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Isotopic niche partitioning in two sympatric howler monkey species

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Abstract

Objectives: Ecological similarity between species can lead to interspecific trophic competition. However, when ecologically similar species coexist, they may differ in foraging strategies and habitat use, which can lead to niche partitioning. As the body tissues of consumers contain a stable isotope signature that reflects the isotopic composition of their diet, stable isotope analysis is a useful tool to study feeding behavior. We measured the isotopic niche width, which is a proxy for trophic niche width, of mantled (*Alouatta palliata*) and black (*A. pigra*) howler monkeys. Specifically, studied populations in allopatry and sympatry to assess whether these species showed niche partitioning.

Materials and Methods: Between 2008 and 2012, we collected hair samples from 200 subjects (113 black and 87 mantled howler monkeys) and used continuous flow isotope ratio mass spectrometry to estimate $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. We described the isotopic niche width of each species in allopatry and sympatry with the Bayesian estimation of the standard ellipse areas.

Results: In allopatry, isotopic niche width and isotopic variation were similar in both species. In sympatry, black howler monkeys had a significantly broader isotopic niche, which was mainly determined by high $\delta^{15}\text{N}$ values, and included the majority of mantled howler monkeys' isotopic niche. The isotopic niche of mantled howler monkeys did not differ between sympatry and allopatry.

Conclusions: The coexistence of these ecologically similar species may be linked to trophic niche adjustments by one species, although the particular features of such adjustments (e.g., dietary, spatial, or sensory partitioning) remain to be addressed.

KEYWORDS

Alouatta, coexistence, stable isotope analysis, standard ellipse area, sympatry, trophic niche

1 | INTRODUCTION

The interaction among individuals of different species in a community can lead to competition for limited resources (Pianka, 1981). In general, the more ecologically similar two species are, the more likely it is

that competition between them will be intense, although the intensity of competition will vary according to the species involved, the size of the interacting populations, inter-individual niche variation, and resource abundance (Araújo, Bolnick, & Layman, 2011; Dammhahn & Kappeler, 2014; Davies, Meiri, Barraclough, & Gittleman, 2007).

Intense interspecific competition may lead to the exclusion of one species (Wisheu, 1998). Still, it is common to find ecologically similar species living together in the same community (Dayan & Simberloff, 2005), indicating that there are strategies that allow for coexistence (Chase & Leibold, 2003).

Resource partitioning minimizes interspecific competition and allows for species coexistence (Schoener, 1974). Resource partitioning is usually based on segregation at one or several dimensions of species niche (*n*-hypervolume *sensu* Hutchinson, 1957), including temporal, spatial, and trophic dimensions. Given the direct influence of food intake on fitness parameters (e.g., Grant & Grant, 2002), it may be expected that strategies allowing for trophic niche (i.e., the way in which animals feed and use their habitat: Pianka, 1974) segregation are under strong selective pressure, and therefore, their study may contribute critical information for understanding species coexistence. Sympatric species may segregate their trophic niches by foraging on different food items or by foraging at diverse locations or times (López-Bao, Mattisson, Persson, Aronsson, & Andrén, 2016; Steinmetz, Garshelis, Chutipong, & Seuaturien, 2011; Vanak et al., 2013; Vernes, 2003). Nevertheless, trophic segregation will be conditional on resource availability, such that high seasonality in food abundance or human-induced scarcity may lead to increased trophic niche overlap (Kuhnen et al., 2017).

