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Tracking host use by bat ectoparasites with stable isotope analysis

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Abstract

We used C and N stable isotopes of nectarivorous bats and their ectoparasites to determine the extent to which parasites depend on the host individual for food. The difference in stable isotope values between parasites and host tissues ($\Delta^{13}\text{C}$ and $\Delta^{15}\text{N}$) was used as a proxy of host use. First, we tested the hypothesis that movement among individual *Choeronycteris mexicana* (Tschudi, 1844) is more likely to occur in winged flies than in mites as indicated by higher host-parasite isotopic Euclidian distance (ED). Second, we tested the hypothesis that ectoparasite species in two coexisting bat species representing the C_3 (*Anoura geoffroyi* (Gray, 1838)) and the CAM (*Leptonycteris yerbabuenae* (Martínez and Villa, 1940)) food chains were monoxenous as indicated by their isotopic values. We also examined $\Delta^{13}\text{C}$ and $\Delta^{15}\text{N}$ of individual parasites in relation to ^{13}C and ^{15}N reference enrichment factors as an indication of host switching. In general, flies in *C. mexicana* had higher ED and wider ranges of individual $\Delta^{13}\text{C}$ and $\Delta^{15}\text{N}$ than mites, suggesting that host switching occurred to a larger extent. Most ectoparasites species collected in both coexisting bats were monoxenous but one fly species appears to be oligoxenous. Individual $\Delta^{13}\text{C}$ and $\Delta^{15}\text{N}$ values varied widely in these parasite species suggesting movements within species hosts.

Keywords: *Anoura geoffroyi*; *Choeronycteris mexicana*; $\delta^{13}\text{C}$; *Leptonycteris yerbabuenae*; monoxenous parasites; $\delta^{15}\text{N}$; nectarivorous bats; oligoxenous parasites; Spinturnicidae; Streblidae.

Introduction

Ectoparasites are vectors of several diseases and tracking their movements among host individuals is particularly important for understanding disease dynamics. Recent incorporation of molecular techniques has helped to trace patterns in the way ectoparasite individuals move among host individuals. For example, using microsatellite genotypes, Levin et al. (2014) found that fly (*Olfersia spinifera* (Leach, 1817)) individuals infected with haemosporidian parasites (*Haemoproteus iwa* (Work and Rameyer, 1996)) were less likely to switch host (*Fregata minor* (Gmelin, 1789)) than uninfected individuals (Levin et al. 2014). Host switching in this study was assumed when the genotypes of the host and the bloodmeal of its flies did not match. Although molecular approaches based on bloodmeal genotyping are promising to track ectoparasite movements, their resolution might be limited by rapid digestion of blood or when bloodmeals originate from different host species (Gómez-Díaz and Figuerola 2010). Stable isotope analysis (SIA) offers a complementary approach that might overcome some of the limitations of molecular tools (Neilson et al. 2005; O'Grady and Dearing 2006; Voigt and Kelm 2006; Stapp and Salkeld 2009; Gómez-Díaz and González-Solís 2010; Clark and Hawke 2012; Sánchez et al. 2013; Demopoulos and Sikkell 2015). SIA is widely used in the study of animal food webs (Boecklen et al. 2011), and it is based on the existence of isotopic differences among food sources, which are incorporated into the consumer tissues in a more or less predictable manner. In the case of blood-feeding ectoparasites, their tissues incorporate the stable isotope values of their host (Schmidt et al. 2011). Therefore, by examining the isotope composition of host and parasite tissues, one can determine the extent to which parasites depend on the host individual for food. The difference in stable isotope values between parasite and host tissues can then be used as a proxy of host use because, as time spent on the host increases, there should be a closer match between the isotopic signals of the host individual and that of its parasites. For example, parasites that have remained long

enough or that are permanent residents on the host individual would have isotopic signals that approach those of the host, whereas recently arrived or accidental parasites would have different isotopic signatures from those of the host. These isotopic patterns might then be used to infer the potential value of parasites as disease vectors.

