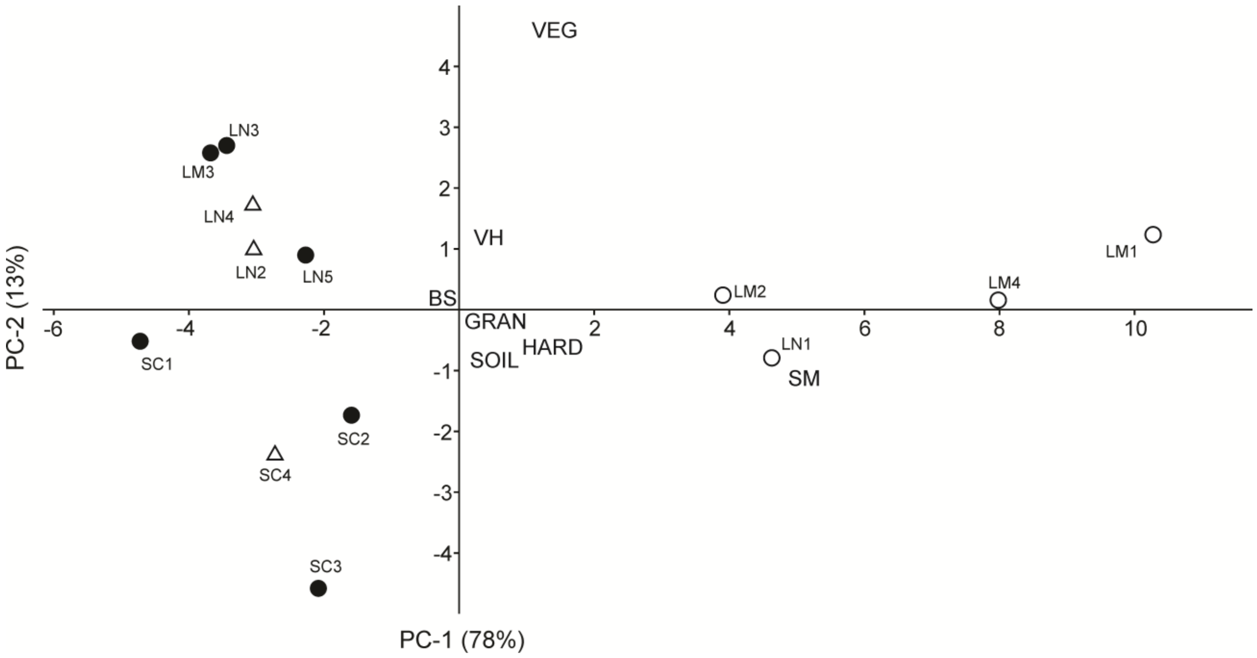

Figure 2: Principal Components Analysis (PCA) of sampled sites with zero individuals of *Ctenomys minutus* (open circles), ≤ 20 individuals (filled circles) and > 20 individuals (open triangles).

Supplementary information

Table A.1. Edaphic variables from each sampled area (SC: Santa Catarina areas; LN: north littoral of Rio Grande do Sul state areas; and LM: medium littoral of Rio Grande do Sul state areas). HARD: soil hardness (kg/cm²); GRAN: soil granulometry (represented by Shannon's heterogeneity index); SM: soil moisture (%); and SOIL: soil chemical composition (pH, Organic matter (%), P - phosphorus (mg/dm^3), K - potassium (mg/dm^3), Ca - calcium (cmolc/dm^3), Mg - magnesium (cmolc/dm^3)).

	HARD	GRAN	SM	SOIL					
				pH	OM	P	K	Ca	Mg
SC1	3.14	1.38	2.0	5.0	1.1	3.3	21	0.5	0.2
SC2	3.97	1.06	5.0	5.0	1.1	16	34	0.5	0.2
SC3	4.59	1.36	5.0	5.1	1.4	3.5	45	1.2	0.7
SC4	2.44	0.92	4.3	5.2	1.0	4.3	23	0.4	0.2
LN1	3.88	1.13	11	5.2	2.3	5.5	19	0.8	0.7
LN2	3.47	1.29	4.0	4.9	0.9	4.0	30	0.5	0.3
LN3	3.30	1.15	3.0	5.0	1.0	3.9	19	0.3	0.2
LN4	3.06	1.34	2.0	5.3	1.0	3.2	46	0.9	0.5
LN5	3.44	1.20	3.0	5.0	0.5	3.4	15	0.2	0.1
LM1	4.45	1.27	16	4.7	2.5	7.0	37	0.4	0.3
LM2	4.94	1.12	10	4.9	1.6	2.9	17	0.5	0.3
LM3	4.39	1.18	2.0	5.1	0.9	7.8	20	0.5	0.3
LM4	4.03	0.93	14	5.1	1.4	22	24	0.9	0.3

Table A.2. Characteristics of the vegetation layer from each sampled area (SC: Santa Catarina areas; LN: north littoral of Rio Grande do Sul state areas; and LM: medium littoral of Rio Grande do Sul state areas). VEG: vegetation species composition; VH: average vegetation height (cm); and BS: proportion of bare soil (%).

VEG	SC1	SC2	SC3	SC4	LN1	LN2	LN3	LN4	LN5	LM1	LM2	LM3	LM4	Relative cover
Axonopus aff. *affinis* Chase	0.00	0.09	0.00	0.04	2.09	2.61	2.69	3.21	3.87	5.40	3.60	4.20	4.53	31.38
Paspalum notatum Fluegge	0.15	0.58	5.33	2.05	0.00	0.03	0.41	0.13	0.22	0.06	0.65	0.02	0.88	10.21
Andropogon lateralis Nees	0.00	0.00	0.00	0.00	1.11	0.07	0.00	1.71	0.04	0.67	1.55	1.37	1.51	7.77
Paspalum pumilum Nees	0.00	0.43	1.34	0.00	1.35	0.07	0.00	0.00	0.00	0.02	0.04	0.39	0.12	3.65
Paspalum arenarium Schrad.	0.73	0.38	0.06	1.45	0.05	0.13	0.03	0.09	0.39	0.00	0.00	0.00	0.00	3.20
Paspalum lepton Schult.	0.10	0.77	0.00	0.01	0.00	0.49	1.09	0.15	0.03	0.28	0.04	0.09	0.07	3.01
Panicum brizanthum Hochst. ex A. Rich.	0.87	0.00	0.00	0.00	1.26	0.00	0.69	0.00	0.00	0.00	0.00	0.00	0.00	2.74
Centella asiatica (L.) Urb.	0.00	0.08	0.00	0.07	0.39	0.21	0.20	0.05	0.03	0.28	0.61	0.19	0.24	2.28
Eragrostis cataclasta Nicora	0.00	0.61	0.00	0.48	0.52	0.01	0.02	0.00	0.06	0.04	0.01	0.03	0.35	2.06
Ischaemum minus J. Presl	0.00	1.34	0.03	0.00	0.02	0.00	0.00	0.00	0.00	0.27	0.00	0.28	0.04	1.91
Dichanthelium sabulorum (Lam.) Gould & C.A. Clark	0.05	0.07	0.03	0.60	0.09	0.07	0.37	0.11	0.01	0.01	0.11	0.05	0.11	1.62
Desmodium incanum DC.	0.00	0.09	0.11	0.09	0.00	0.05	0.16	0.04	0.00	0.53	0.05	0.00	0.13	1.22
Stenotaphrum secundatum (Walter) Kuntze	0.00	1.21	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00	1.20
Croton lanatus Lam.	0.00	0.00	0.00	0.00	0.00	0.33	0.17	0.73	0.00	0.00	0.00	0.00	0.00	1.20
Kyllinga odorata Vahl	0.01	0.19	0.19	0.03	0.06	0.00	0.02	0.00	0.05	0.11	0.17	0.07	0.27	1.14
Sorghastrum setosum (Griseb.) Hitchc.	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.01	0.00	0.83	0.25	0.00	0.03	1.11
Rhynchospora tenuis Link	0.00	0.19	0.00	0.00	0.51	0.05	0.05	0.00	0.01	0.04	0.03	0.24	0.00	1.09
Vernonanthura nudiflora (Less.) H.Rob.	0.00	0.00	0.01	0.00	0.00	0.03	0.15	0.89	0.01	0.02	0.00	0.01	0.00	1.08
VH	7.27	5.97	4.32	5.48	6.94	5.99	6.15	8.53	4.60	7.93	5.05	5.47	5.40	-
BS	3.20	0.96	0.67	3.13	1.03	3.01	1.01	0.49	3.09	0.92	1.80	1.87	0.89	-