The body tissues of consumers contain a stable isotope signature that reflects the isotopic composition of their diet (Hobson, 1999; Hobson & Clark, 1992). Thus, dietary variation should lead to predictable isotopic patterns, which may be assessed with stable isotope analysis (SIA) to investigate the trophic ecology of free-ranging individuals (Crawford, McDonald, & Bearhop, 2008; Newsome, Martínez del Río, Bearhop, & Phillips, 2007). The bidimensional representation of stable carbon isotope ratio ($\delta^{13}\text{C}$) versus stable nitrogen ratio ($\delta^{15}\text{N}$), provides a measure of the isotopic niche (Newsome et al., 2007), and offers quantitative insights into two of the main features of trophic niches: niche width (i.e., habitat use, for instance, in terms of diversity of consumed resources) and trophic position (i.e., the place of the population in the food chain or number of trophic levels occupied; Bearhop, Adams, Waldron, Fuller, & Macleod, 2004; Layman, Arrington, Montaña, & Post, 2007; Newsome et al., 2007; Turner, Collyer, & Krabbenhoft, 2010). Inert tissues, such as hair, are particularly useful for SIA, as their isotopic composition reveals the isotopic values of the diet at the time of tissue synthesis (Oelze, 2016). Furthermore, as the incorporation of isotopes in hair occurs over several days/months and because no turnover takes place after isotope deposition, the isotopic signature of hair allows proxying information on a subject's diet over a long timescale, and this record can be preserved indefinitely (Ayliffe et al., 2004; Ben-David & Flaherty, 2012). For instance, hair isotopic data suggests that sympatric cheirogaleid lemurs (*Cheirogaleus crossleyi* and *C. sibreei*) feed on different canopy heights as a possible mechanism for niche differentiation (i.e., variation in niche width: Crowley, Blanco, Arrigo-Nelson, & Irwin, 2013). On the other hand, variation in $\delta^{15}\text{N}$ levels among chimpanzees (*Pan troglodytes*) suggests that individuals exploit

protein sources from different trophic levels (i.e., occupy different trophic positions: Fahy, Richards, Riedel, Hublin, & Boesch, 2013).

Primates, both extinct and extant, are a good model for exploring species coexistence, because primate communities usually include several taxa and ecologically similar species are frequently found living in sympatry (e.g., Deane, Nargolwalla, Kordos, & Begun, 2013; Ganzhorn, Wright, & Ratsimbazafy, 1999; Peres & Janson, 1999). Howler monkeys (genus *Alouatta*) are a Neotropical primate radiation that includes 12 species (Cortés-Ortiz, Rylands, & Mittermeier, 2015). All species are diurnal, arboreal, live in social groups, and have a frugivorous–folivorous diet (Crockett & Eisenberg, 1987). The distributions of howler monkey species are generally allopatric/parapatric, but several contact zones have been described (Cortés-Ortiz, Agostini, et al., 2015). In particular, mantled (*A. palliata*) and black (*Alouatta pigra*) howler monkeys live in sympatry in southern Mexico (Cortés-Ortiz, Agostini, et al., 2015). In allopatry, both these species have diets that conform to typical frugivore–folivore pattern observed in the genus, spend more time foraging on leaves than on fruits, and consume approximately 20 and 50% of the same plant species and families, respectively (Dias & Rangel-Negrín, 2015). Still, black howler monkeys spend more time eating fruits than mantled howler monkeys (Dias & Rangel-Negrín, 2015), and it has been speculated that they may differ in color perception (Matsushita, Oota, Welker, Pavelka, & Kawamura, 2014). Thus, mantled and black howler monkeys living in sympatry represent a suitable model to explore the responses of ecologically similar species to interspecific trophic competition. Isotope measurements have been conducted in howler monkeys from Central and South America (Schoeninger, Iwaniec, & Glander, 1997; van der Merwe & Medina, 1991), and are comparable to those reported for other primates (e.g., great apes: Oelze, Head, Robbins, Richards, & Boesch, 2014; lemurs: Crowley et al., 2013). This convergence is probably related to the fact that vegetation in tropical forests inhabited by primates mainly relies on a C^3 photosynthetic pathway (Blumenthal, Rothman, Chritz, & Cerling, 2016).

Our aim was to investigate variation in the trophic niche of mantled and black howler monkeys in allopatry and sympatry by measuring their $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopic niche. We examined two hypotheses. First, we hypothesized that given their ecological similarity (i.e., similar diets and arboreal lifestyle); trophic niche should be similar between species in both allopatry and sympatry. We tested two predictions of this hypothesis: (a) $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ranges should be similar among species and allopatry/sympatry conditions and (b) isotopic niche width should be similar among species and allopatry/sympatry conditions. Confirmation of this hypothesis would support previous contentions that a high degree of niche overlap between species, and resulting high potential for interspecific resource competition, is an underlying mechanism for the maintenance of the mostly parapatric distributions of howler monkeys (Agostini, Holzmann, & Di Bitetti, 2010). Second, we hypothesized that, alternatively, trophic niche should diverge between species in sympatry, indicating niche partitioning. Accordingly, we predicted that in sympatry the two species should have: (a) different $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ranges and (b) different isotopic niche widths. Support for this hypothesis would provide evidence in favor of

previous suggestions that behavioral variation between mantled and black howler monkeys has derived in differential competitive success (Baumgarten & Williamson, 2007; Cortés-Ortiz et al., 2003; Ford, 2006). The current geographic distribution of these species would thus be the result of interspecific differences in competitive potential (i.e., *A. palliata* displacing *A. pigra*: Ford, 2006).

