INFLUÊNCIA DE FATORES BIÓTICOS E ABIÓTICOS SOBRE O COMPORTAMENTO, ECOLOGIA E EVOLUÇÃO DA ESPÉCIE *Ctenomys minutus* (RODENTIA: CTENOMYIDAE)

BRUNO BUSNELLO KUBIAK

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Orientador: Dr. Thales Renato O. de Freitas

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Tese aprovada em: _____ / _____ / _____

Banca examinadora:

Dr(a). Gislene Lopes Gonçalves (UFRGS)

Dr. Jorge Reppold Marinho (URI)

Dr(a). Maria João Veloso da Costa Ramos Pereira (UFRGS)

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DID YOU CATCH THE MOUSE?
YOU BET
JIM DAVIS 10-18
WHAT DID YOU DO WITH HI-
TAGGED HIM, AND RELEASED HIM BACK INTO THE WILD

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Com este trabalho encerro um ciclo de quatro anos. No entanto, é obvio que ele não é fruto apenas deste período, mas sim o resultado da busca por um objetivo que se construiu desde o início da graduação, passando pelo mestrado e cuminando aqui. Claro que ao longo de mais de 10 anos tive a colaboração de inúmeras pessoas que foram enssenciais no progresso que obtive como pessoa e (buscando ser um) pesquisador. Sabendo que toda esta caminhada foi dividida em capítulos, se faz necessário agradecer algumas pessoas que foram mais participativas ao longo do me doutoramento, aos demais deixo aqui os meus mais sinceros agradecimentos.

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SUMÁRIO

RESUMO

Os diferentes aspectos relacionados à biologia e história de vida de uma espécie são moldados pela seleção natural relacionada com o ambiente ocupado. Além disso, as espécies não vivem isoladas e interações intra e interespecíficas também são fatores determinantes que podem agir sobre as características das espécies. Contudo, identificar e relacionar os fatores que forjam estes aspectos não é uma tarefa simples e tem sido um dos maiores interesses da biologia. Ao longo deste estudo será utilizado um roedor subterrâneo como alvo para compreender como interações bióticas e abióticas influenciam no comportamento, ecologia e evolução desta espécie. A espécie escolhida foi *Ctenomys minutus, Nhering, 1886*, que pertence à família Ctenomyidae, a qual apresenta o maior número de espécies entre os roedores subterrâneos, aproximadamente 70. As características principais que levaram a escolha desta espécie é que, diferentemente das demais espécies do gênero, ela apresenta distribuição em dois habitats com características distintas (dunas arenosas e campos arenosos) e possui zonas de contato com a espécie *Ctenomys flamarioni*, Travi, 1981 ao longo de sua distribuição, tornando-a um excelente modelo para testar o objetivo proposto neste trabalho. Para isso, este estudo é divido em quatro capítulos que utilizam diferentes ferramentas (modelagem de nicho ecológico, morfometria geométrica e análise do tamanho da área de vida) para alcançar objetivos distintos, fornecendo assim informações que se somam para o esclarecimento do objetivo principal. No cap. 1, utilizando a modelagem de nicho ecológico foi possível perceber que *Ctenomys minutus* e *Ctenomys flamarioni* não se excluem competitivamente. No entanto, a morfometria geométrica nos permite perceber a existência de deslocamento de caracteres em uma das

espécies. *Ctenomys minutus* apresenta menor tamanho quando em contato com *C. flamarioni*. Sendo assim, é possível afirmar que mesmo não sendo detectadas evidências que suportem a exclusão competitiva entre as espécies em um cenário macro geográfico a interação entre elas pode fazer com que possuam diferenciações na seleção do habitat que possivelmente acarretaram na diferenciação morfológica de uma das espécies (*Ctenomys minutus*). Já nos cap. 2 e 3 foi explorado o tamanho da área de vida de *Ctenomys minutus* e se percebe que o tipo de habitat é um fator determinante na diferenciação desta característica. Indivíduos que ocupam o habitat de dunas costeiras possuem áreas de vida significativamente maiores que animais da mesma espécie que ocupam o habitat de campos arenosos. Por outro lado, a coexistência com *Ctenomys flamarioni* não parece influenciar o tamanho da área de vida de nenhuma das espécies, evidenciando que interações bióticas podem não influenciar significativamente nesta característica ecológica e comportamental. Além disso, no cap. 4 são apresentados resultados que evidenciam que o tipo de habitat parece ser um fator importante na determinação da força de mordida e forma do crânio de *Ctenomys minutus*. Os animais que habitam os campos arenosos possuem diferenciações na forma crânio em relação a animais coespecíficos que habitam as dunas costeiras. Isto parece estar intimamente ligado com a diferenciação da força da mordida destes animais, onde indivíduos que habitam os campos arenosos possuem maiores forças de mordida em comparação a indivíduos que habitam as dunas costeiras. De modo geral é possível concluir que as interações bióticas experimentadas por *Ctenomys minutus* possuem influência direta na seleção de habitat da espécie e diferenciação morfológica (deslocamento de caracteres), contudo, não parece influenciar em aspectos comportamentais relacionados ao tamanho da área de vida dos indivíduos. Já as interações abióticas, neste caso a ocupação de diferentes tipos de habitats, influenciam diretamente na diferenciação ecológica e comportamental em relação ao tamanho da área de diferenças morfológicas do crânio, culminando na diferenciação da força da mordida e possivelmente podendo levar a diferenciações evolutivas destas populações.

1. INTRODUÇÃO GERAL

1.1. Interações bióticas e abióticas

Os diferentes aspectos relacionados à biologia e história de vida de uma espécie (e.g. distribuição, comportamento, morfologia, etc.) são moldados pela seleção natural relacionada com o ambiente ocupado (Darwin 1859). Por isso, afirmar que uma espécie é altamente adaptada ao habitat em que vive pode ser considerado uma redundância (Mayr 1970), no entanto, a maneira como encontramos os aspectos de um organismo atualmente, refletem respostas de seus ancestrais às pressões seletivas do passado (Darwin 1859) e compreender os fatores que forjam estes aspectos tem sido um dos maiores interesses da biologia.

Para facilitar a compreensão das interações bióticas e abióticas e suas consequências sobre as espécies se faz necessário a caracterização do termo nicho ecológico. Nicho ecológico pode ser considerado a soma das tolerâncias e necessidades de condições e recursos que uma espécie necessita para ocorrer e persistir (Hutchison 1957). Contudo, pode-se deduzir que dificilmente uma espécie pode ocorrer em todos os locais que apresentem condições favoráveis a sua existência, seja pelo simples fato de não ter acesso ao local ou por existir outra força capaz de impedir que a mesma se fixe em determinada localidade. A compreensão de que existem fatores que podem influenciar no nicho das espécies levou Hutchison (1957) a dividir o nicho em dois tipos distintos: a combinação de condições e recursos que permitem que a espécie mantenha uma população viável sem a presença de competidores é considerado seu (1) nicho fundamental. Contudo, quando na presença de competidores a espécie pode ficar limitada ao seu (2) nicho efetivo ou realizado.

Além disso, já se sabe que espécies com histórias evolutivas similares e morfologicamente semelhantes provavelmente possuem requerimentos ecológicos parecidos, o que pode desta maneira impedir a sua coexistência (Darwin 1859; Gause 1934; MacArthur 1972). Esta teoria se baseia no principio de exclusão competitiva que foi descrito por Gause (1934), onde espécies com mesmos requerimentos ecológicos (nicho ecológico), em locais com recursos limitados, não são capazes de coexistir devido à competição. Neste caso o competidor superior será capaz de excluir competitivamente o competidor inferior, ou as espécies podem modificar seu nicho ecológico, permitindo assim a sua coexistência.

Contudo, modificações nos modos de utilização de recursos, e consequentemente do nicho da espécie, em resposta à competição podem acarretar diferenciações morfológicas, fisiológicas ou comportamentais. Em longo prazo, o processo de partilha de recursos pode ter resultados evolutivos e conduzir ao deslocamento de caracteres nas populações envolvidas (Brown e Wilson 1956). Ou seja, espécies simpátricas que competem por um recurso limitado podem ser favorecidas pela seleção natural a divergir no uso dos recursos e consequentemente no fenótipo. Isso já foi demonstrado em diversos organismos, próximos filogeneticamente, que possuem zonas de contatos onde exibem diferenciação comportamental, de seleção de micro-habitat e de utilização de recursos alimentares para permitir a coexistência (Stoecker 1972; Schoener 1983; Schluter e Mcphail 1992; Dayane e Simmberloff, 1994a, 1994b; Adams e Rohlf 2000; Pfenning e Murphy 2000). Isso tudo pode acarretar em modificações morfológicas dos indivíduos (veja Dayan e Simmberloff 2005; Stuart e Lossos 2013 para revisão). No entanto, para inferir que modificações no nicho e possivelmente o deslocamento de caracteres seja resultado da competição interespecífica, e não somente uma particularidade da espécie, é necessário também à utilização de tratamentos com a presença e ausência do possível competidor. Destacamos aqui duas maneiras possíveis para verificar se as modificações sofridas por uma espécie podem ser interpretadas como fruto da competição: (1) remoção e controle do possível competidor do local de interesse ou (2) analisar as características das espécies em locais de simpatria e alopatria (Stuart e Lossos 2013).

Com base nas informações acima, é possível afirmar que tanto as interações bióticas como abióticas possuem papel fundamental sobre a configuração de características comportamentais, ecológicas e evolutivas das espécies. Contudo, além de possuir o cenário e tratamentos adequados para compreender quais são os fatores e de que maneira as interações bióticas e abióticas influenciam nas características das espécies, é necessário utilizar ferramentas que permitam alcançar os diferentes objetivos que podem ser propostos dentro desta temática. Aqui serão ressaltadas algumas ferramentas que foram utilizados ao longo deste estudo. (1) Modelagem de nicho ecológico é uma ferramenta que se faz passível de ser utilizada devido aos avanços alcançados nos últimos anos, permitindo não somente predizer o potencial de ocorrência das espécies, mas também utilizar abordagens para estudar como as interações bióticas podem influenciar na distribuição das espécies (Anderson 2002; Gutiérrez et al. 2013). A teoria do nicho ecológico é o principal fundamento para o desenvolvimento desta ferramenta (Peterson 2011) considerando que indivíduos de uma mesma espécie possuem requerimentos ecológicos semelhantes que definem sua distribuição (Hutchison 1957). (2) A morfometria geométrica que possibilita acessar informações sobre a forma e tamanho de estruturas das espécies (e.g. crânio, mandíbula, escápula) e desta maneira verificar como elas respondem a interações bióticas e abióticas. Esta ferramenta se baseia no estabelecimento de pontos anatômicos que servem de referência em estruturas homólogas (marcos anatômicos). Estes marcos anatômicos permitem capturar a geometria de dada estrutura em um estudo, podendo ser bi ou tridimensionais e permitem a separação dos componentes de forma e tamanho (Rohlf e Marcus 1993;

Monteiro e Reis 1999). Além disso, uma característica comportamental fundamental de qualquer espécie, a (3) área de vida, que pode ser classificada como o espaço ocupado no processo de movimentação que envolve alimentação, reprodução, cuidado com a prole, entre outras atividades vitais de um indivíduo (Burt 1943). Todas estas ferramentas serão abordadas novamente nos próximos capítulos – modelagem de nicho ecológico (cap.1), morfometria geométrica (cap. 1 e 4) e área de vida (Cap. 2 e 3) – de maneira mais aprofundada na busca por objetivos envolvendo compreender como as interações podem influenciar no comportamento, ecologia e evolução de uma espécie de roedor subterrâneo.

1.2. Roedores subterrâneos: Família Ctenomyidae

A ordem Rodentia é a mais diversificada dentre as linhagens dos mamíferos, ocorre em praticamente todos os continentes com exceção da Austrália, ocupando uma grande variedade de hábitats e é capaz de explorar diferentes hábitos, como terrestre, arborícola e semiaquática (Patton, Pardiñas e D’Elia 2015). Dentre os roedores terrestres, aqueles em que algum momento de sua vida utilizam estruturas abaixo da superfície do solo para realizar alguma atividade vital são denominados fossoriais. Já os grupos que conduzem a maioria de suas atividades vitais em galerias abaixo da superfície terrestre são nomeados de subterrâneos (Lacey, Patton e Cameron. 2000).

O nicho subterrâneo foi invadido independentemente por pelo menos cinco famílias de roedores, distribuídas em todos os continentes com exceção de Austrália e Antártica: Bathyergidae (África), Geomyidae (Ámerica do Norte), Muridae (Ásia, Europa e África), Octodontidae e Ctenomyidae (Ámerica do Sul) (Nevo e Reig 1990; Nowak 1999; Lacey et al. 2000). Habitam, na sua maioria áreas abertas como campos, estepes, savanas ou desertos, porém algumas espécies podem ocorrer em florestas ou mata arbustiva densa, não sendo encontrados em locais com solos saturados de água ou permanentemente congelados (Lacey et al. 2000; Gardner et al. 2015; Stolz et al. 2013). A convergência adaptativa apresentada por roedores subterrâneos em relação ao tamanho, ao modo de vida, as estruturas e as funções está intimamente ligada com estruturas físicas e bióticas do ambiente subterrâneo (Nevo 1979; Lacey et al. 2000). Existem poucos casos registrados de simpatria em roedores subterrâneos, talvez devido as suas semelhanças morfológicas dificultando assim a partilha de recursos e subdivisão de nichos (Cameron 2000). Os roedores subterrâneos são caracterizados por uma mobilidade individual limitada e uma distribuição populacional em manchas (Nevo 1979; Lacey et al. 2000).

A América do Sul possui duas famílias representantes dos roedores subterrâneos: Octodontidae, denominados popularmente de coruros, representados por uma única espécie (*Octodon degus*), que ocorre na parte central do Chile, e Ctenomyidae que é monogenérica (Gênero Ctenomys), sendo os representantes desta família conhecidos popularmente como tuco-tucos. Esta última família é considerada a principal dentre os roedores subterrâneos em relação ao número de espécies, compreendendo aproximadamente 70 espécies descritas (Freitas 2016). Possui a sua origem datada a aproximadamente três milhões de anos (Verzi et al. 2010), apresentando uma das maiores taxas de especiação e evolução cromossômica entre os mamíferos (Reig et al. 1990). É amplamente distribuído, sendo registrado desde o sul da Bolívia e Peru até a Terra do Fogo, na Argentina (Pearson 1959). Os indivíduos do gênero *Ctenomys* constroem e habitam sistemas de galerias, normalmente são solitários com poucos casos de espécies sociais ou semi-sociais (Lacey et al. 1998; Lacey 2000). Estes túneis são mantidos fechados pelos indivíduos ocupantes, o que proporciona não somente a 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 O2 menos elevadas e de CO2 mais elevadas (McNab 1966).

Os tuco-tucos são marcadamente semelhantes entre si, exibindo adaptações morfológicas relacionadas ao seu hábito de vida como redução da cauda e pavilhões auditivos, corpo robusto e cilíndrico, um maior desenvolvimento da musculatura (principalmente dos membros anteriores) e das unhas, e uma abertura bucal atrás dos incisivos que ficam expostos para fora da boca (Nevo 1979; Reig et al. 1990; Nowak 1999). São herbívoros e apresentam uma alimentação preferencial por gramíneas, sendo generalistas na maioria dos casos e influenciam diretamente na comunidade de plantas da região e na modificação das condições do solo, por meio do revolvimento e aeração do mesmo (Zenuto e Busch 1995; Rosi et al. 2000; Del Valle et al. 2001). Assim como os demais roedores subterrâneos, os indivíduos do gênero se caracterizam por distribuir-se em manchas, pela sua alta territorialidade e apresentarem uma baixa dispersão (Busch et al. 2000). Devido a sua similaridade morfológica e ecológica apresentam distribuições majoritariamente alopátricas, onde cada espécie é intimamente ligada ao tipo de habitat que ocupa (Lacey et al. 2000). Somente dois casos de simpatria são conhecidos, um deles ocorre na Argentina, entre as espécies *C. australis* e *C. talarum* na província de Buenos Aires (Contreras e Reig 1965; Comparatore et al. 1992). No Brasil, é descrito o outro caso de simpatria, entre as espécies *C. flamarioni* e *C. minutus*, em dois locais distintos na planície costeira do estado do Rio Grande do Sul (Kubiak, Galiano e Freitas 2015) (Veja Figura 2).

No Brasil, encontram-se descritas oito espécies de tuco-tucos. Uma apresenta distribuição no estado de Rondônia, *Ctenomys bicolor* Miranda Ribeiro, 1914, no estado de Mato Grosso, *Ctenomys rondoni* Miranda Ribeiro, 1914 e *Ctenomys nattereri* Wagner, de 1848. Informações sobre as três espécies acimas ainda são inexistente, sendo desconhecidas informações básicas sobre estas espécies (e.g. distribuição, ecologia, comportamento, etc.) Todas as outras cinco espécies apresentam distribuição para o estado do Rio Grande do Sul (Figura 1), sendo três endêmicas do estado: *Ctenomys flamarioni* Travi, 1981; *Ctenomys lami* Freitas, 2001 e *Ctenomys ibicuiensi* Freitas et al. 2012, as outras duas espécies (*Ctenomys minutus* Nehring, 1887 e *Ctenomys torquatus* Lichtenstein, 1830) apresentam parte de sua distribuição no Estado de Santa Catarina e no Uruguai, respectivamente (Freitas e Lessa 1984; Freitas 1995, 1997).

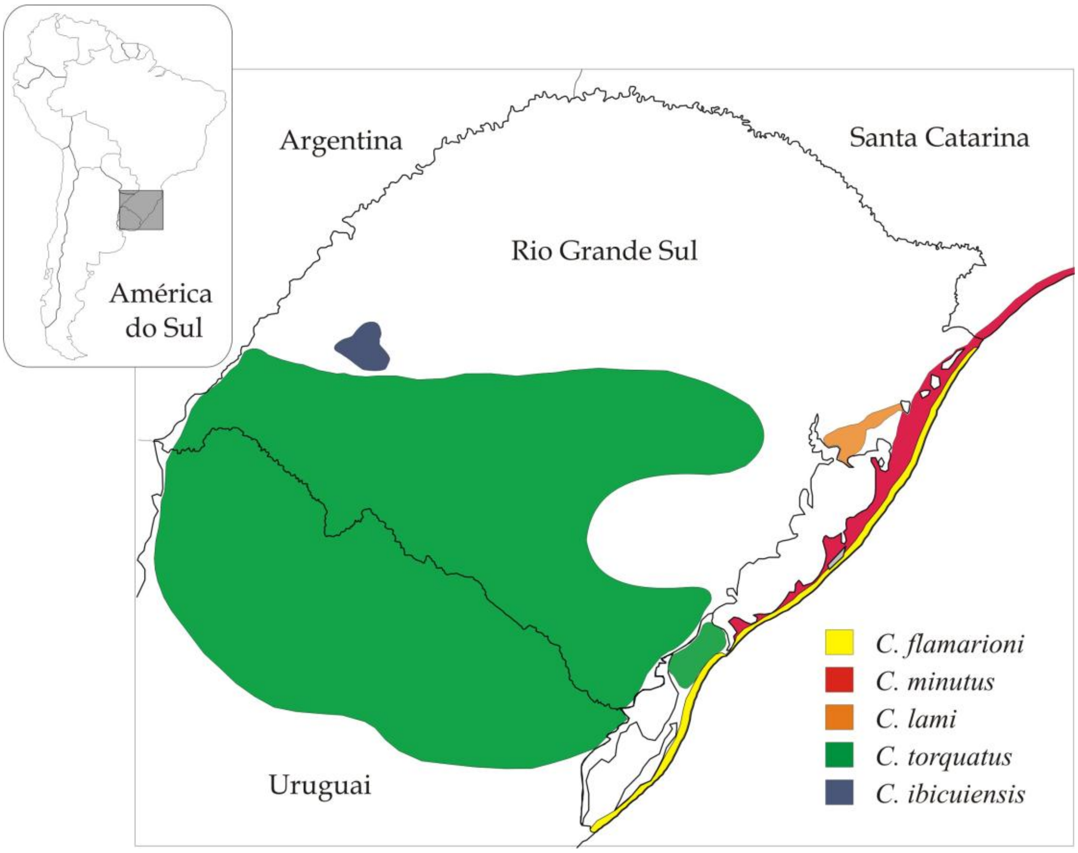

Figura1. Distribuição das espécies do gênero *Ctenomys* no estado do Rio Grande do Sul.

1.3. *Ctenomys minutus*

A espécie *C. minutus* restringe-se à planície costeira da região sul do Brasil, sendo registrada desde o sul de Santa Catarina, nas proximidades da praia de Laguna até a cidade de São José do Norte no Rio Grande do Sul (Freygang et al. 2004) totalizando um distribuição linear de aproximadamente 500 km, fazendo com que ela apresente uma das maiores distribuição longitudinais dentre as espécie do gênero (Figura 2).

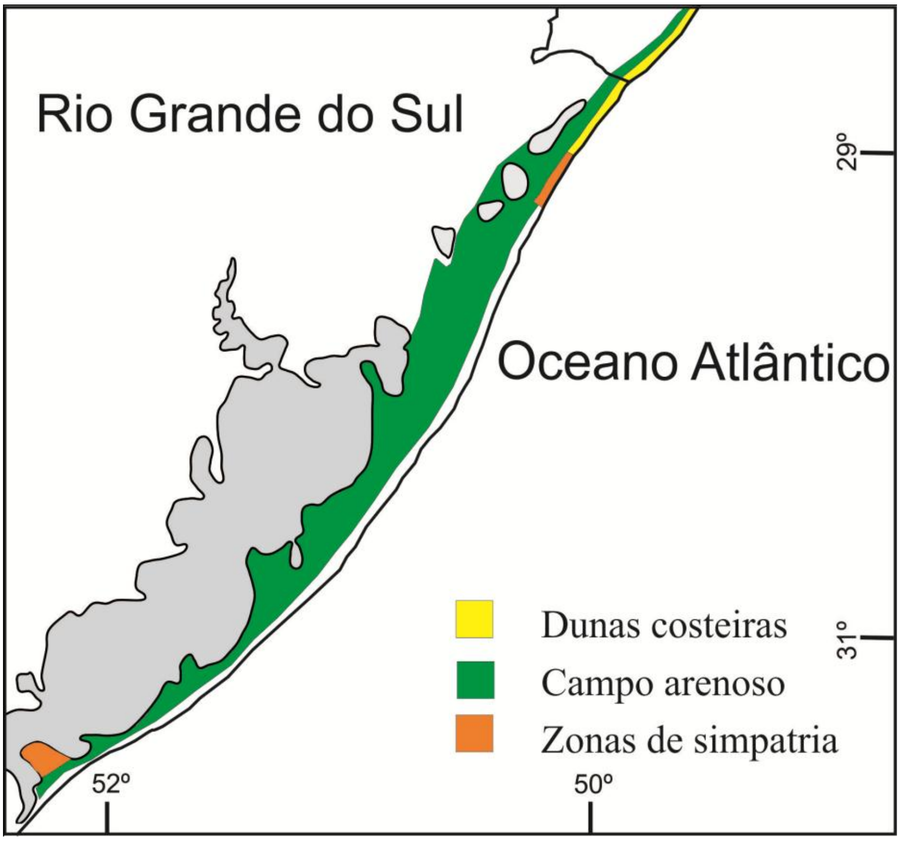

Figura 2. Distribuição geográfica de *Ctenomys minutus* na planície costeira do sul do Brasil nos diferentes tipos de habitats que a espécie ocorre. As zonas de simpatria com a espécie *Ctenomys flamarioni* encontram-se destacadas no mapa.

Ctenomys minutus apresenta uma coloração predominantemente castanho-médio/escura, sendo que apenas a parte inferior de seu corpo apresenta uma coloração castanho-claro com tons da cor areia (Figura 3). A população de *C. minutus* é composta em sua maioria de indivíduos adultos, com uma pequena participação de sub-adultos e jovens (Gastal 1994; Fonseca 2003, Marinho e Freitas 2006), o que sugere uma alta territorialidade dos adultos que pode forçar os jovens à dispersão. 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. 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 (Gastal 1994; Fonseca 2003, Marinho e Freitas 2006). 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 com 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 (Fonseca 2003). Essa espécie é classificada segundo a 'IUCN Red List of Threatened Species' com status de deficiência de dados e como Pouco Preocupante no estado do Rio Grande do Sul (LIVE 2014). Contudo, sabe-se que sofre forte influência da urbanização, do uso de terras para agricultura e pecuária, e da drenagem de solos para cultivo de arroz (Marinho e Freitas 2006) fazendo com que ela seja classificada como Vulnerável na lista de espécies ameaçadas do Brasil (ICMBio 2014).

Figura 3. Exemplar de *Ctenomys minutus* utilizando radio colar na primeira linha de dunas da planície costeira do Rio Grande do Sul

Ao longo de sua ampla distribuição esta espécie apresenta algumas características que se destacam em relação às demais espécies do gênero e de outros roedores subterrâneos. As primeiras características que deve ser ressaltada aqui é a sua distribuição, já que ao norte da sua ocorrência a espécie habita a primeira (dunas propriamente ditas) e segunda linha (campos arenosos) de dunas e a partir da Lagoa dos Barros em direção sul, a sua distribuição é restrita apenas aos campos arenosos, interiorizando-se cerca de 2 km da costa (Freitas 1995) (Figura 2). Ou seja, esta espécie tem capacidade de utilizar tanto o ambiente de dunas costeiras como ambientes de campos arenosos. Estes habitats apresentam uma diferença abrupta em algumas características como biomassa vegetal e dureza do solo, sendo que o habitat de campos arenosos apresenta maior disponibilidade de biomassa vegetal e solos mais duros (Kubiak et al. 2015; Galiano et al. 2016). A ocupação de habitats tão distintos é tão peculiar dentro do gênero que, entre as 70 espécies descritas, somente mais uma espécie apresenta algo similar: *Ctenomys talarum* também se distribui em uma região similar aos campos arenosos e também ocupa regiões de dunas na Argentina (Contreras e Reig 1965; Malizia et al. 1991; Comparatore et al. 1992).

A segunda característica que deve ser destacada é a existência de zonas simpátricas entre *C. minutus* e *C. flamarioni*. Estas zonas de contato ocorrem na planície costeira do estado do Rio Grande do Sul. Uma destas regiões se encontra no extremo sul da ocorrência da espécie, ocorrendo no habitat de campos arenosos na cidade de São José do Norte. Já a outra está localizada ao norte da distribuição da espécie e as espécies compartilham o habitat de dunas costeiras, por aproximadamente 15 km entre os municípios de Capão da Canoa e Arroio Teixeira (Kubiak et al. 2015). Este mesmo estudo apontou que na zona de contato do norte as espécies apresentam segregação de habitat, sendo que *C. minutus* ocupa locais com maior quantidade de biomassa vegetal e com solos mais duros em comparação aos locais ocupados pelos indivíduos da espécie *C. flamarioni.*

Acredita-se que o contexto geográfico é fundamental no processo de especiação de roedores subterrâneos, sendo que o modelo de especiação dos roedores subterrâneos é considerado majoritariamente alopátrico (Steinberg e Patton 2000). No entanto, trabalhos recentes têm demonstrado que espécies de roedores subterrâneos podem ser bons modelos para estudar especiações simpátricas, utilizando espécies que apresentem distribuição em mais de um tipo de habitat, podendo fazer com que demonstrem diferenças morfológicas, comportamentais e genéticas nos diferentes habitats como resposta a possíveis seleções divergentes (Hadid et al. 2013; Li et al. 2015; Lövy et al. 2015, Li et al. 2016). Assim como descrito acima, *C. minutus* apresenta distribuição em habitats com características distintas e ainda apresenta zonas de simpatria com outra espécie do gênero. A soma destes fatores faz com que *C. minutus* seja considerado um bom modelo para desenvolver estudos que buscam compreender como interações bióticas e abióticas influenciam em características comportamentais, ecológicas e evolutivas das espécies.

