Sharing the Space: Distribution, Habitat Segregation and Delimitation of a New Sympatric Area of Subterranean Rodents

Bruno Busnello Kubiak[1]*, Daniel Galiano[2], Thales Renato Ochotorena de Freitas[1,3]

1 Programa de Pós-Graduação em Biologia Animal, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil, 2 Programa de Pós-Graduação em Ecologia, Universidade Regional do Alto Uruguai e das Missões—Campus de Erechim, Erechim, RS, Brazil, 3 Departamento de Genética, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil

* busnelo@hotmail.com

Abstract

Subterranean rodents of the genus *Ctenomys* usually present an allopatric or parapatric distribution. Currently, two cases of sympatry have been recognized for the genus in the coastal dunes of southern Argentina and southern Brazil. In this context, they are ideal models to test hypotheses about the factors that delimit the patterns of space use and to understand interspecific interactions in small mammals. We investigated the vegetation structure, plant biomass and soil hardness selected by two species of subterranean rodents (*Ctenomys flamarioni* and *C. minutus*) when distributed in sympatry and allopatry from nine different areas along the line of coastal dunes in southern Brazil. In addition, our work presents a new record of a third area of sympatry for the genus *Ctenomys*. *Ctenomys flamarioni* and *C. minutus* show habitat segregation in the area where they occur in sympatry. These species show segregation in their selection of microhabitats, differing in relation to soil hardness, plant biomass, and plant cover. *Ctenomys flamarioni* showed a distinction in habitat selection when occurring in allopatry and sympatry, whereas *C. minutus* selected the same habitat characteristics under both conditions. A possible explanation to the observed pattern is that these species have acquired different adaptations over time which allows them the ability to exploit different resources and thus avoid competitive interactions all together.

Introduction

The organization and spatial distribution of organisms play important roles in population stability, thereby directly influencing species coexistence [1]. Biological factors, such as interspecific interactions, may play a key role in the distribution of subterranean rodents [2] and may substantially influence community structure [3]. Different species of subterranean rodents are rarely sympatric, possibly because their morphological similarities make resource partitioning and subdivision of the subterranean niche difficult [4–5]. Instead, these species tend to have allopatric distributions, leading to the initial conclusion that congeneric competition has a minor

effect on subterranean rodent populations [6–7]. Subterranean rodents of the genus *Ctenomys* (tuco-tucos) are characterized by a limited individual mobility and a patchy distribution of the local populations [5]. Due to these characteristics, they are ideal models to test hypotheses about the factors that delimit the patterns of space use and to understand interspecific interactions in small mammals [8].

Species from the genus *Ctenomys* are widely distributed throughout South America [5, 9]. Until now, two cases of sympatry have been recognized for the genus. The first is widely recognized and studied and occurs between the species C. *australis* Rusconi, 1934 and *C. talarum* Thomas, 1989 [9–10] in a coastal dune region in the southern Buenos Aires province of Argentina. These species show segregation in their selection of microhabitats, differing in relation to soil and vegetation characteristics [2, 8, 11]. The second case of sympatry was reported to occur in the southern Brazil coastal plain between C. *flamarioni* Travi, 1981 and *C. minutus* Nehring, 1887 [12]. However, this sympatric zone has been neglected and there is no information regarding the extent of the area, the environmental characteristics or the habitat segregation among species in this location. *C. flamarioni* is narrowly distributed in the first range of mobile sand dunes of this coastal plain of southern Brazil [12]. *Ctenomys minutus* inhabits the sandy fields and dunes of the region and occupies the largest latitudinal gradient among the tuco-tucos in southern Brazil [12–13]. To the south of its distribution, *C. minutus* is found strictly on sandy fields, approximately 2 km from the coast. This species is also found on the range of coastal dunes near Capão da Canoa (29°46'00"S, 50°00'58" W), where it comes into contact with *C. flamarioni* [12].

Information on interspecific interactions among ctenomyids in sympatric areas has been documented in other studies [2, 8, 11]. However, these studies only provided information about habitat segregation in the sympatric areas and did not evaluate how species relate to habitat characteristics when distributed in allopatry. In general, these studies demonstrated that species segregate the environment according to the soil hardness, plant cover and plant biomass. We propose that these relationships of habitat segregation could be better understood by evaluating the environmental characteristics of both species when distributed in allopatry and by comparing this pattern with individuals with sympatric distribution. For this purpose, we evaluated different areas within the distribution of *C. flamarioni* and *C. minutus* by considering areas where the species occur in sympatry and areas where the species occur in allopatry. In addition, this work presents new information about the limits of the sympatric area cited by Freitas [12] and identifies a third sympatric area for the *Ctenomys* genus.