2 | MATERIALS AND METHODS

2.1 | Ethical note

This study adhered to the American Society of Primatologists Principles for the Ethical Treatment of Non-Human Primates. The University of Michigan Committee for the Use and Care of Animals approved the protocols used for animal restraint and sample collection (permit #09319). Permits for collection and transport of samples were provided by Mexican (SGPA/DGVS/03676/07, SGPA/DGVS/06116, SGPA/DGVS/03293/10, SGPA/DGVS/10637/11, and CITES MX 74679), Guatemalan (CESA/mam/Exp.7739), and Spanish (ES-BB-00038-15) authorities. This study adhered to the legal requirements of the Mexican law (NOM-059-SEMARNAT-2010).

2.2 | Study sites

The study was conducted in southeastern Mexico and Guatemala (Figure 1). Allopatry for mantled howler monkeys was located in the state of Veracruz (Los Tuxtlas), where we sampled two groups. Allopatry for black howler monkeys was located in the state of Campeche (Mexico; eight groups) and in El Petén (Guatemala; one group). The sympatry area was located in the state of Tabasco (Macuspana area, Mexico), where we sampled 25 groups (12 *A. palliata* and 13 *A. pigra*; Supplementary Table S1).

Both mean group size (range = 4–6 individuals) and mean size of the habitat occupied by sampled groups were similar between species

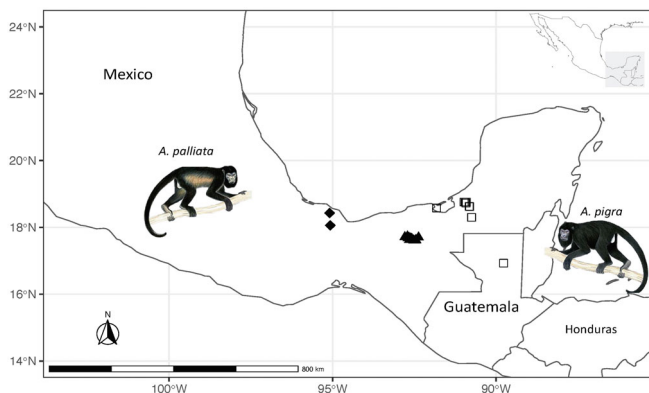


FIGURE 1 Location of the sampled groups: mantled howler monkeys (*Alouatta palliata*) in allopatry as black diamonds; black howler monkeys (*A. pigra*) in allopatry as open squares; both species in sympatry as black triangles. Primate drawings by Stephen Nash

in sympatry (*A. palliata* = 8 ha, *A. pigra* = 10 ha), although notably larger for *A. pigra* in allopatry (16,021 ha; Supplementary Table S1). We know that some individuals in the sympatry area are admixed due to hybridization (Cortés-Ortiz et al., 2007, 2019). However, admixed individuals are the product of multigenerational backcrosses (Cortés-Ortiz et al., 2019) and their morphology (Kelaita, Dias, Aguilar-Cucurachi, Canales-Espinosa, & Cortés-Ortiz, 2011) matches their behavior (i.e., *A. palliata*-like subjects behave as mantled howler monkeys, *A. pigra*-like subjects behave as black howler monkeys: Ho et al., 2014; Kitchen et al., 2017). Thus, for this study, we identified individuals as either mantled or black howler monkeys based on their morphology.

The climate in all conditions is tropical, with variation in annual rainfall: 2,600 mm in Los Tuxtlas, the allopatry area for *A. palliata* (Köppen Af, that is, tropical rainforest climate); 2,400 mm in Tabasco, the sympatry area (Am, that is, tropical monsoon climate); and 1,400 mm in Campeche and northern Guatemala, the allopatry area for *A. pigra* (Aw, that is, tropical wet and dry climate) (Beck et al., 2018; Fick & Hijmans, 2017). In all areas, there is seasonality in rainfall, with a dry season occurring between the months of January to May. Human disturbance has resulted in a significant replacement of original vegetation by agricultural fields and other anthropic land-use covers throughout southern Mexico (Sánchez Colón, Flores Martínez, Cruz-Leyva, & Velázquez, 2009).

