1   Running header: Niche partition in small mammals

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3   **Niche partitioning in small mammals: interspecific and biome-level analyses using stable**

4   **isotopes**

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25   Small mammal assemblages from South America provide a unique opportunity to measure

26   coexistence and niche partitioning between marsupials and placentals. We tested how these two

27   major clades partition environmental resources by comparing stable isotopic ratios of similar

28   sized Didelphidae and Sigmodontinae in four Brazilian biomes: Pampas grassland, Pantanal

29   wetland, Cerrado woodland savanna, and Atlantic Forest. Generally, didelphid isotopic niche

30   follows a scaling law, because we found an association between $\delta^{15}N$ enrichment and body mass.

31   Sigmodontines that primarily partition the environment via forest strata showed a greater intake

32   of C_4 or/and CAM plants than didelphids, as reflected by their wider trophic niche. Values of

33    $\delta^{13}C$ were highest in savannas and grasslands (Cerrado and Pampas biomes), and values of $\delta^{15}N$

34   were highest in the Atlantic Forest (in sigmodontines) and Pampas (in didelphids). While

35   assessing patterns between the two major Brazilian biomes (Atlantic Forest and Cerrado), we

36   found evidence of a broader trophic niche for both clades in the Cerrado. In the Atlantic Forest,

37   niche occupation by Didelphidae was completely enclosed within the Sigmodontinae trophic

38   niche. Both clades showed less overlap in the Cerrado, a less productive environment. Our results

39   highlight the importance of a comparative framework and the use of stable isotopes for testing

40   ecological questions related to how small mammalian communities partition their niche.

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42   Key words: coexistence, Didelphidae, diet, food resource, forest strata use, phylogenetic

43   approach, Sigmodontinae

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45   Na América do Sul, as assembleias de pequenos mamíferos proporcionam uma oportunidade

46   única de analisar a coexistência e partição de nicho entre marsupiais e placentários. Neste estudo,

47   testamos como estes dois clados particionam os recursos do ambiente através da comparação das

48   razões de isótopos estáveis em Didelphidae e Sigmodontinae. O nicho isotópico de didelfídeos

49   segue a regra de escala, uma vez que encontramos associação entre o enriquecimento de $\delta^{15}N$ e

50   massa corporal. Sigmodontíneos, que particionam o ambiente primariamente através do estrato

51   vertical, apresentam maior consumo de plantas C_4 ou/e CAM que didelfídeos, refletindo no seu

52   maior nicho trófico. Os valores de $\delta^{13}C$ são maiores no Cerrado e Pampa, enquanto os valores de

53   $\delta^{15}N$ são maiores na Floresta Atlântica (em sigmodontíneos) e no Pampa (em didelfídeos). Ao

54   verificar estes padrões comparativamente entre dois grandes biomas brasileiros (Floresta

55   Atlântica e Cerrado), encontramos evidências de um nicho ecológico mais amplo para os dois

56   clados no Cerrado. Na Floresta Atlântica a ocupação de nicho de Didelphidae está

57   completamente anexada dentro do nicho de Sigmodontinae. Ambos os clados possuem menor

58   sobreposição de nicho no Cerrado, que é um ambiente menos produtivo. Nossos resultados

59   destacam a importância de estudos comparativos e do uso de isótopos estáveis para entender

60   como comunidades de pequenos mamíferos particionam seus nichos.

61

62   Palavras chave: abordagem filogenética, coexistência, Didelphidae, dieta, recurso alimentar,

63   Sigmodontinae, uso do estrato florestal

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67   South America hosts a large diversity of non-volant small mammals, made up of community

68   assemblages of two evolutionarily distinct clades: Marsupialia and Placentalia. Studies of South

69   American small marsupials, mostly represented by the diverse Didelphidae clade, have shown

70   that diversification rates of these animals are lower than expected compared with other mammal

71   clades (Jansa et al. 2014). In contrast, morphological evidence showed rapid-diversification rates

72   in Sigmodontinae rodents, the most diverse South American clade of placentals (Maestri et al.

73   2016a). The evolutionary history of these two groups was influenced by different and well-

74   known biogeographical events: the Splendid Isolation in which South American marsupials

75   remained isolated over most of the Cenozoic (Simpson 1980), and the Great American

76   Interchange (GABI) when most placental extant groups dispersed from North to South America,

77   facilitated by the formation of the Isthmus of Panama (hypothesized ca. 2.8 Ma—O'Dea et al.

78   2016). Didelphids and sigmodontines often share the same environments and, to some extent,

79   have morphological similarities relative to locomotion (Bubadué et al. 2019).

80   In Brazil, didelphids and sigmodontines overlap across all six biomes recognized by the

81   Instituto Brasileiro de Geografia e Estatística (IBGE 2019): Atlantic Forest, Amazon Rainforest,

82   Pantanal wetland, Caatinga scrubland, Cerrado woodland savanna, and Pampas grassland (Paglia

83   et al. 2012). To cope with coexistence across the wide range of the vegetational domains found in

84   Brazil, small mammals can partition their environment using food resources as well as spatial and

85   temporal differences (Vieira and Monteiro-Filho 2003; Novillo et al. 2017; Ribeiro et al. 2019),

86   none of which are exclusive strategies to avoid competition. Because of the wide range of

87   different biomes where these mammals successfully coexist, we highlight the importance of

88   estimating how they potentially can use these strategies across different biomes.

89   Didelphids and sigmodontines include a wide range of species to some extent displaying

90   similar feeding habits (Vieira and de Moraes 2003; Arregoitia and D'Elía 2020; Bubadué et al.