1.4. Escopo do estudo

O presente estudo apresenta como principal finalidade elucidar como interações bióticas e abióticas influenciam no comportamento, ecologia e evolução da espécie *C. minutus*. Para tal fim este estudo é divido em quatro capítulos com objetivos distintos que se somam, fornecendo assim informações para o esclarecimento do objetivo principal.

No capítulo 1 são analisados quais mecanismos estão envolvidos para que seja possível a coexistência de *C. minutus* e *C. flamarioni,* ao longo de sua zona de contato, utilizando a modelagem de nicho para testar se existe exclusão competitiva em conjunto com a morfometria geométrica para verificar se existe deslocamento de caracteres como fruto da competição.

No capítulo 2 é examinado se interações interespecíficas podem influenciar no tamanho da área de vida das espécies *C. minutus* e *C. flamarioni*. Para isso foi mensurado o tamanho da área de vida das duas espécies no ambiente de dunas costeiras com tratamentos com presença e ausência do possível competidor.

No capítulo 3 é avaliado se diferentes habitats influenciam no tamanho de área de vida de *C. minutus*. Para elucidar este objetivo foi mensurado o tamanho da área de vida de indivíduos que ocupam os habitats de dunas arenosas e campos arenosos.

No capítulo 4 é abordado se diferentes habitats podem gerar divergência adaptativa sobre a força da mordida de *C. minutus*. Para isso utilizamos a morfometria geométrica para acessar informações sobre a forma do crânio e força da mordida nos diferentes habitats.

2. CAPÍTULO I

THE IMPORTANCE OF USING MULTIPLE APPROACHES TO TEST FOR PREDICTIONS OF INTERSPECIFIC COMPETITION: ECOLOGICAL NICHE MODELING, GEOMETRIC MORPHOMETRICS, AND NATURAL HISTORY IN NEOTROPICAL SUBTERRANEAN RODENTS

Bruno B. Kubiak’ [a], Eliécer E. Gutiérrez [b], Daniel Galiano[c], Renan Maestri[d] and Thales R. O. de Freitas[e].

[a] Programa de Pós-Graduação em Biologia Animal, Departamento de Zoologia Universidade Federal do Rio Grande do Sul, Av. Bento Gonçalves 9500, 91501-970, Porto Alegre, Brazil

[b] Programa Nacional de Pós Doutorado em Ecologia, Departamento de Zoologia, Instituto de Ciências Biológicas, Campus UnB, Universidade de Brasília, Asa Norte 70910-900, Brasília, DF, Brazil; and Division of Mammals, Department of Vertebrate Zoology, National Museum of Natural History, Smithsonian Institution, Washington DC.

[c] Programa de Pós-Graduação em Ciências Ambientais – Universidade Comunitária da Região de Chapecó, - Avenida Senador Attílio Fontana, 591-E, CEP 89809-000, Chapecó – SC, Brazil.

[d] Programa de Pós-Graduação em Ecologia, Departamento de Ecologia, Universidade Federal do Rio Grande do Sul – Av. Bento Gonçalves, 9500, CEP 91501-970 Porto Alegre- RS, Brazil.

[e]Programa de Pós-Graduação em Genética e Biologia Molecular, Departamento de Genética, Universidade Federal do Rio Grande do Sul – Av. Bento Gonçalves, 9500, CEP 91501-970 Porto Alegre- RS, Brazil.

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Abstract

Species with similar ecological requirements coexisting in the same geographic region are prone to competitively exclude each other. Alternatively, they may coexist if character displacement acts to change the niche requirements of one or both species. We used two methodological approaches, together with information of the natural history, to assess the hypothesis that interspecific competition explains the parapatric distributions exhibited by two subterranean rodents (*Ctenomys flamarioni* and *C. minutus*). The first approach was based on ecological niche model and aimed to test if the distribution of the two focal species fits the geographic pattern predicted for competitive exclusion. The second approach was based on geometric morphometrics and tested for morphological character displacement (gauged as evidence of competition). We utilized both approaches to test two mutually exclusive hypotheses: (1) given their behavioral, morphological, and ecological similarities, one species competitively excludes the other; or, alternatively (2) character displacement enables their coexistence. The results from the ENM-based approach did not suggest possible competitive exclusion; however, the geometric morphometric analyses demonstrated displacement in skull size of *C. minutus* likely as a result from competition with *C. flamarioni*. This result, combined with observations of microhabitat use and the distributional pattern exhibited by these species, indicates that *C. flamarioni* excludes *C. minutus* from areas with softer soils and higher food availability. We stress the importance for using multiple methodological approaches when testing prediction of competitive exclusion, and discuss the idiosyncrasy of the ENM-based approach when applied to organisms with subterranean habits.

Key words: Biotic interactions, character displacement, competitive exclusion, competitive release, *Ctenomys*.

Introduction

Species' distributions are influenced by history, climate, biotic interactions, and other factors (MacArthur 1972). Among the most studied biotic interactions that affect species' distributions is competition, being competitive exclusion its most extreme manifestation, where one species is completely excluded from a particular habitat by a superior competitor. According to the principle of competitive exclusion, species that exhibit highly similar ecological requirements cannot coexist when resources are limited (Gause 1934; see also Darwin 1859). In fact, the geographic ranges of closely related, morphologically similar species rarely show broad overlaps; however, when they do, the species often use different habitats or differ in behavior, which lessens the strength of competition (Schoener 1974, Pianka 2011; e.g., Stoecker 1972, Peers et al. 2013). These behavioral differences may be followed by morphological differentiation (Brown and Wilson 1956, Grant 1972, Adams and Rohlf 2000), a phenomenon known as character displacement (see below).

Significant advances in ecological niche modeling (ENM) have been achieved in the 21st century, and the development of approaches to study biotic interactions has not been an exception. These efforts have yielded examples of biotic interactions affecting species distribution. Indirect examples of this effect have been provided by studies that showed that incorporating proxies of biotic interactions as predictor variables enhances model predictive ability (e.g. Araújo and Luoto 2007, Heikkinen et al. 2007, Meier et al. 2010, Giannini et al. 2013, González-Salazar et al. 2013). Others, more direct, examples have come from studies employing ENM to unveil evidences of factual, or possible, species interactions (e.g., Anderson et al. 2002, Pellissier et al. 2010, Peers et al. 2013, Gutiérrez et al. 2014).

A method for testing the geographic predictions of competitive exclusion and release has been developed based on analyses of geographic projection of ENMs and occurrence records of a pair of focal, potentially competing species (by Anderson et al. 2002; see also modifications and extensions by Gutiérrez et al. 2014, below). These tests are applicable when the following geographic requirements are met (Anderson et al. 2002, Peterson et al. 2011): (1) the occupied distributional areas (G_O; see Peterson et al. 2011, p. 30) of the two species do not broadly overlap; however, (2) their abiotically suitable areas (G_A) overlap, forming what has been termed 'areas of potential sympatry' (Anderson et al. 2002); (3) within their areas of potential sympatry, contact zones exist, where competition could take place (this allows for testing the geographic prediction of competitive exclusion); and (4) within areas of potential sympatry, zones where only one species is present in absence of the other should exist, where competitive release could take place (this allows for testing the geographic prediction of competitive release, see below). Logically, in addition to these geographic requirements, a credible case for the focal species to potentially be competitors should exist, for example due to a high morphological similarity and a close phylogenetic relationship.

The geographic prediction for competitive exclusion is that the putative superior species is more common—i.e., more than expected by chance in terms of proportion of unique localities—than the other species (i.e., the putatively inferior competitor) in areas of potential sympatry along contact zones (Anderson et al. 2002). The geographic prediction of competitive release states that in zones within the area of potential sympatry where the putative superior competitor is absent, the putative inferior competitor inhabits conditions similar to those in the contact zone (from which it is excluded; Anderson et al. 2002, Peterson et al. 2011). Recently, Gutiérrez et al. (2014) proposed an extension to these tests in which the strength of model predictions are employed to visualize if suitability drives the outcome of the putative competitive exclusion (if any). The idea is to assess whether each species outcompete the other wherever the environmental conditions are more suitable for it than for the other species, or, alternatively, if the putative superior competitor excludes the putative inferior species even from areas more strongly predicted suitable for the latter.

Even in cases in which testing these predictions yields results suggestive of competitive exclusion and release, these analyses based on correlative modeling cannot demonstrate either of these ecological phenomena. Nevertheless, this method can provide directional hypotheses that can then be tested via experimental field and laboratory studies (Anderson et al. 2002; e.g., Brown 1971, Murie 1971, LeBurn et al. 2007). However, when ENM-based analyses do not yield results congruent with the prediction for competitive exclusion, alternative hypotheses are necessary to explain how the focal species maintain their parapatric distributions around contact zones. Because of these limitations of the ENM-based method when used alone, and because experimental studies often are not feasible, here we use the ENM-based method together with a test for morphological character displacement based on geometric morphometric data. Additionally, we used natural history information for the species, including spatial distribution (Freitas 1995, Fernández-Stolz et al. 2007, Lopes et al. 2013, Galiano et al. 2014a Galiano et al. 2014b), diet composition (Lopes et al. 2015) and microhabitats requirements (Kubiak et al. 2015, Galiano et al. 2016).The combination of both techniques (Cáceres et al. 2016), together with natural history information, can provide stronger, and likely complementary evidence of competitive interactions than the use of any of them alone.

Character displacement is an evolutionary phenomenon caused by intense competitive interactions (Brown and Wilson 1956, Grant 1972, Dayan and Simberloff 2005). Brown and Wilson (1956) were the first to use the term “character displacement”, while hypothesizing that the evolution of differences in morphology lead to a reduction in the overlap in resource use and interspecific competition. They suggested that when two species are involved in an intense competitive interaction, they tend to exhibit marked differences in morphology in areas of sympatry, whereas outside of these areas their differences are smaller or even absent. Since then, numerous studies have explored the role of competition in the displacement of morphological traits (e.g., Dayan and Simberloff 1994, Adams and Rohlf 2000, Simberloff et al. 2000, Melville 2002, Benkman 2003; see Dayan and Simberloff 2005, Pfennig and Pfennig 2009).

We studied two species of subterranean rodents, the tuco-tucos *Ctenomys flamarioni* Travi, 1981 and *Ctenomys minutus* Nehring, 1887, and tested two mutually exclusive hypotheses: (1) given their behavioral, morphological, and ecological similarities (reviewed below), one species competitively excludes the other; or, alternatively (2) character displacement enables their coexistence (i.e., competitive exclusion does not take place). Thus, we tested for the geographic predictions of competitive exclusion and release (Anderson et al. 2002; including the extension proposed by Gutiérrez et al. 2014), and employed geometric morphometrics to test if the two focal species are more morphologically distinct in areas of sympatry than in regions where they occur in allopatry. We tested whether the combined use of these techniques allows for a better understanding of competitive interactions than using either of them alone. The results from these analytical approaches were then integrated with available natural history information for both species, focusing on diet and microhabitat partition.

Materials and methods

Focal species and study region

Two species of subterranean rodents of the genus *Ctenomys* represent excellent candidates for testing the geographic predictions of competitive exclusion and displacement of morphological traits. The genus *Ctenomys*, commonly called tuco-tuco, is widely distributed throughout South America, with approximately 70 species (Bidau 2015). These species are predominantly solitary, possess limited mobility and patchy distributions of local populations, and typically present allopatric distributions (Lacey et al. 2000). Only four species of *Ctenomys* are currently known to occur sympatrycally with other congeners, *C. australis* Rusconi, 1934 with *C. talarum Thomas, 1989*, and *C. flamarioni* with *C. minutus* (Contreras and Reig 1965, Reig et al. 1990, Malizia et al. 1991, Comparatore et al. 1992, Kubiak et al. 2015). The latter two, which inhabit the southern Brazilian coastal plain, are the focal species of this study. This region is characterized by its geomorphology being constantly influenced by fluctuations of the Atlantic Ocean, which formed a mosaic of lakes and lagoons in two main environments: sandy dunes (beaches) and sandy fields (Tomazelli et al. 2000). Looking from the east to west, the landscape is formed by the Atlantic Ocean, followed by sandy dunes — beaches that can range from a few meters to about 200 meters — and sandy fields (Fig. 1 and see Electronic Supplementary Material (ESM) A). The climate is mild mesothermal, wet without dry periods, and the vegetation consists of a mosaic of dune vegetation, sandy fields and "restinga" forest (Hesp et al. 2009), with a prevalence of herbaceous species over shrubs (Overbeck et al. 2007, Filho et al. 2013). *Ctenomys flamarioni* is endemic to coastal sand-dune grasslands in the Rio Grande do Sul state, and its range, which extends for about 560 km, is bounded by the city of Arroio Teixeira on the north (Freitas 1995) and by the Chuí River on the south (Fernández-

Stolz et al. 2007). *Ctenomys minutus* inhabits only the sand fields in the southern portion of its range, whereas in its northern portion the species inhabits the first-dune line, predominantly without presence of *Ctenomys flamarioni*. *Ctenomys minutus* occurs from Jaguaruna beach in the Santa Catarina state to the town of São José do Norte in the Rio Grande do Sul state, extending along more than 500 km (Lopes et al. 2013, Galiano et al. 2014a, 2014b). Both species occurs at sea level (Fernadéz-Stolz et al. 2007, Galiano et al. 2014a). Two narrow contact zones have been recently described for these species, one on the northern part of the range of *C. flamarioni*, in an area extending about 15 km on sand dunes; and the other on the southern part of the range of *C. minutus*, in the city of São José do Norte (Kubiak et al. 2015; Fig. 1).

Requirements for testing the geographic predictions of competition exclusion and release

Ctenomys flamarioni and *C. minutus* meet all the requirements for the test of the geographic predictions of competitive exclusion and release. Thus, the distributions of these species do not broadly overlap, but are either allopatric or parapatric with only two contact zones (Fig. 1; Freitas 1995, Kubiak et al. 2015). These contact zones are precisely where competitive exclusion could take place. In several sites along most of the distribution of *C. flamarioni*, *C. minutus* is absent, thus enabling the possibility for competitive release of the former. *Ctenomys minutus* is present in sandy dunes in the northern part of its distribution, where C. *flamarioni* is absent, and therefore competitive release of the former could take place there.

In addition to fulfilling the geographic requirements for testing the geographic predictions of competitive exclusion and release, both species have similar morphologies, making it likely to have similar requirements and preferences regarding resources in the study region (see Galiano et al. 2014b, Kubiak et al. 2015, Galiano et al. 2016). Moreover, based on our fieldwork experience, both species seems equally likely to be captured with the same sampling techniques, in compliance with requirements for conducting the tests proposed by Anderson et al. (2002).

Data sources

To model the species' abiotically suitable areas, we used high-quality occurrence and climatic data. The use of occurrence records (localities) with correct both taxonomic identifications and georeference is critical for satisfactory performance of ecological niche modeling analyses (Romero et al. 2014, Anderson 2015, Costa et al. 2015, Gutiérrez 2016). Hence, we gathered occurrence data only from our own observations in the field or from voucher specimens housed at the collection of the Laboratório de Citogenética e Evolução of the Universidade Federal do Rio Grande do Sul (specimens from our own fieldwork; Freitas 1995, 1997, 2001, Gava and Freitas 2003, Marinho and Freitas 2006, Lopes and Freitas 2012, Lopes et al. 2013, Galiano et al. 2014b, Galiano et al. 2016). Several morphological traits allowed for unmistakable taxonomic identifications (see Freitas et al. 1995). All localities were georeferenced using a GPS Garmin Vista® device at the exact site of collection or observation. We obtained a total of 74 unique localities, 45 for *C. minutus* and 29 for *C. flamarioni* (ESM B). For climatic data, we used 19 Worldclim bioclimatic variables derived from interpolations of precipitation and temperature data, with a resolution of 30 arc-seconds (approximately one kilometer at the Equator; available at http://www.worldclim.org/download; Hijmans et al. 2005). Previous studies have found these variables to be important in determining mammal species distributions (e.g., Gutiérrez et al. 2014, 2015, Soley-Guardia et al. 2016).

Model calibrations

In order to define study regions for model calibrations, we first attempted to use a strategy used in previous studies (e.g., Gutiérrez et al. 2014, 2015), which consists in employing a minimum convex polygons, constructed surrounding large clusters of occurrence records, plus a buffer area outside of this polygon. This operational strategy aims to minimize the inclusion of regions to which the species, does not have access to, due to physical barriers or biotic interactions, but that might contain suitable environmental conditions for it. The inclusion of such inaccessible regions would represent a violation of principles for selecting study areas for model calibration (proposed by Anderson and Raza 2010; see also Barve et al. 2011, Gutiérrez 2016). Nevertheless, because of the truly narrow, small distributions of our focal species, the study areas that resulted from applying the operational strategy just described were too small and did not include enough environmental heterogeneity for Maxent to characterize the abiotically suitable conditions for each species. To solve this problem, we opted for creating a minimum convex polygon surrounding localities of both species, and then delimited a background region by setting a buffer of 50 km around the polygon. The use of a larger buffer seemed unnecessary because ctenomids have limited both mobility and ability of dispersion (Lacey et al. 2000).

To model the species' abiotically suitable areas, we optimized model complexity and predictive power, and conducted model evaluations using a geographically partitioned scheme. The models were constructed with the maximum entropy method implemented in Maxent ver. 3.3.3k (Phillips et al. 2006), a technique that has performed favorably when compared with analytical alternatives for presence-only data (Elith et al. 2006). Recent studies have demonstrated the importance of both balancing model complexity and predictive power and evaluating model’s performance with spatially independent data (see Warren and Seifert 2011, Muscarella et al. 2014, Radosavljevic and Anderson 2014, Warren et al. 2014, Moreno-Amata et al. 2015). Hence, in order produce the best possible model for each species, avoiding overfitting while maximizing predictive power, we employed the R package ENMeval (Muscarella et al. 2014) to select the optimal combination of two important Maxent’s parameters, the value of the regularization multiplier and the combination of feature classes. We tested regularization multiplier values from 0.5 to 6.0 in increments of 0.5, and the following feature classes (or combinations thereof): (1) linear and quadratic; (2) hinge; (3) linear and hinge; (4) quadratic and hinge; and (5) linear, quadratic, and hinge. ENMeval also allowed us to conduct geographically partitioned evaluations, which we did using the “checkerboard1” data-partitioning scheme—this is a variation of the ‘masked geographically structured’ data-partitioning strategy described in Radosavljevic and Anderson (2014). Model performance was assessed using the Akaike Information Criterion corrected for small sample sizes (AICc; Warren and Seifert 2011, Radosavljevic and Anderson 2014). For each species, the final model was constructed employing all unique occurrence records and the combination of regularization multiplier and feature classes that produced the lowest AICc value. To assure that the models selected as optimal performed well, we also inspected omission rate and test AUC. The minimum training threshold was used to classify environmental conditions into suitable or not suitable based on model prediction strengths. This classification is a necessary step for the identification of areas of potential sympatry, i.e. those with suitable conditions for both species.

Specimens and geometric morphometrics

To test for character displacement we used geometric morphometrics based on skull landmarks. We used a total of 85 skulls of adult specimens collected only in the first-dune line—we did not use skulls from specimens from sandy fields to reduce the environmental bias in the test for character displacements (see below). We analyzed data from 39 skulls of *C. flamarioni*, 22 from areas where the other species is absent (hereafter we refer to these sites as 'areas of allopatry') and 17 from areas where the other species is present (hereafter we refer to these sites as 'areas of sympatry'). For *C. minutus*, we analyzed data from 46 skulls, 22 from areas of allopatry and 24 from areas of sympatry. All of the specimens were collected in previous fieldwork (between 2010-2013) and are currently housed at the Laboratório de Citogenética e Evolução, Departamento de Genética, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil (for specimens' locality data and catalogue numbers see ESM C).

We used standards methods of landmark-based 2D geometric morphometrics to remove non-shape differences among our samples. Skulls ventral view images were taken with a Nikon P100 camera with 13.1 megapixel resolution (3648 x 2736) from a standard distance of 75 mm. On each image, 30 landmarks (see ESM D for landmark positions) were digitized using TpsDig2 software (Rohlf 2010), following Fornel et al. (2010). The matrix of landmark coordinates was submitted to a Generalized Procrustes Analysis (GPA) to remove effects not related to shape (position, orientation, and scale) (Bookstein 1991). The resulting matrix (i.e. shape variables) was used in a multivariate regression against centroid size to test for the presence of allometry in the samples (Drake and Klingenberg 2008). Residuals of this analysis were used as a size-corrected shape matrix for all further analyses. The centroid size of each specimen—i.e., the square root of the sum of squared distances of each landmark from the centroid of the configuration—was used as a measure of size (Bookstein 1991). All these procedures were carried out using the software MorphoJ 1.06d (Klingenberg 2011). We assumed that sexual dimorphism is negligible for the present purposes, because interspecific differences are usually greater than the reported sexual dimorphism in both the size and shape of the skull (Fernandes et al. 2009), and the dimorphism is small and constant across geographic locations within species of Ctenomys (Fornel et al. 2010).

Tests of the geographic predictions of competitive exclusion and release

We tested for the geographic predictions of competitive exclusion and release based on ENM and occurrence records. In order to identify areas of potential sympatry, we first projected the final model of each species onto geographic space. These projections were made onto a region in the southern Brazilian coastal plain (extent 27–34º N and 47–53º W) that includes the known ranges of both species. We then overlaid the binary predictions of both models, using the same thresholding rule as in the model evaluations (i.e., the minimum training presence threshold). We analyzed the proportions of species localities in areas of potential sympatry along their known contact zones (described in Kubiak et al. 2015; see also Fig. 1), directly testing the geographic patterns predicted under competitive exclusion (Anderson et al. 2002). Under the assumption that competitive exclusion takes place, we expected two possible geographic scenarios: first, that one species largely predominates in terms of the proportion of unique localities in actual contact zones (this approach is based on binary maps), or, alternatively, that each species predominates wherever the environmental conditions are more suitable for it than for it putative competitor (this approach is based on maps with continuous values of suitability for each species) (see Gutiérrez et al. 2014 for details). We tested for the first of these scenarios using a modification to the method of Anderson et al. (2002) proposed by Gutiérrez et al. (2014), and which aims to avoid likely biases towards the most broadly distributed species. This modification consists in calculating random expectations values of a binomial test using only unique localities of each species from areas of potential sympatry accessible to both species and, to avoid circularity, excluding those in the actual contact zones. For the second scenario, we examined the areas near the contact zones in more detail, determining for each pixel which species had higher values of predicted suitability (following Anderson and Martínez-Meyer 2004, Gutiérrez et al. 2014).

We also tested for the geographic prediction of competitive release. We inspected areas of potential sympatry out of the known contact zones between the species and expected that, in absence of the putative superior competitor, the putative inferior competitor would inhabit conditions similar to those present in the contact zone.

Character displacement

We used a one-way analysis of variance (ANOVA) to test size differences between species in sympatry and allopatry (the categorical variable had one factor with four levels: sympatric *C. minutus*, allopatric *C. minutus*, sympatric *C. flamarioni*, and allopatric *C. flamarioni*). A Tukey's test was conducted to verify pairwise size differences. A between-group principal component analysis (BG-PCA) was used to explore patterns of shape variation among these same four groups. The BG-PCA is an alternative to canonical variate analysis (CVA) when the number of individuals is close to the number of variables (Mitteroecker and Bookstein 2011) as in our case. The BG-PCA consists of a rotation of the shape space in the direction of largest mean group differences, with no distortion of shape distances (as opposed to CVA), and an increase in group discrimination when compared with the ordinary PCA (Mitteroecker and

Bookstein 2011, Seetah et al. 2012). We calculated the significance of mean differences between four groups through pairwise tests with 10,000 permutations, using Procrustes distance between means, in the software MorphoJ 1.06d (Klingenberg 2011). Because we hypothesized that specimens of both species have a higher degree of morphological differentiation in sympatry than in allopatry, we tested if the differences of Procrustes distances between the species in sympatry vs allopatry could not be due to chance alone. To do this, we calculated the observed difference between the Procrustes distance of the species in sympatry minus the Procrustes distance of species in allopatry. The resulting value indicates how strong is the morphological difference in sympatry relative to that in allopatry. We then used a bootstrap procedure to assign random group labels to each specimen (within species, i.e. random assignment of allopatry or sympatry within each species, but not between them), and re-calculated this difference 1,000 times by chance. We then calculated a *p*-value (at α=0.05) assessing the number of times that the random difference (derived from bootstrap sets) was equal or greater than the observed difference.

Results

Species' models and areas of potential sympatry

Our analyses for selecting optimal Maxent's settings to model species' abiotically suitable areas identified Linear and Quadratic features as the best performing combination of feature classes for both species. For *C. flamarioni* the optimal value for the regularization multiplier was 1, whereas for *C. minutus* it was 1.5 (see results of model tuning analyses in ENMeval in ESM E).

Final models of both species identified abiotical suitable areas in the coastal plain region of the southern extreme of Brazil. The final model of *C. minutus* predicted extensive areas as suitable for the species along the coastal plain region, with strong predictions in sandy fields and in sandy dunes. The model identified suitable conditions for this species in the Rio Grande do Sul and southern Santa Catarina states. On the other hand, the abiotically suitable areas identified by the final model of *C. flamarioni* almost exclusively included the sand dunes, where its predictions were stronger to those for *C. minimus*. Only in two regions along the coastal plain *C. flamarioni* had strong predictions in locations away from the sandy dunes (Fig. 2).

After overlapped, binary projections of final models showed an extensive region of potential sympatry in the coastal plain of the Rio Grande do Sul (Fig. 3). The northern boundary of this region is located near the state border between Rio Grande do Sul and Santa Catarina states, whereas the southern boundary is located near the shore of the Patos Lagoon. The environment in this region of potential sympatry is predominantly composed of sandy fields and sandy dunes.

Test for competitive exclusion and release

A total of 61 localities of the focal species were present in areas of potential sympatry. *Ctenomys flamarioni* had a total of 24 localities in areas of potential sympatry, 18 of them away from the contact zones, 4 localities in the northern contact zone, and 2 localities in the southern contact zone. *Ctenomys minutus* had a total of 37 localities in areas of potential sympatry, 33 of them away from contact zones, 3 in the northern contact zone, and 1 locality in the southern contact zone (Fig. 4 and see ESM F). The expected values for the exact binomial tests for *C. flamarioni* and *C. minutus* in the northern contact zone were 2.471 and 4.529, respectively, whereas in the southern contact zone they were 1.059 and 1.941, respectively. Neither in the northern (P =

0.2522) nor in the southern contact zone (P = 0.2858) observed numbers of localities significantly deviated from expectations by chance.

Most records of either species occurred in areas more strongly predicted for that species, but not always. In areas of potential sympatry out of the contact zones, we found a few sites in which a species occurred despite climatic conditions were predicted more suitable for the other species (Fig. 2). That is, in a few sites *C. minutus* occurred despite conditions were more strongly predicted suitable for *C. flamarioni*, and, likewise, in a few sites *C. flamarioni* occurred despite conditions were more strongly predicted suitable for *C. minutus*. By contrast, in contact zones both species occurred in sites always more strongly predicted suitable for *C. flamarioni* (Fig. 3, Fig. 4 and see Appendix S6).