Materials and Methods

Animal capture and delimitation of the sympatric area

Specimens of *C. flamarioni* and *C. minutus* were sampled from nine different areas along the line of coastal dunes in southern Brazil: three areas where the species occur in sympatry (29°43'05S, 49°59'22W; 29°42'20S, 49°58'58W; 29°41'30S, 49°58'32W), three areas where *C. flamarioni* presents allopatric distribution (32°43'54S, 52°27'18W; 29°48'56S, 50°02'31W; 29°47'39S, 50°01'44W), and three areas where *C. minutus* presents allopatric distribution (29°34'12S, 49°53'54W; 29°29'21S, 49°50'20W; 29°23'44S, 49°46'02W). Each area was defined according to the total width of the dunes and had a maximum length of 200 meters. The sampling areas were at least one kilometer apart from each other (Fig 1).

The sampling was performed in two different campaigns between the months of January and February 2012 and between October and November 2012. In each area, we sampled a total of 10 individuals, and in the area of sympatry, five individuals of each species were collected. A total of 45 individuals of *C. flamarioni* (30 distributed in allopatry and 15 sympatrically) and
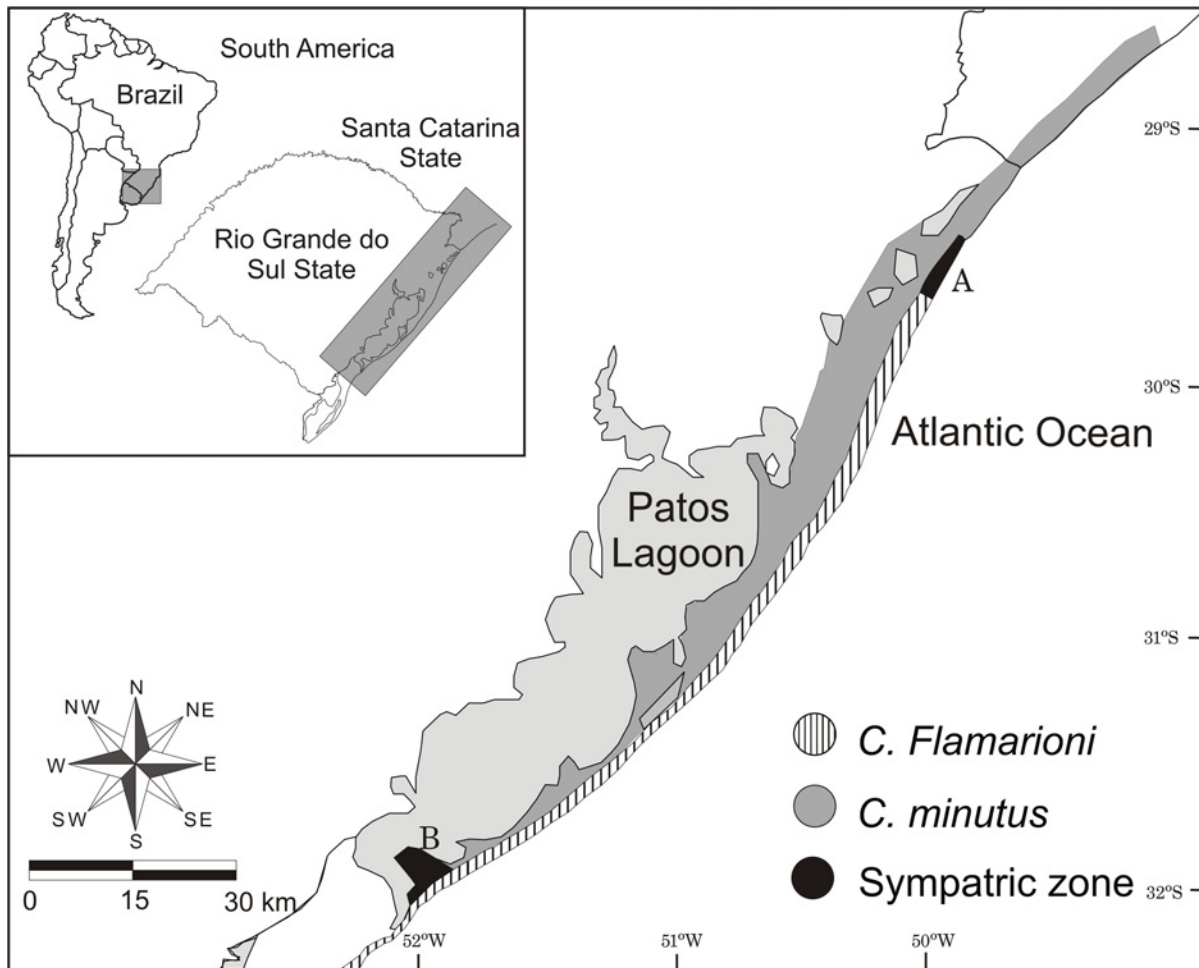
Fig 1. Geographic distribution of *C. flamarioni* and *C. minutus* in the coastal plain of southern Brazil. Two sympatric areas are indicated in the map by letters: A—sympatric area cited by Freitas (1995); B—new sympatric area described by these species. South America map obtained from OpenStreetMap (free available at: http://www.openstreetmap.org/). The image was edited using CorelDraw graphics Suite.