91   2021). Previous dietary studies showed marsupials to be more generalist than sigmodontine

92   rodents because species have a larger variety of food items depending on seasonal and/or

93   environmental availability (Cáceres 2002, 2004; Vieira and de Moraes 2003), even when

94   specialized for the consumption of specific food items in their core diet (Vieira and de Moraes

95   2003). On the other hand, sigmodontines are characterized by broad dietary niches (Arregoitia

96   and D'Elía 2020), larger species diversity and dietary specializations than marsupials (Maestri et

97   al. 2017; Arregoitia and D'Elía 2020), and often presenting more specialized phenotypes (Maestri

98   et al. 2016b; Arregoitia et al. 2017; Missagia et al. 2021). When studying small mammal

99   communities, however, we can encounter particular difficulties relative to species food

100   preferences, because detailed and confident information on species diet is scarce for both clades

101   (Vieira and de Moraes 2003; Arregoitia and D'Elía 2020), often requiring researchers to work

102   with extrapolated and overly simplified data (e.g., Maestri et al. 2017; Chemisquy et al. 2021).

103   As a potential remedy, stable isotope analyses can provide novel information on species use of

104   resources (Ben-David and Flaherty 2012; Missagia et al. 2019). Such studies shed light on the

105   coexistence patterns between rodents and marsupials through quantification of the trophic niche

106   and feeding ecology (Mauffrey and Catzeflis 2003; Galetti et al. 2016; Missagia et al. 2019).

107   Studies on sympatric rodents (Bovendorp et al. 2017a) and marsupials (Kuhnen et al. 2017)

108   support partial feeding overlap between species that might exhibit wide or narrower isotopic

109   niches relative to different degrees of omnivory. In Atlantic Forest communities, Galetti et al.

110   (2016) identified broader isotopic niche space for rodents than marsupials. They also found

111   species locomotory habits to influence isotopic composition in different taxa. Ribeiro et al.

112   (2019) demonstrated that isotopic niches of small mammals from the Brazilian Cerrado biome

113   can vary according to food availability, species dietary preferences, and habitat complexity, while

114   Missagia et al. (2019) recently demonstrated, using a phylogenetic approach in akodontine

115   rodents, that isotopic trophic diversity varies more within than between clades.

116   Stable isotope analysis is a useful tool to study mammalian ecology (Crawford et al.

117   2008), but several factors can affect correct interpretation of its results. In general, δ^{15}N values

118   increase with trophic level, and δ^{13}C differences between C_3 and C_4 plants are reflected in δ^{13}C

119   values of plants that herbivores eat and, subsequently, in their predators (Ben-David and Flaherty

120   2012). In addition, if carefully interpreted, isotopes can be used to investigate the contribution of

121   particular food sources in diverse communities (e.g., Whitney et al. 2018) and for individual

122   preferences within a species (e.g., Voigt and Kelm 2006). Mixed models have been applied to

123   estimate the proportional contributions of food sources to the isotopic composition of the

124   consumer tissues at the local scale (Phillips 2012). Although this approach can be difficult to

125   implement on a broader geographical scale, published data provide a good overview on the

126   isotopic range of small mammals in different areas of the Neotropics and how to interpret them as

127   to their potential dietary sources. For example, higher δ^{15}N values (> 3‰) in small mammals

128   from Atlantic Forest most likely are associated with higher degree of faunivory, particularly

129   consumption of arthropods, but also fungi, whereas low δ^{15}N levels are associated with

130   preference for plant items such as fruits or leaves (Galetti et al. 2016; Bovendorp et al. 2017a).

131   High δ^{13}C values (> –20‰) suggest consumption of C_4 and/or crassulacean acid metabolism

132   (CAM) plants by small mammals in the Atlantic Forest (Galetti et al. 2016). Results and

133   interpretations are similar for Cerrado, but δ^{15}N values seem to vary more than in the Atlantic

134   Forest (Galetti et al. 2016 compared with Ribeiro et al. 2019). To our knowledge, no isotopic

135   study on small mammal communities have incorporated other Neotropical biomes, and no

136 comparative studies have been carried out among biomes, including forest, savannah, wetland,

137 and grassland ecotypes.

138   We combined isotopic and comparative approaches to test how the trophic niche of small

139 mammals varied interspecifically and across four of the six Brazilian biomes: Pampas grassland,

140 Pantanal wetland, Atlantic Forest, and Cerrado woodland savanna. At the interspecific level, we

141 aimed to compare the trophic niche between marsupials and rodents using a

142 comparative/phylogenetic approach. We were interested in the level of phylogenetic structure and

143 amount of isotopic variation within each clade, Didelphidae and Sigmodontinae, and the level of

144 overlap between them. As rodents and marsupials share and partition the environment,

145 particularly based on use of distinct forest strata (Vieira and Monteiro-Filho 2003; Vieira and

146 Camargo 2012) and body mass (see spatial scaling law of Ritchie and Olff 1999), we evaluated

147 the relationship of species average body mass, diet, and locomotor categories, with $\delta^{13}C$ and $\delta^{15}N$

148 values.

149   Considering the variation across biomes, we tested for the impact of local climate on

150 stable isotopes of the sampled small mammals because isotopes can vary depending on

151 environmental conditions of each site (Handley et al. 1999). We also compared the level of

152 overlap between didelphids and sigmodontines within and between the two largest biomes within

153 our sample, Atlantic Forest and Cerrado woodland savanna. Finally, by quantifying trajectories

154 of changes in isotopic values between clades from the Cerrado to the Atlantic Forest, we aimed to

155 identify patterns of trophic shift toward similar environments using the method proposed by

156 Turner et al. (2010). Based on results from previous research (Atlantic Forest: Galetti et al. 2016;

157 Cerrado: Ribeiro et al. 2019), we predicted that Cerrado species would show higher $\delta^{13}C$ and

158 $\delta^{15}N$ values than for both clades in the Atlantic Forest. This is because the Cerrado biome has

159 fewer stable environments where arthropod availability remains constant while fruit availability

160 does not (Ribeiro et al. 2019), and it is also richer in C_4 plants compared with other Brazilian

161 forests. Eventually, this would increase variation in the Cerrado biome, and therefore we

162 predicted the trophic niche to be wider for both clades in this biome than in the Atlantic Forest.