Character displacement

We found significant differences in skull size between groups ($F_{1,81}$= 6,99; P = 0.0035). Specimens of *C. minutus* from areas of actual sympatry with *C. flamarioni* have a smaller size than specimens from areas in which the species is in allopatry (Log centroid size: 2.21 ± 0.09 and 2.27 ± 0.06, respectively; P = 0.036). By contrast, *C. flamarioni* does not present significant differences in size when specimens in sympatry with *C. minutus* are compared with specimens of that species from areas in which the species is in allopatry (Log centroid size: 2.30 ± 0.05 and 2.30 ± 0.07, respectively; P = 0.999) (Fig. 5**.** All pairwise comparisons for shape differences yielded significant results (Fig. 6). In both species, we found that specimens in conditions of sympatry showed significant differences in mean shape when compared with specimens in conditions of allopatry (Procrutes distance = 0.017, P < 0 .0001; Procrutes distance = 0.013, P = 0.001, respectively). More importantly, comparison between species revealed that *C. flamarioni* and *C. minutus* are more different when they are sympatry, than when they are in allopatry (Procrutes distances = 0.046, *P* < 0 .0001; Procrutes distances = 0.034, *P* < 0 .0001, respectively). The bootstrap test showed that this difference is higher than expected by chance (*P* = 0.001).

The BG-PCA analysis showed differences between species and within species in sympatry and allopatry (Fig. 7). The BG-PC1 explains 81.23 % of the total variation. In this axis, differences between the two species are evident, and it is also observed a partial segregation between specimens of *C. minutus* in allopatric and sympatric conditions with respect to each other. Specimens with higher values on BG-PC1 show a more robust posterior part of the skull and increased zygomatic archs (towards the rear), causing the skull to present a slight inclination towards the rear. Moreover, specimens that have lower values on this axis present a relatively smaller posterior part of the skull and a relatively elongated anterior part of the skull. In the BG-PC2 it is possible to verify a difference between specimens of *C. flamarioni* in allopatric and sympatric conditions. BG-PC2 explains 12.58 % of the total variation. In general, shape differences are accentuated for both species in sympatry.

Discussion

Our ENM-based analyses did not support the hypothesis that the distributions of our focal species match the geographic pattern expected under competitive exclusion. Besides, both *C. flamarioni* and *C. minutus* possess records at sites more strongly predicted suitability for the other species, and this is incongruent with the possibility that competitive exclusion (if it would take place) were driven by the climatic conditions. In species pairs with small distributions, it is likely that scarcity of occurrences in areas of potential sympatry away from the contact zones could prevent obtaining expected values that otherwise might allow for the binomial test to yield significant results. However, despite the small, narrow distributions of our focal species, *C. flamarioni* and *C. minutus* possess enough (18 and 24, respectively) occurrences in areas of potential sympatry away from contact zones; hence, we disregard lack of data as potential explanation for the statistically insignificant results obtained. On the other hand, it is possible that the temperature and precipitation variables employed in the ENM analyses do not differentially affect the geographic distributions of the focal species, and that other factors might play a major role in this respect. Given that these species occur in close geographic proximity in a topographically homogeneous region, it is likely that they do not differ substantially in their climatic niches.

Rather than climate, microhabitat characteristics can explain the observed geographic patterns. A recent study found that *C. flamarioni* and *C. minutus* show spatial segregation according to microhabitats characteristics (Kubiak et al. 2015). That study demonstrated that either in sympatry or in allopatry, *C. minutus* selected areas characterized by higher amounts of plant biomass and higher grass cover when compared with areas occupied by *C. flamarioni*. By contrast, *C. flamarioni* showed a distinction in habitat selection when occurring in allopatry and sympatry; in allopatry, the species selects areas with high grass cover and is distributed on less hard soils in comparison to individuals of the same species located in the sympatry with *C. minutus*. The use of soil properties and vegetation types as predicting environmental variables might yield insightful results in future studies aiming to test the geographic prediction of competitive exclusion and release in herbivorous rodents with subterranean habits.

Results from our geometric morphometric analyses documented that the coexistence with *C. flamarioni* led to a reduction in the skull size in *C. minutus*. This finding is congruent with our initial hypothesis stating that *C. flamarioni* and *C. minutus* should present morphological changes to be able to coexist. The fact that *C. minutus* presents a reduction in skull size when in contact with *C. flamarioni* suggests that the former species is an inferior competitor in relation to the latter one. Coincidently, *C. flamarioni* experiences few or no changes in skull size and shape regardless of the presence or absence of *C. minutus*. This result supports the idea that the body size is important in competitive interspecific interactions (Miller 1967), where a bigger body size (*C. flamarioni*) provides competitive advantage over a smaller size (*C. minutus*) (e.g., Diamond et al. 1989, Yom-Tov and Dayan 1996, Melvilee 2002). Browers and Brown (1982) found that granivorous rodents in dessert areas of the southwestern of the US tend to compete intensely when they exhibit similar body sizes (below a 1.5 ratio), and interpreted it as the causal factor for which these species are unlikely to occur in sympatry. Our focal species show a pattern in agreement with this interpretation, as both are parapatric along most of their distributions. The coexistence of both species at the contact zones might be explained by two not mutually-exclusive factors: (1) the focal species forage for food differentially when in sympatry (see Brown et al. 1979), with *C. minutus* changing its diet when in sympatry with *C. flamarioni*, but the latter only decreases the number of consumed plant species when in sympatry with *C. minutus* (Lopes et al. 2015); (2) the focal species use different microhabitats, as previously discussed, in terms of soil hardness and plant biomass (Kubiak et al. 2015).

Importance of multiple methodological approaches

Our results highlight the importance of using multiple methodological approaches when testing predictions of interspecific competition. Because the ENM-based approach did not support the hypothesis that the focal species show a geographic pattern expected under competitive exclusion, the use of this method alone would have fail to reveal the competitive interaction demonstrated via morphometric analyses. Whereas the ENM-based approach is able to detect geographic patterns consistent with competitive exclusion (e.g., Anderson et al. 2002, Gutiérrez et al. 2014), it might fail to do so in cases in which the climatic variables employed are not important—or are substantially less important than other factors—in determining the spatial segregation observed between the focal species. This type I error is expected to occur more frequently in analyses focused on species with highly similar climatic niches, typically with small distributional ranges in topographically homogeneous regions, like is in the case of *C. flamarioni* and *C. minutus*. Although the generality of this possibility requires of additional studies, we encourage the use of additional approaches when studying competitive interactions. Herein, the use of geometric morphometrics, as well as examination of literature on microhabitat use by the focal species, revealed evidence of interspecific competition, which would have been undetected with the use of the ENM approach alone. In fact, *C. flamarioni* and *C. minutus* exhibit a pattern congruent with competitive exclusion, with the latter being excluded from areas with softer soil and higher food availability. No records of *C. minutus* was found for over 20 years of studies in the region of sandy dunes between the two known contact zones (Freitas 1995, Lopes and Freitas 2012, Lopes et al. 2013, Galiano et al. 2014b, Galiano et al. 2016 and Thales de Freitas personal observations). In contrast, *C. flamarioni* is widely recorded sandy dunes in this region (Freitas and Massarini 2005, Fernández-Stolz et al. 2007, Kubiak et al. 2015).

A previous study attempted to analyze results from both ecological niche modelling and geometric morphometrics at a biogeographical scale for two didelphid marsupials (Cáceres et al. 2016). However, the low accuracy in the definition of sympatric and allopatric areas (based on species range maps) probably prevent them to find morphological displacement even if it exists. Moreover, Cáceres et al. (2016) did not explicitly test the predictions of competitive exclusion and release based on ENM, despite discussing it. It is an open question whether competition can be measured in such biogeographical scales based on species range maps.

Underground conditions are known to generate similar selection pressures over rodent linages with subterranean habits. This phenomenon imposes a strong adaptive convergence around the world in which involved species exhibit fusiform body, prominent teeth, reduced eyes, among other similarities (Nevo 1979, Lacey et al. 2000). Because of this similarity, few cases of interspecific sympatry within a genus are recorded for subterranean rodents, and only three are currently known among the approximately 70 species of *Ctenomys* (Contreras and Reig 1965, Reig et al. 1990, Malizia et al. 1991, Comparatore et al. 1992, Kubiak et al. 2015). Clearly, interspecific competition is a dominant factor determining the distribution of subterranean rodents. When applied to these organisms, the ENM approach based on climatic variables-only might be unable to detect patterns congruent with competition, even if competitive exclusion is manifested in the study system. In these systems, it seems necessary use environmental variables more directly associate with underground conditions, as well as multiple approaches that can provide evidence of competition (e.g., geometric morphometrics, and field studies) when it occurs.

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Figures

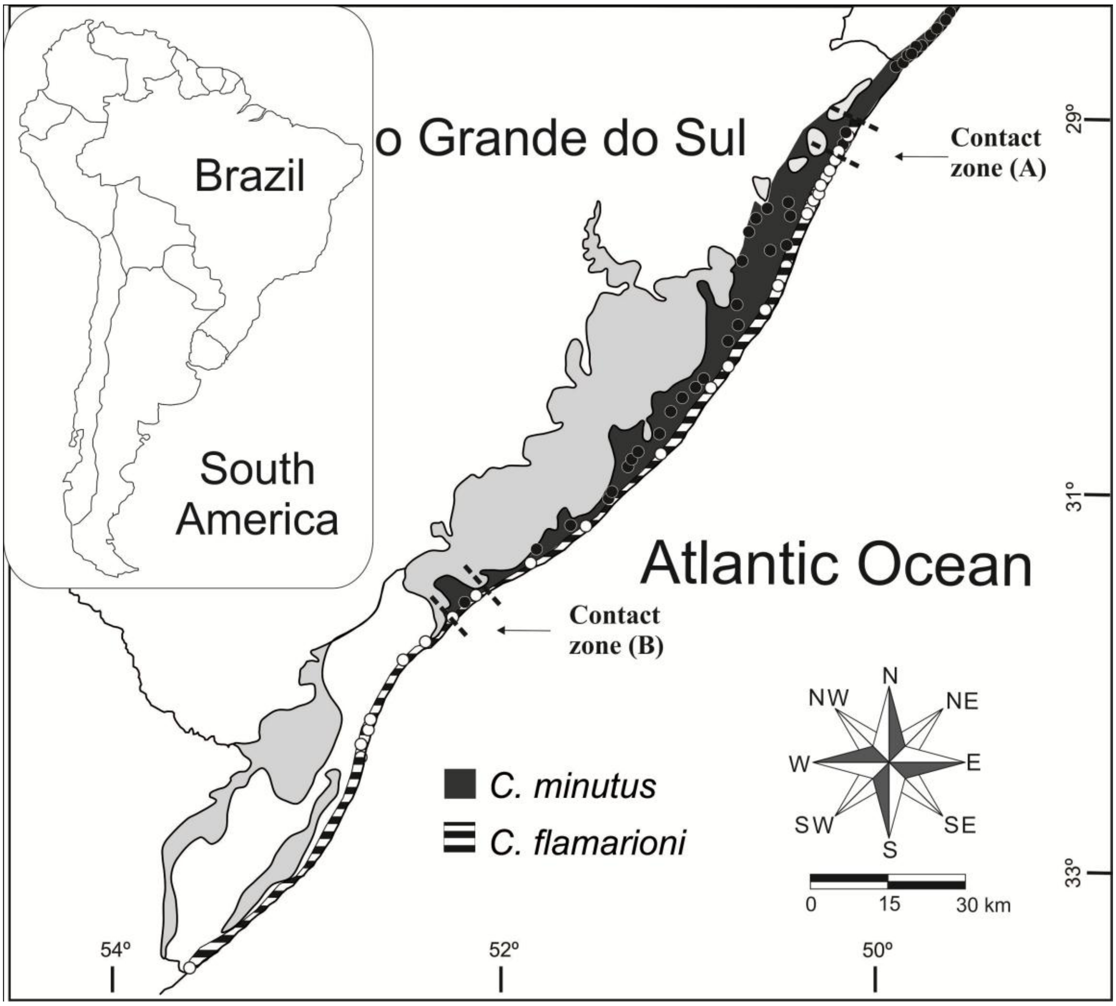


Fig. 1. Localities of *Ctenomys flamarioni* (withe circles) and *Ctenomys minutus* (black circles) and study region used to calibrate models of the abiotically suitable areas. Areas in black and white show the realized distribution of *C. flamarioni*; areas in black represent the realized distribution of *C. minutus*; areas in gray indicate the location of ponds.

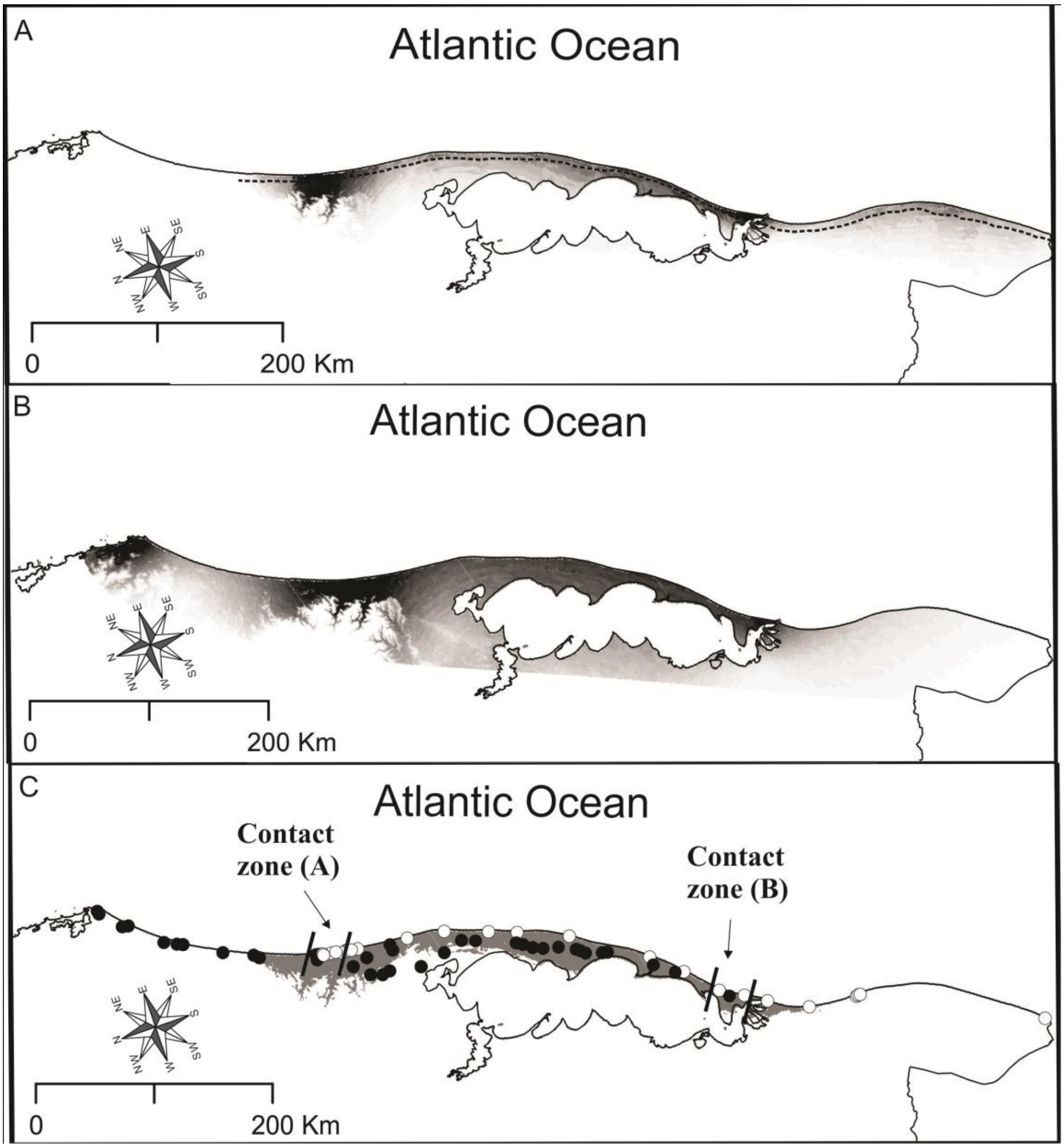


Fig. 2. Ecological niche models projected (in binary format) on the coastal plains of southern Brazil: (A) final Maxent model of abiotically suitable areas for *Ctenomys flamarioni*; (B) final Maxent model of abiotically suitable areas for *C. minutus*, and (C) areas of potential sympatry for both species. Parallel lines indicate location of known contact zone in the north (A) and the south (B). Abiotically suitable areas are indicated with shades of gray; increasingly stronger predictions are indicated with pregressively darker shades. Areas of potential sympatry are those where suitable environmental conditions exist for both species. The dashed line in “A” indicates the approximate limits of the sand dunes. Withe circles represent localities of *C. flamarioni*; black circles reprensent localities of *C. minutus*;.

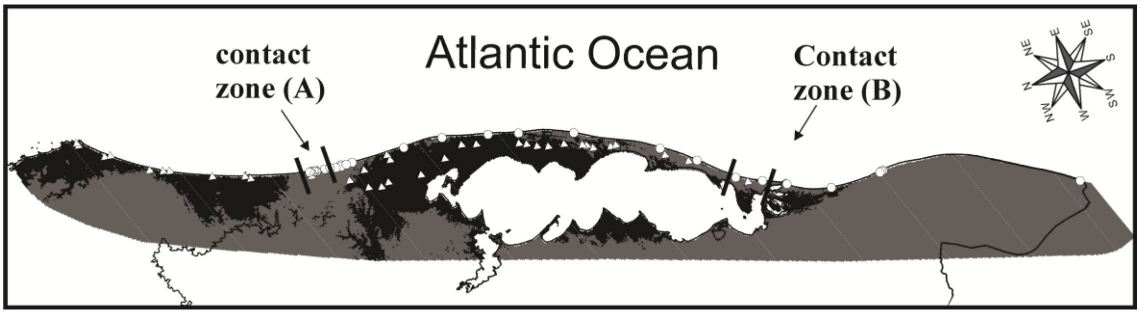


Fig. 3. Binary representation of the climatic suitability for *Ctenomys flamarioni* and *C. minutus* in areas of potential sympatry. Circles represent records of *C. flamarioni*; triangles represent records of *C. minutus*. Sites (image pixels) with higher suitability values for *C. flamarioni* are indicated in grey; sites (image pixels) with higher suitability values for *C. minutus* are indicated with black.

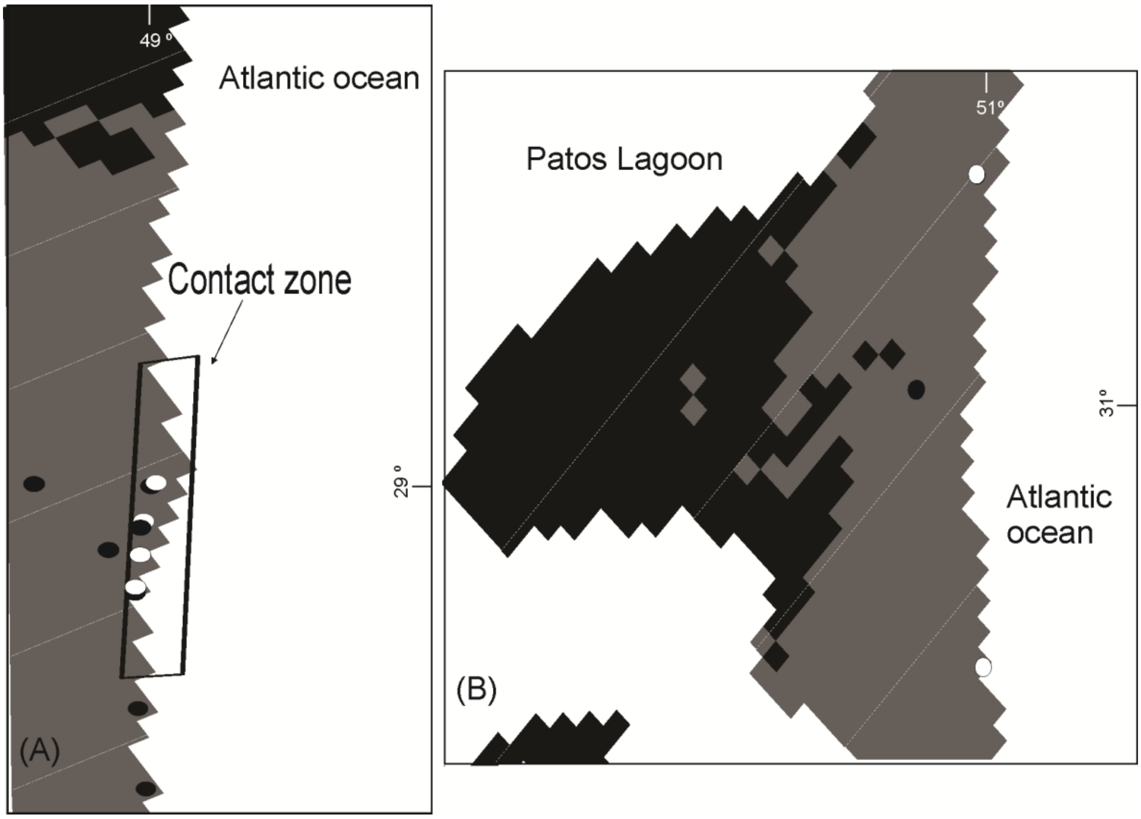


Fig. 4. Comparison strenghts of of predicted environmental suitability for *C. flamarioni* and *C. minutus*. These comparisons are based on final models projected at the known conctact zones of the focal species. Pixels more strongly predicted suitable for either species: pixels in which the species that had higher suitability values was *C. minutus* are indicated with black shading, whereas pixels in which the species with higher suitability values was *C. flamarioni* are indicated with grey shading. (A) shows models projected at the northern area, whereas (B) does so for the southern area. Withe circles reprensent localities of *C. flamarioni* and black circles represent localities of *C. minutus*. The rectangle in the figure (A) represents the limits of the contact zone between the species.

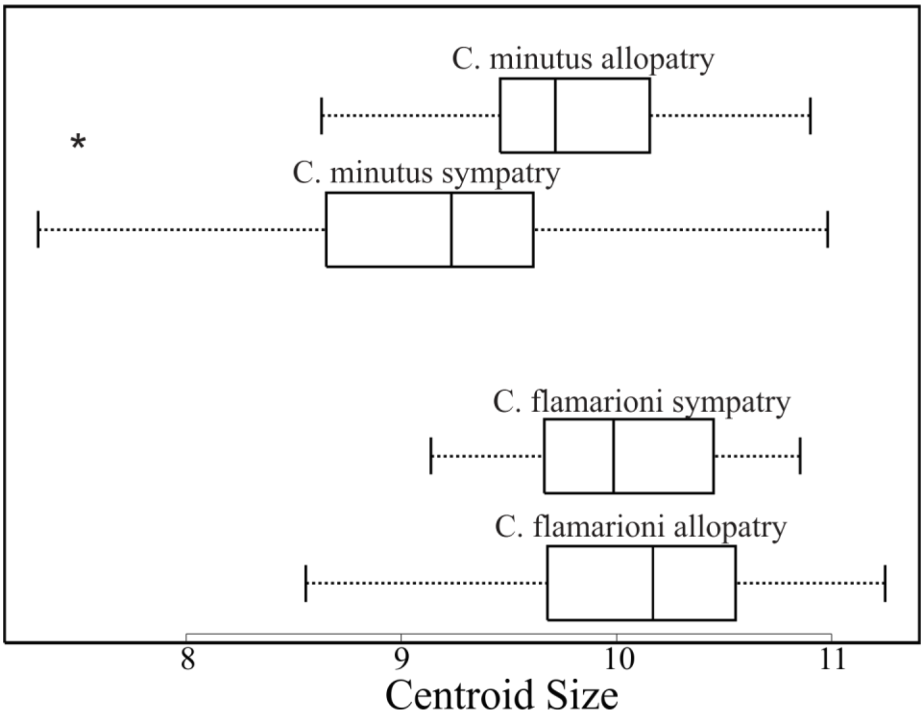


Fig. 5. Boxplot showing skull centroid size variation in *Ctenomys flamarion* and *C. minutus* in sympatry and allopatry. Asterisk indicates a significant difference between groups. The central line show the median, and the square limits are showing the first and thrid quartiles, respectively.

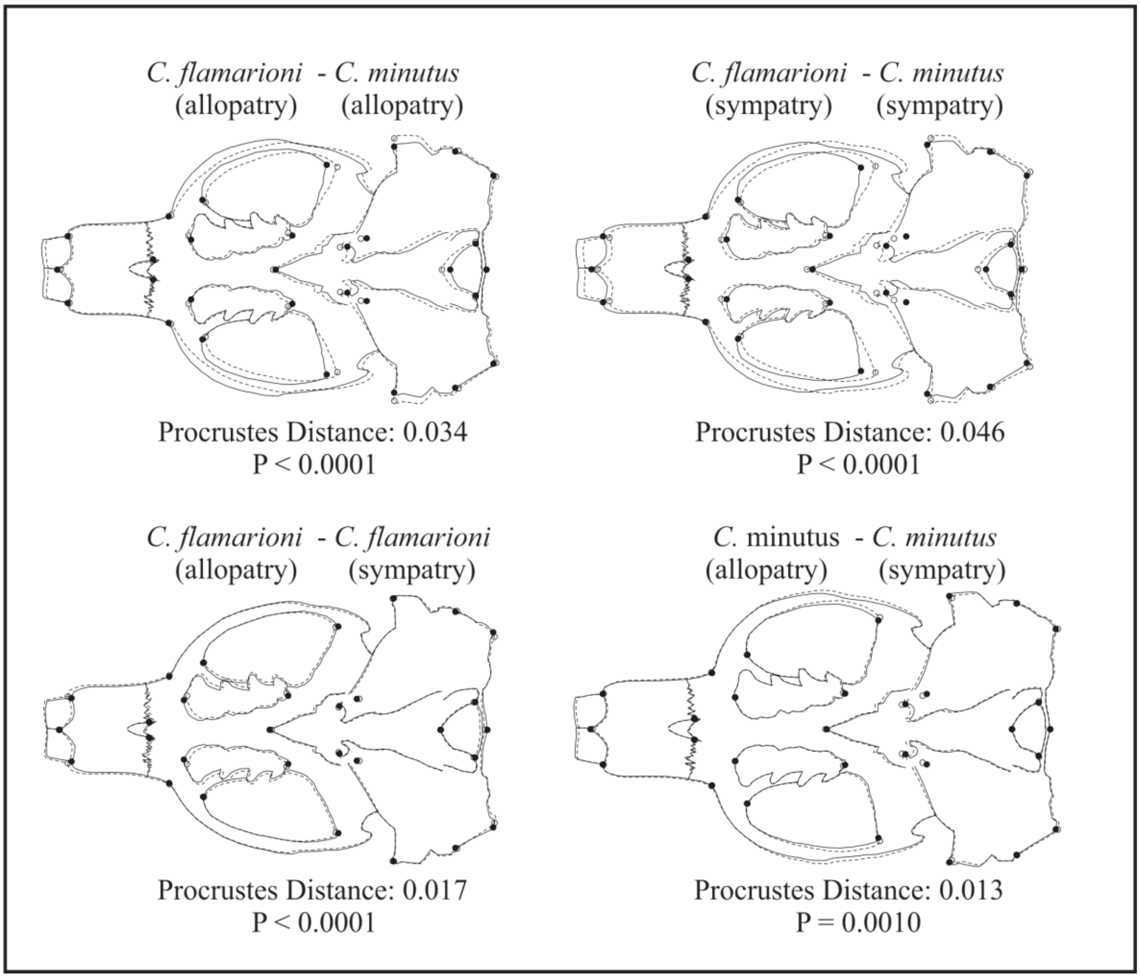


Fig. 6. Procrustes distances between groups. The dotted line represents the first group and the continuous line represents the second. For example, in the first image the dotted line represents *Ctenomys flamarioni* in allopatry, whereas the continuous line represents *C. minutus* in allopatry.