CONSIDERAÇÕES FINAIS

O trabalho apresentado traz dados inéditos e fornece uma contribuição significativa ao conhecimento da ecologia de *Ctenomys minutus*, uma espécie de roedor subterrâneo endêmica da planície costeira do Sul do Brasil, muito estudada do ponto de vista evolutivo e molecular, mas negligenciada quanto a aspectos ecológicos básicos. Os dados obtidos ampliaram o conhecimento ecológico da espécie, no que se refere à ecologia espacial, seleção de habitat, conservação e consequências da atividade deste roedor no ambiente em que habita. As principais conclusões resultantes deste trabalho são:

- Os modelos de distribuição gerados para as espécies *C. minutus* e *C. lami* obtiveram sucesso ao identificar áreas com alta adequabilidade ambiental. O modelo de máxima entropia (Maxent) indicou que a distribuição de *C. minutus* é restrita a planície costeira do Sul do Brasil, sendo que existem áreas de alta adequabilidade ambiental ao longo da primeira linha de dunas e nos campos arenosos ao longo de toda a distribuição da espécie. A distribuição potencial de *C. lami* demostrou-se limitada à região da Coxilha das Lombas, como confirmam as observações de campo;

- O modelo de máxima entropia da espécie *C. minutus* indica não haver uma descontinuidade no potencial de ocorrência da espécie entre os ambientes de duna e campos arenosos;

- A espécie *C. minutus* apresentou uma plasticidade ambiental maior que *C. lami*. Na região onde estas espécies apresentam uma zona de hibridação (margem oeste da

Lagoa dos Barros), as duas espécies apresentam uma probabilidade mediana de ocorrência, sendo esta região adequada para ambas;

- As variáveis que determinam a distribuição potencial de *C. minutus* na planície costeira são o tipo de solo, tipo de vegetação e a altitude;

- A espécie *C. lami* apresentou uma alta adequabilidade ambiental para os quatro blocos cariotípicos propostos por Freitas (2007). Ambientalmente, parece não existir diferenças entre estes blocos cariotípicos;

- Para a espécie *C. minutus*, os sete haplogrupos determinados por Lopes et al. (2013) foram associados com áreas de alta adequabilidade ambiental. Esse padrão parece indicar que maiores valores de diversidade genética estão associados a áreas de maior adequabilidade ambiental. Apesar disso, o índice *Fs* de Fu, que determina expansão ou retração populacional, foi estatisticamente significativo apenas para o haplogrupo Lagoa dos Barros, não sendo possível confirmar a hipótese esperada, onde áreas com maior adequabilidade ambiental poderiam favorecer a expansão populacional;

- Ambas as espécies estudadas neste trabalho, além do alto grau de endemismo, sofrem intensa pressão de fragmentação e perda de habitat, o que é um grande agravante do ponto de vista da conservação, tendo em vista que nossos resultados indicam que ambas são extremamente nicho-específicas. Para a espécie *C. lami*, as principais ameaças são a urbanização e a ocupação humana, associadas a atividades agrícolas, enquanto para *C. minutus* a especulação imobiliária e fragmentação do ambiente de dunas ao norte da distribuição da espécie, e a silvicultura de *Pinus* sp. ao sul, são os principais agravantes;

- A utilização de índices de variabilidade genética associados à adequabilidade ambiental suporta a utilização destas duas técnicas concatenadas para o planejamento de ações de conservação;

- Apesar da atual ampla utilização de marcadores moleculares para estimar a variação genética em diferentes escalas geográficas e originários de diferentes processos evolutivos, ainda é difícil combinar esses dados com parâmetros ecológicos em escala ampla, sendo necessário testar mecanismos alternativos, tais como a fragmentação e os efeitos antrópicos sobre a distribuição das espécies, através da integração de dados genéticos e demográficos em um contexto ecológico;

- A presença de *C. minutus* afeta a biomassa de plantas, cobertura de gramíneas, proporção de solo exposto, dureza do solo e as concentrações de nutrientes nos campos onde habita. A biomassa de plantas e a cobertura de gramíneas são significativamente inferiores nos locais habitados pelos indivíduos, assim como a proporção de solo exposto é maior. O solo se torna menos compacto nos perfis superficiais (10 e 20 cm), mas não em profundidades maiores (50 cm) nos mesmos locais onde os indivíduos estão presentes. Em relação à disponibilidade de nutrientes no solo, os locais habitados por *C. minutus* apresentam maior disponibilidade de P e K, assim como um pH significativamente menor. Todos estes efeitos observados são uma consequência das atividades de forrageio e escavação dos animais.

Consequentemente, os locais onde os animais estão presentes sofrem um efeito significativo na dinâmica e composição da vegetação, assim como nas propriedades do solo, o que vai afetar diretamente processos ecossistêmicos, como decomposição e produtividade;

- A seleção de habitat de *Ctenomys minutus* é determinada principalmente com base em duas variáveis: umidade do solo e vegetação (disponibilidade de recursos ou proteção). Apesar de não haver diferenças óbvias na vegetação e topografia entre as áreas onde a espécie está presente ou ausente, estas duas características ambientais parecem definir a presença de populações quando selecionam as áreas que habitam;

- As descontinuidades de habitat da planície costeira do Sul do Brasil (a estrutura do habitat é bastante heterogênea e proporciona inúmeras barreiras potenciais efetivas para a dispersão de *C. minutus*) é um dos fatores responsáveis por manter os indivíduos em suas áreas natais. Consequentemente, a competição intra-específica dentro de cada população deve aumentar, assim como a endogamia;

- Ao longo da planície costeira, áreas que apresentam uma alta porcentagem das espécies *Centella asiatica* e *Kyllinga odorata* na cobertura da vegetação, parecem ser evitadas por *C. minutus*. Porém, este fato não parece estar relacionado diretamente com as espécies vegetais em si, mas ao tipo de ambiente ocupado pela primeira espécie, e a não utilização como recurso alimentar da segunda (*Ce. asiatica* é característica de ambientes úmidos, evitados por *C. minutus*, e *K. odorata* pertence à família Cyperaceae, ausente na dieta da espécie);

- As características ambientais, tanto superficiais quanto subterrâneas, desempenham um papel importante na ocorrência deste roedor subterrâneo. Acima da superfície do solo, um fator determinante é a oferta de recursos alimentares e a proteção contra a predação, que no caso do presente estudo foi caracterizado pela cobertura das espécies vegetais.

Anexo I

Manuscrito publicado no periódico PLOS ONE

Genetic Pool Information Reflects Highly Suitable Areas: The Case of Two Parapatric Endangered Species of Tuco-tucos (Rodentia: Ctenomiydae)

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Abstract

Conservation of small mammals requires knowledge of the genetically and ecologically meaningful spatial scales at which species respond to habitat modifications. Conservation strategies can be improved through the use of ecological niche models and genetic data to classify areas of high environmental suitability. In this study, we applied a Maxent model integrated with genetic information (nucleotide diversity, haplotype diversity and Fu's *Fs* neutrality tests) to evaluate potential genetic pool populations with highly suitable areas for two parapatric endangered species of tuco-tucos (*Ctenomys minutus* and *C. lami*). Our results demonstrated that both species were largely influenced by vegetation and soil variables at a landscape scale and inhabit a highly specific niche. *Ctenomys minutus* was also influenced by the variable altitude; the species was associated with low altitudes (sea level). Our model of genetic data associated with environmental suitability indicate that the genetic pool data were associated with highly suitable areas for *C. minutus*. This pattern was not evident for *C. lami*, but this outcome could be a consequence of the restricted range of the species. The preservation of species requires not only detailed knowledge of their natural history and genetic structure but also information on the availability of suitable areas where species can survive, and such knowledge can aid significantly in conservation planning. This finding reinforces the use of these two techniques for planning conservation actions.

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Introduction

Subterranean rodents of the genus *Ctenomys* inhabit the southern part of the Neotropical region from the extreme south up to southern Peru, including the entire Patagonian region and showing a wide latitudinal variation. The range of the genus extends from sea level to 4,000 m in the Andean region [1]. These small subterranean herbivores are among the most geographically variable mammals and are also the most speciose of all subterranean rodent groups [2]. Presently, the genus includes approximately 60 species [2]. South American tuco-tucos (*Ctenomys*) have attracted special interest in terms of speciation and evolution by virtue of their patchy distributions, low vagility, territoriality, and extensive karyotypic variation [1].