163 Despite obvious evolutionary differences, studies on small marsupials and placental mammals

164 suggest similar ecological strategies of opportunistic behavior (Vieira and Monteiro-Filho 2003;

165 Vieira and Camargo 2012; Bubadué et al. 2019). Nevertheless, we expected rodents and

166 marsupials not to overlap completely in each biome and the trophic niche of marsupials to be

167 smaller than that of Sigmodontinae, independently of biome.

168

169 **MATERIALS AND METHODS**

170 *Sampling.*—We selected 164 adult specimens of small mammals deposited in the

171 mammal collection of the Universidade Federal de Santa Maria (UFSM–Brazil). These

172 specimens were collected during previous mammal trapping surveys undertaken between 2002

173 and 2010 in the Brazilian states of Goiás, Mato Grosso, Mato Grosso do Sul, São Paulo, Paraná,

174 and Rio Grande do Sul (Cáceres et al. 2007, 2008, 2010, 2011, 2014; Melo et al. 2011, 2013;

175 Sponchiado et al. 2012). Adults were selected based on the associated skull morphology (closed

176 sutures and all permanent teeth erupted). We chose to work only with adults because ontogeny

177 can alter isotopic ratios in mammals due to lactation (Ben-David and Flaherty 2012). The

178 specimens belonged to 45 species: 15 Didelphidae and 30 Sigmodontinae. Specimens originally

179 were collected in 44 different localities distributed within four Brazilian biomes (Fig. 1; Table 1).

180 Field studies have reported an average of 8.2 species captured per site in the Atlantic Forest

181 (Bovendorp et al. 2017b) and 5.8 species in the Cerrado (Mendonça et al. 2018). Sponchiado et

182 al. (2012) reported a total of 9 species captured in an area within the Pampas biome, while 19

183 species were reported in an area of Pantanal by Cáceres et al. (2011). We report isotopic data

184 from 9 (Pampas grassland) to 27 (Atlantic Forest) species per biome.

185   For most specimens, we recorded body mass before death, which we used to estimate

186 mean body mass per species. We also gathered information on locomotion (Astúa 2009; Paglia et

187 al. 2012; Brandão and Nascimento 2015; Smith and Owen 2016; Bubadué et al. 2019) and diet

188 (Paglia et al. 2012; Arregoitia and D'Elía 2020; Bubadué et al. 2021) from the literature for every

189 species included in this study (Supplementary Data SD1).

190   *Stable isotope analysis.*—We collected at least 0.5 g of hair for each specimen from

191 which we measured $\delta^{13}C$ and $\delta^{15}N$. Each sample was submerged in a 2:1 chloroform:methanol

192 mixture (Meier-Augenstein et al. 2011) for 1 h and then left to air-dry in fume hood to clean the

193 samples of body oils and other adhered contaminants. Subsamples of 0.7 mg of the clean hair

194 were weighed into 5 x 3 mm tin capsules and combusted in a Pyrocube elemental analyzer

195 (Elementar, Hanau, Germany). Sample gases were transferred via helium carrier gas to a Thermo

196 Delta XP Plus isotope ratio mass spectrometer (Thermo, Bremen, Germany). Sample data were

197 reported in standard delta per mil notation (δ ‰) relative to V-PDB ($\delta^{13}C$) and AIR ($\delta^{15}N$)

198 international standards. Laboratory reference materials were interspersed within the measurement

199 run to correct for linearity and drift. Analytical precision (*SD*) of GEL (Gelatin) was $\delta^{13}C =$

200 0.06‰ and $\delta^{15}N = 0.09$‰ (full isotopic data in Supplementary Data SD2).

201   *Interspecific variation.*—For comparative analyses, we built a majority-rule consensus

202 tree from Upham et al. (2019) for sigmodontine and didelphid species included in this study.

203 Isotopic values were averaged by species to identify the presence of phylogenetic signal in the

204 data (Blomberg and Garland, Jr. 2002). We used the function "phylosig" to measure the

205 phylogenetic signal (K-statistic), available in the package "phytools" (Revell 2012). We expected

206   closely related species to exhibit similar ecology and behavior, hence similar isotopic data (e.g.

207   Bubadué et al. 2019). To determine if comparative models such as phylogenetic generalized least

208   squares (PGLS) were necessary to be implemented for our models of interspecific variation, we

209   also estimated the phylogenetic signal (K-statistic) in the residual deviations of the predicted

210   variable for the model testing association between isotopes and averaged body mass between

211   species (Revell 2010).

212   We used linear model evaluation with randomized residuals in a 9,999 permutations

213   procedure (function lm.rrpp of the package RRPP—Collyer and Adams 2018) to asses if δ^{13}C

214   and δ^{15}N values differed between Didelphidae and Sigmodontinae. We also used this procedure

215   to test if isotopic ratios differed among diet, log body mass, and species locomotion categories.

216   These analyses were repeated for Didelphidae and Sigmodontinae separately. PGLS was not

217   implemented in these models due to the lack of phylogenetic signal in the residual model values.

218   To measure the level of overlap between clades and test the hypothesis that didelphids'

219   ellipses area would be smaller than in Sigmodontinae (Galetti et al. 2016), we used the R package

220   SIBER (Jackson et al. 2011) to compute and delimit stable isotope Bayesian ellipses, correcting

221   ellipse areas (SEA$_B$) for sample size in each group.

222   *Biome comparisons*.—We extracted bioclimatic variables with a resolution of 10 minutes

223   from the WorldClim raster database (Hijmans et al. 2005) for each locality of small mammal

224   collection. Using averaged values by species and site, we implemented a correlation table to test

225   the association between stable isotopes and climatic condition. We also considered the correlation

226   between bioclimatic variables to select or not select them following a Variance Inflation Criterion

227   (VIF). In the case of multicollinearity, we prioritized the variable with the strongest correlation

228   with our data. Only annual mean precipitation was selected in this procedure.