45 *C. minutus* (30 distributed in allopatry and 15 sympatrically) were sampled. The animals were captured using Oneida Victor No. 0 traps protected with rubber strips to capture the animals without injury. Each trap was introduced into the entrance of a burrow and checked every 10 minutes to reduce stress to the animals caught. This procedure was conducted only to confirm the presence of an animal in the burrow, and all individuals were then released at their respective trapping locations less than five minutes of the capture event.

Additionally, we determined the geographical limits of the sympatric area by sampling the tuco-tucos in a buffer region of 40 km between the south-north known boundaries proposed by Freitas [12] (south: 29°58'20.01" S, 50°07'00.94"W; north: 29°38'46.59"S, 49°56'52.2"W). This procedure allowed us to determine the exact region where both species get in contact. Animals were captured with the same methodology as above. Each captured animal was released at their point of capture and all of the coordinates were recorded using a GPS (Garmins-Vita). This study was carried out in strict accordance with the recommendations of the American Society of Mammalogists [14]. The protocol was approved by the IBAMA—Brazilian Institute for the Environment and for Renewable Natural Resources (Permit number: 14690–1). This work

was not submitted to an institutional Animal Ethics Committee because none of the animals would be injured or sacrificed.

Habitat characteristics and statistical analysis

To evaluate the vegetation and soil characteristics, we considered every point where an individual was captured as a central point, following the methodology proposed by Galiano et al. [15]. At each central point, vegetation structure, plant biomass, and soil hardness were sampled in four sampling units (SU) situated at a distance of 2 m in the four cardinal directions. To evaluate vegetation structure, we used a 1-m^2 quadrat to measure plant cover of grasses and herbs. Plant biomass was measured in these same quadrats with a vertical quadrat sampler with a side length of 16 cm and a side depth of 22 cm (one sample per SU). We removed all above- and below-ground vegetation from inside this quadrat sampler. After removing sand, plant samples were oven-dried to a constant weight at 80°C, and then dry weight was recorded to determine total biomass. We used an impact soil penetrometer (Model IAA / Planalsucar, [16]) to measure soil hardness. At each sampling point, we took four measurements (one per SU), recording the number of strokes necessary to penetrate into the first 50 cm of soil (results are expressed in kg/cm^2). For analysis of vegetation variables and soil hardness, we computed the mean of the four sampling units and then used the mean in the analysis. For plant biomass, the weights of the four samples per unit were summed for the subsequent analysis.

Considering that not all variables had normal distribution, we used a permutational ANOVA analysis (PERMANOVA) to compare habitat characteristics (plant biomass, plant cover, and soil hardness) between species. Each species and condition (allopatry or sympatry) was considered a block in all analyses. The same analysis was used to check for differences in habitat characteristics when species were distributed in sympatry and in allopatry. The tests were performed with matrices based on Euclidean distances, and we ran 9999 permutations. The associations of habitat characteristics used by each species in the sympatric area were assessed using a principal component analysis (PCA) based on a covariance matrix that used the habitat variables collected from the trapped animals. In the results, we present only the information and loadings of the first axis (PC1), since PC2 and other axes had less relevant explanations (≤7%). A Wilcoxon rank test was used to compare the scores of *C. flamarioni* and *C. minutus* for the PC1. All analyses were conducted with the vegan package [17] in the R program for statistical computing [18].

Results

### Delimitation of the sympatric area

The sympatric area where *C. flamarioni* and *C. minutus* are distributed is located in the first range of dunes on the coastal plain of Rio Grande do Sul state in southern Brazil, and covers a length of approximately 15 km. Both species only come into contact in the first range of dunes that extend from Capão da Canoa in the south (29°46'04.41"S, 50°00'59.1"W) to Arroio Teixeira in the north (29°38'23.83"S, 49°56'36.77"W). This area is characterized by the habitat transition of *C. minutus*. In the southern portion of this region, this species is only found in sandy fields, mostly in the interior of the continent, whereas in the northern portion, this species also occupies the dune environment. Moreover, another sympatric area between these species was observed in the Coastal Plain of the Rio Grande do Sul state, but it is located in the southern portion towards the coast. Only a few records of individuals were obtained in the area, in the São José do Norte municipality (32°03'18.15"S, 51°59'43.94"W). This sympatric zone does not occur in the dunes, as both species come into contact in a region of sandy fields. In this area, *C.*

flamarioni occupies the environment where previously there were only records of *C. minutus* (from the sandy fields up to the shores of the Patos Lagoon) (Fig 1).