In the coastal plain of southern Brazil, the species with the widest geographic distribution is *Ctenomys minutus*, which inhabits sandy fields and dunes and has a range extending from Jaguaruna Beach in the state of Santa Catarina to the town of São José do Norte in the state of Rio Grande do Sul. *Ctenomys lami* is an endemic species inhabiting a sandy region named 'Coxilha das Lombas' along a narrow line of old dunes that extends from north of Guaiba Lake to the northwestern sandbanks of Barros Lake [3,4]. A hybrid zone between *C. minutus* and *C. lami* is also present. It is probable that this zone was formed due to habitat alterations. A wide humid zone once separated both species and represented a barrier [5]. *Ctenomys lami* is cited as vulnerable in the Red List of the International Union for Conservation of Nature [6], and *C. minutus* is listed as data deficient. However, given the current state of information about tuco-tucos in Brazil, Fernandes et al. [7] have affirmed that the vulnerability of these subterranean rodents is greater than presently supposed and that any conservation effort should be based on consistent and detailed studies of habitat occupation.

Ecological niche models (ENMs), based on the use of multiple strategies in conservation plans, are an important tool for determining the distribution of threatened species for conservation purposes [8]. In particular cases of restricted distribution or sparse populations of rare or endangered species, the use of ENMs may be necessary to identify a set of areas for protection [9]. Functionally, ENMs could be considered to furnish a specification of the relationship between the points of occurrence of a species and a set of multivariate environmental data [10]. The need to base strategies of ENM development on niche theory facilitates the interpretation and discussion of the resulting models relative to conservation issues. Conservation of small mammals requires knowledge of the genetically and ecologically meaningful spatial scales at which species respond to habitat modification. Understanding this information is important because small mammals represent a major assemblage of species occupying the most varied environments [11]. Large-scale, presence-only models are vital to complement the information obtained by local studies. They reveal otherwise-overlooked ecological requirements by identifying the environmental parameters that influence species distributions on a broad geographical scale [12].

Ctenomys minutus and *C. lami* inhabit the coastal plain of southern Brazil. Several studies have addressed the degradation of this coastal environment, identifying both natural and human-induced factors contributing to long- and short-term change [13,14]. Anthropogenic changes, such as urbanization in active dune areas [14,15], have modified the natural landscape over the years. As a result of human activity, native habitats have become increasingly fragmented or destroyed, changing the patterns of gene flow between populations and modifying the levels of genetic diversity. In our particular case, conservation strategies can be improved with the use of ENMs and genetic data to classify areas of high environmental suitability and to investigate how genetic characteristics diverge in different areas.

In this study, we applied ENMs integrated with genetic information to evaluate potential genetic pool populations with highly suitable potential areas. We developed maximum-entropy (Maxent, Phillips et al. [10]) presence-only distribution models for *C. lami* and *C. minutus* based on the following goals:

1. To conduct the first geographical distribution analysis for both species over their entire distributional range;
2. To determine which ecological factors may be limiting the distributions of the species;
3. To develop an integrated framework to evaluate geographic patterns of genetic diversity within potential genetic pools;
4. To discuss conservation issues in the light of this concatenated approach.

Methods

Study area

The study was conducted on the southern coast of Brazil. The environment consists of sand dunes and relict 'restinga' forests [16]. The landscape of the coastal plain of southern Brazil is characterized by lakes, lagoons, rivers, and dunes that represent natural geographical barriers for different populations of *Ctenomys* [4,17] (Figure 1).

Species records

Current knowledge about the distributions of *C. minutus* and *C. lami* is based on records from the collection of the Laboratório de Citogenética e Evolução of the Universidade Federal do Rio Grande do Sul and collected by researchers [3–5,18–21]. All of the coordinates were recorded using a GPS at the exact point of collection or observation. We considered a total of 74 records for *C. minutus* ($n = 45$) and *C. lami* ($n = 29$), each representing an established population of these tuco-tucos, to generate the ENMs (Figure S1).

Environmental data

We used 19 Worldclim bioclimatic variables obtained through interpolated data from derived rainfall and temperature, with a resolution of 2.5 arc-minutes (five kilometers); one altitude variable [22], available at http://www.worldclim.org/download; and two categorical variables for soil and vegetation composition [23] available at http://mapas.mma.gov.br/i3geo/datadownload.htm. We included those two categorical variables due to their recognized ecological importance to the study species and genus [24–27]. The vegetation and soil maps were converted to rasters at the same level of resolution as the bioclimatic variables using ArcMap v. 10.0. We generated a matrix with the values of each climatic variable for the entire study area. We performed a Principal Components Analysis (PCA) on this matrix to identify correlations between variables, selecting the axes that explained ≥ 95% of the correlation structure. From this result, we selected variables with the highest absolute coefficient in each axis. This procedure yielded seven variables for *C. minutus* (mean temperature of coldest quarter, precipitation seasonality, mean temperature of warmest quarter, mean diurnal range (mean of monthly (max temp - min temp)), soil, vegetation, and altitude) and five variables for *C. lami* (maximum temperature of warmest month, minimum temperature of coldest month, mean temperature of warmest quarter, soil, and vegetation). To avoid information loss, we included all variables represented in the PCAs from both species to generate models for the two species. The models were constructed with a total of nine variables because equal variables were selected for the two species.

Modeling

The models were constructed with Maxent version 3.3.3k [10], available at (http://www.cs.princeton.edu/~schapire/maxent/). Maxent estimates the ecological niche of a species by determining the distribution of maximum entropy (ME), subject to the constraint that the expected value of each environmental variable under this estimated distribution matches its empirical average. The technique was designed as a machine learning algorithm [10,28,29]. The default parameters in Maxent were used to construct the models: automatic features election, regularization multiplier at unity, maximum iterations 500 and convergence threshold 10^{-5}. We produced maps of the potential distribution of the species using the logistic output format [29]. This format is an attempt to ensure the closest possible approach to an estimate of the probability that the species is present given the environment [28]. We chose Maxent because it requires only presence data, because it can process categorical data and model interactions, and because it has performed favorably when compared with alternative approaches [30,31]. The models were validated by calculating the area under the curve (AUC) from a receiver operating characteristic curve (ROC). The relative importance of the variables was assessed with Maxent's built-in Jackknife functionality.

Genetic data

Our basic data consisted of mitochondrial DNA (mtDNA) sequences corresponding to the cytochrome oxidase I gene and the hypervariable control region. The data were obtained from Lopes et al. [20] for *C. minutus* and Lopes and Freitas [19] for *C. lami*, where a detailed description of the genetic data can be obtained. For *C. minutus*, a total of 30 localities were sampled across the entire distribution of the species, and seven principal clades were highlighted in the Bayesian phylogenetic tree. We focused on these seven main genetic haplogroups described by Lopes et al. [20] (North 1, North 2, Coast, Barros Lake, Mostardas, Tavares, and South; see Lopes et al. [20] for details). For *C. lami*, 28 locations were sampled, and the data are presented by the authors based on the four karyotypic blocks proposed for the species (Block A, B, C and D; see Freitas [4], Lopes and Freitas [19]). We chose to present our results for this concatenated approach (genetic data and modeling) based on the haplogroups for *C. minutus* and karyotipic blocks for *C. lami* since we did not directly compare these two different units, but discuss the results independently for each species. We believe that the use of this approach may not overestimate the interpretation of the results.

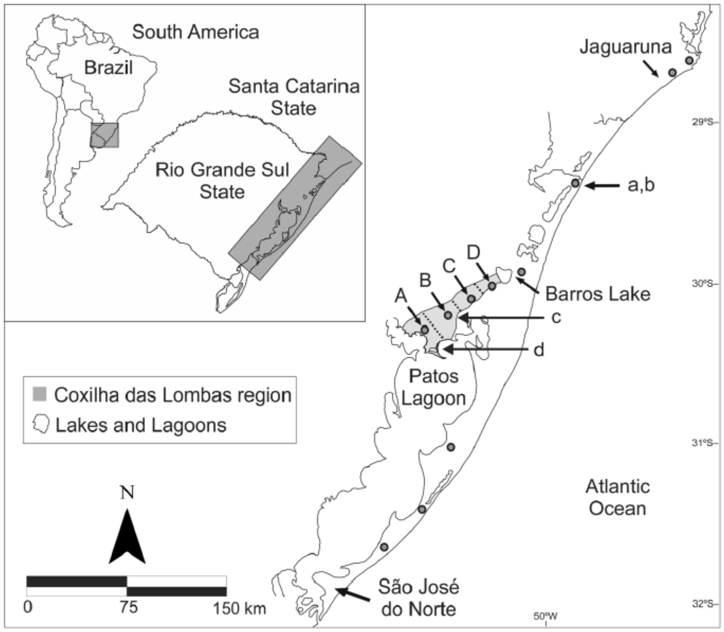

Figure 1. Coastal plain of southern Brazil with geographic details. The most important lakes and rivers are shown. Points (●) represent geographical regions for which genetic data are available from Lopes and Freitas (2012) and Lopes et al. (2013). In the region of Coxilha das Lombas are displayed the four karyotypic blocks described by Freitas (2007) (A, B, C and D). Conservation areas within the distribution of each species are also displayed (a, b: Parque de Itapeva and Parque da Guarita; c: Parque de Itapuã; d: Refugio de Vida Silvestre Banhado dos Pachecos).
doi:10.1371/journal.pone.0097301.g001