Bats are natural hosts of an important number of pathogens (Wibbelt et al. 2006) and their blood-eating ectoparasites might serve as disease vectors within host populations and, less likely, among bat species (Dick and Patterson 2006). Bat hematophagous ectoparasites are characterized mainly by flies (Diptera: Streblidae and Nycteribiidae) and some mites (especially Mesostigmata: Spinturnicidae), representing different life strategies that might affect the form in which they interact with their hosts. For example, streblid flies mate on the host, and females deposit pupae on the walls of the roost where newly hatched flies must immediately infest a new host (Dick and Patterson 2006); in contrast, the life cycle of spinturnicids occurs completely on the bat's body as eggs and pre-larva develop within the mother *in utero* which gives birth to live adult-like protonymphs (Evans 1968; McCoy 2009). Therefore, movement among individual hosts might occur at a higher rate in flies than in mites thus presumably affecting their role as disease vectors. We used C and N SIA to test the hypothesis that flies have higher inter-individual host movements than mites in the nectarivorous Mexican long-tongued bat (Glossophagini: *Choeronycteris mexicana* (Tschudi, 1844)). We predicted that host-parasite isotopic distance would be larger in fly than in mite species implying that they are more important vectors to spread disease among host individuals.

Most blood-eating bat ectoparasite species specialize in a single host species (monoxenous species), and a small fraction are associated with more than one species in the same genus (stenoxenous species) or in the same subfamily or family (oligoxenous species; Herrin and Tipton 1975; Morales-Malacara 1998; ter Hofstede et al. 2004; Dick and Gettlinger 2005; Dick and Patterson 2007). Due to their high host specificity, the role

as pathogen vectors across host species in most bat ectoparasites seems to be limited but there is potential for their use of more than one bat species. Parasite switching to a new host species may have important consequences for disease expansion and epidemics (Woolhouse et al. 2005). When coexisting potential hosts present contrasting isotopic values, SIA has the potential to explore host specificity in relation to host and parasite traits. For example, using C and N SIA, Voigt and Kelm (2006) showed that fly species collected from common vampire (Desmodontinae: *Desmodus rotundus* (Geoffroy, 1810)) and fruit-eating (Caroliinae: *Carollia perspicillata* (L., 1758)) phyllostomid bats coexisting in the same daytime roost were monoxenous parasites. Here we used SIA to evaluate host specificity in ectoparasites of two coexisting nectarivorous phyllostomids belonging to the same subfamily (Glossophagini): the lesser long-nosed bat (*Leptonycteris yerbabuenae* (Martínez and Villa, 1940)) and the Geoffroy's tailless bat (*Anoura geoffroyi* (Gray, 1838)). In summer, both bat species roost in the same cave in an evergreen forest in Central Mexico. *A. geoffroyi* is a permanent resident in this cave and its body tissues reflect the isotopic composition of vegetation that uses the Calvin cycle (C_3) pathway during photosynthesis; in contrast, *L. yerbabuenae* commutes from dry areas dominated by cactus and agaves in which photosynthesis occurs via the crassulacean acid metabolism (CAM) pathway. Because tissues of plants that use C_3 vegetation are depleted in ^{13}C compared to CAM plants, body tissues of coexisting *A. geoffroyi* and *L. yerbabuenae* have dissimilar C stable isotope values. We tested the hypothesis that, similarly to most bat-ectoparasite systems, ectoparasite species in these two bat species are monoxenous. Accordingly, we predicted that ectoparasites would match the isotopic values of the bat species on which they were collected.

Materials and Methods

Study site and sample collection

Bats were captured with mist nets. Individuals of *L. yerbabuenae* and *A. geoffroyi* were captured in a cave near Tlilapan, Veracruz (18°51' N, 96°40'W) between June and September 2011 and 2012. The vegetation in the locality is evergreen forest and riparian vegetation constituted mainly by C₃ plants. This cave holds a population of ~200 individuals of *L. yerbabuenae* that remains only during the summer and that commutes seasonally from the Tehuacán-Cuicatlán Valley as suggested by C SIA (Herrera 1997). We also collected individuals of *L. yerbabuenae* and *C. mexicana* in a cactus forest in Zapotitlán Salinas, Puebla (18°20' N, 97°28' W) between March and June 2012. This site is located in the eastern portion of the Tehuacán-Cuicatlán Valley and its vegetation is characterized by the presence of CAM plants (e.g., columnar cacti *Neobuxbaumia tetetzo* (Weber and Schum.) and *Cephalocereus columna-trajani* (Karw.) Schum.), and agaves *Agave marmorata* (Roezl), *A. macroacantha* (Zucc.), *A. karwinskii* (Zucc.) and *A. kerchovei* (Lem.)—Osorio 1996). We collected approximately 100-200 µl of whole blood from the antebrachial vein of each bat using heparinized capillary tubes. Blood samples were then placed in Eppendorf tubes and they were kept refrigerated during 4-5 days before being dried on a hotplate (Thermo Scientific- SP131635) at 60C°. Dried blood was pulverized in a small mortar.