229   Biplots of δ^{13}C and δ^{15}N were used to visualize the dispersion of isotopic values between

230 marsupials and rodents in our data within the four biomes (Atlantic Forest, Cerrado, Pantanal,

231 and Pampas). The Layman et al. (2007) community-wide measures of trophic structure required

232 at least 5 species per group to be compared in each community (in our case, biomes). Thus, we

233 selected the largest biomes for which we had enough data to undertake comparative analyses:

234 Atlantic Forest (12 Didelphidae spp., 15 Sigmodontinae spp.) and Cerrado (11 Didelphidae spp.,

235 16 Sigmodontinae spp.). For Atlantic Forest, we added the isotope ratios of hair published for

236 small mammals by Galetti et al. (2016), whose values were similar to those in our data for the

237 repeated species in the same biome, to meet the sampling criteria for that area. We averaged our

238 data per species and biome (Layman et al. 2007), and used the R package SIBER (Jackson et al.

239 2011) to compute the Bayesian estimates of the following metrics for the Atlantic Forest and

240 Cerrado: δ^{15}N range as a measure of trophic length (NR), δ^{13}C range as an estimate of diversity

241 of basal resources (CR), and mean distance to centroid as a measure of trophic diversity taking

242 the degree of species spacing into account (CD).

243   We delimited stable isotope Bayesian ellipses in SIBER (Jackson et al. 2011) correcting

244 ellipse areas (SEA$_B$) for sample size for each clade, and we separated data from the Atlantic

245 Forest and Cerrado samples. Standard ellipse core isotopic niche areas (SEA$_C$) allowed

246 comparisons of groups by disregarding outliers. We also calculated the total convex hull area

247 (TA) as an indicator of the whole niche width for each clade within each biome. A similar

248 approach was used and described in detail by Missagia et al. (2019).

249   We measured the probability of the trophic niche (= SEA) in didelphids to be smaller than

250 that of sigmodontines in each biome. Within each clade, we measured the probability of trophic

251 niche to be smaller in the Atlantic Forest than in the Cerrado. We also measured and compared

252 trophic niche overlap between Didelphidae within Sigmodontinae and vice-versa for each biome.

253 To make sure our data were not biased because of the different composition of species between

254 biomes, we selected a subsample containing only the species that we had in common for each

255 biome and repeated the overlapping estimations for comparing both biomes (*Didelphis*

256 *albiventris*, *Gracilinanus agilis*, *Akodon montensis*, *Cerradomys maracajuensis*, *Necromys*

257 *lasiurus*, *Oecomys bicolor*). In this last procedure, we did not separate clades because we did not

258 have enough samples for each clade.

259 Turner et al. (2010) provided an R script used to adapt phenotypic trajectory analysis for

260 isotopes. This final analysis allowed us to evaluate the magnitude and direction of change of

261 stable isotope ratios from Cerrado to the Atlantic Forest between the clades. For an empirical *P*-

262 value, the ranked percentile of observed differences between clades were used. This was

263 computed using 9,999 random permutations of residuals from reduced linear models generated

264 by a residual permutation procedure (Collyer and Adams 2018; Turner et al. 2010).

265

266 **RESULTS**

267 *Interspecific variation*.—In both clades, we did not find a phylogenetic signal for $\delta^{13}C$

268 values (Didelphidae $K = 0.416$, $P = 0.324$; Sigmodontinae $K = 0.268$, $P = 0.938$) or $\delta^{15}N$ values

269 (Didelphidae $K = 0.112$, $P = 0.826$; Sigmodontinae $K = 0.353$, $P = 0.795$). The probability of the

270 didelphid trophic niche (SEA = 8.721) being smaller than that of sigmodontines (SEA = 17.632)

271 was 98.5%. No difference in stable isotopes was detectable for $\delta^{13}C$ values with respect to clades,

272 diet, locomotor categories, and body mass ($P > 0.05$, Table 2). Relative to $\delta^{15}N$ values, we found

273 differences between clades ($R^2 = 0.079$, $F = 5.230$, $P = 0.027$), with the highest values for

274 sigmodontines, and among locomotor categories ($R^2 = 0.283$, $F = 3.942$, $P = 0.010$, Table 2).

275 Significant interaction between clades and diet was also detectable for $\delta^{15}N$ ($R^2 = 0.098$, $F =$

276 4.757, $P = 0.036$, Table 2).

277   The plot of δ^{13}C vs δ^{15}N showed greater variation within Sigmodontinae (extreme values

278   are within Sigmodontinae for both isotopes) than within Didelphidae, particularly for δ^{13}C (#16

279   *Akodon azarae* and #20 *A. reigi* with the greatest δ^{13}C values), although considerable overlap

280   occurred between clades (Overlap area = 6.965, Fig. 2A). Insectivorous species showed a wide

281   range of isotopic variation, and overlapping values occurred among all dietary categories (Fig.

282   2A). When samples are labeled based on locomotor categories, we detected high interspecific

283   variation of isotopic values within the terrestrial category followed by the arboreal category (Fig

284   2B). Analyzing the clades separately, we found no association within either clade between δ^{13}C

285   and diet, mean body mass, or locomotion categories. For δ^{15}N, we identified a positive and

286   significant association with body mass for didelphids (Fig. 3) and with locomotion categories for

287   sigmodontines (Table 3). Diet was significantly associated with δ^{15}N in didelphids, but this factor

288   lost its significance when incorporated within an interaction model with body mass (Table 3).

289   Locomotor category differences among sigmodontines are the same as described in Fig 2B for all

290   species.

291   *Biome comparisons.*—Annual precipitation was negatively correlated with δ^{13}C values

292   (Spearman's R = –0.232, P = 0.018) and positively correlated with δ^{15}N values (Spearman's R =

293   0.239, P = 0.015). High δ^{13}C values were present in open and dry areas (Cerrado and Pampas

294   biomes), especially for Sigmodontinae. Didelphidae δ^{15}N values were highest in the Pampas and

295   the Cerrado and lowest in the Pantanal and Atlantic Forest biomes. Sigmodontinae δ^{15}N values

296   were highest in Atlantic Forest and Cerrado and lowest in the Pampas and Pantanal (Fig. 4).