Habitat characteristics and spatial distribution of the species

Ctenomys flamarioni and *C. minutus* use different habitat characteristics when they occur in sympatry. *Ctenomys minutus* selects areas with higher amounts of plant biomass and grass cover relative to the areas occupied by *C. flamarioni* ($F = 6.45$, $p = 0.015$ and $F = 9.32$, $p = 0.005$, respectively). However, there is no difference in the selection of areas in relation to soil hardness and herb cover ($F = 1.58$, $p = 0.22$ and $F = 0.96$, $p = 0.34$, respectively) (Fig 2).

With respect to the differentiation in the selection of habitats, we observed that when *C. flamiarioni* occurs in allopatry, this species selects areas with high grass cover and are distributed on less hard soils in comparison to individuals of the same species located in the sympatric
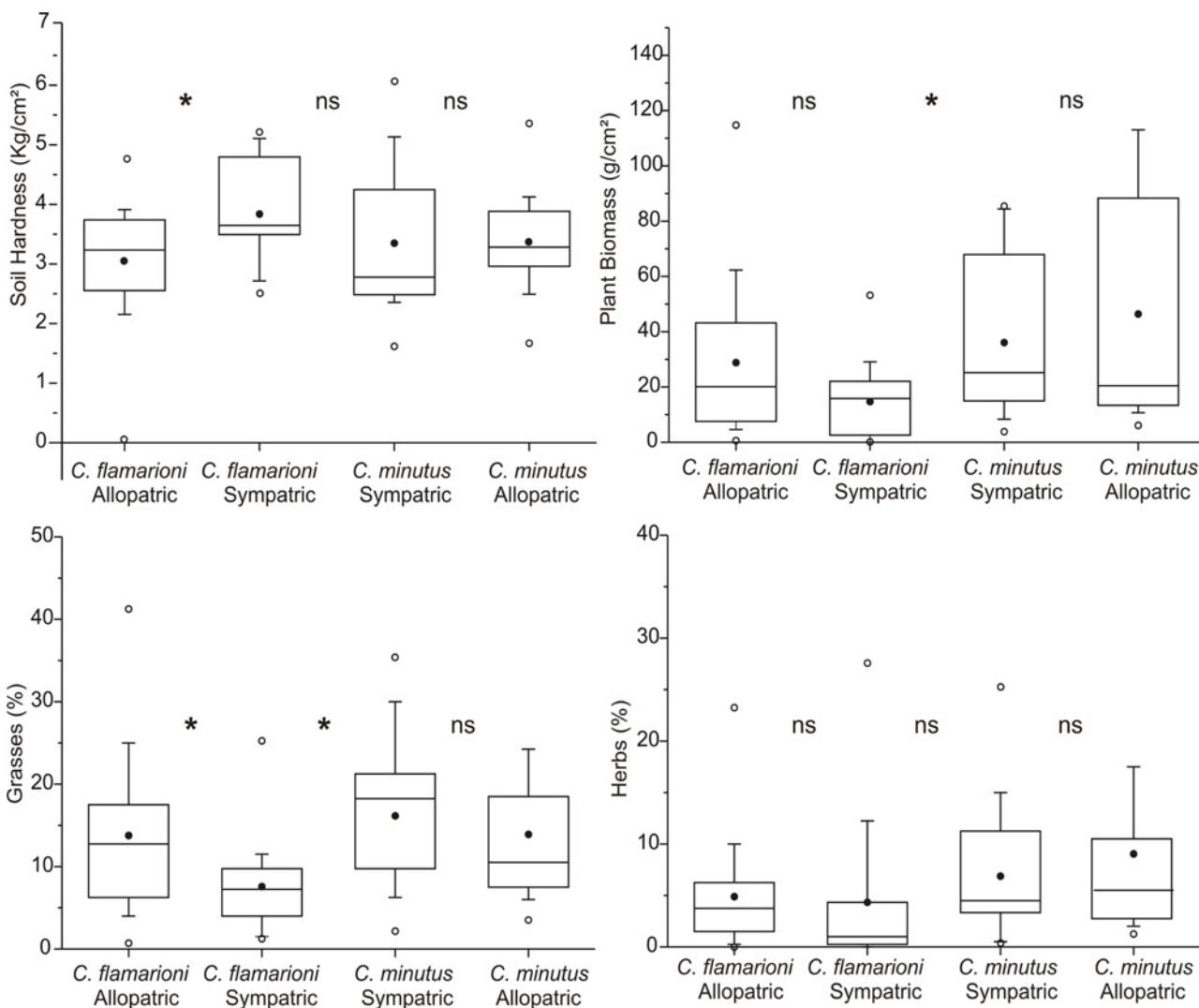
Fig 2. Comparisons of soil hardness, plant biomass, grass cover and herb cover between *C. flamarioni* and *C. minutus* distributed in sympatric and allopatric areas. Top and lower horizontal lines of boxes represent standard deviations. Black points and the center horizontal lines represent the mean and the median, respectively. The results of the statistical tests are displayed in the text. Asterisk indicates a significant difference and "ns"non-significant. For all the variables, the middle "*/ns" reflects a test between species, and the first and third "*/ns" reflect comparisons within species.
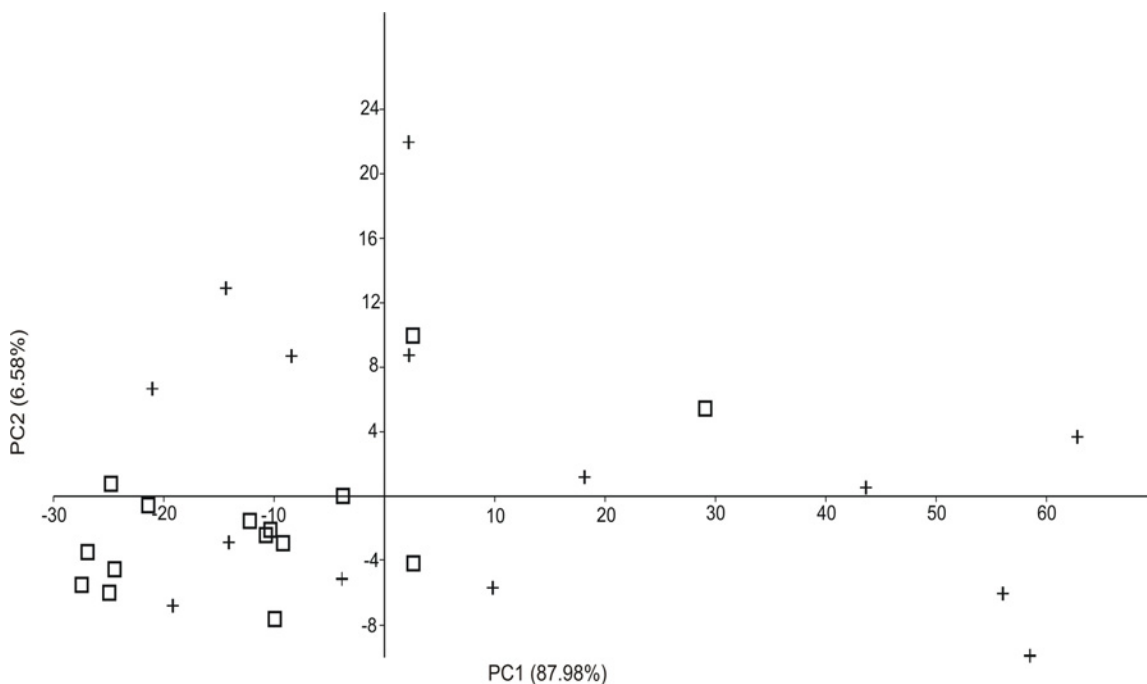
doi:10.1371/journal.pone.0123220.g002