Ectoparasites collection and identification

Captured bats were maintained in individual cloth bags before being examined. The whole body of each bat was inspected under an optical microscope (Olympus SF20) for 30 minutes, and ectoparasites were collected using dissection forceps and preserved in 80% ethanol until identification. We used taxonomic guides to identify streblid (Wenzel et al. 1966; Wenzel 1976) and mite species (Morales-Malacara 1998; Morales-Malacara and López-Ortega 2001). We recorded the number of individuals of each ectoparasite species found on each individual host, and determined prevalence (*i.e.* percent parasitized individuals) and intensity (*i.e.* number of ectoparasites in each parasitized individual). Bats

were provided fruit juice with a dosing syringe before being released after processing at the site of capture. We followed Canadian Council on Animal Care guidelines for bat handling.

Stable isotope analysis

One sample of each ectoparasite species collected on each individual host was placed in a 3.5 x 5 mm tin cup (Anachem, Fort Lauderdale, Florida). When possible, individual ectoparasites were capsulated separately, but samples represent the integrated values of several parasite individuals collected from the same host individual in cases where individual ectoparasites were too small for SIA. Number of individual parasites per capsule ranged from 1 to 38 (4.4 ± 6.2 , mean $\pm$ SD) in flies, and from 3 to 31 (12.1 ± 10.3) in mites. Amount of sample ranged from 0.1 to 1 mg (0.3 ± 0.2 mg) per capsule in flies, and from 0.1 to 1 mg (0.4 ± 0.3 mg) in mites. Similarly, one sample of blood (~1 mg) from each individual host was placed in a tin capsule for SIA. Samples were analyzed at the Department of Biology of the University of Miami. Samples were combusted in a EurovectorTM (GV Instruments Ltd., Manchester UK) elemental analyzer. Resultant gases were separated and analyzed in a GV IsoprimeTM (GV Instruments Ltd., Manchester UK) continuous-flow isotope ratio-mass spectrometer for stable-C and -N isotope ratios on the same sample. We used two laboratory standards (egg albumen) for every five unknowns in sequence. Stable-isotope ratios were expressed in δ -notation as parts per thousand (‰) deviations from the international standards Peedee belemnite marine limestone (carbon) and air (nitrogen) according to the equation: $\delta X = ([R_{\text{sample}}/R_{\text{standard}}] - 1)$, where X was ^{13}C or ^{15}N , and R was the corresponding ratio $^{13}\text{C}:^{12}\text{C}$ or $^{15}\text{N}:^{14}\text{N}$. On the basis of several hundred replicates of laboratory standards, we estimated laboratory measurement error to be $\pm 0.1\%$ for carbon and nitrogen.

Data and statistical analyses

We used one-sample *t*-tests in all bat species to compare mean ^{13}C and ^{15}N enrichment factors (e.g. the difference in isotopic values between ectoparasites and their hosts) with reference enrichment factors experimentally derived for blood-eating ectoparasites (Schmidt et al. 2011): 0.7 and -0.1 ‰ for C, and 3.85 ‰ (the average of 3.8 and 3.9 ‰) for N. Additionally, we graphically examined individual ^{13}C and ^{15}N enrichment factors in relation to reference enrichment factors. If ^{13}C and ^{15}N enrichment factors derived for a particular parasite sample matched reference enrichment factors, we consider that those parasites have *most likely* been feeding on the host for long enough to acquire its isotopic signal. If ^{13}C and ^{15}N enrichment factors were not similar to reference enrichment factors, we considered that those individual parasites have *most likely* switched host. Similarly to molecular movement tracking studies (Levin et al. 2014), we did not intend to identify the individual from which parasites that switched host originated because we did not have the isotopic signal of all potential hosts. Our approach just focused on establishing if parasites were permanent residents of or if they had just moved to the host from which they were collected. Because enrichment factors may be affected by variables that we cannot control (e.g., age, feeding rate; Roth and Hobson 2000; Gaye-Siessegger et al. 2004; Schmidt et al. 2011), we are cautious and keep our interpretations of host switching at a qualitative level.