297   Small mammals CR was larger in the Atlantic Forest (0.904) than in the Cerrado (0.882),

298   while the opposite trend was encountered for NR (Cerrado = 1.386, Atlantic Forest = 0.584).

299   Centroid Distance was higher in Cerrado (0.821) than in the Atlantic Forest (0.538). SEA, SEA$_C$,

300   and TA, were smaller in Didelphidae than in Sigmodontinae for both biomes. Didelphidae from

301   the Atlantic Forest showed a smaller niche than in the Cerrado, while the opposite trend occurred

302   in Sigmodontinae (Table 4, Fig. 5). The probability of SEA$_B$ being smaller in didelphids than

303   sigmodontines was 100% in the Atlantic Forest and 85% in the Cerrado. The probability of

304   didelphids SEA$_B$ in the Atlantic Forest being smaller than in Cerrado was 100%. Within

305   sigmodontines, the probability of SEA$_B$ being smaller in the Atlantic Forest than in the Cerrado

306   was 13%. SEA overlap between clades in the Atlantic Forest was 3.48 (Didelphidae AF area:

307   3.48; Sigmodontinae AF area: 21.94) and 2.66 in the Cerrado (Didelphidae CER area: 9.66;

308   Sigmodontinae CER area: 14.02). SEA overlapping area among biomes was 1.359 for didelphids

309   and 7.086 for sigmodontines. A biplot generated adding data from Galetti et al. (2016) visually

310   showed these trends (Fig. 5A). When adding only the common species shared between biomes in

311   the overlapping estimations, we found that the ellipse area of Cerrado was larger (29.545) than in

312   the Atlantic Forest (11.914) and that 89.59% of the Atlantic Forest isotopic niche was within the

313   Cerrado isotopic niche.

314   Isotopic trajectories validated a shared tendency between clades of decreasing δ^{13}C from

315   the Cerrado to the Atlantic Forest and increasing δ^{15}N in the same direction. The magnitude

316   (trajectory size difference = 0.22; $P > 0.05$) and direction (angle between vectors = 22.22; $P >$

317   0.05) of isotopic trends from Cerrado to the Atlantic Forest were the same for both clades (Fig.

318   5B).

319

320   **DISCUSSION**

321   Our results showed that variation of δ^{15}N in our sampled small mammals can be predicted by

322   locomotion categories (explaining 33% of δ^{15}N variation). Highest values of δ^{15}N were reported

323   for the semifossorial insectivores *Oxymycterus quaestor* and *Brucepattersonius iheringi* and for

324   the semiaquatic carnivorous *Chironectes minimus*, while terrestrial species (such as the

325   insectivores *Akodon reigi* and *Deltamys kempi)*, omnivorous *Cerradomys maracajuensis*, and the

326   arboreal insectivore *Oecomys roberti*, had the smallest measures. The locomotory abilities of

327   small mammals and their associated morphological adaptations can determine how species forage

328   and move within the vertical strata (Bubadué et al. 2019). Vertical stratification has been

329   consistently pointed out as one of the main resource partitioning strategies used by small

330   mammals in their natural environment (Vieira and Monteiro-Filho 2003; Vieira and Camargo

331   2012; Camargo et al. 2018). In comparison, and although our general comparative models were

332   not controlled by site, our results were congruent with the findings of Galetti et al. (2016), who

333   found that locomotion predicted trophic niche segregation of small mammals in Atlantic Forest

334   sites. Galetti et al. (2016) also reported high levels of δ^{15}N in semifossorial and the smallest

335   levels in arboreal and terrestrial small mammal species (their data were not included in our

336   interspecific analysis, so the shared results are independent and comparable).

337   We found that our attempt to categorize small mammal diets was not useful in explaining

338   variation in δ^{15}N or δ^{13}C isotopic ratios. This can be an effect of the lack of knowledge about

339   these species' biology resulting in a failure to accurately categorize their diet (Paglia et al. 2012;

340   Arregoitia & D'Elía 2020; Bubadué et al. 2021). This categorization problem was previously

341   noted by Missagia et al. (2019) for Akodontini rodents. Another issue when analyzing stable

342   isotopes is the fact that factors other than diet can influence ratios in animal tissues (see Ben-

343   David and Flaherty 2012 for a review on mammals), such as environmental particularities

344   (Handley et al. 1999; Newton 2010), species differences in metabolic rates (McAvoy et al. 2006),

345   or both.

346   Averaging our data per site and species, we found that, to some extent, stable isotope

347   variation indeed is impacted by climatic conditions, because our isotopic data correlated with

348   annual precipitation. Studies on foliar and soil δ15N showed that water availability, associated

349 with temperature, and latitudinal variation can increase local $\delta^{15}N$ ratios (Handley et al. 1999).

350 Our results for small mammals also showed an increase of $\delta^{15}N$ in areas with higher annual

351 precipitation. Morphological studies on cranial shape variation associated with a climatic gradient

352 in rodents and marsupials also support these results, which are interpreted by some authors as an

353 effect of resource availability (Cáceres et al. 2016; Magnus et al. 2017, 2018; see also Resource

354 Rule description of McNab 2010).

355   In contrast, drier environments present higher levels of $\delta^{13}C$, suggesting the presence of

356 C_4 vegetation in the diet of small mammals when these resources are available. C_4 plants occur at

357 higher percentages in grassland and savanna regions, which tend to have lower precipitation

358 throughout the year (Still et al. 2003; Powell and Still 2008), which might have a direct impact on

359 the increase of trophic niche diversity within our data. Thus, we argue that $\delta^{13}C$ values, at least in

360 our study model, can be related to whether the environment presents C_4 or CAM plants or not,

361 rather than species-specific preferences to include them or not in their diet. To give some specific

362 examples, C_4 or CAM plants ($\delta^{13}C > -20‰$) only are implied in the diet of the terrestrial

363 didelphid *Lutreolina crassicaudata* (-17.8‰) and the akodontines *Necromys lasiurus* (-19.2‰),

364 *Akodon reigi* (-13.2‰) and *A. azarae* (-12.5‰—Bubadué et al. 2019; Missagia et al. 2019).