zone ($F = 5.819$, $p = 0.018$ and $F = 7.507$, $p = 0.008$, respectively). On the other hand, there is no difference in the environmental characteristics of the habitats selected by *C. minutus* individuals when they occur in sympatry or in allopatry (Fig 2).

The PCA results showed that plant biomass and grass and herb cover were positively correlated with the PC1 axis (factor loadings: 0.9717, 0.2151 and 0.095, respectively). Plant biomass had the highest correlation value with this axis, in contrast to soil hardness, which showed a negative correlation (factor loading: -0.0190). The PC1 axis explained 87.98% of the total variation. Through the PCA results, it was possible to identify two groups; the first group consisted of *C. minutus* individuals, characterized by being distributed in places with higher plant cover and biomass, and the second group, formed by *C. flamarioni*, was found in places with lower plant cover and biomass (Fig 3). We found a significant difference between the scores of *C. flamarioni* and *C. minutus* for PC1 axis ($Z = 1.931$, $p = 0.0267$).

Discussion

Ctenomys minutus and *C. flamarioni* show habitat segregation in the area where they occur in sympatry. This has already been shown in congeneric species, such as *C. australis* and *C. talarum* [2, 8, 11], and other species of subterranean rodents [19–21]. The habitat segregation described for species of subterranean rodents is most likely related to the similarity of the species' habits [5], as closely related species often devise strategies to avoid competition [22–23]. In the present study, we observed that individuals of *C. flamarioni* select different sites in the sympatric zone from the places commonly used when they are distributed in allopatry. However, *C. minutus* does not show any changes in the selected environments when it occurs in sympatry or allopatry. Several studies demonstrate interspecific competition as one of the main causes of allopatric distribution (usually found in subterranean rodents), where the species that presents competitive superiority selects the habitat of its preference, suppressing other species

Fig 3. Principal component analysis (PCA) of habitat features selected by *C. flamarioni* (□) and *C. minutus* (+) species in sympatric area in the southern Brazilian coastal plain.

which occupy less favorable habitats [19, 20, 24, 25]. Our results suggest that *C. minutus* selects environments with the same characteristics (soil hardness, biomass, and plant cover) in both sympatric and allopatric distributions. Thus, it seems that *C. flamarioni* individuals might be pushed into different environments than they usually use when found in allopatry.

Moreover, Malizia et al. [11] demonstrated that in the sympatric area of tuco-tucos in Argentina, *C. australis*, originally distributed in the coastal dune region, is the dominant species in the dune environment. Vassallo [26] observed that interspecific interactions and displacement by *C. australis* could be significant but nonetheless marginal in sustaining the pattern of microhabitat segregation between the species. These differences may be related to physiological factors, where the largest species present a better thermoregulatory capacity and a lower resting metabolic rate, as reported for other subterranean rodents [27, 28]. In our case, the species which has a mean larger body size is *C. flamarioni* in relation to *C. minutus* (266 mm ± 30.98 and 250 mm ± 35.13, respectively; t = 3.45, *p* = 0.0004; information based on records from the collection of the Laboratório de Citogenética e Evolução of the Universidade Federal do Rio Grande do Sul). However, we did not find the same pattern described for *C. australis* and *C. talarum*. Thus, it is possible to assume that other factors, such as aggressiveness, might be responsible for the observed pattern. The aggressive behavior in subterranean rodents is a known mechanism that contributes to competitive exclusion [3]. This behavior was studied among ctenomyids in laboratory (*C. australis* and *C. talarum*), with the larger-bodied *C. australis* dominating the smaller-bodied (see [29] for more details). Similarly, Hickman [30] recorded that *Thomomys bottae*, *Geomys bursarius*, and *Pappogeomys castanops* present aggressive interactions, with larger individuals being behaviorally dominant in interspecific encounters.

Nevertheless, the distribution of these two species in the coastal plain may be related to historical factors of occupation of the range of coastal dunes that they inhabit. During the development of the coast of southern Brazil in the Pleistocene and early Holocene, four barrier systems arose, related to four transgressive and regressive events [31, 32]. The first and second barriers appeared at the beginning and middle of the Pleistocene, respectively. Then, at late Pleistocene, a third barrier increased the Coastal Plain, forming a multiple barrier system, which is inhabited by the species *C. minutus* [31, 32, 12]. Finally, at the beginning of the Holocene, the last movement originated the fourth barrier, corresponding to the first dune line, and extends along the entire coastline of the states of Rio Grande do Sul and southern Santa Catarina [31,32], which is occupied by *C. minutus* above the sympatric zone, and by *C. flamarioni* below this contact region. Moreover, the evolutionary history of the species shows that *C. flamarioni* is closely related to the species present in Uruguay and Argentina, and show a pattern of occupation South-North [12, 33,34], whereas *C. minutus* shows a pattern of North-South occupation, from Santa Catarina to Rio Grande do Sul state [35]. Consequently, the geological development of the coast of southern Brazil associated with the evolutionary history of both species may have had a significant influence in the way that these species are distributed nowadays.

Our results demonstrate that the vegetation characteristics (plant biomass and grass cover) appear to be closely related to the habitat segregation of *C. flamarioni* and *C. minutus*. Between the characteristics of the vegetation, grass cover appears to be the most important variable for both species, probably related to the fact that grasses are the major food source for both species [15, 36, 37]. Additionally, Lopes et al. [36] demonstrated that thirteen plant families, mainly represented by grasses, were identified in the diet of *C. minutus*, and 10 families, also with a predominance of grasses, were present in diet of *C. flamarioni*. These authors found differences in diet composition between species, suggesting some level of diet partitioning between *C. flamarioni* and *C. minutus* in the sympatric region. They concluded that these differences might have been developed to avoid competition in the region of co-occurrence (see Lopes et al. [36] for more details). Additionally, vegetation not only supplies food but also affects patterns of

ventilation and heat flow within burrow systems [38]. Therefore, the environmental characteristics related to vegetation may have a significant influence on the spatial distribution of subterranean rodents when distributed in sympatry.