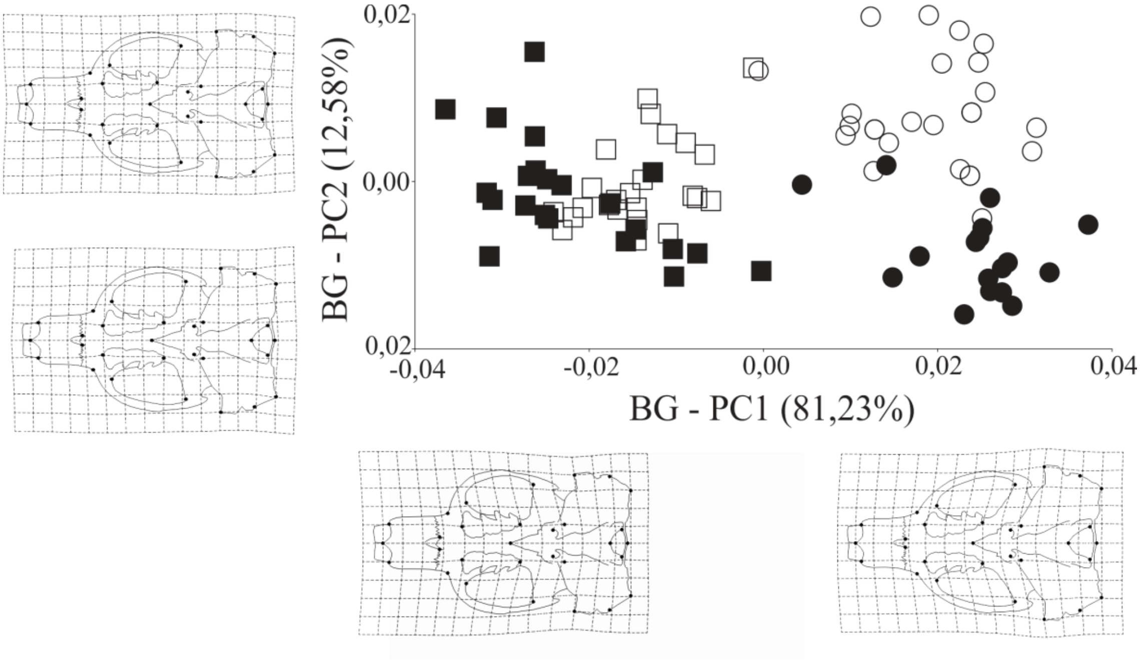
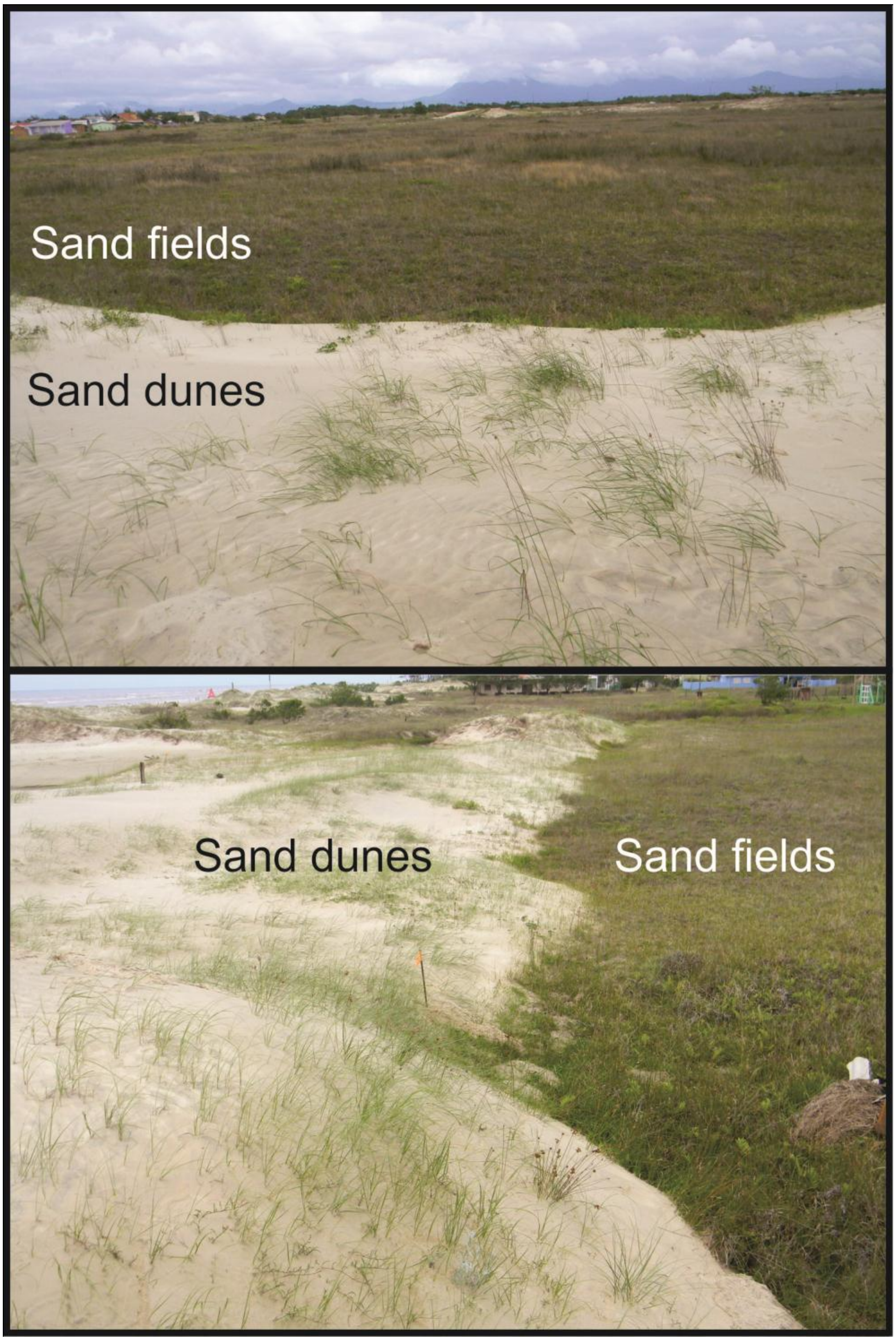


Fig. 7. Scatter plot of the two first axes of a between-group principal component analysis for the ventral view of the skull for C. *flamarioni* and C. *minutus*. The predicted shape change along each axis is given. Solid circles represent *C. flamarioni* in allopatry; open circles represent *C. flamarioni* in sympatry; solid squares represent *C. minutus* in allopatry; open squares represent *C. minutus* in sympatry.

ELECTRONIC SUPPLEMENTAL MATERIAL

ESM A Typical habitats of the coastal plain of southern Brazil. The sandy dunes is habitated by *C. flamarioni* and *C. minutus*, whereas only *C. minutus* inhabits the sandy fields.



ESM B - Below we list the localities of *Ctenomys flamarioni* and *C. minutus* used in this study (Fig. 1). All locations are located in the Brazilian states of Rio Grande do Sul (RS) and Santa Catarina (SC).

Ctenomys flamarioni - 27 unique georeferenced localities: 1. Praia do Barco/RS (29º43'41"S, 50º00'18"W), TR 1626; 2. Praia do Barco/RS (29º42'11"S, 49º59'13"W), TR 1853; 3. Imbé/RS (29º57'11"S, 50º06'50"W), TR 2812; 4. Albatroz/RS (29º53'35"S, 50º05'20"W), TR 2819; 5. Santa Terezinha do Norte/RS (29º52'55"S, 50º04'34"W), TR 050; 6. Atlântida Sul/RS (29º52'36"S, 50º05'06"W), TR1919; 7. Mariapolis/RS (29º50'42"S, 50º 04' 19"W), TR 1584; 8.ESEC Taim/RS (32º43'44"S, 52º27'18”W), TR 1721; 9. ESEC Taim/RS (32º43'16"S, 52º27'14"W), TR 1736; 10. Atlântida/RS (29º46'41"S, 50º01'48"W), TR 048; 11. Xangri-lá/RS (29º48'43"S, 50º02'46"W), TR 056; 12. Praia da harmonia/RS (29º54'40"S, 50º05'53"), TR 1535; 13. Magistério/RS (30º17'24"S, 50º15'32"W), TR 029; 14. Vila São Simão/RS (30º57'43"S, 50º40'48"W), TR 215; 15. Bojuru/RS (31º38'42"S, 51º22'19"W), TR 203; 16. Praia do Mar Grosso/RS (32º02'54"S, 51º59'14"W), TR 2213; 17. Cassino (32º09'40"S, 52º06'47"W), TR 500; 18. Santa Vitória do Palmar/RS (32º42'14"S, 52º27'11"W), TR 474; 19. Barra do Chuí/RS (33º43'41"S, 53º21'50"W), TR 062; 20. Praia do Cassino/RS (32º22'50"S, 52º19'25"W), TR 689; 21. São José do Norte (31º55'12"S, 51º51'18"W), TR 2215; 22. Farol da Conceição (31º47'17"S, 51º35'46"W), TR 2192; 23. Mostardas (31º15'23"S, 50º54'59"W), TR 203; 24. Farol da Solidão/RS (30º47'42"S, 50º33'18"W), TR 2074; 25. Lagoa Bacopari (30º32'13"S, 50º22'19"W), TR 213; 26. Praia do Barco (29º42'58"S, 49º59'46"W), TR 499; 27. Praia do Barco (29º41'10"S, 49º58'48"W), TR 1618.

Ctenomys minutus: 45 unique georeferenced localities: 1. São José do Norte/RS (31º57'21S, 51º55'54"W), TR 2224; 2. Bojuru/RS (20 Km South) (31º43'59"S, 51º34'00"W), TR 2227; 3. Bojuru/RS (31º37'59"S, 51º25'59"W), TR 2239; 4.

Tavares/RS (31º22'59"S, 51º09'W), TR 1941; 5. Tavares/RS (31º16'59"S, 51º04'59"W), TR 2089; 6. Mostardas/RS (26 Km South) (31º15'46"S, 51º02'34"W), TR 1099; 7. Mostardas/RS (17 Km South) (31º14'30"S, 51º01'12"W), TR 1091; 8. TR 688 – Mostardas/RS – 31º08'48"S, 50º56'11"W; 9. Mostardas/RS (Km 115) (31º08'48"S, 50º56'11"W), TR 852; 10. BR 101 – KM108/RS (31º08'48"S, 50º56'11"W), TR 856; 11. Mostardas/RS (Lagoa das Figueiras) (30º59'36"S, 50º49'27"W), TR 847; 12. BR 101 – KM101/RS (30°56'55"S, 50º46'26"W), TR 199; 13. Capivaria do Sul/RS (30º54'45"S, 50º44'16"W), TR 848; 14. Mostardas/RS (Fazenda Ressaca – KM 96) (30º40'59"S, 50º33'22"W), TR 845; 15. Mostardas/RS (Granja Passo Fundo) (30°36'01"S, 50º29'40"W), TR 840; 16. BR101 – KM35/RS (30º27'10"S, 50º29'40"W), TR 836; 17. Palmares do Sul/RS – (30º15'22"S, 50º28'49"W), TR 2040; 18. BR101 – KM35/RS – (30º27'10"S, 50º29'40"W), TR 839; 19. Palmares do Sul/RS (30º15'22"S, 50º28'49"W), TR 2092; 20. Pitangueira/RS (30º03'11"S, 50º22'38"W), TR 442; 21. Passinhos/RS (30º01'24"S, 50º22'45"W), TR 2035; 22. Lagoa dos Barros/RS (29º55'27"S, 50º19'05"W), TR 209; 23. Lagoa dos Barros/RS (29º59'44"S, 50º22'20"W), TR 407; 24. Lagoa Fortaleza/RS (30º09'37"S, 50º13'36"W), TR 2002; 25. Lagoa Suzana/RS (30°09'23"S, 50º15'58"W), TR 453; 26. Lagoa Fortaleza/RS (30º09'37"S, 50º13'36"W), TR 2102; 27. Nova Tramandaí/RS (29º59'06"S 50º12'27"W), TR 166; 28. Lagoa Emboaba/RS (29º58'28"S, 50º12'26"W), TR 020; 29. Tramandaí/RS (29º51'08"S, 50º12'05"W), TR 033; 30. Lagoa Emboaba/RS (29º58'28"S, 50º12'26"W), TR 023; 31. Tramandaí/RS (lagoa Traíras) (29º51'08"S, 50º12'05"W), TR 039; 32. Praia do Barco/RS (29º42’26”S, 49º59’03”W), TR 2814; 33. Praia do Barco/RS (29º42’22”S, 49º58’58”) TR 2815; 34. Praia do Barco/RS (29º40'53"S 49º58'08"W), TR 1620; 35. Torres/RS (29º21'12"S, 49º43'56"W), TR 2347; 36. Passo de Torres/SC (29º19'04"S, 49º42'45"W), TR 212; 37. Praia da Gaivota/SC (29º09'21"S, 49º34'27"W), TR 2379; 38. Morro dos Conventos/SC (28º56'38"S, 49º21'50"W), TR 1216; 39. Ilhas/SC (28º54'25"S, 49º20'27"W), TR 1166; 40. Lagoa dos Esteves/SC (28º50'15"S, 49º16'45"W), TR 1170; 41. Balneário Arroio Corrente/SC (28º41'56"S, 49º01'26"W), TR 004; 42. Jaguaruna/SC (28º39'29"S, 49º00'46"W), TR 007; 43. Praia de Jaguaruna/SC (28º41'56"S, 49º01'26"W), TR 1164; 44. Farol de Santa Marta/SC (28º35'53"S, 48º50'19"W), TR 1200; 45. Farol de Santa Marta/SC (28º35'49"S, 48º49'08"W), TR 1214.

ESM C 2 – Below we list the specimens' locality data and catalogue numbers of *Ctenomys flamarioni* and *C. minutus* used in geometric morphometric analysis. All locations are located in the Brazilian states of Rio Grande do Sul (RS) and Santa Catarina (SC).

Ctenomys flamarioni**: Allopatry** – 1. PUC 39 – Tramandaí/RS; 2. PUC 46 – Tramandaí/RS; 3. PUC 80 Praia do Cassino/RS; 4. PUC 82 – Praia do Cassino/RS; 5. PUC 83 – Praia do Cassino/RS; 6. PUC 232 – Praia rei do peixe, Palmares/RS; 7. PUC 384 – Farol da Solidão/RS; 8. PUC 613 – Rainha do Mar/RS; 9. TR 30 – Cidreira/RS; 10. TR 32 – Cidreira/RS; 11. TR 47 – Santa Terezinha/RS; 12. TR 61 – Fazenda Caçapava, Taim/RS; 13. TR 62 – Fazenda Caçapava, Taim/RS; 14. TR 64 – Fazenda Caçapava, Taim/RS; 15. TR 65 – Fazenda Caçapava, Taim/RS; 16. TR 66 – Fazenda Caçapava, Taim/RS; 17. TR 67 – Fazenda Caçapava, Taim/RS; 18. TR 230 – Farol da Solidão/RS; 19. TR 485 – Tramandaí/RS; 20. TR 494 – São Simão/RS; 21. TR – Fazenda Caçapava, Taim/RS 495; 22. TR 500– Praia do Cassino/RS.

Sympatry – 1. PUC 45 – Arroio Teixeira/RS; 2. PUC 47 – Capão Novo/RS; 3. TR 44 – Praia do Barco/RS; 4. TR 45 – Praia do Barco/RS; 5. TR 54- Capão Novo/RS ; 6. TR 1842 – Praia do Barco/RS; 7. TR 1845 – Praia do Barco/RS; 8. TR 1859 – Praia do Barco/RS; 9. TR 1852 – Praia do Barco/RS; 10. TR 1853 – Praia do Barco/RS; 11. TR 1849 – Praia do Barco/RS; 12. TR 1861 – Praia do Barco/RS; 13. TR 1863 – Praia do Barco/RS; 14. TR 1864 – Praia do Barco/RS; 15. TR 1865 – Praia do Barco/RS; 16. TR 1866 – Praia do Barco/RS; 17. TR 1867 – Praia do Barco/RS.

Ctenomys minutus: **Allopatry** – 1. PUC 49 – Praia da Cal, Torres/RS; 2. PUC 281 – Praia de Jaguaruna/SC; 3. PUC 288 – Praia de Jaguaruna/SC ; 4. PUC 290 – Praia de Jaguaruna/SC; 5. PUC 291 – Praia de Jaguaruna/SC; 6. PUC 293 – Praia de

Jaguaruna/SC; 7. PUC 312 – Passo de Torres/SC; 8. PUC 313 – Passo de Torres/SC; 9. PUC 414 – Morro dos Conventos/RS; 10. PUC 426 – Morro dos Conventos/RS– Morro dos Conventos/RS ; 11. PUC 427– Morro dos Conventos/RS; 12. PUC 610 – Morro dos Conventos/RS; 13. TR 1 – Praia de Jaguaruna/SC; 14. TR 2 – Praia de Jaguaruna/SC; 15. TR 4 – Praia de Jaguaruna/SC; 16. TR 5 – Praia de Jaguaruna/SC; 17. TR 6 – Praia de Jaguaruna/SC; 18. TR 7 – Praia de Jaguaruna/SC; 19. TR 8 – Praia de Jaguaruna/SC; 20. TR 201 – Morro dos Conventos/SC; 21. TR 544 – Praia da Gaivota/RS; 22. TR 554 – Praia da Cal, Torres/RS; 23. TR567 – Praia de Jaguaruna/SC; 24. TR 632 – Praia da Cal, Torres/RS.

Sympatry – 1. TR 40 – Praia do Barco/RS; 2. TR 41 – Praia do Barco/RS; 3. TR 42 – Praia do Barco/RS; 4. TR 43 – Praia do Barco/RS; 5. TR 46 – Praia do Barco/RS; 6. TR 52 – Capão Novo/RS; 7. TR 55 – Capão Novo/RS; 8. TR 56 – Capão Novo/RS; 9. TR 1841 – Praia do Barco/RS; 10. TR 1843 – Praia do Barco/RS; 11. TR 1844 – Praia do Barco/RS; 12. TR 1846 – Praia do Barco/RS; 13. TR 1847 – Praia do Barco/RS; 14. TR 1848 – Praia do Barco/RS; 15. TR 1850 – Praia do Barco/RS; 16. TR 1851 – Praia do Barco/RS; 17. TR 1855 – Praia do Barco/RS; 18. TR 1856 – Praia do Barco/RS; 19. TR 1857 – Praia do Barco/RS; 20. TR 1858 – Praia do Barco/RS; 21. TR1860 – Praia do Barco/RS; 22. TR1862 – Praia do Barco/RS.

ESM D – Description of landmark positions.

L1: Anterior tip of suture between premaxillas; L2,3: lateral extremity of incisor alveolus; L4,5: lateral edge of incisive foramen in the suture between premaxilla and maxilla; L6,7: anteriormost point of the root of zygomatic arch; L8,9: anteriormost point of premolar alveolus; L10,11: anteriormost point of the orbit in inferior zygomatic root; L12,13: posterior extremity of third molar alveolus; L14: posterior extremity of suture between palatines; L15,16: anteriormost point of intersection between jugal and squamosal; L17,18: posteriormost point of pterygoid; L19,20: anteirior extremity of tympanic bulla; L21,22: anterior tip of external auditory meatus; L23,24: posterior extremity of mastoid apophysis; L25,26: posterior extremity of paraoccipital apophysis; L27: anteriormost point of foramen magnum; L28: posteriormost point of foramen magnum at midsagittal plane; L29,30: posterior extremity of occipital condyle in foramem magnum.

ESM E – Results of model tuning analyses conducted in ENMeval.

Our analyses to select optimal Maxent's settings identified the use of Linear and Quadratic features as the best combination of features classes for both species. For *C. flamarioni* the best performing value for regularization multiplier was 1, whereas for *C. minutus* it was 1.5. These optimal settings (LQ 1, for *C. flamarioni*; LQ 1.5 for *C. minutus*) showed the lowest mean AICc, which was our primary optimality criterion. They were also the best performing settings considering the mean diff.AUC (i.e. mean training minus testing AUC values), and Mean.ORmin (omission rate calculated with the the minimum training presence threshold). Other settings possessed higher mean AUC values, but the differences were small. See tables with values for all evaluation metrics below.

Table 1. Results from tuning experiments using ENMeval for *Ctenomys flamarioni*. Matrix of evaluation criteria sorted by AICc, the optimality criterion used for model tuning in this study. The combination of feature class and regularization multiplier with the lowest AICc was considered the 'best' model and used for final model calibration and all subsequent analysis; here the best combination of settings were Linear and Quadratic and regularization multiplier = 3. The default settings (Linear, Quadratic, and Hinge; regularization = 1) led to models that were substantially worse. Mean omission rate was calculated using the minimum training presence threshold.

Feature Class	**Regularization Multiplier**	**Mean Test AUC**	**Variance Test AUC**	**Mean AUC DIFF**	**Variance AUC DIFF**	**Mean Omission Rate**	**Variance Omission Rate**	**AICc**	**Delta AICc**	**Model Parameters**
H	0.5	0.94837	0.00001	0.03474	0.00001	0.26136	0.00013	NA	NA	50
QH	0.5	0.94694	0.00001	0.03604	0.00001	0.35227	0.01046	NA	NA	33
LQ	0.5	0.93494	0.00006	0.02648	0.00023	0.24432	0.01424	529.0159	31.54638421	14
LH	0.5	0.94839	0.00000	0.03440	0.00001	0.26136	0.00013	NA	NA	37
LQH	0.5	0.94654	0.00000	0.03624	0.00001	0.26136	0.00013	NA	NA	45
H	1	0.93853	0.00011	0.02234	0.00025	0.18182	0.03306	563.586132	66.11661619	17
QH	1	0.94017	0.00003	0.02007	0.00014	0.18182	0.03306	558.6894797	61.21996396	17
LQ	**1**	**0.93188**	**0.00013**	**0.01847**	**0.00020**	**0.13636**	**0.01860**	**497.4695158**	**0**	**7**
LH	1	0.93765	0.00013	0.02291	0.00027	0.18182	0.03306	580.5217055	83.05218972	18
LQH	1	0.93956	0.00004	0.02082	0.00016	0.18182	0.03306	578.0532946	80.58377887	18
H	1.5	0.92347	0.00041	0.02563	0.00066	0.13636	0.01860	516.8429289	19.37341316	11
QH	1.5	0.93121	0.00011	0.01770	0.00015	0.13636	0.01860	509.624857	12.15534122	10
LQ	1.5	0.92921	0.00020	0.01676	0.00028	0.13636	0.01860	502.4284193	4.958903501	7
LH	1.5	0.92706	0.00028	0.02204	0.00049	0.13636	0.01860	523.1460541	25.67653837	12
LQH	1.5	0.93040	0.00012	0.01910	0.00018	0.13636	0.01860	509.5695492	12.10003346	10
H	2	0.91645	0.00057	0.02767	0.00077	0.13636	0.01860	519.8563936	22.38687784	10
QH	2	0.93001	0.00018	0.01504	0.00023	0.09091	0.00826	513.0998754	15.63035959	9
LQ	2	0.92781	0.00021	0.01673	0.00028	0.09091	0.00826	503.1318155	5.662299742	6
LH	2	0.92216	0.00033	0.02212	0.00049	0.13636	0.01860	524.6147077	27.14519193	11
LQH	2	0.92840	0.00023	0.01673	0.00028	0.09091	0.00826	513.2646974	15.79518165	9
H	2.5	0.90936	0.00064	0.02880	0.00083	0.09091	0.00826	522.1891694	24.71965364	9
QH	2.5	0.92746	0.00017	0.01527	0.00023	0.09091	0.00826	506.3315215	8.862005717	6
LQ	2.5	0.92577	0.00021	0.01714	0.00029	0.09091	0.00826	506.3322411	8.862725321	6
LH	2.5	0.91800	0.00028	0.02112	0.00045	0.09091	0.00826	515.6319032	18.16238746	8

Feature Class	**Regularization Multiplier**	**Mean Test AUC**	**Variation Test AUC**	**Mean AUC DIFF**	**Variation AUC DIFF**	**Mean Omission Rate**	**Variation Omission Rate**	**AICc**	**Delta AICc**	**Model Parameters**
LQH	2.5	0.92586	0.00021	0.01714	0.00029	0.09091	0.00826	506.3337549	8.864239119	6
H	3	0.90571	0.00055	0.02624	0.00069	0.09091	0.00826	519.4917576	22.02224186	7
QH	3	0.92490	0.00013	0.01539	0.00024	0.09091	0.00826	509.2339791	11.76446333	6
LQ	3	0.92262	0.00017	0.01752	0.00031	0.09091	0.00826	509.207253	11.73773724	6
LH	3	0.91376	0.00024	0.02007	0.00040	0.09091	0.00826	510.9101229	13.44060708	6
LQH	3	0.92301	0.00018	0.01752	0.00031	0.09091	0.00826	509.1947311	11.72521534	6
H	3.5	0.90222	0.00051	0.02564	0.00066	0.09091	0.00826	519.2173194	21.74780358	6
QH	3.5	0.92304	0.00009	0.01446	0.00021	0.09091	0.00826	508.3388378	10.86932202	5
LQ	3.5	0.91941	0.00011	0.01712	0.00029	0.09091	0.00826	508.3387823	10.86926654	5
LH	3.5	0.91013	0.00022	0.01917	0.00037	0.09091	0.00826	509.5815466	12.11203086	5
LQH	3.5	0.92077	0.00014	0.01712	0.00029	0.09091	0.00826	508.3420784	10.87256262	5
H	4	0.90057	0.00043	0.02409	0.00058	0.09091	0.00826	515.9500787	18.48056294	4
QH	4	0.92044	0.00007	0.01441	0.00021	0.09091	0.00826	509.7825473	12.31303151	5
LQ	4	0.91648	0.00005	0.01582	0.00025	0.09091	0.00826	509.7793672	12.30985144	5
LH	4	0.90598	0.00023	0.01975	0.00039	0.09091	0.00826	511.1268383	13.65732255	5
LQH	4	0.91912	0.00009	0.01582	0.00025	0.09091	0.00826	509.7831384	12.31362262	5
H	4.5	0.89659	0.00037	0.02383	0.00057	0.09091	0.00826	521.8822364	24.41272066	5
QH	4.5	0.91651	0.00004	0.01441	0.00021	0.09091	0.00826	511.2517444	13.78222866	5
LQ	4.5	0.91310	0.00002	0.01542	0.00024	0.09091	0.00826	511.2517792	13.78226341	5
LH	4.5	0.90181	0.00020	0.01993	0.00040	0.09091	0.00826	512.7184373	15.24892157	5
LQH	4.5	0.91560	0.00005	0.01542	0.00024	0.09091	0.00826	511.2521696	13.78265378	5
H	5	0.89381	0.00036	0.02327	0.00054	0.09091	0.00826	524.7994548	27.32993903	5
QH	5	0.91288	0.00002	0.01444	0.00021	0.09091	0.00826	509.6667252	12.19720944	4
LQ	5	0.91064	0.00002	0.01521	0.00023	0.09091	0.00826	509.666575	12.19705926	4
LH	5	0.89802	0.00021	0.01885	0.00036	0.09091	0.00826	511.254697	13.78518123	4
LQH	5	0.91187	0.00003	0.01521	0.00023	0.09091	0.00826	509.6665806	12.19706481	4
H	5.5	0.89488	0.00032	0.02060	0.00042	0.09091	0.00826	524.2352078	26.76569206	4
QH	5.5	0.91054	0.00002	0.01452	0.00021	0.09091	0.00826	508.3143847	10.84486893	3
LQ	5.5	0.90900	0.00001	0.01483	0.00022	0.09091	0.00826	508.3143943	10.84487852	3
LH	5.5	0.89691	0.00025	0.01772	0.00031	0.09091	0.00826	510.0263267	12.5568109	3
LQH	5.5	0.91014	0.00002	0.01483	0.00022	0.09091	0.00826	508.3143989	10.84488309	3
H	6	0.89642	0.00026	0.01710	0.00029	0.09091	0.00826	526.5701355	29.10061971	4
QH	6	0.90822	0.00002	0.01477	0.00022	0.09091	0.00826	509.6387042	12.16918838	3
LQ	6	0.90822	0.00002	0.01477	0.00022	0.09091	0.00826	509.6387113	12.16919549	3
LH	6	0.89747	0.00023	0.01588	0.00025	0.09091	0.00826	511.4667886	13.99727277	3
LQH	6	0.90822	0.00002	0.01477	0.00022	0.09091	0.00826	509.6387035	12.16918777	3
H	6.5	0.89710	0.00024	0.01556	0.00024	0.09091	0.00826	526.2050644	28.73554859	3
QH	6.5	0.90844	0.00004	0.01465	0.00021	0.09091	0.00826	513.767408	16.29789221	4
LQ	6.5	0.90740	0.00003	0.01465	0.00021	0.09091	0.00826	513.7687181	16.29920232	4
LH	6.5	0.90120	0.00039	0.01556	0.00024	0.09091	0.00826	512.9377918	15.468276	3