2.3 | Hair collection

During the dry seasons of 2008–2012, we collected hair samples from all individuals belonging to 36 groups, in a total of 200 howler monkeys: 49 black howler monkeys in allopatry, 64 black howler monkeys in sympatry, 75 mantled howler monkeys in sympatry, and 12 mantled howler monkeys in allopatry. We followed the capturing and handling procedures described in Canales-Espinosa et al. (2011). For each captured animal, hair collection followed a standard protocol: a patch of hair was cut from the abdominal area as close to the skin as possible with fine-tipped surgical scissors, taking care not to damage the skin. Each hair sample was placed into a paper envelope, which in turn was inserted in a plastic bag and stored in a cool, dark environment until analysis.

2.4 | Stable isotope analysis

Prior to analysis, we finely cut (ca. 1 mm in length) 10–15 hairs from each collected hair patch with the same length (approximately 5 cm, sufficient to assess isotopic variation over several months; Supplementary Table S2). We then homogenized hair with our fingers and placed it into Eppendorf tubes to be cleaned sequentially with acetone, ethanol, and distilled water in a rotator for about 1 hr each, to remove attached lipids or dirt. We dried all samples in an oven (60°C) until a constant weight was reached. This method was designed following the cleaning procedures reported in diverse studies that used

hair samples for isotopic analysis (e.g., Ayliffe et al., 2004; Codron et al., 2013; Crowley, 2012; Schoeninger et al., 1997).

We weighed subsamples of hair (ca. 0.4 mg for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) and placed them in tin capsules. Isotopic analysis was carried out by elemental analysis-isotope ratio mass spectrometry using a Thermo Finnigan Flash 1112 coupled to a Delta isotope ratio mass spectrometer via ConFlo III interface at the Centres Científics i Tècnics of the University of Barcelona. Stable isotope signatures were reported in conventional δ notation (‰) relative to Pee Dee Belemnite for $\delta^{13}\text{C}$ and atmospheric nitrogen for $\delta^{15}\text{N}$. The $\delta^{13}\text{C}$ standard was Vienna Pee Dee Belemnite calcium carbonate, and the $\delta^{15}\text{N}$ standard was atmospheric nitrogen (N_2). International standards (ammonium sulfate, potassium nitrate, glutamic acid for $\delta^{15}\text{N}$ and polyethylene, sucrose, and glutamic acid for $\delta^{13}\text{C}$) were inserted after every 12 samples to calibrate the system and compensate for any drift over time. Precision was $\leq 0.1\text{‰}$ for $\delta^{13}\text{C}$ and $\leq 0.3\text{‰}$ for $\delta^{15}\text{N}$.

2.5 | Data analysis

Sex did not have significant effects (linear mixed models using group identity as a random factor) on either $\delta^{13}\text{C}$ (*A. palliata*: $F^2_{88} = 0.03$, $p = .859$; *A. pigra*: $F^3_{112} = 3.34$, $p = .071$) or $\delta^{15}\text{N}$ (*A. palliata*: $F^2_{88} = 1.97$, $p = .163$; *A. pigra*: $F^3_{112} = 0.75$, $p = .390$) hair values. Therefore, we did not include this factor in further analysis.

We used statistical analyses that account for the small and unbalanced sample sizes we had in our study. To characterize the isotopic niche of each species in each condition (i.e., allopatry vs. sympatry), we calculated: (a) mean isotopic values, and compared isotopic values with Kruskal–Wallis tests followed by Mann–Whitney post-hoc tests with significance levels corrected for multiple comparisons (Bonferroni corrected $p < .004$); (b) the range of $\delta^{13}\text{C}$ values, which is a proxy for the diversity of consumed resources (Layman et al., 2007); (c) the range of $\delta^{15}\text{N}$ values, which is a proxy for the number of trophic levels occupied (Layman et al., 2007); (d) the isotopic niche width using multivariate ellipse-based metrics of $\delta^{13}\text{C} \times \delta^{15}\text{N}$ values (Jackson, Inger, Parnell, & Bearhop, 2011). Specifically, we calculated standard ellipse areas corrected for small sample size (SEA_c), which represent the core mean of the population's isotopic niche, regardless of sample size (Jackson et al., 2011). We also generated Bayesian estimates of standard ellipse areas (SEA_b) to test for differences in isotopic niche width via SEA_b 95% credible interval comparisons; and (e) pairwise overlapping of SEA_c between species in sympatry and in allopatry as the proportion of the sum of the nonoverlapping areas of the ellipses (function “bayesianOverlap” in SIBER). We calculated standard ellipse areas and metrics with the R package “SIBER” (Jackson & Parnell, 2019).