Although our study species occur in sympatry, they are segregated microspatially by different habitat preferences, and the observed vegetation and soil preferences might be related to the quality and quantity of food in the inhabited area. Because the tuco-tucos occur in a coastal environment, the relationship between soil hardness and plant cover contrasts with other variables not measured in this study. One possible explanation to the observed pattern is that these species have acquired different adaptations over time which allows them the ability to exploit different resources [39] and thus avoid competitive interactions all together. It should be emphasized that the sympatric area located in the south was not included in our analysis considering that there are no records of allopatric distribution of *C. flamarioni* in sand fields. Nevertheless, a great sampling effort is still needed to characterize the limits of this second area and to verify the relationship between the spatial distribution of this species and its habitat characteristics.

Acknowledgments

Author Contributions

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

References

1. Tilman D, Kareiva P (1997) Spatial ecology: the role of space in population dynamics and interspecific interations. Princenton University Press, Princenton, New Jersey. 416 p.

2. Comparatore VM, Agnusdei M, Bush C (1992) Habitat relations in sympatric populations of *Ctenomys australis* and *Ctenomys talarum* (Rodentia, Octodontidae) in a natural grassland. Z. für Säugetierkunde 57: 47–45. doi: 10.1177/0961203308089428.A PMID: 18625634

3. Cameron GN (2000) Community ecology of subterranean rodents. In: Laceys EA, Patton JL, Cameron G N, editors. Life underground: the biology of subterranean rodents. University of Chicago Press, Chicago. pp. 227–256

4. Nevo E (1979) Adaptive convergence and divergence of subterranean mammals. Annu Rev Ecol Evol Syst 10: 269–308.

5. Lacey EA, Patton JL, Cameron GN (2000) Life Underground. The University of Chicago Press, Chicago, Illinois, USA. 449 p.

6. Kernnely TE (1959) Contact between the ranges of two allopatric species of pocket gophers. Evolution 13: 247–263.

7. Pearson OP (1984) Taxonomy and natural history of some fossorial rodents of Patagonia, southern Argentina. J Zool 202: 225–237.

8. Cutrera AP, Mora MS, Antinucci CD, Vassallo AI (2010) Intra-and interspecific variation in home-range size in sympatric tuco-tucos, *Ctenomys australis* and *Ctenomys talarum*. J Mammal 91: 1425–1434.

9. Reig OA, Bush C, Ortellis MO, Contreras JL (1990) An overview of evolution, systematica, population biology and molecular biology. In: Nevo E, Reig OA, editors. Evolution of subterranean mammals at the organismal and molecular levels. New York: Wiley-Liss.pp. 71–96. PMID: 2309010

10. Contreras JR, Reig OA (1965) Dados sobre la distribuición de género Ctenomys talarum (Rodentia: Octodontidae) en la zona costera de la Provincia de Buenos Aires entre Neocochea y Bahía Blanca. Physis 25: 169–186.

11. Malizia AI, Vasssallo AI, Bush C (1991) Population and habitat characteristics of two sympatric species of Ctenomys (Rodentia:Octodontidae). Acta Theriol 36: 87–94.

12. Freitas TRO (1995) Geographic distribution and conservation of four species of the genus Ctenomys in Spothern Brasil. Stud Neotraop Fauna Environ 30: 53–59.

13. Freygang CC, Marinho JR, Freitas TRO (2004) New karyotypes and some considerations of Ctenomys minutus (Rodentia: Ctenomyidae) on the coastal plain of the Brazilian State of Rio Grande do Sul. Genetica 121: 125–132. PMID: 15330112

14. Animal care and use commintee 1998. Guidelines for the capture, handling, and care of mammals as approved by the American Society of Mammalogists. J Mammal 79: 1416–1431.

15. Galiano D, Kubiak BB, Gerhard EO, Freitas TRO (2014) Effects of rodents on plant cover, soil hardness, and soil nutrient content: a case study on tuco-tucos (Ctenomys minutus). Acta Theriol. 59: 583–587.

16. Stolf R (1991) Teoria e teste experimental de fórmulas de transformação dos dados de penetrômetro de impacto em resistência do solo. Rev Bras Ciênc Solo 15: 229–235.

17. Oksanen J, Kindt R, Legendre P, O'Hara RB (2012) Vegan: community ecology package version 2.0–5. http://cran.r-project.org.