Feature Class	Regularization Multiplier	Mean Test AUC	Variation Test AUC	Mean AUC DIFF	Variation AUC DIFF	Mean Omission Rate	Variation Omission Rate	AICc	Delta AICc	Model Parameters
LQH	6.5	0.90699	0.00003	0.01472	0.00022	0.09091	0.00826	513.7677093	16.29819348	4
H	7	0.89710	0.00024	0.01556	0.00024	0.09091	0.00826	528.6896644	31.22014864	3
QH	7	0.90635	0.00004	0.01422	0.00020	0.09091	0.00826	512.2939445	14.8244287	3
LQ	7	0.90635	0.00004	0.01422	0.00020	0.09091	0.00826	512.2939254	14.82440959	3
LH	7	0.89710	0.00024	0.01556	0.00024	0.09091	0.00826	514.416428	16.94691221	3
LQH	7	0.90589	0.00005	0.01440	0.00021	0.09091	0.00826	512.2939345	14.82441876	3
H	7.5	0.89710	0.00024	0.01556	0.00024	0.09091	0.00826	528.4610819	30.99156611	2
QH	7.5	0.90455	0.00007	0.01445	0.00021	0.09091	0.00826	513.5224976	16.05298183	3
LQ	7.5	0.90455	0.00007	0.01445	0.00021	0.09091	0.00826	513.5224935	16.05297776	3
LH	7.5	0.89710	0.00024	0.01556	0.00024	0.09091	0.00826	515.9202609	18.45074511	3
LQH	7.5	0.90455	0.00007	0.01446	0.00021	0.09091	0.00826	513.5226606	16.05314481	3
H	8	0.89710	0.00024	0.01556	0.00024	0.09091	0.00826	530.7013669	33.23185111	2
QH	8	0.90270	0.00010	0.01491	0.00022	0.09091	0.00826	514.7650402	17.29552442	3
LQ	8	0.90270	0.00010	0.01491	0.00022	0.09091	0.00826	514.7650603	17.29554456	3
LH	8	0.89710	0.00024	0.01556	0.00024	0.09091	0.00826	517.4412625	19.97174673	3
LQH	8	0.90270	0.00010	0.01492	0.00022	0.09091	0.00826	514.7650317	17.29551596	3

Table 2. Results from tuning experiments using ENMeval for *Ctenomys minutus*. Matrix of evaluation criteria sorted by AICc, the optimality criterion used for model tuning in this study. The combination of feature class and regularization multiplier with the lowest AICc was considered the 'best' model and used for final model calibration and all subsequent analysis; here the best combination of settings were Linear and Quadratic and regularization multiplier = 3. The default settings (Linear, Quadratic, and Hinge; regularization = 1) led to models that were substantially worse. Mean omission rate was calculated using the minimum training presence threshold.

Feature Class	Regularization Multiplier	Mean Test AUC	Variance Test AUC	Mean AUC DIFF	Variance AUC DIFF	Mean Omission Rate	Variance Omission Rate	AICc	Delta AICc	Model Parameters
H	0.5	0.90785	0.00003	0.03632	0.00015	0.24741	0.00324	NA	NA	43
QH	0.5	0.91125	0.00009	0.03368	0.00021	0.24741	0.00324	NA	NA	43
LQ	0.5	0.89295	0.00046	0.01800	0.00032	0.02174	0.00047	876.3307155	0.609794966	14
LH	0.5	0.90962	0.00007	0.03445	0.00017	0.20393	0.00018	NA	NA	44
LQH	0.5	0.91121	0.00009	0.03302	0.00021	0.22567	0.00124	NA	NA	46
H	1	0.88804	0.00015	0.02497	0.00034	0.04762	0.00227	991.8439511	116.1230306	28
QH	1	0.89049	0.00025	0.02244	0.00050	0.06936	0.00067	1029.437882	153.7169616	30
LQ	1	0.88192	0.00033	0.01860	0.00035	0.02174	0.00047	879.018572	3.297651506	11
LH	1	0.88694	0.00020	0.02199	0.00046	0.04762	0.00227	1081.59954	205.8786192	32
LQH	1	0.89068	0.00022	0.02224	0.00049	0.06936	0.00067	1028.3259	152.6049799	30
H	1.5	0.87926	0.00037	0.01935	0.00037	0.02381	0.00057	944.5806888	68.85976822	23
QH	1.5	0.87947	0.00030	0.02175	0.00047	0.04555	0.00000	890.8727757	15.15185514	16
LQ	**1.5**	**0.87441**	**0.00032**	**0.01834**	**0.00034**	**0.02174**	**0.00047**	**875.7209205**	**0**	**7**
LH	1.5	0.87783	0.00029	0.02116	0.00045	0.04555	0.00000	902.2065736	26.48565307	18
LQH	1.5	0.87996	0.00029	0.02114	0.00045	0.04555	0.00000	908.6964734	32.97555286	19
H	2	0.87779	0.00054	0.01947	0.00038	0.02381	0.00057	888.2566675	12.53574694	12
QH	2	0.87219	0.00028	0.02169	0.00047	0.04555	0.00000	895.8804474	20.15952689	15
LQ	2	0.86997	0.00033	0.01929	0.00037	0.02174	0.00047	876.7125293	0.991608729	5
LH	2	0.87550	0.00035	0.02128	0.00045	0.02381	0.00057	900.1935018	24.47258123	16
LQH	2	0.87225	0.00028	0.02170	0.00047	0.04555	0.00000	895.7805567	20.05963618	15
H	2.5	0.87493	0.00063	0.01906	0.00036	0.02381	0.00057	892.2575501	16.53662955	12
QH	2.5	0.86578	0.00045	0.02199	0.00048	0.04555	0.00000	896.6624395	20.94151901	14
LQ	2.5	0.86301	0.00041	0.01964	0.00039	0.02174	0.00047	879.897867	4.176946462	5
LH	2.5	0.87496	0.00042	0.02007	0.00040	0.04555	0.00000	888.4234946	12.70257408	12
LQH	2.5	0.86693	0.00042	0.02162	0.00047	0.04555	0.00000	896.4571878	20.73626731	14
H	3	0.87088	0.00060	0.01819	0.00033	0.02381	0.00057	895.4892945	19.76837396	12
QH	3	0.86473	0.00056	0.02046	0.00042	0.04555	0.00000	896.2441842	20.52326362	13

Feature Class	Regularization Multiplier	Mean Test AUC	Variation Test AUC	Mean AUC DIFF	Variation AUC DIFF	Mean Omission Rate	Variation Omission Rate	AICc	Delta AICc	Model Parameters
LQ	3	0.85966	0.00041	0.01933	0.00037	0.02174	0.00047	882.3332969	6.61237632	5
LH	3	0.87365	0.00047	0.01900	0.00036	0.04555	0.00000	888.2856446	12.5647241	11
LQH	3	0.86523	0.00054	0.02021	0.00041	0.04555	0.00000	896.258926	20.53800544	13
H	3.5	0.86492	0.00064	0.01823	0.00033	0.00000	0.00000	891.2730418	15.55212126	10
QH	3.5	0.86299	0.00058	0.01935	0.00037	0.04555	0.00000	892.3780476	16.65712709	11
LQ	3.5	0.85701	0.00036	0.01833	0.00034	0.02174	0.00047	886.7619543	11.04103377	6
LH	3.5	0.86970	0.00047	0.01857	0.00034	0.02174	0.00047	891.8372557	16.11633521	11
LQH	3.5	0.86361	0.00056	0.01914	0.00037	0.04555	0.00000	899.6045853	23.8836648	13
H	4	0.85817	0.00081	0.01834	0.00034	0.00000	0.00000	901.8511442	26.13022363	12
QH	4	0.85942	0.00049	0.01821	0.00033	0.02174	0.00047	884.9717473	9.250826778	8
LQ	4	0.85446	0.00030	0.01786	0.00032	0.02174	0.00047	886.0142458	10.2933253	5
LH	4	0.86586	0.00046	0.01808	0.00033	0.04348	0.00189	887.850229	12.12930845	9
LQH	4	0.86023	0.00047	0.01813	0.00033	0.02174	0.00047	887.9186091	12.19768854	9
H	4.5	0.85323	0.00083	0.01862	0.00035	0.00000	0.00000	894.1940607	18.47314021	9
QH	4.5	0.85661	0.00042	0.01741	0.00030	0.02174	0.00047	890.9857329	15.26481239	9
LQ	4.5	0.85335	0.00026	0.01733	0.00030	0.02174	0.00047	886.791698	11.07077746	5
LH	4.5	0.86475	0.00043	0.01740	0.00030	0.04348	0.00189	887.3947532	11.67383269	8
LQH	4.5	0.85760	0.00042	0.01733	0.00030	0.02174	0.00047	894.1011084	18.38018786	10
H	5	0.85092	0.00073	0.01848	0.00034	0.00000	0.00000	908.3273434	32.60642288	12
QH	5	0.85503	0.00037	0.01683	0.00028	0.02174	0.00047	894.1595066	18.43858606	9
LQ	5	0.85309	0.00027	0.01681	0.00028	0.02174	0.00047	890.5608819	14.83996133	6
LH	5	0.86313	0.00040	0.01702	0.00029	0.04348	0.00189	887.2458597	11.52493917	7
LQH	5	0.85571	0.00036	0.01681	0.00028	0.02174	0.00047	893.7177553	17.99683475	9
H	5.5	0.84966	0.00067	0.01784	0.00032	0.00000	0.00000	908.4147147	32.69379419	11
QH	5.5	0.85362	0.00032	0.01637	0.00027	0.02174	0.00047	894.5227855	18.80186495	8
LQ	5.5	0.85314	0.00027	0.01630	0.00027	0.02174	0.00047	892.12601	16.40508946	6
LH	5.5	0.86203	0.00038	0.01671	0.00028	0.04348	0.00189	890.3461978	14.62527722	7
LQH	5.5	0.85441	0.00031	0.01630	0.00027	0.02174	0.00047	897.0715163	21.35059581	9
H	6	0.84502	0.00062	0.01776	0.00032	0.00000	0.00000	908.7867485	33.06582799	10
QH	6	0.85244	0.00029	0.01643	0.00027	0.02174	0.00047	894.7017716	18.98085108	7
LQ	6	0.85334	0.00027	0.01608	0.00026	0.02174	0.00047	893.7659183	18.04499772	6
LH	6	0.86073	0.00035	0.01651	0.00027	0.04348	0.00189	893.1403298	17.41940923	7
LQH	6	0.85330	0.00027	0.01611	0.00026	0.02174	0.00047	894.1341817	18.41326118	7
H	6.5	0.84195	0.00061	0.01823	0.00033	0.00000	0.00000	902.8460284	27.12510786	7
QH	6.5	0.85256	0.00029	0.01640	0.00027	0.02174	0.00047	897.8872356	22.16631503	7
LQ	6.5	0.85330	0.00027	0.01619	0.00026	0.02174	0.00047	895.4272301	19.70630956	6
LH	6.5	0.85987	0.00036	0.01655	0.00027	0.04348	0.00189	890.02867	14.30774942	5
LQH	6.5	0.85331	0.00027	0.01616	0.00026	0.02174	0.00047	897.2604871	21.53956652	7
H	7	0.83684	0.00080	0.01947	0.00038	0.00000	0.00000	903.5956801	27.87475952	6
QH	7	0.85267	0.00029	0.01633	0.00027	0.02174	0.00047	898.0100836	22.28916303	6

Feature Class	**Regularization Multiplier**	**Mean Test AUC**	**Variation Test AUC**	**Mean AUC DIFF**	**Variation AUC DIFF**	**Mean Omission Rate**	**Variation Omission Rate**	**AICc**	**Delta AICc**	**Model Parameters**
LQ	7	0.85330	0.00028	0.01630	0.00027	0.02174	0.00047	897.1707226	21.44980209	6
LH	7	0.85741	0.00038	0.01644	0.00027	0.04348	0.00189	892.5178036	16.79688311	5
LQH	7	0.85330	0.00028	0.01634	0.00027	0.02174	0.00047	897.1694777	21.44855716	6
H	7.5	0.83553	0.00073	0.01947	0.00038	0.00000	0.00000	904.4153554	28.69443486	5
QH	7.5	0.85275	0.00029	0.01620	0.00026	0.02174	0.00047	899.7955127	24.07459212	6
LQ	7.5	0.85340	0.00028	0.01615	0.00026	0.02174	0.00047	898.9889768	23.26805623	6
LH	7.5	0.85554	0.00038	0.01645	0.00027	0.04348	0.00189	893.7690877	18.04816717	5
LQH	7.5	0.85341	0.00028	0.01616	0.00026	0.02174	0.00047	899.1378701	23.4169496	6
H	8	0.83443	0.00067	0.01947	0.00038	0.00000	0.00000	904.76157	29.04064945	4
QH	8	0.85289	0.00029	0.01614	0.00026	0.02174	0.00047	901.7766302	26.05570969	6
LQ	8	0.85341	0.00028	0.01608	0.00026	0.02174	0.00047	898.0328373	22.31191681	5
LH	8	0.85413	0.00036	0.01629	0.00027	0.04348	0.00189	895.05597	19.33504948	5
LQH	8	0.85342	0.00028	0.01607	0.00026	0.02174	0.00047	898.0569498	22.33602927	5

3. CAPÍTULO II

INTERSPECIFIC INTERACTIONS MAY NOT INFLUENCE HOME RANGE SIZE IN SUBTERRANEAN RODENTS: A CASE STUDY OF TWO TUCO-TUCO SPECIES (RODENTIA, CTENOMYIDAE)

BRUNO B. KUBIAK[1], RENAN MAESTRI[2], LEANDRO R. BORGES[1], DANIEL GALIANO[3] AND THALES R. O. DE FREITAS[1,2,4]

[1]Programa de Pós-Graduação em Biologia Animal, Universidade Federal do Rio Grande do Sul, Av. Bento Gonçalves 9500, 91501-970 Porto Alegre, Brazil.

[2]Programa de Pós-Graduação em Ecologia, Universidade Federal do Rio Grande do Sul, Av. Bento Gonçalves 9500, 91501-970 Porto Alegre, Brazil.

[3]Programa de Pós-Graduação em Ciências Ambientais, Área de Ciências Exatas e Ambientais, Universidade Comunitária da Região de Chapecó, av. Senador Attílio Fontana 59, 89809-000 Chapecó, Brazil.

[4] Programa de Pós-Graduação em Genética e Biologia Molecular, Universidade Federal do Rio Grande do Sul Av. Bento Gonçalves 9500, 91501-970 Porto Alegre, Brazil.

Running Heading: Interspecific interaction in home range size

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Abstract

Coexistence between species with similar niche requirements is often facilitated by displacement of morphological, behavioral, or physiological characteristics of potential competitors, usually in opposite directions. Experiments comparing treatments with and without the presence of potential competitors are ideal for testing hypotheses of interspecific competition. Here we investigate a fundamental aspect in the life history of a species: the home range. Our main objective was to investigate whether interspecific interactions can influence the home range size of two subterranean rodent species, *C. flamarioni* and *C. minutus*. We evaluated home range size in populations of both species in allopatric and sympatric sites along the coastal plain of southern Brazil. Animals were radio-tracked, and the home range size of each individual was estimated using grid cells (GCs) and minimum convex polygon (MCP) methods. We found no significant differences in home range size between collection sites or species, and the interaction was non-significant. We also found no relationship between home range size and body mass or sex. Our results suggest that interspecific interactions do not significantly influence home range size in these species, perhaps due to environmental adaptations that facilitate coexistence (e.g., microhabitat segregation and dietary modifications). Further, the peculiar characteristics of the sandy dune habitat seem to act as environmental filters, favoring similar home range sizes for both species.

Key words: Competition; *Ctenomys*; Radio telemetry.

Introduction

Species with similar ecological requirements cannot occupy the same habitat when resources are limited (Gause 1934; Tilman 1977). This condition commonly leads to modification of morphological, behavioral, or physiological characteristics in one or more species, which reduces competition and facilitates coexistence (Stoecker 1972; Schoener 1974; see Dayan and Simberloff 2005). These changes are likely directly related to differences in resource utilization due to interspecific interactions such as competition (Stuart and Losos 2013). However, accessing information to make reliable inferences about these interactions requires an appropriate experimental design.

Tests of hypotheses related to competition must contain treatments with and without possible competitors. Two different approaches can be utilized: experimental and natural. The experimental approach will generate evidence of competition through controlled artificial conditions, via comparison of the species responses among treatments with a possible competitor and in isolation (i.e., controls) (some examples: Turkington and Merhoff 1990; Vassallo 1993). Natural experiments can provide us with similar information by studying the distributions of potentially competing species in areas where they co-occur (sympatry) and areas where they do not (allopatry) (Adams and Rohlf 2000; Simberloff et al. 2000). The experimental approach requires effort to ensure that species distributions in control groups do not overlap with potential competitors. Further, depending on the purpose of the study and the taxonomic group, the expected responses may take a considerable amount of time; this makes the experimental approach at times impractical. The natural approach requires that potentially competing species have both sympatric and allopatric populations. These environments should also differ only in the presence or absence of the potential competitor, with minimal differences in the physical environment (See Stuart and Losos 2013).

We investigate here a fundamental aspect in the life history of a species: the home range. Home range can be defined as the area used by an animal to perform the basic required activities (i.e., care of offspring, resource acquisition, mating, use of refuge, etc.) (Burt, 1943; Powell, 2012). Subterranean rodents present a peculiarity in this aspect, as they occupy systems of tunnels that are constructed by one individual, and this tunnel system is thought to be the home range of that animal (Nevo 1979; Lacey et al. 2000). These rodents are thus interesting experimental models for testing hypotheses concerning factors that delimit the use of space in small mammals (Cutrera et al. 2010). Previous studies have demonstrated that home range can vary with environmental characteristics such as soil hardness (Cutrera et al. 2006; Šumbera et al. 2008), plant biomass (Heth 1989; Reichman et al. 1982), as well as with animal body size (Šumbera et al. 2003, 2008; Cutrera et al. 2010). However, few studies have explored the influence of interspecific interactions on home range in subterranean rodents (Cutrera et al. 2010).

The paucity of studies addressing this issue is linked to life history characteristics of these animals, including low mobility, patchy distribution, usually solitary habits, and lack of sympatry (Nevo 1979, Lacey et al., 2010). The scarcity of sympatric zones in particular makes studies of interspecific interactions rare. The subterranean rodent family with the greatest number of species is Ctenomyidae, with approximately 69 described species (Freitas 2016). Among ctenomyids, only three zones of sympatry are recognized: one in Argentina with *Ctenomys australis* and *Ctenomys talarum* (Contreras and Reig, 1965); and the other two in Brazil with *Ctenomys flamarioni* and *Ctenomys minutus* (Kubiak et al. 2015). In this study we investigate whether interspecific interactions can influence the home range size in subterranean rodents. We measured home range size in allopatric and sympatric populations of *C. flamarioni* and *C. minutus* along the coastal plain of southern Brazil.

Materials and Methods

Focal species and study Area

Ctenomys flamarioni Travi, 1981 and *Ctenomys minutus* Nehring, 1887 are subterranean rodents, commonly called “tuco-tucos”. Both species inhabit the southern Brazilian coastal plain at sea level, and both exhibit solitary habits (Fernández-Stolz et al. 2007; Galiano et al. 2014b). *Ctenomys flamarioni* is endemic to coastal sand-dune grasslands in the state of Rio Grande do Sul. The species geographical range includes a linear distribution along 560 km of the coastal plain (Fig. 1). The range limits are the city of Arroio Teixeira on the northern boundary (Freitas 1995) and the Chuí River on the southern boundary (Fernández-Stolz et al. 2007). *Ctenomys minutus* inhabits sand fields in the southern portion of its geographical range, and co-occurs with *C. flamarioni* in the sand-dune grasslands in the northern portion of its distribution. In the north, *C. minutus* occurs predominantly in areas without *C. flamarioni*. *Ctenomys minutus* occurs from Jaguaruna beach in Santa Catarina to the city of São José do Norte in Rio Grande do Sul, extending linearly along more than 500 km (Lopes et al. 2013; Galiano et al. 2014a).

The coastal plain of Rio Grande do Sul has geomorphology under constant influence of Atlantic Ocean fluctuations, formed by an unstable mosaic of lakes, lagoons, beaches, sand dunes, and sand fields (Tomazelli et al. 2000). The climate is mild mesothermal, without dry periods. The average summer temperature in 2016 varied between 13.6 and 34.1 ºC. In winter, the average temperature varied between and 2.1 and 29.5 ºC (Inmet, 2016). The vegetation consists of a mosaic of dune vegetation, sand fields, and “restinga” forests (Hesp et al. 2009). There is a prevalence of herbaceous species but shrubs are also common, and trees become more frequent along a gradient from east to west. Poaceae, Asteraceae, Cyperaceae, and Fabaceae are the predominant plant families in the region (Overbeck et al. 2007; Filho et al. 2013).

Two narrow contact zones have been recently described for these two rodent species. One zone is located on the northern limit of *C. flamarioni* distribution, in a sand dune area extending about 15 km; the second is located on the southern limit of *C. minutus* distribution, in the city of São José do Norte (Kubiak et al. 2015; Fig. 1). In this study, we investigated the home-range size of both species only in the sand-dune grasslands habitat (the sandy dunes area). Three treatments were used: (i) *C. minutus* in allopatry, (ii) *C. flamarioni* in allopatry, and (iii) both species in the sympatric zone located in the north (Fig. 1).

Radio-Tracking

Ctenomys flamarioni and *C. minutus* were sampled in three different sites along the line of coastal dunes in southern Brazil: one site where the species occur in sympatry (centroid: 29°41‘57”S, 49°58‘44”W), one site where *C. flamarioni* has allopatric distribution (centroid: 30°04‘51”S, 50°09‘53”W), and one site where *C. minutus* has allopatric distribution (centroid: 29º37’06” S 49º55’51” W) (Fig. 1).

We captured and radio-tagged 20 *C. flamarioni* (10 in allopatry and 10 in sympatry, equal numbers of males and females) and 20 *C. minutus* (10 in allopatry and 10 in sympatry, equal numbers of males and females), for a total of 40 animals. Animals were captured using Oneida Victor No. 0 traps. After the capture, animals were anesthetized (Ketamine, 100mg/ml), weighed, sexed, fitted with a radio collar (M 1640, Advanced Telemetry Systems, USA), and released at their respective trapping locations. The animals were released only when fully recovered from the effects of the anesthetic. We used radio-collars that weighed 6.0 grams (<5% of the body weight of the smallest animal used in the study). All procedures involving capture, handling, and use of radio collars were approved by the Institutional Animal Care and Use Committee of the Universidade Federal do Rio Grande do Sul - Biological Sciences Research Committee (nº282360). This study was conducted in strict accordance with the recommendations of the American Society of Mammalogists (Sikes et al.

2016). The protocol was approved by the Brazilian Institute for the Environment and for Renewable Natural Resources (IBAMA - Permit number 14690–1).

Radio-tracking began 24-48 hours after animal release. We used a receiver (IC - R20, Icom America Inc.) and a two-element Yagi antenna to locate animals. Radio fixes were taken in 12 hours sessions (06:00 to 19:00) for a period of five days. To ensure independence of data collection, there was an approximate one hour time interval between fixes (Kenward, 1987; Swihart and Slade, 1985), for a total of 60 radio locations per individual. We estimated the asymptote of accumulation curves to determine the minimum number of radio locations required to stabilize individual home range sizes (White and Garrot, 1990; Gehring and Swihart, 2004). Prior to field work, radio fixes were taken at known locations to determine the accuracy of the procedure, which was less than one meter. Of the 40 captured animals, it was not possible to complete the radio-tracking for eight. Of these eight, three were not detectable by radio-tracking after release (one *C. minutus* sympatric male, one *C. flamarioni* sympatric male, and one *C. flamarioni* allopatric female). We had issues receiving the transmitter signal in five other individuals. The animals with transmitter issues were recaptured and the radio collars were removed, and data from the eight individuals with missing or incomplete transmission were not used in downstream analyses.

Home range size and statistical analyses

The home range size of each individual was estimated using grid cells (GCs) and minimum convex polygon (MCP). We defined home range sizes by using a set of 2 x 2 meter grid cells (GCs) covering all of the radio fixes. For data sets that are relatively complete with little error (as in most studies of subterranean rodents), excellent home range estimates can be generated with the GCs method (Powell and Mitchell 2012). When necessary, we used cells to connect the GCs using the shortest possible link (Šklíba et al. 2009). We also calculated the minimum convex polygon (MCP 100%) using all points from each individual for comparison with other studies. Analyses were performed using QUANTUM GIS software (Quantum GIS Development Team, 2013), and Biotas 2.0 (Ecological Software Solutions).

We performed an analysis of covariance (ANCOVA) to test whether home range size differs between sites (sympatric or allopatric), species (*C. minutus* or *C. flamarioni*), and whether the relationship of home range size between sites or within species differs between the two species (i.e., by testing the interaction between site and species). We also used (log) body mass and sex as covariables to test their relationships with home range size for both estimators independently (formulas: GCs = body mass + species + sex + sites + species × sex × sites; MCP = body mass + species + sex + sites + species × sex × sites). Two of the males captured (FAM2 and MAM4, see Table 01) presented signs of reproductive activity, and were excluded from statistical analyses. All analyses were conducted in the R program for statistical computing (R Development Core Team, 2016).

Results

We successfully completed radio-tracking for 32 animals. Only one individual was captured in each tunnel system, and species home ranges did not overlap. The contour area estimator for the 100% MCP method stabilized in approximately 3 - 4 days for both species (*C. flamarioni* = 42.57 ± 7.59 and *C. minutus* 43.07 ± 5.34 radio locations).