365 These high $\delta^{13}C$ values only were detectable in terrestrial species that were typical of open

366 habitats, where C_4 plants are available commonly (Still et al. 2003; Powell and Still 2008). Our

367 visual empirical data of carbon ratios support this because they are the highest for South

368 American savanna and grassland (Cerrado and Pampas biomes, Fig. 4). A mixture of C_3 and C_4

369 plants are available commonly in these biomes (Wagner et al. 2018; Ribeiro et al. 2019),

370 although we highlight that high values of $\delta^{13}C$ also were present in specimens from the Atlantic

371 Forest biome, as reported by Galetti et al. (2016).

372   Within clades, Sigmodontinae showed the same pattern as the overall small-mammal

373   model (discussed previously), in which locomotory category was the main predictor of δ^{15}N

374   ratios. Sigmodontines showed greater separation among locomotory categories than didelphids

375   with respect to δ^{15}N ratios, with the highest trophic level being secured by semifossorial

376   insectivores and a terrestrial insectivore (*A. paranaensis*) and the lowest either to arboreal,

377   semiaquatic, and other terrestrial phytophagous or insectivorous species. Didelphidae, on the

378   other hand, supported resource partitioning following a scaling law. The scaling law was

379   described by Ritchie and Olff (1999) to predict how organisms of different body sizes find and

380   partition food resources within the same community. By way of summary, smaller and larger

381   animals differ significantly in how they find and forage for different food sources, as well in their

382   capability to incorporate larger prey into their diet more frequently (Bubadué et al. 2021). We

383   potentially identified this trend in didelphids because they vary more in size than sigmodontines

384   within our sample. Didelphids' body size variation ranges from 11 g to 620 g in our sample,

385   while sigmodontines range from 15 g to 202 g. Moreover, some of the largest marsupials we

386   sampled have preferences for animal food sources, as reflected by their high δ^{15}N. These species

387   are, in order of increasing mass, *Philander canus*, *L. crassicaudata*, *Didelphis albiventris* (well

388   known to strategically include more animal food items in its diet when fruits are scarce; Cáceres

389   2002), and *C. minimus*. All these species are considered the most carnivorous South American

390   marsupials, particularly *L. crassicaudata* and *C. minimus,* although they generally are categorized

391   as omnivorous (Cáceres 2002, 2004; Santori and Moraes 2012).

392   In comparison, the overall trophic niche of Didelphidae we sampled is smaller than in

393   Sigmodontinae, and it completely overlaps the rodent isotopic space, supporting the idea of a

394   narrower diversity of niches in marsupials. This can be explained by niche conservatism in

395   Didelphidae (Chemisquy et al. 2021) compared to Sigmodontinae, which presents broader overall

396   species richness, phylogenetic diversity, and possibly functional diversity in the Neotropics

397   (Maestri and Patterson 2016; Maestri et al. 2016a). Nevertheless, we sampled fewer didelphid

398   species than sigmodontines, and therefore the smaller trophic niche in didelphids could be related

399   to a simple effect of sample size. Within biome comparisons and with a comparable number of

400   species between clades, we always identified the same trend: trophic niche is smaller in

401   didelphids than sigmodontines (Galetti et al. 2016). On the other hand, between biomes,

402   sigmodontines and didelphids show some similarities, suggesting that, despite their differences,

403   these animals' diets depend primarily on resource availability.

404   Didelphids and rodents show the largest SEA in the Cerrado (Neotropical savanna)

405   compared with their own clades' SEA in the Atlantic Forest. This is not an effect of species

406   composition differences between biomes because our SEA measurements in Cerrado and Atlantic

407   Forest that only focused on the common species subsample showed similar results. Moreover,

408   environmental differences between Cerrado and Atlantic Forest shifts the trajectories of

409   sigmodontines' and didelphids' isotope ratios in the same direction (increase of δ^{15}N and

410   decrease of δ^{13}C toward the forest biome). These environmental shifts are congruent with the

411   previously discussed correlations of our isotopic data with precipitation—where annual

412   precipitation was positively correlated with δ^{15}N and negatively with δ^{13}C—because the Cerrado

413   is a dryer and less stable environment than the Atlantic Forest (IBGE 2019).

414   Because of these environmental particularities, the comparison between trophic niches

415   within Atlantic Forest and Cerrado biomes show that the two clades partition the environment

416   very differently in these biomes. The Atlantic Forest supports 100% of didelphids' trophic niche

417   within sigmodontines. Still, only a portion of the trophic niche of didelphids is shared within the

418   wide range of sigmodontines. Community studies in the Atlantic Forest have shown that small

419   mammals partition environmental resources based on vertical strata (Vieira and Monteiro-Filho

420   2003; Grelle 2003; Melo et al. 2011) and that spatial heterogeneity is not as important for species

421   composition in a forest biome as it is in the savanna (Melo et al. 2011; Camargo et al. 2018).

422   Conversely, and while didelphid SEA still is smaller than that of sigmodontines, the trophic niche

423   overlap area in the Cerrado is considerably smaller than in the forest, suggesting a more direct

424   partition of food resources in the savanna than in the forest biome (Fig. 5). Considering that the

425   Atlantic Forest is more productive than the Cerrado (Raich et al. 1991), it is possible that food

426   availability and stability in the Atlantic Forest would support greater overlap between clades.