3 | RESULTS

We found significant variation in $\delta^{13}\text{C}$ ($\chi^2_3 = 92.3$, $p < .001$) and $\delta^{15}\text{N}$ ($\chi^2_3 = 56.4$, $p < .001$). Specifically, $\delta^{13}\text{C}$ was the highest in black

howler monkeys in allopatry and $\delta^{15}\text{N}$ was the highest in black howler monkeys in sympatry ($p < .004$ in all pairwise comparisons; Table 1; Figure 2). The range of $\delta^{13}\text{C}$ was very similar across sympatry and allopatry in both species, although marginally smaller in mantled howler monkeys living in allopatry. In allopatry, $\delta^{15}\text{N}$ range was the same in black howler monkeys and mantled howler monkeys. The range of $\delta^{15}\text{N}$ in black howler monkeys in sympatry doubled that of both species in allopatry, whereas mantled howler monkeys in sympatry had an intermediate range.

Black howler monkeys living in allopatry and mantled howler monkeys living in both allopatry and sympatry had similar isotopic niche widths, as attested by SEA_c and SEA_b values (Table 1; Figure 3). Black howler monkeys in sympatry had the widest isotopic niche (Figure 4).

The area of overlap was the highest between the isotopic niches of both species living in sympatry, which was twice the overlap area between black howler monkeys in allopatry and mantled howler monkeys in sympatry (Figure 5). SEA_c of both species in sympatry and of mantled howler monkeys in allopatry overlapped more than SEA_c of black howler monkeys in allopatry and both black howler monkeys in sympatry and mantled howler monkeys in allopatry.

4 | DISCUSSION

We took advantage of a natural area of sympatry between two species of howler monkeys to examine if the coexistence of ecologically similar species is associated with trophic niche partitioning, assessed through isotopic niche measurements. In allopatry, isotopic niche width and isotopic variation were similar in mantled and black howler monkeys, confirming their ecological similarity at the trophic level. In sympatry, we found evidence of trophic niche partitioning between these two species, supporting the second hypothesis. This partitioning was not linked to isotopic niche segregation, as defined by a high proportion of nonoverlapping isotopic niches. Rather, black howler monkeys had a significantly broader isotopic niche in sympatry, which was mainly determined by high $\delta^{15}\text{N}$ values, and included the majority of mantled howler monkeys' isotopic niche. Additionally, the isotopic niche of mantled howler monkeys did not differ between sympatry and allopatry. Therefore, the coexistence of these two species may be linked to trophic adjustments by black howler monkeys, supporting previous contentions that they may differ in adaptive choices (Ford, 2006).

The isotopic values that we found are consistent with those reported in previous studies of howler monkeys from Central and South America (Schoeninger et al., 1997; van der Merwe & Medina, 1991), but also with values reported for other primates (e.g., Crowley et al., 2013; Oelze et al., 2014). This consistency is likely because howler monkeys, as most other primates (Harcourt, 2006), inhabit tropical forests dominated by vegetation with a C_3 photosynthetic pathway (Blumenthal et al., 2016). Still, black howler monkeys showed a wider isotopic range than that reported in previous studies for the genus, especially in $\delta^{15}\text{N}$ values. In addition to its possible relationship

TABLE 1 Hair stable isotopic values from two howler monkey species living in allopatry and in sympatry in southern Mexico and Guatemala

Isotopic niche attributes	<i>A. pigra</i>		<i>A. palliata</i>	
	Allopatry N = 49	Sympatry N = 64	Sympatry N = 75	Allopatry N = 12
Mean $\delta^{13}\text{C}$ (‰)	-24.5	-25.5	-25.5	-25.3
$\delta^{13}\text{C}$ range (‰)	-25.9–(-23.8)	-26.4–(-24.2)	-26.4–(-24.7)	-25.9–(-24.7)
Mean $\delta^{15}\text{N}$ (‰)	3.7	5.4	4.0	3.9
$\delta^{15}\text{N}$ range (‰)	2.6–5.0	3.4–8.0	2.6–5.9	2.5–4.9
SEA _c ^a	0.76	1.46	0.86	0.70
SEAb _b ^a	0.75	1.44	0.85	0.67
95% C.I. ^a	0.55–0.97	1.11–1.84	0.66–1.06	0.42–1.01