Using both estimators, no significant differences in home range size was detected between sites or species (using either estimator), and the interaction was not significant. Home range size for *C. flamarioni* at sympatric sites did not differ from that of conspecifics occupying allopatric sites (averages: GCs = 38, MCP = 214.77 and GC = 38.75, MCP = 278.08 for sympatric and allopatric individuals, respectively). Similar results were obtained for *C. minutus* at sympatric and allopatric sites (averages: GGs = 38.86, MCP = 247.72 and GCs = 38.9, MCP = 293.25, respectively). Furthermore, there were no significant differences in home range size among sites (sympatric or allopatric) or between species (*C. flamarioni* or

C. minutus). We found no relationships between home range size and body mass or sex. Results from the ANCOVA are summarized in Tables 1 and 2.

Discussion

Our results suggest that interspecific interactions do not influence the home range size of *C. flamarioni* and *C. minutus*. Home range size is usually linked to the size of the animal, where larger animals require larger areas to meet their resource needs (e.g., food, mating, etc.) (McNab, 1963). This difference is expected to be more evident in comparisons between species with obvious size differences. For example *C. australis* is 2 to 3 times larger than *C. talarum*, and has a home range size ~19 times larger (Cutrera et al., 2010). However, the two species in the current study do not present significant size differences in sand dune areas. *C. minutus* occupying sandy field habitat are indeed smaller than conspecifics occurring in the sand dunes (Kubiak et al., 2017), however *C. minutus* and *C. flamarioni* occurring in allopatry on the dunes do not differ in size. This may be due to convergence in size by means of environmental filtering. In addition to the difference in body size, *C. minutus* that inhabit sand dunes have home range sizes 1.75 times larger than congeneric individuals inhabiting sand fields (Kubiak et al., 2017).

This suggests that interspecific interactions may not influence the size of the home range size of subterranean rodents. Further, these species seem to have undergone adaptive convergence regarding the home range size and body mass in the sandy dunes habitat. This potential adaptive convergence was likely driven by environmental characteristics, including scarce food availability and low soil hardness (Kubiak et al 2015, Galiano et al., 2014b), a higher risk of tunnel collapse, and a wind regime that directly influences the conformity of the landscape (i.e., modifies the position and shape of the dunes); these factors could have favored environmental adaptations that resulted in similar home range size and body mass along their distribution in the sand dunes.

Many environmental and ecological factors have been shown to influence home range size in subterranean rodents, including body size (Sumbera et al., 2003, 2008; Cutrera et al, 2010), extent of search for mates (Zuri and Terkel 1997), plant production among habitats (Heth 1989; Reichman et al. 1982), differences in soil hardness (Cutrera et al. 2006; Sumbera et al. 2008; (Lövy et al. 2015) and habitat type (Kubiak et al. 2017). However, interspecific interaction does not appear be a key factor for determining the home range size for *C. minutus* and *C. flamarioni*. We should consider that these species may have other (adaptive) behavioral modifications that may reduce or eliminate the need to change home range size when in contact with other congenerics. For example, recent studies have shown that these two ctenomyids exhibit spatial segregation (Kubiak et al., 2015) and differentiation in diet (Lopes et al. 2015) in the same contact zone of the current study. Kubiak et al. (2015) found that *C. flamarioni* selected habitats differently when occurring in allopatry versus sympatry; in allopatry, the species selects areas with high grass cover and is distributed on less hard soils compared to conspecifics located in sympatry with *C. minutus*. By contrast, *C. minutus* selected areas with higher plant biomass and greater grass cover compared to *C. flamarioni* in sympatry or in allopatry. The focal species also forage differently when in sympatry. *C. minutus* modifies its diet when in sympatry with *C. flamarioni*, but *C. flamarioni* only decreases the number of consumed plant species when in sympatry with *C. minutus* (Lopes et al., 2015).

In general, the home range size of these species does not seem to be related to body mass, and neither species are sexually dimorphic. Other studies have shown that the relationship between the home range size and body mass or sex is species-dependent (Cutrera et al. 2006, 2010). Further, males that are sexually active tend to have larger home range size, as demonstrated in *Spalax galili* (Lövy et al. 2015; Šklíba et al. 2015) and emphasized for *C.*

minutus (Kubiak et al., 2017). We found the same pattern in *C. flamarioni* for a sexually active male in the current study (FAM2).

In summary, our results suggest that interspecific interactions do not significantly influence the home range size of these subterranean rodent species. This may be due to life history adaptations that enable coexistence in the sandy dunes, e.g., microhabitat segregation and dietary modifications. Further, the peculiar characteristics of this habitat seem to result in a similar home range size for both species. Additional studies are needed to better understand home range dynamics in these species, specifically regarding patterns of home range occupation, distance between neighbors, and individual distributional conformity in areas of sympatry. Future studies should include sampling of entire populations in order to thoroughly assess these characteristics.

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Tables and Figures

Table 1. Individual ID, body mass, and estimated home range sizes using the grid cell (GC) and minimum convex polygon (MCP) methods for *Ctenomys flamarioni* and *Ctenomys minutus* in sympatry and allopatry along the coastal plain of southern Brazil.

Ctenomys flamarioni

	Sympatry				**Allopatry**		
ID	**Body mass (g)**	**GCs**	**MCP (m^2)**	**ID**	**Body mass (g)**	**GCs**	**MCP (m^2)**
FSF1	230	32	168.05	FAF1	200	38	241.57
FSF2	150	33	162.89	FAF2	210	28	94.78
FSF3	160	48	346.25	FAF3	195	25	231.77
FSF4	200	42	218.35	FAF4	210	44	243.75
FSF5	230	26	91.27	FAM1	310	31	129.1
FSM1	295	50	357.89	FAM2	260	67	710.29
FSM2	240	35	158.71	FAM3	140	32	172.99
-	-	-	-	FAM4	260	45	400.4
Mean	**215.00**	**38.00**	**214.77**	**Mean**	**223.13**	**38.75**	**278.08**
SD	**49.92**	**8.89**	**100.87**	**SD**	**51.89**	**13.50**	**197.58**

Ctenomys minutus

	Sympatry				**Allopatry**		
ID	**Body mass (g)**	**GCs**	**MCP (m^2)**	**ID**	**Body mass (g)**	**GCs**	**MCP (m^2)**
MSF1	230	40	489.72	MAF1	160	37	269
MSF2	250	30	110.04	MAF2	195	23	121.5
MSF3	200	37	176.4	MAF3	210	43	333
MSF4	170	32	118.39	MAF4	200	27	163
MSM1	295	53	436.57	MAF5	205	35	229
MSM2	210	34	149.71	MAM1	245	41	302
MSM3	200	46	253.24	MAM2	240	23	100
-	-	-	-	MAM3	240	37	267
-	-	-	-	MAM4	295	69	689.5
-	-	-	-	MAM5	260	54	458.5
Mean	**222.14**	**38.86**	**247.72**	**Mean**	**225.00**	**38.90**	**293.25**
SD	**40.81**	**8.21**	**155.26**	**SD**	**38.51**	**14.24**	**174.86**

Table 2. ANCOVA results for the following models: GCs = body mass + species + sex + sites + species × sex × sites; MCP = body mass + species + sex + sites + species × sex × sites.

CGs	Df	*F*	*P*	MCP	Df	*F*	*P*
Body mass	1	2.021	0.17	**Body mass**	1	1.886	0.184
Species	1	0.02	0.888	**Species**	1	0.445	0.512
Sex	1	1.141	0.298	**Sex**	1	0.201	0.659
Sites	1	1.167	0.292	**Sites**	1	0	0.993
Species: sex	1	0.294	0.593	**Species: sex**	1	0.048	0.829
Species: sites	1	0.005	0.943	**Species: sites**	1	0.004	0.949
Sex: sites	1	0.319	0.578	**Sex: sites**	1	0.013	0.91
Species: sex: site	1	0.018	0.894	**Species: sex: site**	1	0.003	0.954
Residuals	21			**Residuals**	21		

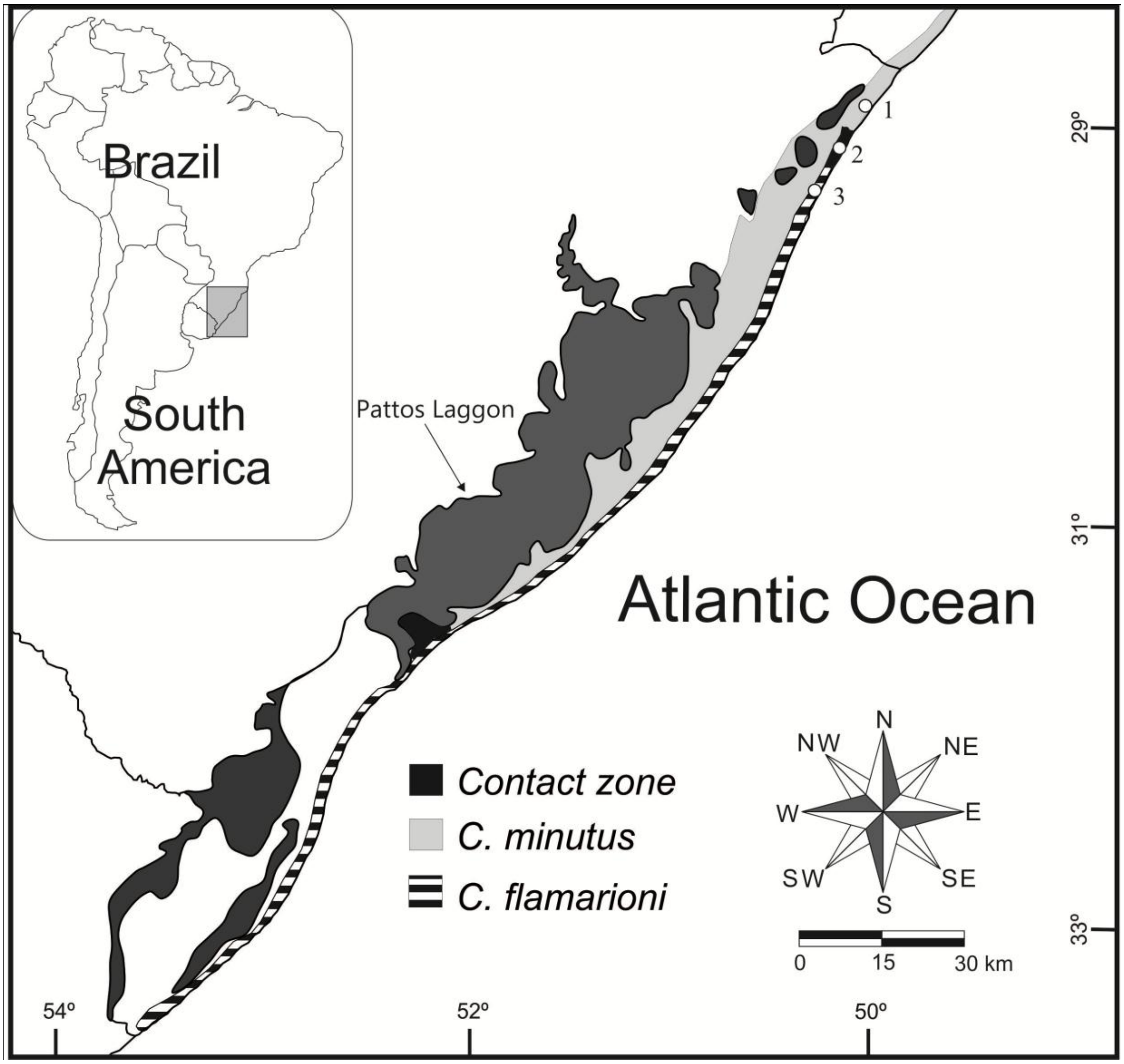

Fig.1. Geographic distribution of *C. flamarioni* and *C. minutus* along the coastal plain of southern Brazil. The collection sites are as follows: 1 - *C. minutus* in allopatry; 2 - *C. flamarioni* and *C. minutus* in sympatry; and 3 - *C. flamarioni* in allopatry.

4. CAPÍTULO III

CAN THE ENVIRONMENT INFLUENCE SPECIES HOME RANGE SIZE? A CASE STUDY ON *Ctenomys minutus* (RODENTIA, CTENOMYIDAE)

BRUNO B. KUBIAK[1], DANIEL GALIANO[2] AND THALES R. O. DE FREITAS[1,3]

[1]Programa de Pós-Graduação em Biologia Animal, Universidade Federal do Rio Grande do Sul, Av. Bento Gonçalves 9500, 91501-970 Porto Alegre, Brazil.

[2] Programa de Pós-Graduação em Ciências Ambientais, Área de Ciências Exatas e Ambientais, Universidade Comunitária da Região de Chapecó, av. Senador Attílio Fontana 59, 89809-000 Chapecó, Brazil.

[3] Programa de Pós-Graduação em Genética e Biologia Molecular, Universidade Federal do Rio Grande do Sul Av. Bento Gonçalves 9500, 91501-970 Porto Alegre, Brazil.

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Abstract

The environment physically limits animal movement and use of space. In the case of subterranean rodents, natural selection is expected to favor burrow structures that minimize energetic costs of digging. However, in some cases the same species is found in habitats that strongly differ in resource availability and soil characteristics, as in Ctenomys minutus. This species is thus a good model in which to estimate the influence of habitat on behavioral characteristics such as home-range size. We evaluated the home-range sizes of two C. minutus populations that inhabit different habitats: sand dunes and sand fields. We radio tracked 19 adult animals, and estimated the home-range size of each individual using the grid cell (2 9 2 m covering all of the radio fixes), and minimum convex polygon (MCP) methods. Our results show that home ranges of C. minutus differ in the two habitats, with average size 1.75 times larger for individuals inhabiting sand dunes. This difference in home-range size between habitats is likely associated with differences in resource availability (plant biomass) or soil conditions.

Key words: radio telemetry; Ctenomys minutus; tuco-tucos; subterranean rodents; home-range size; soil conditions.

Introduction

Home range can be defined as an area routinely used by an individual to satisfy its daily needs, e.g., food gathering, mating, and parental care (Burt, 1943; Powell, 2012). However, movement and use of space within the home range area can be limited by physical properties of the habitats where the animals occur. Subterranean rodents perform most activities underground using burrow systems that are occupied by solitary individuals or colonies (Nevo, 1999; Lacey et al. 2000). Subterranean rodent herbivores use burrow systems to access and store plants, and as a consequence these systems are constantly changing in size and shape as new tunnels are built and older ones collapse. Additionally, some tunnels can be actively blocked (backfilled) by the animals, and can make up a large part of the burrow system of some individuals (Davis & Jarvis, 1986; Andersen, 1987; Zuri & Terkel 1996). The extent of the burrow system used by an individual may thus serve as a good approximation of its home range size (Šklíba et al. 2009).

There is substantial energy expenditure associated with tunnel excavation (Vleck, 1981; Luna & Antenucci, 2006; Zelová et al. 2010). Natural selection is expected to favor burrow structures that minimize the energetic costs of digging (Lacey et al. 2000). As a consequence, the amount of resources and soil hardness of a given area (which may vary seasonally) can directly influence the total size and shape of burrow systems in different habitat types (Reichman et al., 1982; Heth, 1989; Rosi et al. 2000; Šumbera et al. 2003; Romañach, Reichman & Seabloom, 2006; Lövy et al. 2015).

Among the subterranean rodents Ctenomyidae is the most speciose group, and currently includes one genus (*Ctenomys*) and approximately 70 species (Gardner et al. 2014; Bidau, 2015). *Ctenomys* sp. (tuco-tucos) are usually solitary (with some social species) (Lacey, Braude & Wieczorek, 1997; Lacey, Braude & Wierczorek, 1998; Lacey et al., 2000), are typically distributed in small patches of suitable habitats, and have low mobility (Lacey et al. 2000; Galiano et al. 2016). Species usually use habitats with similar soil characteristics and availability of resources (Lacey et al. 2000). However, some species are found in habitats with sharp differences in resource availability and soil characteristics, as is the case for *Ctenomys minutus* Nehring, 1887 (Galiano et al. 2014a, 2014b; Kubiak et al. 2015; Galiano et al. 2016). This species has a solitary habits and its distribution is restricted to the coastal plains of southern Brazil, where populations occur along a linear extension of approximately 500 km (Freitas, 1995; Freygang, Marinho & Freitas, 2004). Along its distribution *C. minutus* is found in two distinct habitats: sand dunes and sand fields, which have marked differences in habitat characteristics including plant biomass, vegetation cover, and soil hardness (see Galiano et al. 2014b; Kubiak et al. 2015 for details of habitat characteristics for the species in the region) (see Fig. 1).

Recent studies have demonstrated that environmental characteristics can be a key factor affecting behavior in *Spalax galili* in parapatric populations of a species (Hadid et al. 2013; Lövy et al. 2015; Šklíba et al. 2016). *C. minutus* is an excellent model for studying how differences in environmental factors affect behavior of subterranean rodents. We estimated the home range size in two populations of *C. minutus* populations in different habitats: sand dunes and sand fields. We hypothesized that individuals inhabiting sand dunes would have larger home ranges than individuals inhabiting sand fields due to differences in soil hardness and resource availability. Our hypothesis is based on empirical knowledge of the species, and information from the literature (Galiano et al. 2014b; Kubiak et al. 2015). Because sand dunes have softer soils and lower availability of resources, we predicted that 1) individuals in this habitat require larger home range sizes to access resources, and that 2) animals inhabiting sand fields should have smaller home ranges due to higher resource availability above tunnels.

Material and Methods

STUDY AREA

The study was conducted in the coastal plain of Rio Grande do Sul state in southern Brazil, at two different areas (sand dunes: 29º37’06” S 49º55’51” W; and sand fields: 29º40’38” S 50º01”28” W, respectively). The region is characterized by having geomorphology under constant influence of Atlantic Ocean fluctuations, formed by an unstable mosaic of lakes, lagoons, beaches, sand dunes, and sand fields (Tomazelli et al. 2000). The climate is mild mesothermal, without dry periods. The average summer temperature in the year of 2016 varied between 34.1 and 13.6 ºC. In winter, the average temperature varied between 29.5 and 2.1 ºC (Inmet, 2016).

The vegetation consists of a mosaic of dune vegetation, sand fields and “restinga” forests (Hesp et al. 2009). There is a prevalence of herbaceous species but shrubs are also common, and trees are increasingly frequent from east to west. Poaceae, Asteraceae, Cyperaceae and Fabaceae are the predominant families in the region (Overbeck et al. 2007; Filho et al. 2013). The species *C. minutus* feeds underground, and consumes mainly plants of the family Poaceae (51.81%), Fabaceae (15.18%), Araliaceae (15.06%), Asteraceae (12.06%), and Apocynaceae (2.78%) (Lopes et al. 2015). Average plant biomass and soil hardness in the *C. minutus* dune habitat is 46.30 ± 51.59 g, and 3.37 ± 0.78 Kg/cm², respectively (Kubiak et al. 2015); in sand fields plant biomass is 91.21 ± 59.13 g, and the soil hardness is 3.64 ± 0.74 Kg/cm² (Galiano et al. 2014b) (Fig 1). Plant biomass evaluated in both studies consisted of dry plant biomass (above and below ground), potentially consumable by the ctenomyds as demonstrated for other species (Comparatore et al. 1995, Del Vale et al. 2001, Albanese et al. 2010).

RADIO-TRACKING

Radio-tracking was carried out in November and December 2015. We sampled 10 adult *C. minutus* at each site (five males and five females). Animals were captured using Oneida Victor No. 0 traps. After capture animals were anesthetized (Ketamine, 100mg/ml), weighed, sexed, fitted with a radio collar (M 1640, Advanced Telemetry Systems, USA), and released at their respective trapping locations. The animals were released only after they fully recovered from the effects of the anesthetic. We used radio-collars that weighed 6.0 grams (<5% of the body weight of the smallest animal used in the study). At the end of the sampling period, we removed the radios and re-weighed individuals to test whether the transmitter affected body mass. All procedures involving, capture, handling and use of radio collars on animals were approved by the Institutional Animal Care and Use Committee of the Universidade Federal do Rio Grande do Sul - Biological Sciences Research Committee (nº282360). This study was carried out in strict accordance with the recommendations of the American Society of Mammalogists (Sikes & The Animal Care and Use Committee of the American Society of Mammalogists, 2016). The protocol was approved by the Brazilian Institute for the Environment and for Renewable Natural Resources (IBAMA - Permit number 14690–1).

Radio-tracking began 24-48 hours after animal release. We used a receiver (IC - R20, Icom America Inc.) and a two-element Yagi antenna to locate animals. Radio fixes were taken in 12 hours sessions (06:00 to 19:00) for a period of five days. To ensure independence of data collection, there was an approximate one hour time interval between fixes (Kenward, 1987; Swihart & Slade, 1997), for a total of 60 radio locations per individual. We estimated the asymptote of accumulation curves to determine the minimum number of radio locations required to stabilize individual home range sizes (White & Garrot, 1990; Gehring & Swihart, 2004). Prior to field work, radio fixes were taken at known locations to determine the accuracy of the procedure, which was less than one meter. Of the 20 sampled animals, one male from the sand field habitat was not detectable by radio-tracking after release. This male weighed 270g, and was not included in any of the analysis.

STATISTICAL ANALYSES

We estimated the home range size of each individual using two different methods. First, we defined home range sizes by using a set of 2 x 2 m grid cells (GCs) covering all of the radio fixes. For data sets that are relatively complete with little error, as obtained is most studies with subterranean rodents, excellent home range estimates can be generated with the GCs method (Powell & Mitchell, 2012). When necessary, we used cells to connect the GCs in the shortest way (Šklíba et al. 2009). We also calculated the minimum convex polygon (MCP – 100%) using all points from each individual for purposes of comparison with other studies. Analyses were performed using QUANTUM GIS software (Quantum GIS Development Team, 2013), and BIOTAS^{TM} version 2.0 (Ecological Software Solutions LLC, Hegymagas, Hungary). We performed an analysis of covariance (ANCOVA) using body mass and sex as covariates to associate home range size with the different habitat type (HRsizeGC ~ body mass + sex + habitat type; HRsizeMCP ~ body mass + sex + habitat type). This allowed us to evaluate the influence of body mass on individual home range areas, and to test for sexual dimorphism in home range area. We applied an analysis of variance (ANOVA) to test the differences between male and female body mass. We also tested the results of MCP with other studies using a Student’s T Test. The largest male in the sample, who also had the largest home-range, was the male Md04 from sand dunes (Table 1; Fig. 3). Because its home-range size and shape suggest some reproductive activity, we excluded it from the main statistical analyses. All analyses were conducted in the R program for statistical computing (R Development Core Team, 2012) using the vegan package (Oksanen et al. 2013).

Results

Individual home ranges did not overlap in either habitat, and only one individual was captured in each tunnel system. The contour area estimator for the 100% MCP method stabilized in approximately 3-4 days in the sand dunes and sand fields (46 ± 6.54; 43.87 ± 6.15 radio locations, respectively, no significant difference: $t = 0.6875$, $P = 0.25$). By the end of the sampling period, there were no significant differences in individual body mass due to radio collar use ($t = 0.2294$, $P = 0.41$). We found a significant statistical difference between male and female body mass, but no significant difference of animals' body mass from different habitats ($F_{1,16} = 27.758$, $P \leq 0.001$; $F_{1,16} = 2.018$, $P = 0.175$, respectively).

Individual home range size in *C. minutus* is significantly influenced by the habitat in which the animal lives, for both estimators (ANCOVA results: GCs: $F_{1,13} = 13.293$, $P = 0.00203$; MCP: $F_{1,13} = 12.167$, $P = 0.00362$). Home range size of animals inhabiting sand dunes (GCs = 32.1 ± 9.6; MCP = 293.25 ± 174.86 m²) were 1.75 times larger than those inhabiting sand fields, according GCs method, and 3.18 according MCP method (GCs = 18.33 ± 4.58; MCP = 94.17 ± 41.12 m²) (Fig. 2 and Fig. 3). Home range size was not influenced by body mass within each habitat (ANCOVA results: GCs: $F_{1,13} = 3.078$, $P = 0.10122$; MCP: $F_{1,13} = 2.730$, $P = 0.12074$). We found no significant differences in home range size between sexes (ANCOVA results: GCs: $F_{1,13} = 0.350$, $P = 0.56340$; MCP: $F_{1,13} = 0.456$, $P = 0.51052$). The results from the analysis of home range sizes and individual body mass from both habitats are summarized in Table 1.

Discussion

Our results showed that individual home range size in *C. minutus* differs between sand dune and sand field habitats. The GC method indicated that average home range size of animals in sand dunes animals was 1.75 times larger than that of animals in sand fields, corroborating our initial prediction. This difference is probably associated with differences in plant biomass or soil conditions between habitats, as these variables were previously indicated as responsible for modifications in tunnel system architecture - and consequently the home ranges - in other subterranean rodents (Heth, 1989; Rosi et al. 2000, Spinks et al. 2000; Šumbera et al. 2003; Romañach et al. 2005; Lövy et al. 2015). It is probable that animals that inhabit sand dunes need longer tunnels (covering larger areas) to access sufficient food resources, as this habitat have significant less plant biomass than sand fields, and consequently a small amount of food resources. Additionally, soil hardness was similar in both sampled areas in our case. Thus, the soil constraints of burrowing which determines the home range size of this tuco-tuco in both habitats probably do not consist in the soil hardness. Moreover, one important factor that should be considered is the risk of collapsing tunnels, which in the habitat of sand dunes seems to be greater. In habitats where the risk of collapse is higher, it is probable that the burrow systems are larger too, for the maintenance of a viable home range area.

In addition, when we compare our study to Gastal (1994), which estimated the home range size of individuals of *C. minutus* from a sand field habitat based on the MPC method, we found no statistical differences between range sizes (99.25 ± 88.18; $t = 0.15$, $P = 0.44$). Further, stabilization of home ranges size from both habitats was similar despite having statistical differences in size. This may be an indication that the animals in sand dunes have a higher movement rate than those in sand fields.

Moreover, we cannot dismiss the influence of other (untested) factors on the home range size of subterranean rodents. For example, aggressiveness (Lövy et al. 2015) may vary within the same species in different habitats, or among populations with different densities (Zenuto, Lacey & Busch, 1999a; Zenuto, Malizia & Busch, 1999b; Zenuto, Vassallo & Busch, 2002), features (Wolff, 1985), and metabolic costs related to digging activity (Vleck, 1981; Ebensperger & Bozinovic, 2000; Luna & Antinuchi, 2006). The search for potential mates is also a factor that influences the spatial organization of tunnels and consequently, individual home ranges (Clutton-Brock, 1989; Komers & Brotherton, 1997). Although we did not measured these factors in our study, some of the fixes of one male individual (Md04) were located in a region near a female (Fd02), and outlying the portion of agglomeration of fixes, suggesting reproductive activity (effort to encounter females). Search for potential mates would be facilitated by prolonged burrow systems, which seem to be the case of individual Md04. This kind of activity was already described in other subterranean rodents too, e.g., *Spalax galili* (Šklíba et al. 2015). For subterranean rodents, larger home ranges and higher outside-nest activity could occur in males searching for sexual partners. In our case, the largest male in the sample also had the largest home-range, and such large males can be rare in the population. We believe that this male could mate more frequently than other males in the population because he can access females more easily, since he has a larger home range area.