Briefly, the geographical genetic structure of *C. minutus* was characterized by examining 340 individuals over the entire distributional range and using information from cytochrome oxidase I gene and control region sequences. For *C. lami*, a total of 178 specimens were sampled, using information from the same mitochondrial DNA markers. Our analyses were based primarily on three different genetic parameters obtained from genetic data estimated by these authors [19,20]. The first parameter, nucleotide diversity (π), is defined by the equation

$$\pi=\sum_{ij} x_i x_j \pi_{ij},$$

where π_{ij} is the proportion of nucleotide differences between the *ith* and *jth* types of DNA sequences and x_i and x_j are the respective frequencies of these sequences. Nucleotide diversity is a measure of genetic variation. The second parameter, haplotype diversity (H_d), is a measure of the uniqueness of a particular haplotype in a given population. H_d is defined by the equation

$$Hd=\frac{N}{N-1}(1-\sum i x^2 i),$$

where x_i is the (relative) haplotype frequency of each haplotype in the sample and N is the sample size. Haplotype diversity is given for each sample. The third parameter, Fu's *Fs*, is the probability of observing a random sample with a number of alleles equal to or smaller than the observed value given the observed level of diversity and the assumption that all of the alleles are selectively neutral. If we call this probability $\hat{S}$, then

$$Fs=\ln\left(\frac{\hat{S}}{1-\hat{S}}\right),$$

so that a negative value of *Fs* would be expected from a recent population expansion or from genetic hitchhiking. A positive value of *Fs* is evidence for a deficiency of alleles, as would be expected from a recent population bottleneck or from overdominant selection [32]. To display the genetic information on a map, we established a geographic coordinate associated with each haplogroup for *C. minutus* and with each karyotypic block for *C. lami*.

Results

The models

The models constructed with Maxent produced distributional predictions for each species. The map with the widest distributional area was generated for *C. minutus*, whereas *C. lami* presented a very narrow distribution probability (Figure 2).

According to the ME model, the occurrence of *C. lami* was most strongly associated with soil and vegetation. The variable with the highest gain when used in isolation was vegetation. For this reason, vegetation appears to be the single variable that furnishes the most useful information. The variable that decreased the gain most markedly when omitted was soil. For this reason, soil appears to furnish the greatest amount of information not present in the other variables. For *C. minutus*, occurrence was strongly associated with altitude, vegetation, and soil. The variable with highest gain when used in isolation was vegetation, and the variable that decreased the gain most markedly was also vegetation (Figure 3).

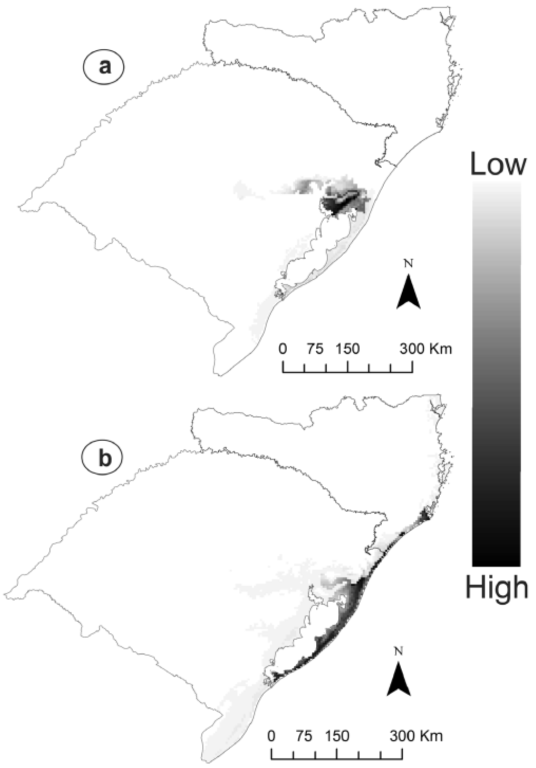

Figure 2. Suitable areas for (a) *Ctenomys lami* and (b) *Ctenomys minutus*, according to Maxent (maximum entropy) model in southern Brazilian coastal plain. Darker regions indicate greater ecological suitability.
doi:10.1371/journal.pone.0097301.g002

Both species were strongly influenced by vegetation and soil variables. Model performance, defined as the area under the curve, was highly discriminative for both species (AUC values for *C. lami* = 0.997 and *C. minutus* = 0.993), indicating that they inhabit a highly specific landscape niche.

Genetic pool data

The three local genetic parameters estimated showed no apparent patterns for *C. lami*. The suitability values calculated between the blocks were the same (high), and block C showed the highest value of π; however, the same pattern was not followed by the H_d values, which were higher in blocks B and D. The *Fs* values were not significant for any of the analyzed blocks (Figure 4).

For *Ctenomys minutus*, areas of high suitability were in accordance with the seven principal genetic haplogroups found for the species (see Lopes et al. [20] for details). For π values, the northern portion of the distribution presented higher values (except for North 1). H_d presented the same trend, with the center of the distribution showing greater diversity. For the *Fs* index, only the Barros Lake haplogroup showed a significant result (−5.30), as would be expected from a recent population expansion or from genetic hitchhiking (Figure 4).

Discussion

Maximum entropy models and environmental variables

This is the first study to use ENM methods to test parameters that influence the occurrence of the genus *Ctenomys* and one of the first to use these techniques on subterranean mammals. The models presented here were successful in identifying regions that are suitable for the species. Both models showed few commission errors, such as the areas of medium environmental suitability on the east side of the Coxilha das Lombas region for *C. lami* and the areas of low suitability in the countryside along Patos Lagoon for *C. minutus*. In addition, model performance was highly discriminative for both species. Although we identified these commission errors, we must consider that is very difficult to incorporate the dispersal abilities of the species and geographical accessibility to the model (landscape configuration) at present or through history; however, this factor is necessary for the actual presence of species [33]. In our particular case, geographical accessibility is a very important issue because the tuco-tucos are characterized by limited individual mobility and a patchy distribution of local populations [25,34]. *Ctenomys lami* appears to be restricted to the region of Coxilha das Lombas and showed a potential distribution at low to medium levels of occurrence beyond the limits of this region. The species was shown to be associated with local vegetation and soil type and to be geographically limited by the presence of wetlands and geographical inaccessibility, factors that characterize this region.

The distribution of *Ctenomys minutus* was restricted to the coastal plain of southern Brazil, including the region's first line of dunes and extending to sandy fields. This species shows a sympatric zone with another tuco-tuco species (*C. flamarioni*) in a range of approximately 15 km [35]. In an area extending to the northern portion of this region, *C. minutus* inhabits the first line of dunes. Below the sympatric zone, *C. minutus* occupies only the regions of sandy fields, although the ME model indicates that it can potentially occur up to the line of dunes. The reason for this apparent discrepancy is that near the southern limits of this sympatric zone, the first line of dunes is already occupied by *C. flamarioni* up to the southern boundary of Rio Grande do Sul state. This finding is, most likely, related to the historical patterns of occupation by these species. *Ctenomys minutus* shows a pattern of north-south occupation extending from Santa Catarina to Rio Grande do Sul state [20], and *C. flamarioni* shows a pattern of occupancy from south to north [36,37]. This transition between the sand fields and dune line represents an environmental discontinuity along the range of *C. minutus*. According to Fornel et al. [38], who have found significant differences in skull morphology between specimens of *C. minutus* that inhabit sand fields and those that inhabit dunes, environmental characteristics such as soil hardness produce morphological adaptations in tuco-tucos. These authors found some variation among different populations belonging to different habitats in cranial shape, and these intraspecific phenotypic differences appear to arise as a combination of selection and drift acting as diversifying forces. However, according to our model of environmental suitability, this discontinuity may not inhibit free dispersal by specimens from different habitats. Therefore, these different environments may also be associated with distinct levels of environmental suitability, although we could not measure this difference in our models.