^aStandard ellipse areas corrected for small sample size (SEA_c), Bayesian standard ellipse areas (SEA_b), and SEA_b 95% credible intervals were calculated with SIBER (see Jackson et al., 2011 for more details on these metrics).

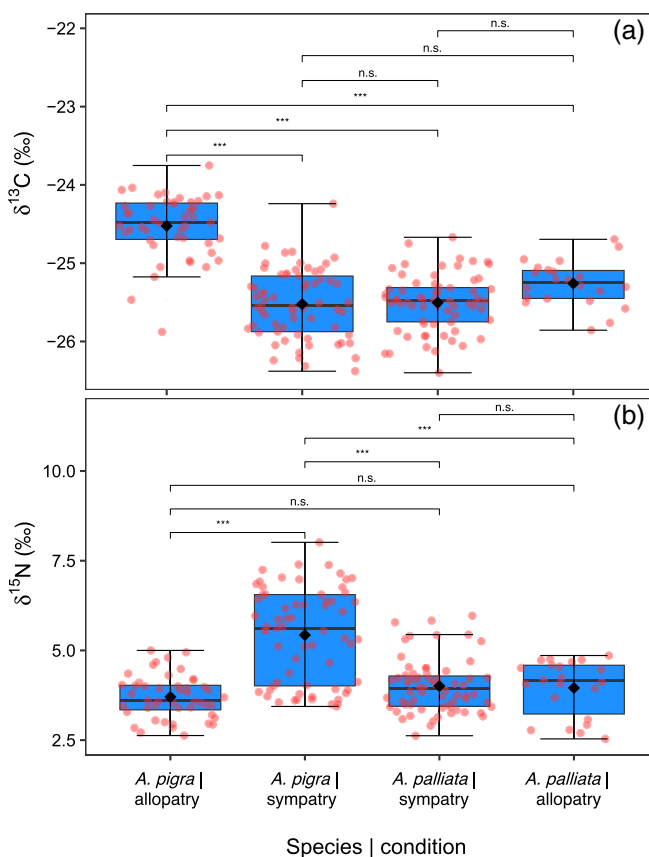


FIGURE 2 Variation in $\delta^{13}\text{C}$ (a) and $\delta^{15}\text{N}$ (b) values among two species of howler monkeys living in allopatry and sympatry. Thick lines inside the boxes are the medians; black diamonds are the means; box limits are the 25th and 75th percentiles; whiskers indicate 1.5*interquartile ranges; data points are plotted as red circles. Pairwise comparisons performed with Mann-Whitney univariate tests: n.s., nonsignificant; ***, $p < .004$

with a high degree of trophic flexibility by this species when in sympatry (discussed below), it is important to note that our results are based on a sample size ($N = 200$ subjects) considerably larger than that of previous studies ($N = 12$ in Schoeninger et al., 1997; $N = 1$ in van der Merwe & Medina, 1991).

Black howler monkeys living in sympatry with mantled howler monkeys had the widest isotopic niche. There was, however, extensive niche overlap between species in sympatry. Niche overlap among species basically defines species exclusion or coexistence when competition takes place (De Roos, Schellekens, van Kooten, & Persson, 2008). Niche overlap can cause adjustments in habitat use and trophic niche (e.g., Lush, Ward, & Wheeler, 2017; Merkle, Polfus, Derbridge, & Heinemeyer, 2017; Oelze et al., 2014), but also species marginalization and disappearance (e.g., Beaudrot et al., 2013; Simon & Townsend, 2003; Steinmetz et al., 2011). In contrast, niche diversification, in which competing species focus on different resources or exploit the same resources at diverse places or moments (i.e., spatial or temporal niche partitioning), favors species coexistence (Chesson, 2000; Tilman, 1982). Our results suggest that the coexistence of mantled and black howler monkeys in sympatry is probably favored by isotopic niche diversification by the latter. Long-term observations on patterns of species distribution in sympatry will confirm whether coexistence or exclusion is occurring (Agostini et al., 2010; Baumgarten & Williamson, 2007; Cortés-Ortiz et al., 2003; Ford, 2006), although habitat disturbance in the area is critically threatening the viability of both populations (Dias, Alvarado, Rangel-Negrín, Canales-Espinosa, & Cortés-Ortiz, 2013).