We measured host-parasite isotopic distance in *C. mexicana* as the Euclidean distance using both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values ($\text{ED} = \sqrt{[\delta^{13}\text{C}_{\text{host}} - \delta^{13}\text{C}_{\text{parasite}}]^2 + (\delta^{15}\text{N}_{\text{host}} - \delta^{15}\text{N}_{\text{parasite}})^2}$). $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of parasites were corrected with reference enrichment factors (0.7 ‰ for C and 3.85 ‰ for N; Schmidt et al. 2011) to estimate ED. Parasite samples containing 0.1 mg yield $\delta^{15}\text{N}$ values that are 0.75 ‰ lower than samples containing 0.65 and 0.28 mg (Schmidt et al. 2011). Because 42% of our mite and 45% of our fly samples contained 0.1 mg of mass, we made an additional correction to $\delta^{15}\text{N}$ values of those samples and found no significant difference with ED values estimated

without this correction (paired t -test; $t_{27} = 1.2$, $P = 0.2$). We also estimated ED using -0.1 ‰ as enrichment factor for C (Schmidt et al. 2011) but no significant difference was found between both estimates (paired t -test; $t_{27} = 0.3$, $P = 0.7$). Therefore, we used the first estimate of ED without correcting $\delta^{15}\text{N}$ in small samples for comparisons. We compared ED among flies and mites in *C. mexicana* using one-way analysis of variance (ANOVA) followed by Tukey's HSD test for unequal sizes. We compared $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values between *A. geoffroyi* and *L. yerbabueae* individuals collected in Tlilapan, and among their ectoparasites using one-way ANOVA followed by Tukey's HSD tests for unequal sizes. We compared $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of parasites and those of *L. yerbabuenae* and *A. geoffroyi* individuals from which they were collected using paired t -tests. We estimated the contribution of *A. geoffroyi* and *L. yerbabuenae* as sources of food for each major ectoparasite species using a Bayesian model in the SIAR (stable-isotope analysis in R) package (Parnell et al. 2010). The model incorporates sources of uncertainty in isotope composition and element concentration of food sources. We considered mean ($\pm$ SD) of $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, % C, and % N of each host species. We used 0.7 ‰ and 3.85 ‰ as enrichment factors for C and N, respectively (Schmidt et al. 2011). All statistical analyses were conducted in Statistica version 7 (StatSoft 2004), and statistical significance was set at $P \leq 0.05$.

Results

Host-parasite isotopic distance: flies vs. mites

We collected individuals from three species of ectoparasites in individuals of *C. mexicana*: two winged fly species (*Trichobius dugesii* (Townsend, 1891): 70%, 1.3 ± 0.8 , prevalence, mean intensity $\pm$ SD; *Paratrachobius longicrus* (Ribeiro, 1907): 47%, 1.7 ± 3.8) and one mite species (*Periglischrus leptosternus* (Kolenati, 1857): 47%, 20.3 ± 12.3). Mean ^{13}C enrichment factors were not significantly different than reference enrichment factors in any parasite species (Table 1). Mean ^{15}N enrichment factors were not

significantly different than the reference enrichment factor in *P. leptosternus* and *P. longicrus*, but it was significantly different in *T. dugesii* (Table 1). When individual enrichment factors were examined, all parasite species presented a wide range of values that in several cases deviated from ^{13}C and ^{15}N reference enrichment factors, and that included positive and negative values (Fig. 1A). In general, dispersion from reference enrichment factors was higher in fly than mite species (Fig. 1A). There were significant differences in ED ($F_{2,25} = 5.8$, $P = 0.009$) among *C. mexicana* ectoparasites: host-parasite isotopic distances for *T. dugesii* ($P = 0.03$) and *P. longicrus* ($P = 0.01$) were higher than for *P. leptosternus*, and no significant differences in ED were found between both fly species ($P = 0.9$; Fig. 2A). ED from parasites collected from the same host were lower for *P. leptosternus* in three individuals, and in two individuals ED values were almost identical for fly and mite species (Fig. 2B).