Although they are morphologically similar, both species studied here occupy distinct areas of the coastal plain. According to the ME models, *C. minutus* appears to show higher environmental plasticity than *C. lami*. The models proposed for these two species showed a low overlap of areas of high suitability. This

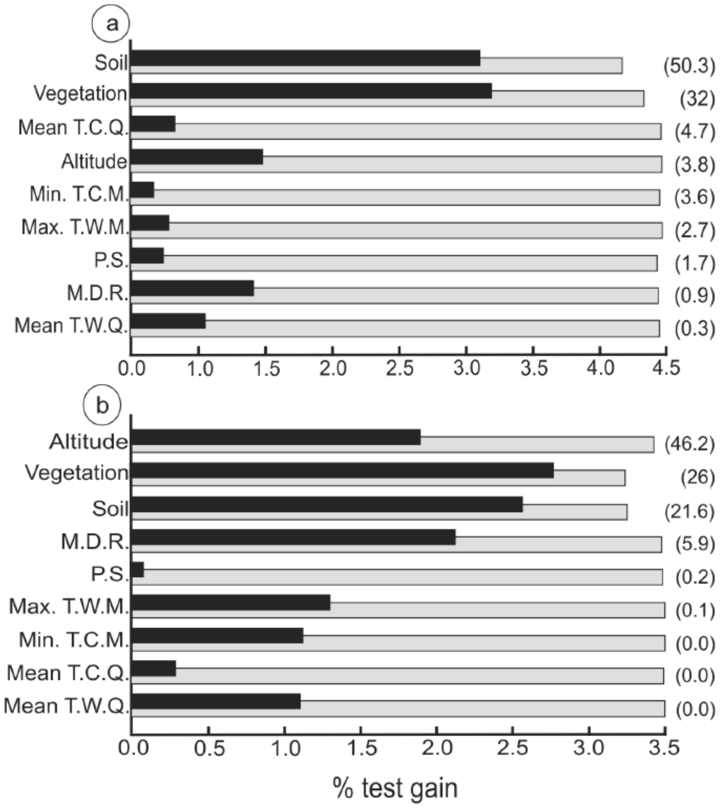

Figure 3. Jackknife analyses of the importance of environmental variables in maximum entropy modeling of *C. lami* (a) and *C. minutus* (b) occurrence. A heuristic estimate of the relative contribution of each variable to the global model is given in parentheses, with variables listed in descending order of importance. Grey bars show the performance of the global model (known as test gain) without each variable, and black bars show the influence of each variable in isolation (derived from a univariate model only). The variables are the following: Mean T.C.Q.: mean temperature of coldest quarter; Min. T.C.M.: minimum temperature of coldest month; Max. T.W.M.: maximum temperature of warmest month; P.S.: precipitation seasonality; M.D.R.: mean diurnal range (mean of monthly (max temp - min temp)); Mean T.W.Q.: mean temperature of warmest quarter.
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characteristic appears to be defined primarily by the presence of the Coxilha das Lombas region and the geographical inaccessibility of this region. A hybrid zone between *C. lami* and *C. minutus* is present in the northern portion of this coxilha. This zone has been the subject of previous study. According to Gava and Freitas [5], it was formed by modifications of the environment because a humid zone that separated these species was drained for rice cultivation. Environmentally, our results suggest a medium probability of occurrence for both species in the region where they hybridize.

Moreover, edaphic conditions can be very important for the definition of a species' fundamental niche [39,40]. The occurrence of *C. lami* and *C. minutus* was strongly influenced by soil and vegetation. *C. minutus* was also influenced by altitude, as it was associated with low altitudes (sea level). According to Kubiak [35], *C. minutus* is associated with areas with higher vegetation and biomass, which strengthens the evidence for the importance of this variable to the species. Given that tuco-tucos are exclusively subterranean, the use of variables that are directly linked to the species niche demonstrated to be extremely important in building the models, regardless of soil and vegetation variables, might have suffered from their degree of generalization. It is recommended that the choice of predictors in ENMs should consider the ecological relevancy of the predictors to the target species [41]. Such considerations are extremely important to improve conservation actions.

Genetic information and conservation actions

Our analyses of the genetic data associated with the ME model for *C. lami* showed that there are differences in the levels of genetic diversity (π and H_d) between populations, although the suitability is currently the same for all of the blocks. Lopes and Freitas [19] found that the genetic structure associated separately with each of the four different karyotypic blocks was inconsistent for mtDNA and microsatellite data. Environmentally, we also found no differences among these four karyotypic blocks. Thus, the presence of chromosomal rearrangements or distinct karyotypes between individuals noted by Freitas [4] does not appear to be limited by the environment.

Lopes et al. [20] recovered a pattern of genetic structure of the sampling sites subdivided into seven main haplogroups for *C. minutus*. In the current study, these haplogroups, represented by three genetic parameters, were associated with areas of high

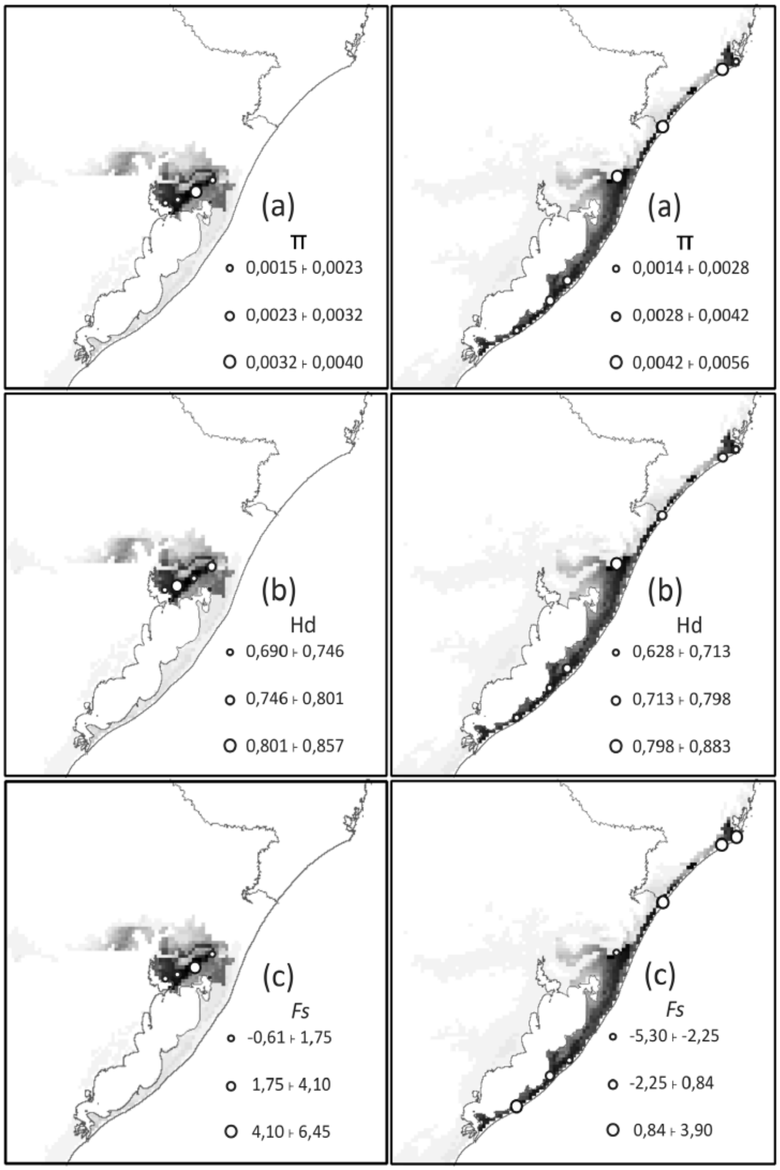

Figure 4. Geographical patterns of genetic parameters estimated within phylogeographic groups (*C. minutus*) and karyotype block (*C. lami*). Nucleotide diversity, Π (a); Haplotype diversity, *Hd* (b); Fu's neutrality parameter, *Fs* (c), overlapping the potential distribution of *C. lami* (lef column) and *C. minutus* (right column). Darker regions indicate greater ecological suitability.
doi:10.1371/journal.pone.0097301.g004

environmental suitability. This pattern may result from the occurrence of higher genetic diversity in the areas of higher suitability for the species. According to Lopes et al. [20], a relatively ancient genetic structure was present in the northern area of the geographical distribution, whereas the southern sampling sites may exhibit a founder effect of more recent occurrence. However, areas with high suitability showed different values of π and H_d, thus other extrinsic (historical or contemporary) or intrinsic (behavioral) factors could be affecting the patter of genetic diversity and structure.

The only value of the *Fs* index that was statistically significan for *C. minutus* was from the Barros Lake haplogroup, which wa associated with a highly suitable area. As this pattern was no found for *C. lami* or for the other haplogroups of *C. minutus*, it is no possible to support our hypothesis that highly suitable areas favo population expansion or genetic hitchhiking, as indicated by significant negative value of the *Fs* index.