In the absence of behavioral observations and of isotope quantification in the foods consumed by howler monkeys, we can only speculate on the causes of the observed variation in isotopic values. Whereas it is plausible to suggest that the high $\delta^{13}\text{C}$ of black howler monkeys in allopatry, the driest condition in our sample, could be associated with a negative relationship between rainfall levels and $\delta^{13}\text{C}$ that has been consistently reported (e.g., Schoeninger, Most, Moore, & Somerville, 2016), it is more difficult to explain the high $\delta^{15}\text{N}$ of this species in sympatry. Mean $\delta^{15}\text{N}$ values of black howler monkeys in sympatry were similar to those of omnivorous chimpanzees (*P. troglodytes*: Oelze et al., 2014), insectivorous-frugivorous mouse lemurs (*Microcebus* spp.: Crowley et al., 2013), or frugivorous spider monkeys (*Ateles geoffroyi*: Schoeninger et al., 1997). Therefore, it is possible that black howler monkeys are consuming more nitrogen enriched foods than mantled howler monkeys, such as legumes (Schoeninger et al., 1997) or animal prey (Crowley et al., 2013; Fahy

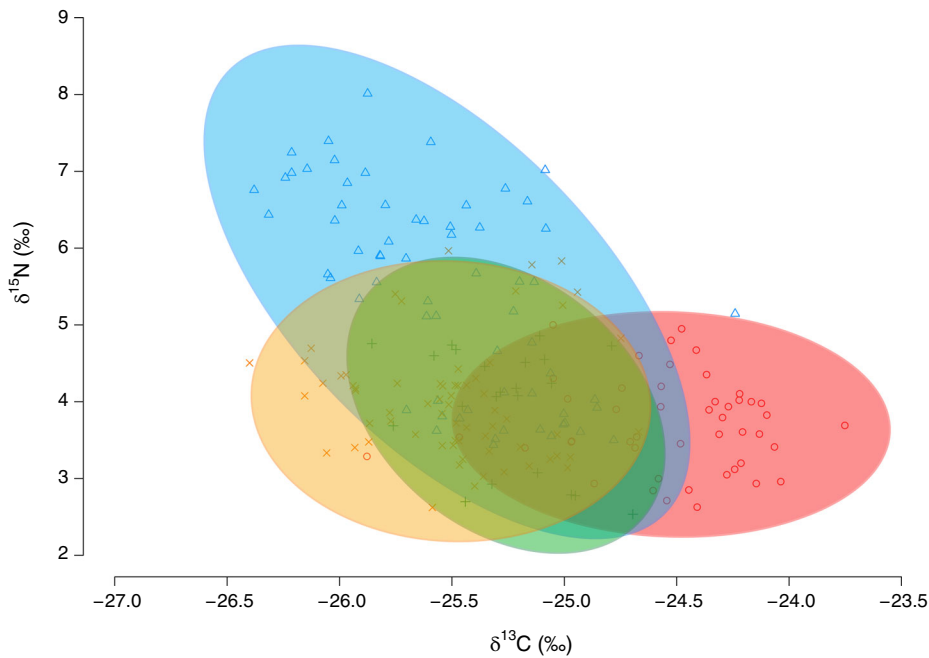


FIGURE 3 Variation in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ from howler monkey hair samples and bivariate ellipses (SEA_c) estimated by SIBER analysis (Stable Isotope Bayesian Ellipses in R): *A. pigra* in allopatry = red oval and open circles; *A. pigra* in sympatry = blue oval and open triangles; *A. palliata* in sympatry = orange oval and times signs; *A. palliata* in allopatry = green oval and plus signs. SEA_c were calculated from the variance and covariance of the isotope ratios and reveals the core nucleus of the isotopic dietary niche of the study animals