Although *C. minutus* males are larger than females in both habitats, we did not detect sexual dimorphism in home range sizes. Additionally, we did not find a significant correlation between body mass and home range size. These correlations may vary between *Ctenomys* species. For example, *C. talarum Thomas, 1989* present sexual dimorphism in home range size depending in the habitat they inhabit (sand dunes or sand fields) (Cutrera et al. 2006, 2010). Body mass does not appear to be a determining factor for home range of *C. minutu*s. This suggests that although it may influence home range for some species, body mass alone does not explain the intraspecific variation in the home ranges of Ctenomyids (Cutrera et al. 2010). Further, when we analyze home ranges individually we find a great deal of variation within the same habitat. This difference may be directly associated with the occupation time of each burrow system, as there is a known positive correlation between time of occupation and system length (Gastal, 1994).

In summary, our study provides evidence that the habitat can influence the home range size of the same species. Consequently, behavioral changes associated with habitat features may lead to a profound differentiation of aspects related to genetics, morphology, and physiology of a species, as was demonstrated for other subterranean rodents such as *Spalax galili* (Polyakov et al. 2004; Hadid et al. 2013; Li et al. 2015; Lövy et al. 2015; Šklíba et al. 2016), *Spalax ehrenbergi* (Heth, 1989), and *C. mendocinus* (Rosi et al. 2000). Future studies including estimates of features associated with home range behavior in this species would be particularly informative.

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Tables and Figures

Table 1. Individual ID, body mass, and home range sizes estimated from the grid cell (GC) and minimum convex polygon method (MCP) for *Ctenomys minutus* in two different environments (sand dunes and sand fields) along the coastal plain of southern Brazil.

Sand dunes				**Sand fields**			
ID	**Body mass (g)**	**GCs**	**MCP (m^2)**	**ID**	**Body mass (g)**	**GCs**	**MCP (m^2)**
Md1	245	41	302	Mf1	210	19	66.5
Md2	240	23	100	Mf2	260	20	91
Md3	240	37	267	Mf3	220	30	179.5
Md4	295	69	689.5	Mf4	250	15	68
Md5	260	54	458.5	-	-	-	-
Fd1	160	37	269	Ff1	220	25	133.5
Fd2	195	23	121.5	Ff2	165	22	116.5
Fd3	210	43	333	Ff3	210	16	69
Fd4	200	27	163	Ff4	150	16	63
Fd5	205	35	229	Ff5	170	16	60.5
Mean	225.17 ± 38.51	38.8 ± 14.24	293.25 ± 174.86	**Mean**	206.11 ± 37.73	19.89 ± 5.05	94.17 ± 41.12

Figure 1. Examples of sand dunes and sand fields found along the coastal plain of southern Brazil, both typically inhabited by *Ctenomys minutus*.

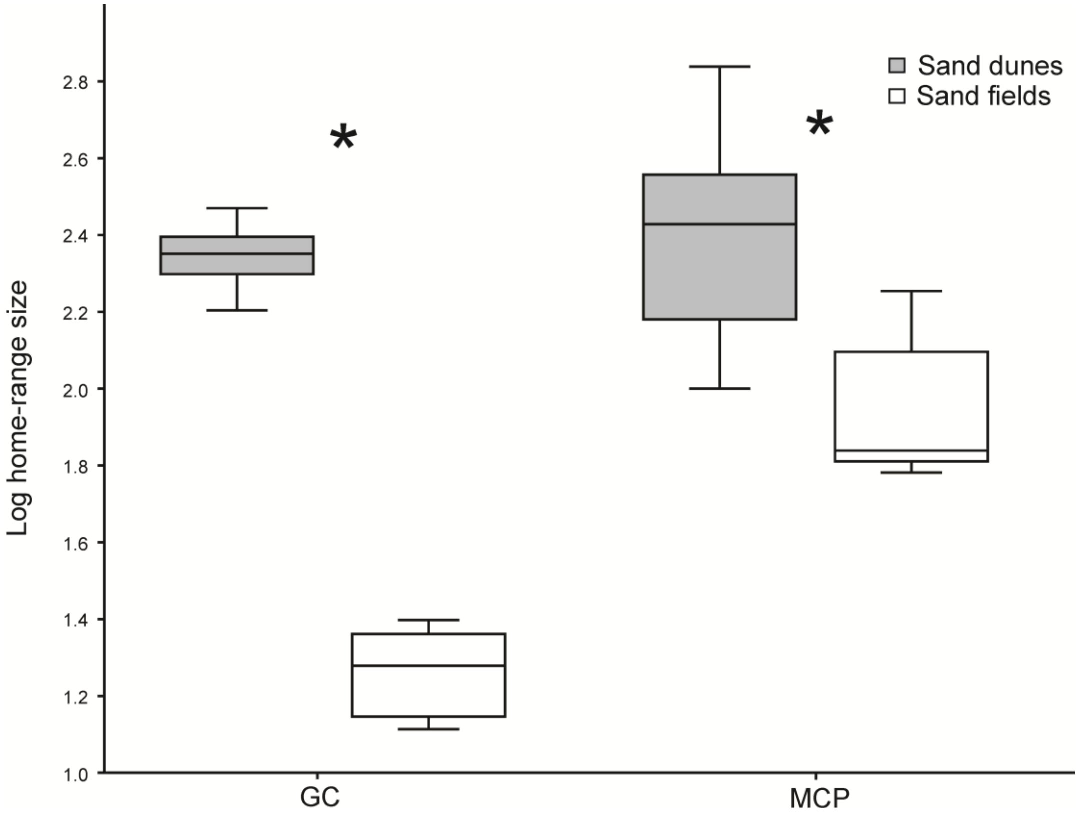

Figure 2. Mean home range sizes of *Ctenomys minutus* in sand dunes and sand fields estimated by grid cell (GC) and 100% minimum convex polygon (100% MCP) methods. Asterisks indicate a significant difference.

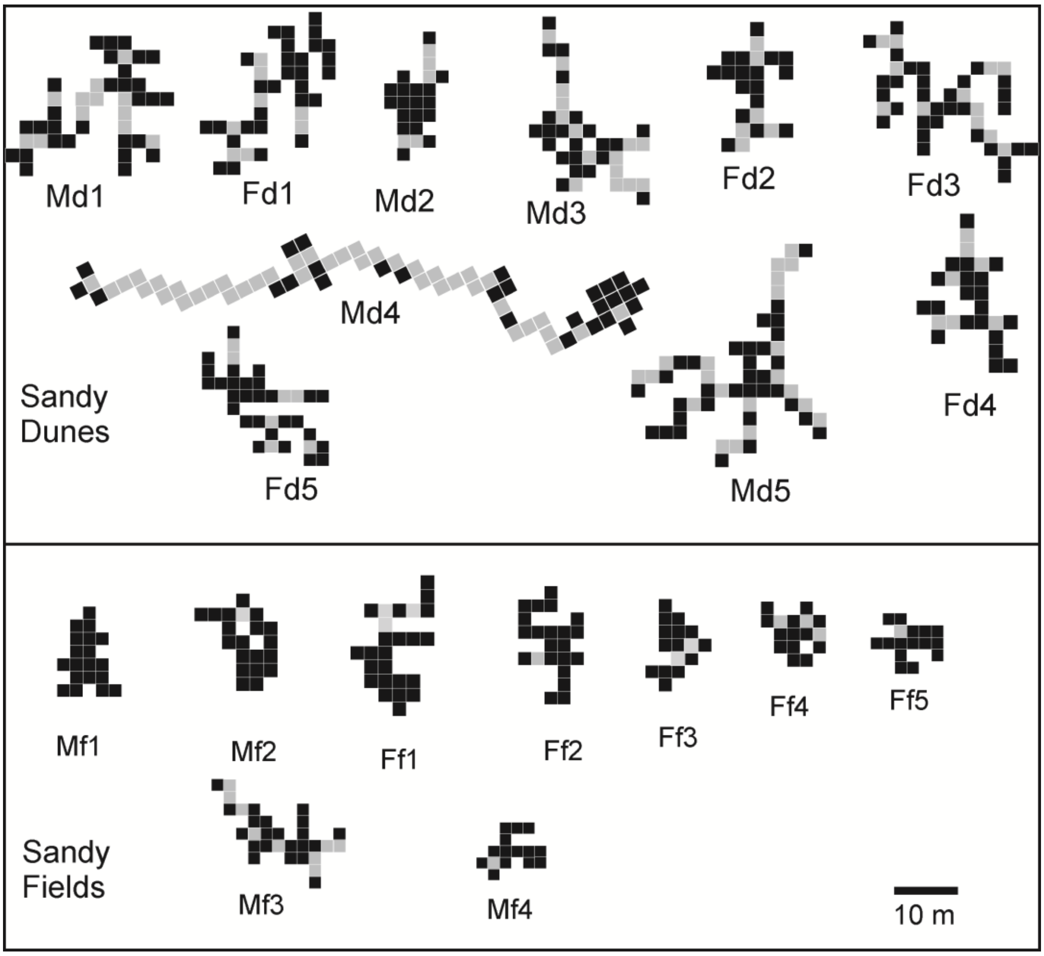

Figure 3. Representation of home range sizes estimated by grid cell method (GC) for radio tracked *Ctenomys minutus* in sand dunes and sand fields in southern Brazil (N=19). Cell sizes are 2 x 2 m. Black squares represent sites where individuals were radio fixed, and gray squares represent cells used to link the home ranges when necessary. Relative positions of the burrow systems were not retained in the figure.

5. CAPÍTULO IV

IT IS EASIER TO BITE WHEN SOFT: SOIL HARDNESS INFLUENCES BITE FORCE IN A SUBTERRANEAN RODENT (RODENTIA: CTENOMYIDAE)

BRUNO B. KUBIAK[1], RENAN MAESTRI[2], THAMARA S. DE ALMEIDA[3], LEANDRO R. BORGES[1],

DANIEL GALIANO[4], RODRIGO FORNEL[5] AND THALES R. O. DE FREITAS[1,2,3]

[1]Programa de Pós-Graduação em Biologia Animal, Universidade Federal do Rio Grande do Sul, Av. Bento Gonçalves 9500, 91501-970 Porto Alegre, Brazil.

[2]Programa de Pós-Graduação em Ecologia, Universidade Federal do Rio Grande do Sul, Av. Bento Gonçalves 9500, 91501-970 Porto Alegre, Brazil.

[3]Departamento de Genética, Instituto de Biociências, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, 91501-970, Brazil.

[4] Programa de Pós-Graduação em Genética e Biologia Molecular, Universidade Federal do Rio Grande do Sul Av. Bento Gonçalves 9500, 91501-970 Porto Alegre, Brazil.

[5]Programa de Pós-graduação em Ecologia, Universidade Regional do Alto Uruguai e das Missões , Campus de Erechim, RS, 99709-910, Brazil.

Running Title: Divergent selection in bite force

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ABSTRACT

One of the major interests of evolutionary biologists is understanding which environmental features are best associated with morphological and behavioral characteristics of a species. Among the various morphological features of a species, we investigate here bite force, which is widely studied in interspecific contexts yet seldom explored among populations within species. Subterranean rodents present unique characteristics, one of which is the use of jaws and incisors to excavate soil for building of tunnels; skull morphology may thus be related to soil hardness. *Ctenomys minutus* occupies two distinct types of habitat: sand fields and sand dunes. Here, we evaluate whether bite force differs between *C. minutus* populations from sand fields and sand dunes, and whether these habitats differ in soil hardness. We use a total of 88 skulls and mandibles from *C. minutus* from both habitats to estimate the bite force and generate morphometric data. Our results indicate a significant difference in bite force between individuals from the two different habitats, whereby individuals inhabiting sand fields have a bite force 6.11 times higher than those inhabiting sand dunes. This difference is most likely a consequence of the harder soils in sand fields which are consequently more difficult to excavate. Our results suggest the possibility of divergent selection in the bite force of populations occupying the two different habitats. This study also reinforces the importance of soil hardness in shaping the bite force in subterranean species, demonstrating that factors beyond those related to feeding strategies and diet may influence differentiation in bite force.

KEYWORDS: Digging adaptations – Divergent selection – Dune habitat – Geometric morphometrics – Mammalian skull – Natural selection – Phenotypic plasticity – Soil hardness.

INTRODUCTION

It is well understood that environmental features are associated with morphological and behavioral changes of many species (Schluter, 2000; Herrel *et al.*, 2008; Losos & Mahler, 2010; Silva *et al.*, 2016). One of the major interests of evolutionary biologists is understanding the factors that guide these changes. In this context, the skull is one of the most studied structures within vertebrates, mainly because this structure is involved in many functions (e.g., feeding, sensory system). Several intrinsic (e.g., evolutionary history) and extrinsic factors (e.g., environmental variables, including biotic interactions) may influence morphological variation of the skull (Viguier, 2002; Caumul & Polly, 2005; Dollion *et al.*, 2016). Among the various functions associated with the skull we highlight bite force, which has already been widely studied in mammals and is demonstrated to be closely correlated with morphological variation in several species (Anderson, Mcbrayer, & Herrel, 2008; Maestri *et al.*, 2016).

Variation in bite force is largely related to body size (Freeman & Lemen, 2008) and the shape and size of the masticatory apparatus (Herrel *et al.*, 2005a, 2005b). Dietary composition, variation in feeding modes among animals, and phylogenetic relationships have also been investigated in response to variation in bite force, with the suggestion that selection on bite force is related to body size but that evolutionary tendencies are linked to dietary specialization (Christiansen & Wroe 2007; Nogueira *et al.*, 2009). These studies generate data that allow inferences about macroevolutionary patterns associated with selection for different bite forces, and indicate that diet is an important factor for selection on this feature in different groups (e.g., bats and carnivores). However, few studies have addressed the differentiation of bite force in a single species, or analyzed the microevolutionary scenario in distinct populations (Sagonas *et al.*, 2014; Sustaita & Rubega, 2014). These single-species studies are important for generation of complementary information regarding the factors that influence selection on bite force.

Among the mammals, subterranean rodents possess characteristics that make them ideal experimental models for testing factors that influence the bite force. First, they perform most of their vital activities below the soil surface, constructing and inhabiting tunnel systems that are excavated predominantly using the teeth (Nevo, 1979; Lacey, Patton & Cameron, 2000). The genus *Ctenomys* is among the subterranean rodent genera with the highest richness, with approximately 70 species (Freitas, 2016). These species have several adaptations to accommodate their specific life histories and habits, many of them related to excavation (Nevo 1979, Lacey *et al.*, 2000). One of the main characteristics related to excavation is the use of the jaw and incisors to remove soil and cut through plant roots (Hildebrand, 1985; Nevo, 1999, Stein, 2000). Skull morphology and bite force among species is thus related to soil hardness in their respective habitats (Borges *et al.,* 2017), and usually results in development of a stronger bite force than expected for the body size of these animal (Becerra *et al.*, 2011, 2014) .

Ctenomyids are herbivorous rodents, feeding on both aerial and underground plants collected along the galleries of their tunnels (Lacey *et al*. 2000, Lopes *et al*., 2015). They are usually distributed in regions with open vegetation (savannas, deserts, and dunes), and each species typically occurs in only a single habitat type (Lacey *et al*., 2000). *Ctenomys minutus* Nehring, 1887 is distributed along a 512 km extension of the southern Brazilian coastal plain, presenting one of the largest longitudinal distributions among species in the genus (Freitas 1995, see Fig. 1). However, unlike the general distribution patterns for the genus, this species occupies more than one type of habitat: sand fields and sand dunes (Freitas 1995; Lopes *et al.*, 2013; Galiano *et al.*, 2014, 2016) (Fig. 01). These habitats have marked differences in plant biomass, with sand fields tending to have higher above and below ground plant biomass

(Galiano *et al*. 2014; Kubiak *et al*. 2015). Moreover, it is known that the home range size of this species changes according to habitat, with animals living in sand dunes tending to have larger home ranges (Kubiak *et al*., 2017); the selection of food items also differs between the two habitat types (Lopes *et al*., 2015).

In this study we evaluate whether bite force of *C. minutus* differs in populations from different habitats (sand dunes vs. sand fields). We hypothesized that individuals inhabiting sand fields may have a stronger bite force than individuals inhabiting sand dunes, based on the different habitat features (plant biomass and soil hardness) that have potential to influence selection on bite force.

MATERIAL AND METHODS

SAMPLE

We evaluated a total of 88 skulls and mandibles of adult *C. minutus* (juveniles were not used in this study) to estimate the bite force and generate morphometric data. We used 38 skulls and mandibles from specimens collected at six locations within the sand dune habitat (13 males, 17 females, and 8 without sex identification). We also evaluated 50 skulls and mandibles from individuals collected from eight locations in the sand field habitat (28 males and 22 females) (Fig. 1). We excluded juveniles from the sample based on the small size of the skull. All skulls and mandibles were submitted to the collection at the Laboratório de Citogenética e Evolução of the Departamento de Genética, Universidade Federal do Rio Grande do Sul. Collection numbers and locations of each skull and mandible are presented in the supplementary material (Supporting Information - Appendix S1).

BITE FORCE MEASUREMENT

Bite force was estimated for each individual using methods proposed by Freeman & Lemen (2008). Two measurments are taken of the lower incisor: (1) length (anterior-posterior length), and (2) width (medial-lateral width), and subsequently applied to the following formula: Zi = ((anterior-posterior length) × 2 (medial-lateral width)) / 6, where Zi is the index of incisor strength. Freeman & Lemen (2008) found that this index is highly correlated with individual bite force measurements *in vivo*, with a correlation coefficient of 0.96. After determining the Zi we used the regression equation provided by the same authors to transform values to Newtons (N). See Supporting Information - Appendix S1 for individual bite force values.

GEOMETRIC MORPHOMETRICS APPROACH

We used the same skulls and mandibles to obtain shape variables. Briefly, we used a digital camera (Nikon Coolpix P100, 13.1 megapixels, 3648 x 2736 resolutions) to produce images of the mandible and the dorsal, ventral, and lateral view of the skull of each specimen. The position and distance between the camera and the subjects were the same for all specimens. We chose 29 landmarks that were digitalized in the dorsal view of the skull, 30 in the ventral view, 21 in the lateral view (Fernandes *et al.*, 2009), as well as 13 in the mandible (Fornel, Cordeiro-Estrela, & De Freitas, 2010) (see Supporting Information - Appendix S2 for landmark positions). The anatomical landmarks were digitized using TPSDig2 version 2.17 (Rohlf, 2015). The resulting matrices of coordinates were superimposed through Generalized Procrustes Analysis (GPA), which removes the effects of scale, orientation, and position. Geometric morphometric procedures were performed with the geomorph package (Adams & Otárola-Castillo, 2013).

STATISTICAL ANALYSES

We used the data generated by Galiano *et al.* (2014, 2016) and Kubiak *et al.*, (2015) to investigate differences in soil hardness at nine different locations: six areas in sand fields and three areas in sand dunes (see Fig. 1). A minimum of 10 measurements of soil hardness were made in each area (all were occupied by *C. minutus*) using a soil penetrometer (see details in Galiano *et al*., 2014, 2016; Kubiak *et al*., 2015). We used mean hardness values for comparison among areas to avoid pseudo-replication. We used soil hardness at 10 cm and 20 cm depth in analyses, as these depths correspond to the portion of the soil that this species inhabits (Galiano *et al*., 2014). We performed an analysis of variance (ANOVA) to test the difference in soil hardness and vegetation cover in the two habitat types.

We also used an analysis of covariance (ANCOVA) to test for relationships between bite force and the habitat type. Bite force is significantly correlated with body size (Freeman & Lemen, 2008) and may exhibit sexual dimorphism, thus we use size (skull length) and sex as covariates to mitigate this effect (Bite force ~ habitat type + sex + skull length). We used a series of partial least-squares regressions (PLS) to investigate the relationship between bite force and shape in the dorsal, ventral and lateral views of the skull and the mandible. All analyses were done using the R program (R Core Team, 2016) with the vegan (Oksanen *et al*. 2013) and geomorph packages (Adams & Otárola-Castillo 2013).

RESULTS

The comparison of the soil hardness showed that sand fields have harder soils at both 10 and 20 cm depth compared to sand dunes ($F_{1,7}$ = 10.68, P = 0.013; and $F_{1,7}$ = 18.59, P = 0.003, respectively) (Fig. 3). Individuals from sand fields have a stronger bite force than individuals from sand dunes ($F_{7,72}$ = 15,348; $P < 0.001$), and males have a stronger bite force than females ($F_{7,72}$ = 12.470; $P < 0.001$). We also found a positive correlation between body size and bite force ($F_{7,72}$ = 140,637; $P < 0.001$). However, there was no interaction between sex and habitat because males have a stronger bite force in both habitat types. The bite force and mean skull size data are summarized in Table 1.

The PLS indicated that the shape of all skull and mandible are strongly correlated with bite force (Dorsal: r = 0.81; Ventral: r = 0.74; Lateral: r = 0.74 and Mandible: r = 0.60) (Fig. 02). Visualization of the changes in shape described by the PLS shape vector (derived from skull data) showed the highest values of bite force were associated with rostral enlargement and retraction of the skull base in dorsal and ventral view. At the opposite end of the same shape vector, lower values of bite force are associated with shortening of the rostrum and zygomatic arch, and increase in the skull base (Fig. 02 - A, B). In the lateral view of the skull and mandible, higher bite force values are associated with an increase in skull and mandible height, and greater incisor procumbency angle (Fig. 02 - C, D).

DISCUSSION

Our results demonstrate a significant inter-habitat differences in *C. minutus* bite force. Individuals occupying sand fields have an average bite force 6.11 times higher than those inhabiting sand dunes. Thus, as already suggested by Borges *et al*. (2017) species of the genus *Ctenomys* seem to present a positive relationship between bite force and soil hardness. Our results suggests that the differences in bite force in populations of the same species inhabiting different habitats likely occurred in response to different selective pressures (e.g. soil hardness).

Variation in bite force can be related to differences in diet or feeding habits (Nogueira *et al*., 2009, Da Silva *et al*., 2016, Maestri *et al*., 2016, Dollion *et al*., 2017). Our results demonstrated that the bite force can be shaped by other factors that are related to life history and behavior. The subterranean lifestyle requires *ctenomids*, among others with similar habits, to use their teeth as tools for tunnel building (Stein, 2000), and harder soils require more energy expenditure for excavated than do soft soils (Luna & Antinuchi, 2006). Species may differ in digging strategies, and are perhaps able to reduce the use of teeth in favor of increased use of the anterior and posterior limbs (Hildebrand, 1998; Stein, 2000), which may result in the differences in bite force that we found in our study populations.

It is known that soil hardness and plant cover are closely linked to the ecology of subterranean rodents, influencing their distributions (Miller 1964), excavation strategies, (Hildebrand, 1998; Stein, 2000), and bite force on a macroevolutionary scale (Borges *et al*., 2017). Furthermore, the occupation of different habitat types influences behavioral aspects of *C. minutus*, such as the home range size (Kubiak *et al*., 2017). Animals occupying sand dunes have larger home range sizes than animals occupying sand fields. Based on our results, we can infer that soil hardness also influences differentiation in skull shape and bite force between populations. These results also suggest that soil characteristics are closely related to the vital activities of subterranean animals and a key factor for selection on some characteristics like size, shape, home range area (Heth, 1989, Lövy *et al*., 2015), and distribution (Miller, 1964), possibly influencing species divergence.

Differences in bite force of animals occupying different habitats is closely related to the changes in individual skull shape. Animals with stronger bite force in sand fields have a more robust mandible, a higher degree of insertion of the incisors, and a wider skull compared to those with lower bite force occupying sand dunes. This pattern of differentiation is similar to what has been described for other mammals (Christiansen & Adolfssen, 2005; Nogueira *et al*., 2009; Maestri *et al*., 2016; Borges *et al*., 2017). Furthermore, Mora *et al*. (2003) described changes in the angle of incisor procumbency based on a sample of 23 species of *Ctenomys*; the authors associated these changes with the rostral allometry. Indirectly, we found differences in the degree of rostrum slope in *C. minutus* and consequently, in the angle of incisor procumbency (see Fig. 2C), corroborating the idea that there is an association between rostrum-incisor procumbency and soil hardness (Reig & Quintana 1992; Mora *et al*., 2003; Lessa *et al*., 2008; Marcy *et al*., 2016).

Ctenomy minutus presented sexual dimorphism in relation to bite force. In addition to inter-habitat differentiation, males exhibit greater bite force than females in both habitats. Similar results were described for *C. talarum* (Becerra *et al.*, 2011) corroborating the authors' idea that differentiation in bite force between sexes should be a result of sexual selection. This selection is likely primarily associated with male hierarchy, due to the polygynous mating system of the genus *Ctenomys* in which males engage in aggressive interactions with other males. (Zenuto *et al*., 1999a, 1999b). Genetic data corroborate these results: while *C. minutus* present the same karyotype (2n=46a) in both sand fields and sand dunes (Freitas 1997), molecular markers such as mtDNA show that they have different haplotypes between habitats. The same was described using microsatellite DNA, which suggests lack of gene flow between the habitats (Lopes *et al*., 2013).

In summary, our data point to a possible divergent selection in bite force in populations occupying sand dunes and sand fields. This study also reinforces the importance of soil hardness in shaping bite force in subterranean species, demonstrating that factors besides those related to feeding strategies can influence differentiation of this characteristic. In addition, our results together with those described by Kubiak *et al*., (2017) indicate that *C. minutus* presents a two-character differentiation (home range size and bite force) that is directly associated with habitat. Therefore, we can infer that habitats with differences in soil hardness and vegetation cover directly influence behavioral characteristics in subterranean rodents, as has been proposed for other species (Heth, 1989; Rosi *et al*. 2000, Spinks *et al*. 2000; Šumbera *et al*. 2003; Romañach *et al*. 2005). Consequently, this divergent selection could lead to sympatric speciation, as reported in recent studies (Polyakov *et al*. 2004; Hadid *et al*. 2013; Li *et al.*, 2016; Lövy *et al*. 2015; Šklíba *et al*. 2015). Unfortunately, the results generated here do not allow us to accurately discriminate between adaptation and phenotypic plasticity, the two likely outcomes of selection driven by habitat. Future studies should be developed to address whether differences in *C. minutus* bite force is a result of adaptation or simply an expression of phenotypic plasticity (e.g., by evaluating bite force in newborns and juveniles from both habitats).

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Tables and Figures

Table 1. Mean values (±SD) for bite force and skull lengths in *Ctenomys minutus* from sand dunes and sand field habitats.