427   This would explain why resource partitioning in the Cerrado is much more obvious between

428   clades. Compared with our results, Camargo (2016) found that marsupials tend to decrease their

429   isotopic niche in gallery forest compared with woodland savanna forests, while the opposite

430   occurs for rodent species. He argued that in environments where resource availability is greater,

431   marsupials are able to shift their diet. Our results also support this because didelphids have

432   smaller isotopic niche in the Atlantic Forest (more productive) than in the Cerrado (less

433   productive), while sigmodontines have the opposite trend.

434   In summary, our results are in agreement with the common notion of opportunistic

435   feeding characteristics in Neotropical small mammals' ecology, along with their niche plasticity,

436   which probably is the primary factor that allowed their successful coexistence in a number of

437   different environments. Our study, in addition to previous isotopic (Galetii et al. 2016; Ribeiro et

438   al. 2019), morphological (Bubadué et al. 2019, 2021), and field research (Vieira and Monteiro-

439   Filho 2003; Vieira and Camargo 2012; Grelle 2003; Melo et al. 2011; Camargo et al. 2018),

440   supports the idea that both clades, most likely because of their different evolutionary and

441   biogeographical backgrounds, cope with coexistence by using two main partitioning strategies:

442   scaling law (didelphids) and vertical partitioning of resources (particularly sigmodontines). When

443   dealing with different environments (e.g., Atlantic Forest vs. Cerrado biomes), marsupials and

444 rodents either can decrease their trophic niche, most likely based on food abundance of preferable

445 food items throughout the year (habitat homogeneity), or increase niche partitioning, responding

446 to habitat productivity and seasonality (Camargo 2016; Ribeiro et al. 2019). Eventually, our

447 approach using stable isotopes can be used as a guideline to study trophic variation and

448 coexistence patterns in different clades.

449

458

459   SUPPLEMENTARY DATA

460 Supplemental data are available at the *Journal of Mammalogy* online.

461   **Supplementary Data SD1**.—Stable isotope data for each species included in this study

462 with information on taxonomy, locomotory categories, diet categories, and mass (common

463 logarithm).

464   **Supplementary Data SD2**.—Stable isotope data for each specimen included in this study

465 with information on taxonomy, municipality, biome, geographical coordinates, sex, and mass.

466

467

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654   *Submitted _____. Accepted _____ .*

655   *Associate Editor was _____ .*

656

657   **Tables**

658   **Table 1.**—Number of specimens and species separated by clade and biome. In the

659   Atlantic Forest, number of species in parentheses corresponds to the number after isotopic values

660   in Galetti et al. (2016) were added.

	Didelphidae		Sigmodontinae	
Biome	Specimens	Species	Specimens	Species
Atlantic Forest	5 (16)	4 (12)	27 (34)	11 (15)
Cerrado	38	12	54	14
Pantanal	6	4	21	8
Pampas	4	2	9	7

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664   **Table 2.**—Linear Model Evaluation with Randomized Residuals in a 9,999 Permutation

665   Procedure using clades (didelphid and sigmodontines), diet, body mass (BM), and locomotor

666   categories (LC) as predictors of isotopic signatures in the full sample. Significant or marginally

667   significant results are in bold.

Y = δ^{13}C	df	SS	R^2	F	P
Clades	1, 43	2.260	0.007	0.309	0.618
Diet	3, 41	5.830	0.018	0.256	0.837
BM	1, 43	2.010	0.006	0.274	0.607
LC	4, 40	45.970	0.145	1.693	0.157
Clades:Diet	1, 39	8.020	0.036	1.496	0.230
Clades:BM	1, 41	20.33	0.064	2.837	0.102
Clades:LC	3, 36	4.270	0.013	0.192	0.882
Y = δ^{15}N					
Clades	1, 43	10.597	0.079	5.230	**0.027**
Diet	3, 41	3.863	0.029	0.403	0.745
BM	1. 43	4.477	0.033	0.728	0.234
LC	4, 40	38.103	0.283	3.942	**0.010**
Clades:Diet	1, 39	13.216	0.098	4.757	**0.036**
Clades:BM	1, 41	6.889	0.051	2.442	0.137
Clades:LC	3, 36	4.647	0.035	0.765	0.524

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670 **Table 3.**—Linear Model Evaluation with Randomized Residuals in a 9,999 Permutation
671 Procedure using body mass (BM), and locomotor categories (LC) as predictors of isotopic
672 signatures separating between clades.

Didelphidae	df	SS	R^2	F	P
Simple models					
$\delta^{13}C{\sim}BM$	1, 13	2.928	0.055	0.763	0.408
$\delta^{13}C{\sim}Diet$	2, 12	11.485	0.217	1.667	0.178
$\delta^{13}C{\sim}LC$	3, 11	7.542	0.143	0.611	0.598
$\delta^{15}N{\sim}BM$	1, 13	9.060	0.328	6.342	**0.027**
$\delta^{15}N{\sim}Diet$	2, 12	11.253	0.408	4.122	**0.035**
$\delta^{15}N{\sim}LC$	3, 11	7.533	0.273	1.374	0.310
Diet*BM model					
$\delta^{15}N{\sim}BM$	2, 10	11.253	0.407	3.662	0.051
$\delta^{15}N{\sim}Diet$	1, 10	0.599	0.022	0.390	0.554
$\delta^{15}N{\sim}Diet*BM$	1, 10	0.416	0.015	0.271	0.629
Sigmodontinae					
$\delta^{13}C{\sim}BM$	1, 28	18.501	0.070	2.123	0.156
$\delta^{13}C{\sim}Diet$	3, 26	10.505	0.040	0.361	0.741
$\delta^{13}C{\sim}LC$	4, 25	40.628	0.158	1.145	0.329
$\delta^{15}N{\sim}BM$	1, 28	2.042	0.021	0.605	0.444
$\delta^{15}N{\sim}Diet$	3, 26	7.065	0.073	0.684	0.555
$\delta^{15}N{\sim}LC$	4, 25	43.691	0.453	5.168	**0.005**

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675   **Table 4.**—Values of Standard Ellipse Area (SEA), Standard Core Ellipse Area (SEA$_C$),

676   and Total Area calculated for each clade in each biome: AF = Atlantic Forest, CER = Cerrado, D

677   = Didelphidae, S = Sigmodontinae.