18. R Development Core Team (2012). R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org

19. Miller MA (1964) Ecology and distribution of pocket gophers (Geomyidae) in Colorado. Ecology 45: 256–272.

20. Borghi CE, Giannoni SM, Martinez-Rica JP (1994) Habitat segregation on three sympatric fossorial rodents in the Spanish Pyrenees. Z für Säugetierkunde 59: 52–57. doi: 10.1177/0961203308089428.A PMID: 18625634

21. Lovy M, Sklíba J, Burda H, Chitaukali WN, Sumbera R (2011) Ecological characteristics of two African mole-rats species with different social system in an area of sympatry implications for the mole-rat social evolution. J Zool 286: 145–153.

22. Gause GF (1934) Struggle for Existence. Williams & Wilkins Co.: Baltimore. 163p.

23. Schoner TW (1974) Resource partitioning in ecological communities. Science 185: 27–39. PMID: 17779277

24. Thaeler CS (1968) An analysis of the distribution of pocket gopher species in north-eastern California (genus Thomomys). Univ California Publ Zool 86: 1–46.

25. Reichman OJ, Baker RJ (1972) Distribution and movements of two species of pocket gophers (Geomyidae) in an area of sympatry in the Davis Montains, Texas. J Mammal 53: 21–33.

26. Vassallo AI (1993) Habitat shift after experimental removal of the bigger in sympatric Ctenomys talarum and Ctenomys australis (Rodentia: Octodontidae). Behaviour 127: 247–263.

27. Contreras LC, McNab BK (1990) Thermoregulation and energetics in subterranean mammals. In: Nevo E, Reig OA, editors. Biology of subterranean mammals at the organismal and molecular levels. New York, Wiley-Liss. pp. 231–250. PMID: 2309010

28. Busch C (1989) Metabolic rate and thermoregulation in two species of tuco-tuco Ctenomys talarum and Ctenomys australis (Caviomopha, Octodontidae). Com Biochem Physiol 93: 345–347. PMID: 2573453

29. Vassallo AI, Busch C (1992) Interspecific agonism between two sympatric species of Ctenomys (Rodentia: Octodontiae) in captivity. Behaviour 120: 40–50.

30. Hickmann GC (1977) Geomyid interactions in burrow system. Tex J Sci 29: 235–243.

31. Villwock JA, Tomazelli LJ, Los EL, Dehnhardt EA, Horn Filho NO, Bachi FA, et al. (1986) Geology of the Rio Grande do Sul coastal province. In: Rabassa J editors. Quaternary of South America and Antarctic Peninsula. Balkema AA: Rotterdam. pp 79–97.

32. Tomazelli LJ, Dillenburg SR, Villwock JA (2000). Late Quaternary geological history of Rio Grande do Sul coastal plain, southern Brazil. Rev Bras Geociências 30: 474–476.

33. Freitas TRO (1994) Geographical variation of heterochromatin in Ctenomys flamarioni (Rodentia: Octodontidae) and its cytogenetic relationship with other species of the genus. Cytogenet Cell Genet 67: 193–198. PMID: 8062596

34. Massarini AI, Freitas TRO (2005) Morphologival and cytogenetics comparison in species of the mendoci-nus—group (genus: *Ctenomys*) with emphasis in *C. australis* and *C. flamarini* (Rodentia-Ctenomyidae). Caryologia (Firenze) 58: 21–27.

35. Lopes CM, Ximenes SSF, Gava A, Freitas TRO (2013) The role of chromosomal rearrangements and geographical barriers in the divergence of lineages in a South American subterranean rodent (Roden-tia: Ctenomyidae: *Ctenomys minutus*). Heredity 111: 293–305. doi: 10.1038/hdy.2013.49 PMID: 23759727

36. Lopes CM, Barba M, Boyer f, Mercier C, Filho PJSS, Heidtmann LM, et al. (2014) DNA metabarcoding diet analysis for species with parapatric versus sympatric distribution: a case study on subterranean ro-dents. Heredity (Edinburgh. Print), in press. doi: 10.1038/hdy.2014.109

37. Galiano D, Bernardo-Silva J, Freitas TRO (2014) Genetic Pool Information Reflects Highly Suitable Areas: The Case of Two Parapatric Endangered Species of Tuco-tucos (Rodentia: Ctenomiydae). Plos One 9: e97301. doi: 10.1371/journal.pone.0097301 PMID: 24819251

38. Busch C, Antinuchi CD, del Valle JC, Kittlein MJ, Malizia AI, Vassallo AI, et al. (2000). Population ecolo-gy of subterranean rodents. In: Lacey EA, Patton JL, Cameron GN, Editors. Life Underground: The Biol-ogy of Subterranean Rodents. The University Chicago Press, Chicago, pp. 183–226.

39. Lessa EP, Thaeler CSJR (1989) A reassessment of morphological specializations for digging in pocket gophers. Journal of Mammalogy 70:689–700.