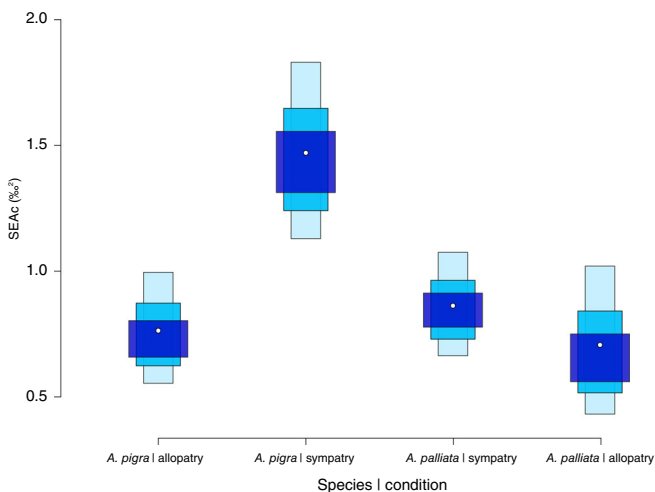


FIGURE 4 Density plots showing the credibility intervals (50% pale blue, 75% light blue, and 95% dark blue) of the standard ellipse areas for howler monkeys living in allopatry and sympatry estimated by SIBER analysis (Stable Isotope Bayesian Ellipses in R). White points are the mean standard ellipse areas of each species in each condition corrected for small sample size (SEA_c)

et al., 2013; Oelze et al., 2011). Still, mean differences in $\delta^{15}\text{N}$ between black howler monkeys and mantled howler monkeys did not reach the 3‰ threshold, which is usually considered to reflect a change in trophic position (Ben-David & Flaherty, 2012; Blumenthal et al., 2016; Crowley, 2012; DeNiro & Epstein, 1981; Post, 2002), suggesting that the consumption of animal protein, if it occurs at all, may be infrequent or restricted to particular contexts (e.g., animals that have access to eggs: Bicca-Marques, Muhle, Prates, de Oliveira, & Calegario-Marques, 2009).

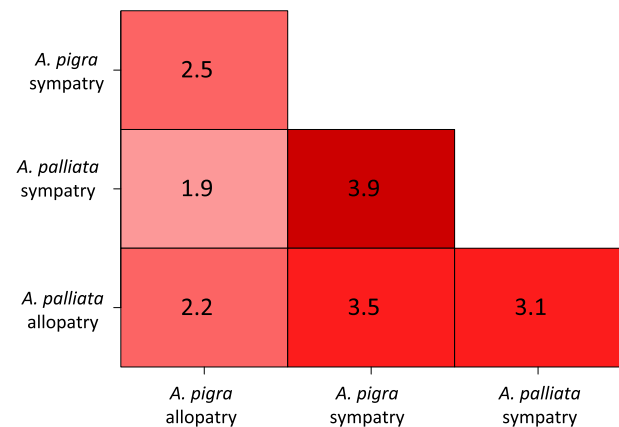


FIGURE 5 Overlap (numbers inside boxes) of the standard ellipse areas (SEA_c) of howler monkeys living in allopatry and sympatry estimated by SIBER analysis (Stable Isotope Bayesian Ellipses in R). Color intensity increases with SEA_c overlap

If isotopic niche variation between howler monkey species living in sympatry is actually linked to trophic niche variation, its underlying mechanisms remain to be determined. Variation in the feeding habits of howler monkeys has been linked to rainfall, group size, and habitat size (Dias & Rangel-Negrin, 2015). Given that these factors did not vary between groups of the two species in the sympatry area, it is unlikely that they explain the observed differences in isotopic niches. Still, we know that, compared to mantled howler monkeys, black howler monkeys on average devote more time to consuming fruits than leaves (Dias & Rangel-Negrin, 2015) and that there may be variation between species in color vision (Matsushita et al., 2014). Small-scale variation in the proportions of consumed foods and sensory variation could facilitate species coexistence (Falk et al., 2015; Lush et al.,

2017), even in the absence of spatial or temporal niche partitioning (Carothers & Jaksic, 1984; Schoener, 1974), so future research into the mechanisms of niche partitioning could focus on these factors.

In sum, we found evidence that the isotopic niche of two howler monkey species varies from allopatry to sympatry via the broadening of the niche by one of the species when in sympatry. Therefore, we suggest that the coexistence of black and mantled howler monkeys is linked to trophic niche adjustments, although the particular features of such adjustments (e.g., dietary, spatial, or sensory partitioning) remain to be addressed by future investigation.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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