	Sand dunes		Sand fields	
	Bite force (N)	Skull length (mm)	Bite force (N)	Skull length (mm)
Males	50.94 ± 10.19	44.27 ± 2.57	59.31 ± 10.41	44.72 ± 1.94
Females	44.53 ± 7.72	43.08 ± 2.87	45.08 ± 6.83	41.78 ± 1.79
Total	46.93 ± 9.43	43.48 ± 2.68	53.04 ± 11.43	43.22 ± 2.47

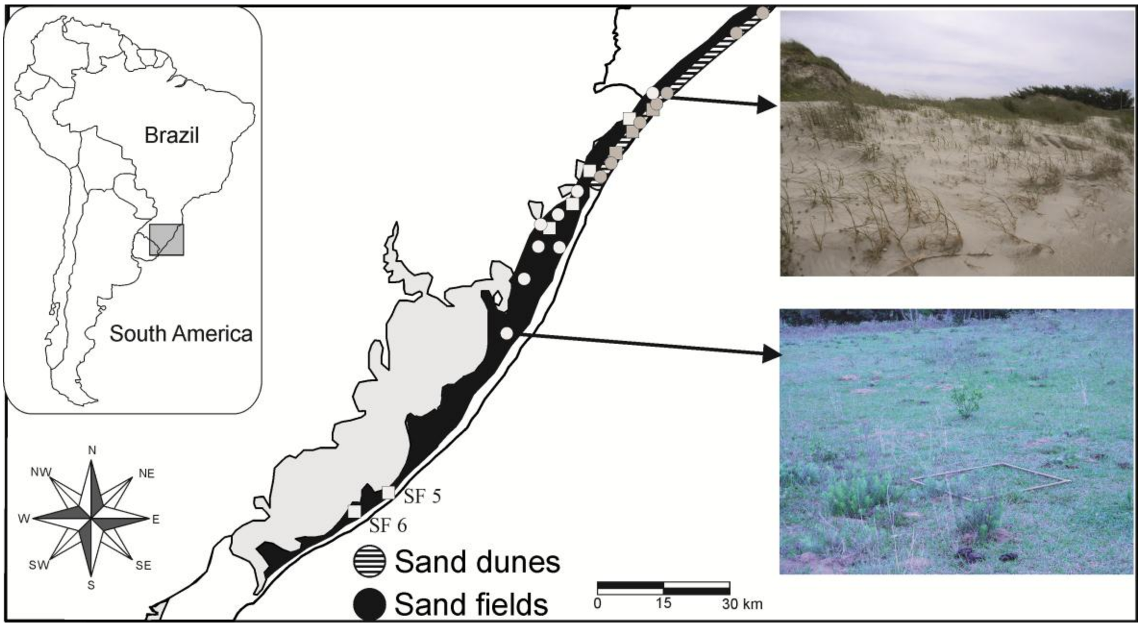

Fig. 1. Geographic distribution of *C. minutus* in the coastal plain of southern Brazil in sand dunes and sand fields. The squares represent the sampling sites for soil hardness in sand fields (white squares) and sand dunes (gray squares), and the circles represent the sample locations for skulls in sand fields (white circles) and sand dunes (gray circles).

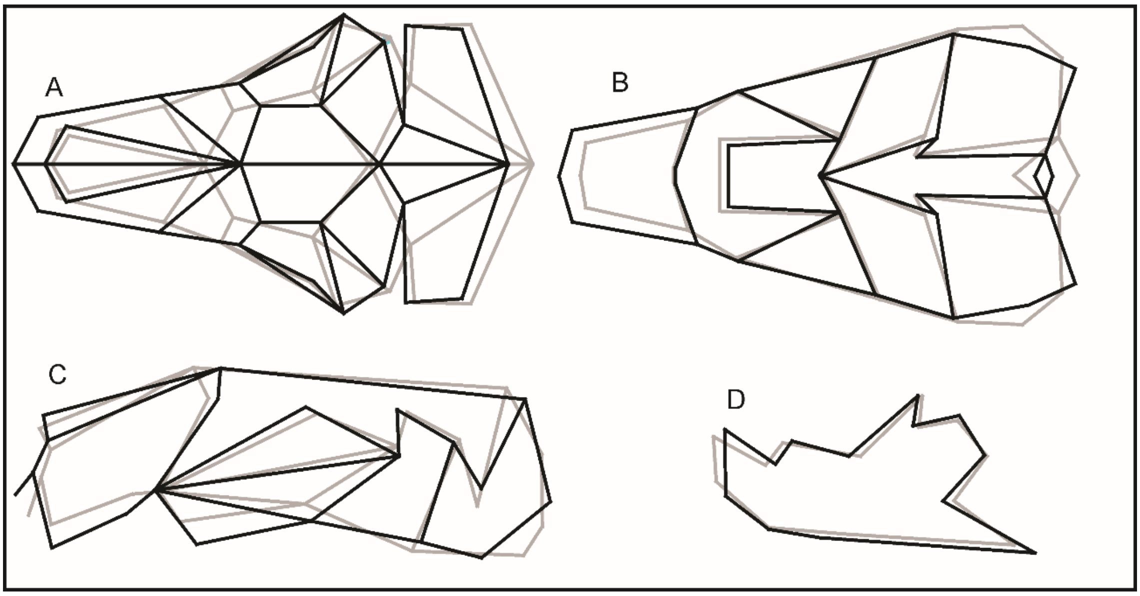

Fig. 2. Representation of conformational changes associated with negative and positive PLS vectors in dorsal (A), ventral (B), and lateral view (C), and mandible (D). Solid black lines correspond to the shape associated with positive scores— indicating higher bite forces—and solid grey lines correspond to the shape associated with negative scores—indicating lower bite forces.

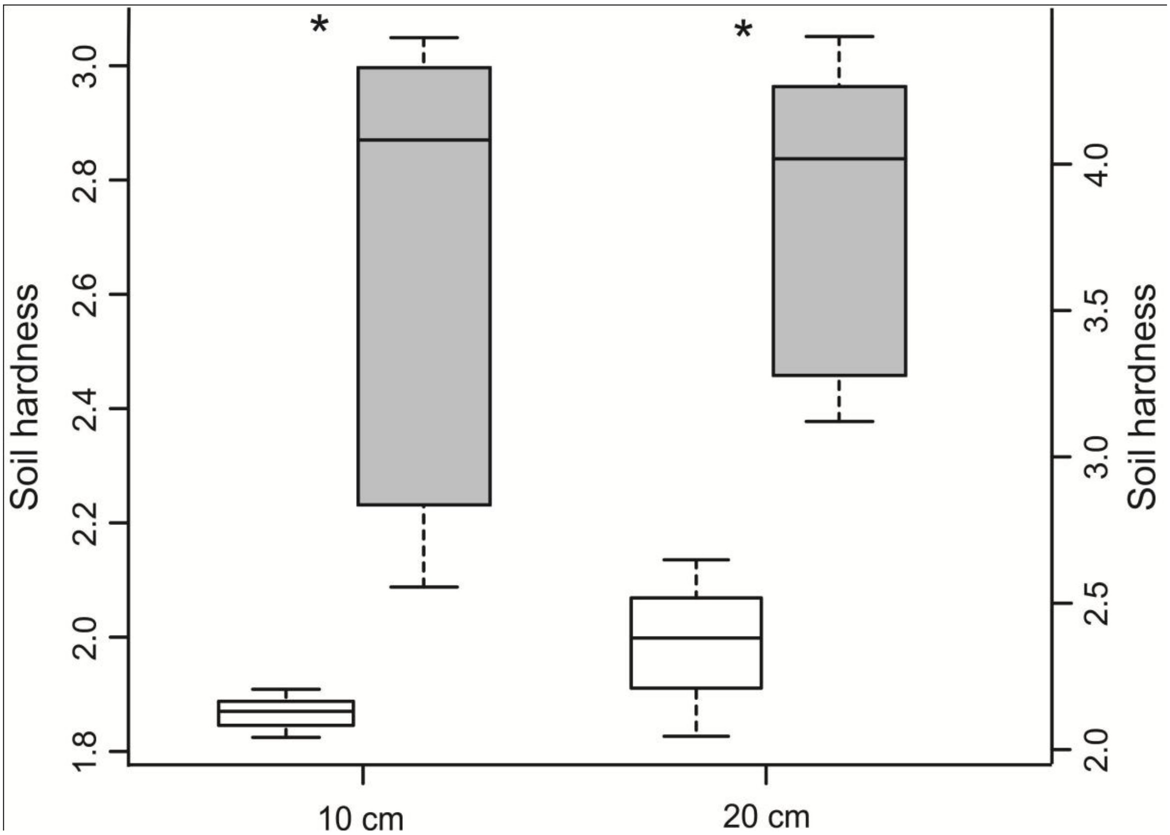

Fig. 3. Soil hardness (kg/cm^2) at two different depths (cm) from sampling sites in sand dunes (white boxes) and sand fields (gray boxes). The left scale indicates hardness values at 10 cm depth, and the right scale indicates hardness values at 20 cm depth. Results from statistical tests are displayed in the text. Asterisks indicate significant differences between habitat types.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article:

Appendix S1. List with identification numbers for *Ctenomys minutus* skull specimens deposited in the collection of Laboratório de Citogenética e Evolução da UFRGS. Information of sex, habitat, locality, and estimated bite force in Newtons for all individuals are provided.

ID	Sex	Habitat	Locality	Bite force (N)
TR.20	F	Sand fields	Lagoa Emboaba/RS	37.0480393
TR.33	F	Sand fields	Tramandai/RS	43.27631002
TR.37	F	Sand fields	Tramandai/RS	39.85140394
TR.167	F	Sand fields	Osório/RS	45.50814485
TR.168	F	Sand fields	Osório/RS	68.67801269
TR.171	F	Sand fields	Osório/RS	46.78601285
TR.175	F	Sand fields	Osório/RS	48.869085
TR.212	F	Sand fields	Passo de Torres/SC	42.91371753
TR.394	F	Sand fields	Capivari do Sul / RS	36.22501155
TR.395	F	Sand fields	Capivari do Sul / RS	38.97974571
TR.397	F	Sand fields	Capivari do Sul / RS	45.03646942
TR.402	F	Sand fields	Capivari do Sul / RS	40.84074858
TR.407	F	Sand fields	Lagoa dos Barros/RS	42.76687927
TR.409	F	Sand fields	Lagoa dos Barros/RS	44.65890333
TR.419	F	Sand fields	Lagoa dos Barros/RS	50.31837463
TR.428	F	Sand fields	Lagoa dos Barros/RS	55.49863746
TR.429	F	Sand fields	Lagoa dos Barros/RS	42.15438736
TR.430	F	Sand fields	Lagoa dos Barros/RS	43.51723509
TR.431	F	Sand fields	Estancia Velha – Osório/RS	45.54106483
TR.432	F	Sand fields	Estancia Velha – Osório/RS	47.19331475
TR.433	F	Sand fields	Estancia Velha – Osório/RS	44.91806898
TR.436	F	Sand fields	Estancia Velha – Osório/RS	41.12190083
TR.01	F	Sand dunes	Jaguaruna/SC	49.80473705
TR.02	F	Sand dunes	Jaguaruna/SC	38.26791018
TR.07	F	Sand dunes	Jaguaruna/SC	63.73716451
TR.40	F	Sand dunes	Praia do Barco/RS	42.71052319
TR.201	F	Sand dunes	Morro dos Conventos/SC	45.34922668
TR.632	F	Sand dunes	Torres/RS	48.89411297
TR.1841	F	Sand dunes	Praia do Barco/RS	35.40503581
TR.1844	F	Sand dunes	Praia do Barco/RS	49.80278435
TR.1847	F	Sand dunes	Praia do Barco/RS	37.98449494
TR.1848	F	Sand dunes	Praia do Barco/RS	38.21220054
TR.1850	F	Sand dunes	Praia do Barco/RS	35.38651517
TR.1851	F	Sand dunes	Praia do Barco/RS	38.19863716
TR.2011	F	Sand dunes	Jaguaruna/SC	46.04256548

TR.2013	F	Sand dunes	Jaguaruna/SC	40.44983715
TR.2015	F	Sand dunes	Jaguaruna/SC	56.93684194
TR.2016	F	Sand dunes	Jaguaruna/SC	46.36267217
TR.2108	F	Sand dunes	Morro dos Conventos/SC	43.48224639
TR.21	M	Sand fields	Lagoa Emboaba/RS	43.67481941
TR.22	M	Sand fields	Lagoa Emboaba/RS	61.8605837
TR.24	M	Sand fields	Lagoa Emboaba/RS	54.83570394
TR.34	M	Sand fields	Tramandai/RS	58.41802321
TR.35	M	Sand fields	Tramandai/RS	60.47705656
TR.38	M	Sand fields	Tramandai/RS	56.26425454
TR.166	M	Sand fields	Osório/RS	68.45864522
TR.169	M	Sand fields	Osório/RS	55.25768099
TR.170	M	Sand fields	Osório/RS	67.52242989
TR.173	M	Sand fields	Osório/RS	54.09905188
TR.209	M	Sand fields	Lagoa dos Barros/RS	37.04344095
TR.391	M	Sand fields	Capivari do Sul / RS	69.47132791
TR.392	M	Sand fields	Capivari do Sul / RS	94.52051568
TR.393	M	Sand fields	Capivari do Sul / RS	68.75156701
TR.398	M	Sand fields	Capivari do Sul / RS	59.19582713
TR.399	M	Sand fields	Capivari do Sul / RS	60.88187879
TR.400	M	Sand fields	Capivari do Sul / RS	62.50956318
TR.401	M	Sand fields	Capivari do Sul / RS	59.88399286
TR.410	M	Sand fields	Lagoa dos Barros/RS	63.59753932
TR.411	M	Sand fields	Lagoa dos Barros/RS	61.0167504
TR.412	M	Sand fields	Lagoa dos Barros/RS	52.28639287
TR.415	M	Sand fields	Lagoa dos Barros/RS	44.69878928
TR.416	M	Sand fields	Lagoa dos Barros/RS	58.75900399
TR.418	M	Sand fields	Lagoa dos Barros/RS	63.24238196
TR.425	M	Sand fields	Lagoa dos Barros/RS	54.61259444
TR.435	M	Sand fields	Estancia Velha – Osório/RS	66.31981731
TR.438	M	Sand fields	Estancia Weber – Osório/RS	53.84078956
TR.440	M	Sand fields	Estancia Weber – Osório/RS	49.07414283
TR.41	M	Sand dunes	Praia do Barco/RS	52.68192958
TR.46	M	Sand dunes	Praia do Barco/RS	49.566214
TR.52	M	Sand dunes	Capão Novo/RS	51.66107808
TR.55	M	Sand dunes	Capão Novo/RS	53.51166469
TR.56	M	Sand dunes	Capão Novo/RS	48.77638556
TR.554	M	Sand dunes	Jaguaruna/SC	67.04238646
TR.567	M	Sand dunes	Jaguaruna/SC	62.4457462
TR.544	M	Sand dunes	Praia da Gaivota/SC	37.62311981
TR.2088	M	Sand dunes	Morro dos Conventos/SC	30.03030826
TR.5	M	Sand dunes	Jaguaruna/SC	59.89995266
TR.2087	M	Sand dunes	Morro dos Conventos/SC	55.72188288
TR.2080	M	Sand dunes	Morro dos Conventos/SC	40.15629845
TR.2082	M	Sand dunes	Morro dos Conventos/SC	53.16544953
TR.1843	NA	Sand dunes	Praia do Barco/RS	40.94764612
TR.1855	NA	Sand dunes	Praia do Barco/RS	34.6941847
TR.1856	NA	Sand dunes	Praia do Barco/RS	33.09842198
TR.1857	NA	Sand dunes	Praia do Barco/RS	50.78828715
TR.1858	NA	Sand dunes	Praia do Barco/RS	49.39207331

TR.1860	NA	Sand dunes	Praia do Barco/RS	48.77638556
TR.1862	NA	Sand dunes	Praia do Barco/RS	40.90446757
TR.6	NA	Sand dunes	Jaguaruna/SC	65.42853941

Appendix S2. Descriptions of landmarks positions with numbers and locations for each view of the cranium and the mandible of *Ctenomys minutus*.

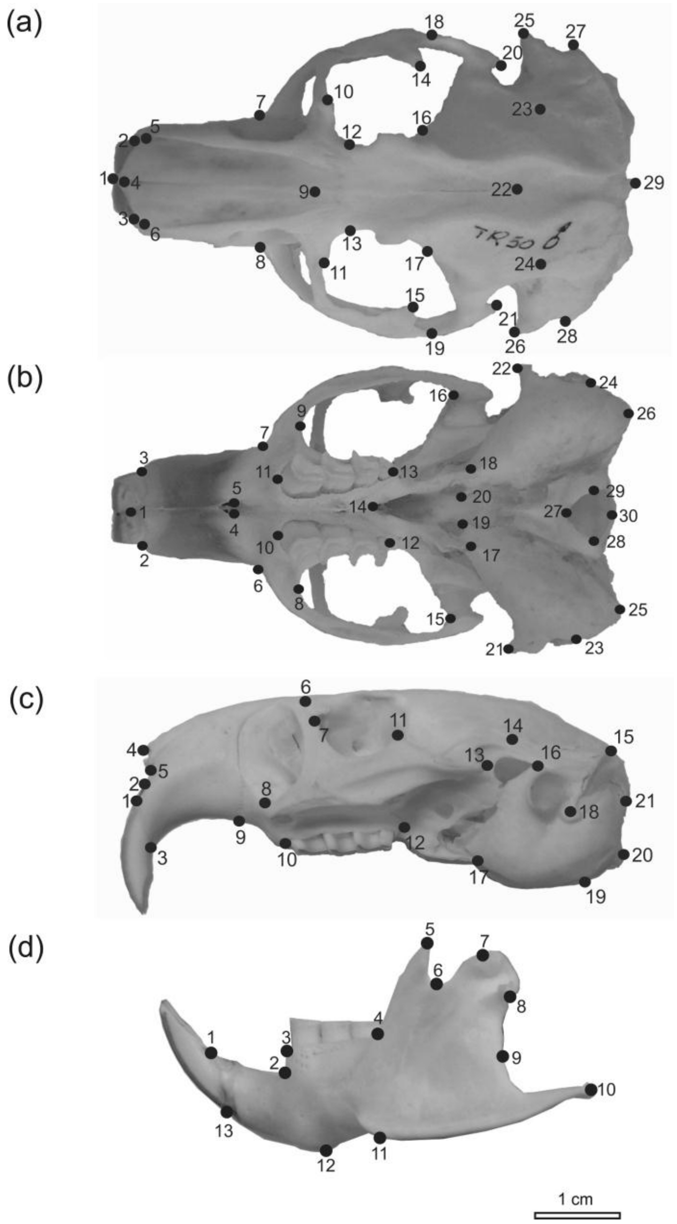

Figure S2. Landmarks used to capture shape from the dorsal (a), ventral (b) e lateral (c) view of the skull and the left side view of the mandible (d) in *Ctenomys minutus*.

Dorsal view of the cranium: 1. anterior tip of the suture between premaxillas; 2-3.anterolateral extremity of incisor alveolus; 4. anterior extremity of suture between nasals; 5-6. anteriormost point of suture between nasal and premaxilla; 7-8. anteriormost point of root of zygomatic arch; 9. suture between nasals and frontals; 10-11. anterolateral extremity of lacrimal bone; 12-13. narrowest point between frontals; 14-15. tip of extremity of superior jugal process; 16-17. anterolateral extremity of suture between frontal and squamosal; 18-19. lateral extremity of suture between jugal and squamosal; 20-21. tip of posterior process of jugal; 22. suture between frontals and parietals; 23-24. anterolateral extremity of suture between parietal and squamosal; 25-26. anterior tip of external auditory meatus; 27-28. point of maximum curvature on mastoid apophysis; 29. posteriormost point of occipital along midsagittal plane.

Ventral view of the cranium: 1. anterior tip of suture between premaxillas; 2-3. anterolateral extremity of incisor alveolus; 4-5. lateral edge of incisive foramen in suture between premaxilla and maxilla; 6-7. anteriormost point of root of zygomatic arch; 8-9. anteriormost point of orbit in inferior zygomatic root; 10-11. anteriormost point of premolar alveolus; 12-13. posterior extremity of III molar alveolus; 14. posterior extremity of suture between palatines; 15-16. anteriormost point of intersection between jugal and squamosal; 17-18. posteriormost point of pterygoid; 19-20. anterior extremity of tympanic bulla; 21-22. anterior tip of external auditory meatus; 23-24. posterior extremity of mastoid apophysis; 25-26. posterior extremity of paraoccipital apophysis; 27. anteriormost point of foramen magnum; 28-29. posterior extremity of occipital condyle in foramen magnum; 30. posteriormost point of foramen magnum along midsagittal plane.

Lateral view of the cranium: 1. anteriormost point of premaxilla; 2. posteriormost point of incisor alveolus; 3. inferiormost point of incisor alveolus; 4. anterior tip of nasal; 5. anteriormost point of suture between nasal and premaxilla; 6. suture between premaxilla,

maxilla and frontal in superior zygomatic root; 7. inferiormost point of suture between lacrimal and maxilla; 8. inferiormost point of infraorbital foramen in inferior zygomatic root; 9. inferiormost point of suture between premaxilla and maxilla; 10. anteriormost point of premolar alveolus; 11. extremity of superior jugal process; 12. extremity of inferior jugal process; 13. tip of posterior jugal process; 14. medial point of suture between parietal and squamosal; 15. superior extremity of lambdoidal crest; 16. posterior extremity of postglenoid fossa; 17. inferior extremity in suture between pterygoid and tympanic bulla; 18. inferior extremity of mastoid apophysis; 19. anteriormost margin of paraoccipital apophysis; 20. posteriormost margin of paraoccipital apophysis; 21. posterior extremity of intersection between occipital and tympanic bulla.

Lateral view of the mandible: 1. upper extremity of anterior border of incisor alveolus; 2. extremity of diastema invagination; 3. anterior edge of premolar alveolus; 4. intersection between molar alveolus and coronoid process; 5. tip of coronoid process; 6. maximum of curvature between coronoid and condylar processes; 7. anterior edge of articular surface of condylar process; 8. tip of postcondyloid process; 9. maximum curvature between condylar and angular processes; 10. tip of angular process; 11. intersection between mandibular body and masseteric crest; 12. posterior extremity of mandibular symphysis; 13. posterior extremity border of incisor alveolus.

6. CONSIDERAÇÕES FINAIS

Os resultados e interpretações gerados ao longo deste trabalho são possíveis devido aos diversos estudos realizados com a espécie *Ctenomys minutus*, fazendo com que esta seja a espécie mais estuda entre os *Ctenomys* que possuem distribuição registrada no Brasil. A espécie possui estudos e definições robustas sobre aspectos evolutivos, moleculares, padrões filogeográficos, rearranjos cromossômicos, dinâmica populacional, dieta alimentar, ecologia espacial, seleção de habitat e distribuição (Freitas 1995, 1997; Fonseca 2003; Gava et al. 2004; Marinho e Freitas 2006; Fornel et al. 2010; Lopes et al. 2013; Galiano et al. 2014; Kubiak et al. 2015; Lopes et al. 2015; Galiano et al. 2016). Além disso, os resultados apresentados neste trabalho ampliaram o conhecimento da espécie, ajudando a elucidar como as interações bióticas e abióticas influenciam em aspectos comportamentais, ecológicos e evolutivos de *Ctenomys minutus*.

A utilização de múltiplas ferramentas se mostrou muito eficaz ao longo deste trabalho, fornecendo assim uma visão ampliada dos processos e respostas que a espécies *Ctenomys minutus* apresenta em relação às interações. Utilizando a modelagem de nicho ecológico foi possível perceber que as *Ctenomys minutus* e *Ctenomys flamarioni* não se excluem competitivamente. No entanto, a morfometria geométrica nos permite verificar que existe deslocamento de caracteres em uma das espécies. Já que, *C. minutus* apresenta menor tamanho quando em contato com *C. flamarioni*. Sendo assim, é possível afirmar que mesmo não sendo detectado evidencia que suportem a exclusão competitiva entre a espécie em um cenário macro geográfico, a interação entre elas pode fazer com que possuam diferenciações na seleção do habitat e possivelmente isso ocasiona a diferenciação morfológica de uma das espécies (*Ctenomys minutus*).

Quando foi explorado o tamanho da área de vida de *C. minutus* se percebe que o tipo de habitat é um fator determinante na diferenciação desta característica. Sendo que indivíduos que ocupam o habitat de dunas costeiras possuem áreas de vida significativamente maiores que animais da mesma espécie que ocupam o habitat de campos arenosos. Por outro lado, a coexistência com *C. flamarioni* não parece influenciar o tamanho da área de vida de nenhuma das espécies, evidenciando que interações bióticas podem não influenciar significativamente nesta característica ecológica e comportamental.

De maneira similar ao tamanho da área de vida, o tipo de habitat também parece ser um fator importante na determinação da força de mordida e forma do crânio de *Ctenomys minutus*. Os animais que habitam os campos arenosos possuem diferenciações na forma do crânio em relação a animais coespecíficos que habitam as dunas costeiras. Isto parece estar intimamente ligado com a diferenciação da força da mordida destes animais, onde indivíduos que habitam os campos arenosos possuem maiores forças de mordida em comparação a indivíduos que habitam as dunas costeiras.

De modo geral é possível concluir que as interações bióticas experimentadas por *C. minutus* possuem influência direta na seleção de habitat da espécie e diferenciação morfológica (deslocamento de caracteres). Contudo, parece não influenciar em aspectos comportamentais relacionados ao tamanho da área de vida dos indivíduos. Já as interações abióticas, neste caso a ocupação de diferentes tipos de habitats, influenciam diretamente nas diferenciações ecológicas e comportamentais em relação ao tamanho da área de vida e diferenças morfológicas do crânio, culminando na diferenciação da força da mordida e possivelmente podendo levar a diferenciações evolutivas destas populações. Finalizando, espero que as informações geradas aqui possam ser extrapoladas além da espécie alvo deste estudo, abrangendo o gênero *Ctenomys*, roedores subterrâneos e outros organismos, ajudando a elucidar questões maiores envolvendo fatores limitantes na distribuição das espécies, resultados de interações sobre o comportamento e ecologia das espécies e seleção divergente entre populações simpátricas.

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8. ANEXOS

Anexo 01

Normas para citações e referências Segundo o periódico Journal of Mammalogy. Informações retiradas disponíveis em: <https://academic.oup.com/jmammal/pages/General_Instructions>

Literature Cited.—List all works cited in the text in the Literature Cited, including authority citations for scientific names in systematic papers but excluding references used only in Supporting Information. Works not cited should not be listed. Submitted manuscripts must be in press or removed before manuscript acceptance. Unpublished data and reports cannot be cited in the manuscript or listed in the Literature Cited, use pers. comm. or pers. obs. instead. The number of references cited should suffice to lead readers to key literature; use the lowest number of references necessary; rarely are more than 3 citations needed for a given point.

Personal communications should be cited parenthetically in the text; the citation should include the source's name and affiliation and the date of the communication: (Henry J. Smith, [university or other affiliation, city, state], personal communication, [month and year of communication]). Submit letters from authors of personal communications giving permission to use the material. Manuscripts submitted for publication but not yet accepted may not be cited.

Cite literature in text using the "Name-Year" format as presented in the CSE guidelines. Multiple in-text citations are ordered chronologically (Author 1998; Author 1999, 2000); if a reference was published in a different year than that presented in the book insert the correct year in square brackets; e.g., "Gardner [2008]" (see below for formatting these in the Literature Cited). Use the 1st author's last name and "et al." for in-text citation of works with more than 2 authors or editors, and cite in chronological order by lead author (e.g., if Jones, Smith, and Andrews 2000 and Jones, Andrews, and Smith 2001 were cited simultaneously, this should be cited as Jones et al. 2000, 2001). For multiple works by an author in the same year, cite as “a”, “b”, etc. (e.g., Author 2010a, 2010b) with the 1st article cited in text denoted as “a” (note that Jones, Smith, and Andrews 2001 and Jones, Andrews, and Smith 2001 would be cited as Jones et al. 2001a, 2001b, with the letters allocated in the order that these are presented in the Literature Cited).

In the Literature Cited, list the name of every author or editor, unless there are more than 7, in which case use “Author, et al. date” (see examples below). References are presented in alphabetical order by all authors (unless “et al.” is used), and chronologically for references with identical author lines. Capitalize only the 1st word and proper nouns of a reference and use italics only for scientific names. The style of some journals (i.e., the American Midland Naturalist, older issues of the Annals and Magazine of Natural History and of Mammalian Species) was to set scientific names in the titles of articles in Roman font, not italics; neither CSE nor the Chicago Manual provide guidance on how to reflect this in citation. This issue was raised with CSE personnel, and their recommendation (in litt. to DAK, 4 April 2016), which should be followed for both the Journal and Mammalian Species, was to always italicize scientific names in the Literature Cited, regardless of the original presentation. Provide the full names of all journals.