The preservation of species requires not only detailed knowledge of their natural history and genetic structure but also information on the availability of suitable areas where species can survive; such knowledge can aid significantly in conservation planning. Both species analyzed here have suffered intense pressure from the fragmentation and reduction of their habitats. This process results in the death or isolation of individuals because these species are extremely niche-specific. The principal threats to *C. lami* are the progressive urbanization and human settlement in its territory, associated, for example, with agricultural activities [7,19]. In the northern portion of the coast, *C. minutus* is threatened by land speculation associated with urban constructions along the coast and on the shores of the lagoon and by the fragmentation of the dune habitat. In the south, silviculture involving *Pinus* sp. directly affects the populations of the species ([7], authors' personal observations). A study by Bernardo-Silva et al. [42] focused on the conservation of two endangered species of red-bellied toads, and the authors suggested five important areas for species conservation based on a hotspot analysis. These areas included a significant number of threatened species, including *C. minutus*. Our results underscore that the areas proposed by those authors should be considered for reserve planning because they cover three highly suitable areas within the range of *C. minutus* (see Bernardo-Silva et al. [42]). Although their results did not focus on underground rodents, these hotspot areas are of great interest for *C. minutus* because considering conservation of habitats as a priority, the protection of sandy field areas with high suitability for underground tunneling is an interesting solution for protecting the species. Additionally, *C. minutus* has only two protected areas within its entire distribution (Parque de Itapeva and Parque da Guarita). These areas are in neighboring locations in the northeastern portion of the distribution. However, none of the southern populations are located in protected areas. Two state conservation units (Parque de Itapuã and Refugio de Vida Silvestre Banhado dos Pachecos) are located within the range of *C. lami*, but only parts of these areas overlap the distribution of the species. Over the past year, extensive field work has been conducted to search for *C. lami*, but few populations have been found outside these two units (authors' personal observations).

Our models of genetic data associated with environmental suitability indicated that genetic pool data were associated with highly suitable areas for *C. minutus*. This result supports the use of these two techniques to plan conservation actions. This pattern was not evident for *C. lami*, perhaps due to the restricted range of the species. Despite the current wide use of molecular markers to estimate genetic variation at different geographical scales and originating from different evolutionary processes [43,44], it is still difficult to match these data with ecological parameters at broad scales. Moreover, despite many studies testing the relationship between genetic diversity and geographical populations, it is necessary to test alternative mechanisms, such as fragmentation and human effects on species distributions, by integrating genetic and demographic data in an ecological context.

Supporting Information

Figure S1 Input points given to the software (Maxent).
(TIF)

Acknowledgments

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Author Contributions

Conceived and designed the experiments: DG JBS TROF. Performed the experiments: DG JBS. Analyzed the data: DG JBS. Contributed reagents/materials/analysis tools: DG JBS TROF. Wrote the paper: DG JBS TROF. Acquired the data: DG TROF.

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Anexo II

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SHORT COMMUNICATION

Effects of rodents on plant cover, soil hardness, and soil nutrient content: a case study on tuco-tucos (*Ctenomys minutus*)

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Gerhard E. Overbeck · Thales R. O. de Freitas**

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Abstract We analyzed the effects of tuco-tucos (*Ctenomys minutus*, Ctenomyidae) on plant cover, plant biomass, soil hardness, soil pH, and variables related to nutrient disposition (P, K, Mg, and Ca), using data from three areas in the South Brazilian coastal plain. In each area, samples were taken from sites with and without *C. minutus* and results are presented in a concatenate way. Our results show that the presence of *C. minutus* modifies total plant biomass, grass cover, bare soil, soil hardness, soil pH, and nutrient content. Soils horizons at the depths of 10 and 20 cm are significantly softer in sites with *C. minutus* and phosphorus and potassium had higher concentrations. The content of magnesium and calcium were not affected. Soil pH was significantly lower where tuco-tucos occurred. Altogether, our results show that these animals may have a significant effect on vegetation composition and dynamics as well as on soil properties.

Keywords Animal interactions · Coastal grasslands · Subterranean mammals · Southern Brazil

Introduction

Tuco-tucos (Ctenomyidae) belong to a highly specialized mammalian family of subterranean rodents endemic to South-America, with approximately 60 species (Woods and Kilpatrick 2005). This family is characterized by a conspicuous ecological and morphological diversity (Busch et al. 2000; Lacey et al. 2000; Mora et al. 2003), and species occur across extensive areas (Lacey et al. 2000). They can, for instance, be found in deserts or semideserts in the Andean region, as well as in open areas in subtropical forests (Reig et al. 1990). *Ctenomys minutus* Nehring, 1887 is a medium-sized herbivorous rodent restricted to the southern Brazilian coastal plain. This species inhabits sandy grasslands and dunes, and it occupies the longest latitudinal gradient among tuco-tucos in southern Brazil (Freitas 1995; Freygang et al. 2004).

Subterranean rodents excavate and inhabit extensive burrow systems, and changes in plant diversity, abundance, and community composition are typical consequences of the many activities of these animals (Andersen 1987; Contreras and Gutiérrez 1991; Huntly and Reichman 1994; Malizia et al. 2000; Campos et al. 2001; Reichman and Seabloom 2002; Kerley et al. 2004; Lara et al. 2007; Hagenah and Bennett 2013). They also may alter soil conditions (Schauer 1987; Cox and Roig 1986; Borghi et al. 1990; Malizia et al. 2000; Lara et al. 2007; Šklíba et al. 2009; Hagenah and Bennett 2013). These effects of subterranean rodents on vegetation and soil can arise from burrow dynamics, diet selection, or foraging behavior (Huntly and Reichman 1994). Because of the great impact they have on entire ecosystems, including on water and air in soil, decomposition processes of plant material, nutrient cycling, and composition of local biota (Hole 1981), subterranean rodents are regarded as ecosystem engineers (Cameron 2000; Reichman and Seabloom 2002; Reichman 2007). Because of the magnitude of their effects, their actions can constitute a major factor in soil and vegetation dynamics.

Several studies have focused on the effects of *Ctenomys* on vegetation (Malizia et al. 2000; Campos et al. 2001; Tort et al. 2004; Lara et al. 2007) and soil conditions (Malizia et al. 2000; Lara et al., 2007). In most of these studies, *Ctenomys* alters vegetation and nutrient content of soil, although there is some

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variation in magnitude of these effects among studies. Like other subterranean rodents, *Ctenomys* spends most of its life in burrows that provide safety and foraging opportunities (Lacey et al. 2000). While direct observation of the species in the field is impossible, the investigation of the influence of *Ctenomys* on vegetation and soil conditions may provide valuable information about the magnitude of the effects of these animals in the ecosystem they inhabit. In this context, the aim of our study was to analyze the effects of a subterranean rodent (*C. minutus*) on its environment by comparing vegetation cover, plant biomass, soil hardness, and nutrient concentration in the soil between areas with and without this tuco-tuco.

Material and methods

Study area

The study was conducted between May and July 2013 in the coastal plain of the state of Rio Grande do Sul, southern Brazil. We sampled three areas where *C. minutus* occurred, situated in the municipalities of Torres (29°23' S, 49°45' W), Capão da Canoa (29°40' S, 50°01' W) and Osório (29°57' S, 50°13' W). Climate at these sites is mild mesothermal, wet, with no dry season. Average annual temperature ranges between 16 and 20ºC and annual rainfall varies between 1,000 and 1,500 mm (Nimer 1977; Vieira and Rangel 1998). The region is a mosaic of lakes, lagoons, sand dunes, and sand fields, and its geomorphology is continuously influenced by the fluctuations of the Atlantic Ocean (Tomazelli et al. 2000).

The vegetation in the region consists of mosaics of dune vegetation, coastal grasslands, and "restinga" forests (Hesp et al. 2009). Our study sites were on grasslands under cattle grazing with rather low stocking rates, as typical in the region. Feces produced by cattle covered less than 3 % of the area (patches with *C. minutus*: 2.02±2.03 %, and areas without *C. minutus*: 1.86±1.96 %; *t*=0.85, without significant differences between areas). Grasslands in the region are dominated by C4 grasses and are rich in plant species, with high importance especially of species from the Poaceae, Asteraceae, Cyperaceae, and Fabaceae (see Overbeck et al. 2007 for a review on South Brazilian grassland vegetation). The most dominant species at the study sites was the prostrate grass *Axonopus* aff. *affinis* Chase. The vegetation also contains some small grassland shrubs (e.g. *Baccharis* sp., *Croton lanatus* lam.). Total vegetation cover is approximately 50 to 80 %, and proportion of bare sandy substrate is high.