	SEA	SEAc	TA
AF D	3.167	3.484	8.123
AF S	20.645	21.935	44.495
CER D	8.778	9.655	19.416
CER S	12.943	14.021	31.274

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681   **Fig. 1.**—Map of South America, separating biomes and with collection localities of our dataset.

682   **Fig. 2.**—Averaged per species scatter plot of δ^{13}C (‰) vs. δ^{15}N (‰) stable isotopes. Polygons

683   were used to calculate total niche area, and standard ellipses areas were used to compare trophic

684   niche between groups. Species were grouped according to A) clades and diet and B) locomotor

685   categories (legends embedded). Didelphidae: 1. *Caluromys philander*, 2. *Chironectes minimus*, 3.

686   *Cryptonanus agricolai*, 4. *Cryptonanus chacoensis*, 5. *Cryptonanus guahybae*, 6. *Didelphis*

687   *albiventris*, 7. *Gracilinanus agilis*, 8. *Lutreolina crassicaudata*, 9. *Marmosa budini*, 10. *Marmosa*

688   *murina*, 11. *Marmosa paraguayana*, 12. *Monodelphis domestica*, 13. *Monodelphis kunsi*, 14.

689   *Philander canus*, 15. *Thylamys macrurus*. Sigmodontinae: 16. *Akodon azarae*, 17. *Akodon*

690   *lindberghi*, 18. *Akodon montensis*, 19. *Akodon paranaensis*, 20. *Akodon reigi*, 21.

691   *Brucepattersonius iheringi*, 22. *Calomys callosus*, 23. *Cerradomys maracajuensis*, 24.

692   *Cerradomys marinhus*, 25. *Cerradomys scotti*, 26. *Deltamys kempi*, 27. *Euryoryzomys nitidus*,

693   28. *Holochilus chacarius*, 29. *Hylaeamys megacephalus*, 30. *Juliomys pictipes*, 31. *Necromys*

694   *lasiurus*, 32. *Nectomys rattus*, 33. *Nectomys squamipes*, 34. *Oecomys bicolor*, 35. *Oecomys*

695   *mamorae*, 36. *Oecomys roberti*, 37. *Oligoryzomys chacoensis*, 38. *Oligoryzomys nigripes*, 39.

696   *Oxymycterus nasutus*, 40. *Oxymycterus quaestor*, 41. *Rhipidomys macrurus*, 42. *Scapteromys*

697   *tumidus*, 43. *Sooretamys angouya*, 44. *Thaptomys nigrita*, 45. *Wilfredomys oenax*.

698   **Fig. 3.**—Regression plot between δ^{15}N (‰) and log (body mass). Regression line and the 95%

699   confidence interval (shaded area) are only plotted for Didelphidae's significant relation between

700   variables. Dietary categories are plotted with different symbols in accordance to legend.

701   Sigmodontinae plotted for comparison (legend embedded). Didelphidae: 1. *Caluromys philander*,

702   2. *Chironectes minimus*, 3. *Cryptonanus agricolai*, 4. *Cryptonanus chacoensis*, 5. *Cryptonanus*

703   *guahybae*, 6. *Didelphis albiventris*, 7. *Gracilinanus agilis*, 8. *Lutreolina crassicaudata*, 9.

Marmosa budini, 10. *Marmosa murina*, 11. *Marmosa paraguayana*, 12. *Monodelphis domestica*, 13. *Monodelphis kunsi*, 14. *Philander canus*, 15. *Thylamys macrurus*. Sigmodontinae: 16. *Akodon azarae*, 17. *Akodon lindberghi*, 18. *Akodon montensis*, 19. *Akodon paranaensis*, 20. *Akodon reigi*, 21. *Brucepattersonius iheringi*, 22. *Calomys callosus*, 23. *Cerradomys maracajuensis*, 24. *Cerradomys marinhus*, 25. *Cerradomys scotti*, 26. *Deltamys kempi*, 27. *Euryoryzomys nitidus*, 28. *Holochilus chacarius*, 29. *Hylaeamys megacephalus*, 30. *Juliomys pictipes*, 31. *Necromys lasiurus*, 32. *Nectomys rattus*, 33. *Nectomys squamipes*, 34. *Oecomys bicolor*, 35. *Oecomys mamorae*, 36. *Oecomys roberti*, 37. *Oligoryzomys chacoensis*, 38. *Oligoryzomys nigripes*, 39. *Oxymycterus nasutus*, 40. *Oxymycterus quaestor*, 41. *Rhipidomys macrurus*, 42. *Scapteromys tumidus*, 43. *Sooretamys angouya*, 44. *Thaptomys nigrita*, 45. *Wilfredomys oenax*.

Fig. 4.—Scatter plots of δ^{13}C (‰) vs δ^{15}N (‰) separated by biomes and clades. Inset maps show location of biomes in Brazil.

Fig. 5.—Plots of δ^{13}C (‰) vs. δ^{15}N (‰) comparing Didelphidae and Sigmodontinae from Atlantic Forest and Cerrado. A) Standard ellipses isotopic niche area and convex hulls are plotted for each group. A smaller plot is available showing the range of the Bayeasian ellipses generated, the Standard Bayaesian Ellipse average and Standard Core Ellipse Area (SEA$_C$) in white. Each group is labeled as legend embedded. AF D = Atlantic Forest Didelphidae; AF S = Atlantic Forest Sigmodontinae; CER D = Cerrado Didelphidae; CER S = Cerrado Sigmodontinae. B) Scatter plot with all sampled species in each biome and phenotypic trajectories showing the changing path tendencies between Cerrado (squares) and Atlantic Forest (circles). D = Didelphidae (in black); S = Sigmodontinae (in white).