Sampling methodology and statistical analysis

To evaluate the influence of *C. minutus* on vegetation and soil, we sampled ten burrow sites of active individuals at each of the three study sites ("presence points"). We sampled the burrow systems found in each area which presented the activity of tuco-tucos, and the first 10 sites in which animals were captured were considered the presence points. In addition, ten randomly allocated points without presence of tuco-tucos were established in the same grasslands where animals were captured ("control points"). Control points were a minimum of 80 m from presence points containing *C. minutus*. No obvious differences in vegetation or topography were apparent between points of presence and absence of tuco-tucos, other than the presence or absence of burrows of *Ctenomys*. In total, we had 60 sample points (20 at each study site). We used live traps (Oneida Victor No. 0 traps protected with rubber strips) to capture the animals in burrows systems. Each trap was introduced into the entrance of a burrow, checked every 10 min, and animals were released at their point of capture. Trapping was conducted under the IBAMA (Brazilian Institute for the Environment and for Renewable Natural Resources) permit number 14690–1.

At each of the presence and control points, vegetation structure, plant biomass, and soil hardness were sampled in four sampling units (SU) situated at a distance of 2 m in the four cardinal directions. To evaluate vegetation structure, we used a 1-m^2 quadrat to measure percent cover of grasses, herbs, and bare ground. The side of the quadrat was placed 2 m away from each presence/control point. Plant biomass was measured in these same quadrats with a vertical quadrat sampler with a side length of 16 cm and a side depth of 22 cm (one sample per SU). We removed all above- and belowground vegetation from inside this quadrat sampler. After removing sand, plant samples were oven-dried to a constant weight at 80ºC, and then dry weight was recorded to determine total biomass. We used an impact soil penetrometer (Model IAA / Planalsucar, Stolf 1991) to measure soil hardness. At each presence and control point, we took four measurements (one per SU), recording the number of strokes necessary to penetrate to three soil depths: 10, 20, and 50 cm (results are expressed in kg/cm^2). For analysis of vegetation variables and soil hardness, we computed the mean of the four sampling units and then used the mean in the analysis (60 data points; 10 from presence points, 10 from control points, for each of three study sites). For plant biomass, the weights of the four samples per unit were summed for the subsequent analysis.

We also collected soil samples to evaluate the influence of *C. minutus* on soil pH, phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg). Soil samples were collected from the top 10 cm of soil at each of the 60 points. Phosphorus and potassium were determined by Mehlich-1 method (expressed in g/m^3), and calcium and magnesium were extracted with potassium chloride (KCl, 1 mol/L) (expressed in g/m^3) (Mehlich 1953; de Pesquisa Agropecuária) 1997).

Differences between areas with and without tuco-tucos were analyzed regarding variables of vegetation structure, mean dry biomass (g/sample), soil hardness, and soil nutrient concentration. Each area (Torres, Capão da Canoa e Osório) was considered a block in all analyses. Considering that not all variables had normal distribution, we used a permutational ANOVA analysis (PERMANOVA) to compare variables between presence and control sites (Underwood 1998; Zar 1999). The tests were performed with matrices based on Euclidean distances, and we ran 9,999 permutations. All analyses were conducted using the vegan package in R (R Development Core Team 2012; Oksanen et al. 2012).

Results

Total plant biomass and grass cover were significantly lower at sites where tuco-tucos were present, but herb cover did not differ between sites with and without tuco-tucos. There also was significantly more bare ground at sites with tuco-tucos (Table 1).

Soil hardness was significantly lower at 10 cm ($F=6.13$, $P=0.0016$) and 20 cm ($F=2.08$, $P=0.049$) in areas containing tuco-tucos. There was no significant difference in soil hardness at 50 cm between sites with and without tuco-tucos ($F=0.52$, $P=0.375$) (Fig. 1).

Areas with tuco-tucos had significantly higher concentrations of phosphorus and potassium than areas without tuco-tucos, but there was no significant difference in calcium and magnesium between sites with and without tuco-tucos. Soil pH was significantly lower where tuco-tucos occurred (Table 1).

Discussion

Our results indicate that tuco-tucos exert significant effects on vegetation and soil conditions. The influence of *C. minutus* on plant biomass (a 37 % reduction) is within the range of other herbivorous subterranean rodents (plant biomass reduced by 25 to 50 %; Reichmand and Smith 1985). This reduction in plant biomass also was similar to that measured for other species of *Ctenomys* (*C. mendocinus*: 44 % reduction of biomass on a plant community in the southern Puna Desert, Lara et al. 2007; *C. talarum*: 31 % reduction in biomass in grasslands of Buenos Aires Province, Malizia et al. 2000). We also observed a decrease in grass cover on sites with tuco-tucos. The reduction in grass cover and plant biomass at sites with *C. minutus* is likely a result of foraging activity of individuals, since this species feeds mostly on grasses (C.M. Lopes, pers. comm.), which are the dominant species group in the vegetation at our study sites. This reduction also can be a direct result from activity related to burrow construction. Albanese et al. (2010) also found that grasses were the dominant life form consumed by C. *mendocinus* (79 %), and grass leaves were the most representative item among consumed plant parts (89.5 %). Decreased densities of grasses in areas with *Ctenomys* also were reported by Campos et al. (2001) and Lara et al. (2007). This pattern suggests an effect of *Ctenomys* foraging on the abundance of grasses, which may then, in turn, have further effects on community dynamics, e.g., by relaxing competition and effects on different plant types, thus changing, for example, the ration of species with sexual and vegetative propagation (see also Lara et al. 2007).

There was significantly more bare ground at areas where tuco-tucos were present (61 % more bare ground). Bare ground is created by both burrowing and foraging activities of tuco-tucos. However, the direct effect of the creation of burrow systems and the resultant deposition of soil on the surface that can bury existing vegetation is easily visible and likely much more important in quantitative terms.

Values from the soil penetrometer provide a clear indication of the effect of tuco-tucos on soil hardness at soil depths

Table 1 Mean and standard deviation of vegetation variables (plant biomass, grass cover, herb cover, and bare soil) and soil variables (pH, and nutrient concentrations: phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg)). The results of permutational ANOVA (PERMANOVA) tests for differences between areas with and without tuco-tucos (*Ctenomys minutus*) are displayed

	Without tuco-tucos ($n=30$) Mean±SD	With tuco-tucos ($n=30$) Mean±SD	F	p level
Vegetation				
Plant biomass (g)	146.64±75.16	91.21±59.13	5.88	0.0014
Grasses (%)	56.88±18.51	35.14±19.01	12.84	0.0001
Herbs (%)	8.01±7.01	5.98±4.47	0.95	0.2192
Bare Soil (%)	18.01±18.44	47.39±24.52	19.38	0.0001
Soil				
pH	5.62±0.95	5.28±0.38	6.18	0.0013
P (g/m^3)	4.32±2.08	5.84±4.09	2.43	0.0315
K (g/m^3)	17.30±10.58	22.95±9.28	3.75	0.0033
Ca (g/m^3)	146.66±383.06	106.66±77.07	0.14	0.9701
Mg (g/m^3)	33.61±33.26	44.95±26.34	1.08	0.1801

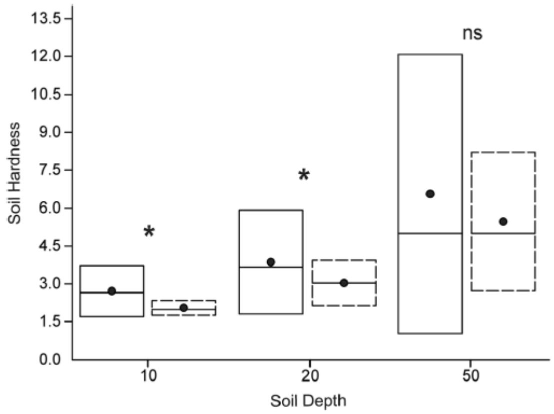

Fig. 1 Soil hardness (kg/cm^2) at three different depths (cm) from sites without (*solid boxes*) and with (*dashed boxes*) individuals of *Ctenomys minutus*. *Top and lower horizontal lines of boxes* represent standard deviations. *Black points* and the *center horizontal lines* represent the mean and the median, respectively. The results of the statistical tests are displayed in the text. *Asterisk* indicates a significant difference and "ns" non-significant

of 10 and 20 cm. According to Gastal (1994), the average depth of the burrow systems built by *C. minimus* is 25 cm and the maximum depth is 29.1 cm. This range of burrow depths is consistent with our finding of a significant effect of tuco-tucos on soil hardness at depths of 10 and 20 cm, but not at 50 cm. Sampling areas situated 2 m from the burrow opening could present some undiscovered burrows that were nearby sampling units, thus affecting soil hardness. During burrowing, the animals redistribute soil among different horizons which contributes to aeration, irrigation, and fertilization of soils (Reichmand and Smith 1990). The latter can be observed from higher P and K concentrations just below the soil surface, but not concerning Ca and Mg. According to Malizia et al. (2000), the formation of new mounds by tuco-tucos (*C. talarum*) increased the levels of N, P, Na, K, and Mg, but higher levels of Ca and pH were found in undisturbed areas. Lara et al. (2007) also found that the disturbing activity of *C. mendocinus* increased nutrient concentration (N, K, P) in bare soil compared to bare soil in undisturbed areas. Even though effects on different nutrients vary between studies, it is well known that tuco-tucos and other subterranean mammals (pocket gophers, Geomyidae) modify the distribution of nutrients (Abaturov 1972; Mielke 1977; Grant and Mc Bryer 1981; Hole 1981; Spencer et al. 1985; Inouye et al. 1987; Reichman and Seabloom 2002). In our case, this was shown even for patches within the same grassland area. Thus, we can assume that sites close to *C. minutus* burrows are nutrient hotspots (for P and K), resulting from the animals' activities moving, mixing, or bringing soil to the surface from lower levels and the incorporation of nutrients into the soil. In contrast, the more acid pH found on sites with *C. minutus* may be a consequence of the dead vegetal material resulting from foraging activity.

Altogether, our results show that *C. minutus* affects plant biomass, grass cover, the proportion of bare soil, soil hardness, concentrations of P and K, and pH in the grasslands under study. In addition, we demonstrated that this tuco-tuco affects soil hardness and vegetation characteristics in an area greater than just above the burrowing mount (two meters), probably because of the tunneling and feeding of individuals. Still, if we measured these characteristics above the opening or over the tunnel itself, this difference could be greater. These animals may thus have a significant effect on vegetation composition and dynamics as well as on soil properties. While this obviously will affect ecosystem processes such as decomposition or productivity, the consequence of all the effects that tuco-tucos have on ecosystems are difficult to predict. Studies with pocket gophers have revealed that the extensive excavations and their associated impacts generate a dynamic mosaic of nutrients and soil conditions that promote diversity and maintain disturbance-dependent components of plant communities (Reichman and Seabloom 2002). The possible importance of herbivory by small rodents on plant composition and ecosystem properties in coastal grassland communities in South Brazilian has been neglected and detailed effects of *C. minutus* still have to be investigated.

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Anexo III

Normas de publicação do periódico Acta Theriologica

Instructions for Authors

Mammal Research, formerly published as Acta Theriologica, is an international journal of mammalogy, covering all aspects of mammalian biology. Long-since recognized as a leader in its field, the journal was founded in 1954, and has been exclusively published in English since 1967.

The journal presents work from scientists all over the world, covering all aspects of mammalian biology: genetics, ecology, behaviour, bioenergetics, morphology, development, reproduction, nutrition, physiology, paleontology and evolution.

Title Page

The title page should include:

The name(s) of the author(s)

A concise and informative title

The affiliation(s) and address(es) of the author(s)

The e-mail address, telephone and fax numbers of the corresponding author

Abstract

Please provide an abstract of 150 to 250 words. The abstract should not contain any undefined abbreviations or unspecified references.

Keywords

Please provide 4 to 6 keywords which can be used for indexing purposes.

Text

Text Formatting

Manuscripts should be submitted in Word.

Use a normal, plain font (e.g., 10-point Times Roman) for text.

Use italics for emphasis.

Use the automatic page numbering function to number the pages.

Do not use field functions.

Use tab stops or other commands for indents, not the space bar.

Use the table function, not spreadsheets, to make tables.

Use the equation editor or MathType for equations.

Save your file in docx format (Word 2007 or higher) or doc format (older Word versions).

References

Citation

Cite references in the text by name and year in parentheses. Some examples:

Negotiation research spans many disciplines (Thompson 1990).

This result was later contradicted by Becker and Seligman (1996).

This effect has been widely studied (Abbott 1991; Barakat et al. 1995; Kelso and Smith 1998; Medvec et al. 1999).

Reference list

The list of references should only include works that are cited in the text and that have been published or accepted for publication. Personal communications and unpublished works should only be mentioned in the text. Do not use footnotes or endnotes as a substitute for a reference list.

Reference list entries should be alphabetized by the last names of the first author of each work.

Journal article

Gamelin FX, Baquet G, Berthoin S, Thevenet D, Nourry C, Nottin S, Bosquet L (2009) Effect of high intensity intermittent training on heart rate variability in prepubescent children. Eur J Appl Physiol 105:731-738. doi: 10.1007/s00421-008-0955-8

Ideally, the names of all authors should be provided, but the usage of “et al” in long author lists will also be accepted:

Smith J, Jones M Jr, Houghton L et al (1999) Future of health insurance. N Engl J Med 965:325–329

Article by DOI

Slifka MK, Whitton JL (2000) Clinical implications of dysregulated cytokine production. J Mol Med. doi:10.1007/s001090000086

Book

South J, Blass B (2001) The future of modern genomics. Blackwell, London

Book chapter

Brown B, Aaron M (2001) The politics of nature. In: Smith J (ed) The rise of modern genomics, 3rd edn. Wiley, New York, pp 230-257

Online document

Cartwright J (2007) Big stars have weather too. IOP Publishing PhysicsWeb. http://physicsweb.org/articles/news/11/6/16/1. Accessed 26 June 2007

Dissertation

Trent JW (1975) Experimental acute renal failure. Dissertation, University of California

Always use the standard abbreviation of a journal's name according to the ISSN List of Title Word Abbreviations, see

ISSN.org LTWA

If you are unsure, please use the full journal title.

For authors using EndNote, Springer provides an output style that supports the formatting of in-text citations and reference list.

EndNote style (zip, 2 kB)

Tables

All tables are to be numbered using Arabic numerals.

Tables should always be cited in text in consecutive numerical order.

For each table, please supply a table caption (title) explaining the components of the table.

Identify any previously published material by giving the original source in the form of a reference at the end of the table caption.

Footnotes to tables should be indicated by superscript lower-case letters (or asterisks for significance values and other statistical data) and included beneath the table body.

Artwork And Illustrations Guidelines

Color Art

Color art is free of charge for online publication.

If black and white will be shown in the print version, make sure that the main information will still be visible. Many colors are not distinguishable from one another when converted to black and white. A simple way to check this is to make a xerographic copy to see if the necessary distinctions between the different colors are still apparent.

If the figures will be printed in black and white, do not refer to color in the captions.

Color illustrations should be submitted as RGB (8 bits per channel).

Figure Lettering

To add lettering, it is best to use Helvetica or Arial (sans serif fonts).

Keep lettering consistently sized throughout your final-sized artwork, usually about 2–3 mm (8–12 pt).

Variance of type size within an illustration should be minimal, e.g., do not use 8-pt type on an axis and 20-pt type for the axis label.

Avoid effects such as shading, outline letters, etc.

Do not include titles or captions within your illustrations.

Figure Numbering

All figures are to be numbered using Arabic numerals.

Figures should always be cited in text in consecutive numerical order.

Figure parts should be denoted by lowercase letters (a, b, c, etc.).

If an appendix appears in your article and it contains one or more figures, continue the consecutive numbering of the main text. Do not number the appendix figures,

"A1, A2, A3, etc." Figures in online appendices (Electronic Supplementary Material) should, however, be numbered separately.

Figure Captions

Each figure should have a concise caption describing accurately what the figure depicts.

Include the captions in the text file of the manuscript, not in the figure file.

Figure captions begin with the term Fig. in bold type, followed by the figure number, also in bold type.

No punctuation is to be included after the number, nor is any punctuation to be placed at the end of the caption.

Identify all elements found in the figure in the figure caption; and use boxes, circles, etc., as coordinate points in graphs.

Identify previously published material by giving the original source in the form of a reference citation at the end of the figure caption.

Figure Placement and Size

When preparing your figures, size figures to fit in the column width.

For most journals the figures should be 39 mm, 84 mm, 129 mm, or 174 mm wide and not higher than 234 mm.

For books and book-sized journals, the figures should be 80 mm or 122 mm wide and not higher than 198 mm.

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If you include figures that have already been published elsewhere, you must obtain permission from the copyright owner(s) for both the print and online format. Please be aware that some publishers do not grant electronic rights for free and that Springer will not be able to refund any costs that may have occurred to receive these permissions. In such cases, material from other sources should be used.

Accessibility

In order to give people of all abilities and disabilities access to the content of your figures, please make sure that

All figures have descriptive captions (blind users could then use a text-to-speech software or a text-to-Braille hardware)

Patterns are used instead of or in addition to colors for conveying information (colorblind users would then be able to distinguish the visual elements)

Any figure lettering has a contrast ratio of at least 4.5:1