Host specialization

Most parasite individuals collected from *L. yerbabuenae* in Tlilapan corresponded to two fly species (*Nycterophilia coxata* (Ferris, 1916): 89.1%, 8.3 ± 8.9 ; *Trichobius sphaeronotus* (Jobling, 1939): 59.4%, 1.7 ± 1.1) with a few individuals from one mite species (*Periglischrus paracaligus* (Herrin and Tripton, 1975): 1.3%, 7.4 ± 3.3). In addition to that, two wingless fly species (*Anastrebla modestini* (Wenzel, 1966) and *Exastinion clovisi* (Pessoa and Guimaraes, 1937)) were collected separately from two individual *L. yerbabuenae*. Ectoparasite composition in *L. yerbabuenae* examined in Zapotitlán was similar except that *A. modestini* and *E. clovisi* were not present, and one individual host presented *N. parnelli* (Wenzel, 1966). Most parasite individuals collected from *A. geoffroyi* corresponded to two fly species (*A. modestini*: 66.6%, 3 ± 2.28 ; *E. clovisi*: 88.8%, 5.2 ± 2.5), and one mite species (*Pe. vargasi* (Hoffmann, 1944): 66.6%, 3.5 ± 2.1). Additionally, we collected individuals of one fly species (*N. coxata*) in two individual *A. geoffroyi*.

There were significant differences in isotopic composition between both bat species collected in Tlilapan: *L. yerbabuenae* had higher $\delta^{13}\text{C}$ ($F_{1,81} = 144.8$, $P < 0.0001$) and lower $\delta^{15}\text{N}$ ($F_{1,80} = 6.5$, $P = 0.01$) mean values than *A. geoffroyi* (Fig. 3A-B). There were significant differences in $\delta^{13}\text{C}$ values among parasite species ($F_{5,126} = 201.8$, $P < 0.0001$): *L. yerbabuenae* ectoparasites had higher mean values than *A. geoffroyi* ectoparasites (Fig. 3C). There were significant differences in $\delta^{15}\text{N}$ values among parasite species ($F_{5,126} = 10.5$, $P < 0.0001$): mean value in *E. clovisi* was higher than in *N. coxata*, *Pe. paracaligus*, and *Pe. vargasi*, and *T. sphaeronotus* had a higher mean value than *Pe. paracaligus* (Fig. 3D). Mean $\delta^{15}\text{N}$ in *A. modestini* was not different that mean values in the other parasite species (Fig. 3D). $\delta^{15}\text{N}$ values of *A. modestini* (10.4 ‰) and *E. clovisi* (10.3 ‰) collected in single individuals of *L. yerbabuenae* were within the range of values of other parasites found in this host; however, $\delta^{13}\text{C}$ was enriched in *E. clovisi* (-16.7 ‰) and depleted in *A. modestini* (-21.5 ‰). $\delta^{15}\text{N}$ values of *N. coxata* found in two individuals of *A. geoffroyi* (11.5 ‰, 11 ‰) were within the range of other parasites found in this host but their $\delta^{13}\text{C}$ values were enriched (-14.6 ‰, -16 ‰).

Leptonycteris yerbabuenae captured in Zapotitlán had higher $\delta^{13}\text{C}$ values (-12.7 ± 1.7 ‰, $N = 8$) than in Tlilapan ($F_{1,73} = 13.6$, $P < 0.001$) but both groups did not differ in $\delta^{15}\text{N}$ values (Zapotitlán: 8.6 ± 1.3 ‰, $P = 8$; $F_{1,73} = 2.6$, $P = 0.1$). Ectoparasites collected in Zapotitlán followed the isotopic composition of *L. yerbabuenae* individuals (*N. coxata*: $\delta^{13}\text{C} = -13.4 - -16$ ‰, $\delta^{15}\text{N} = 10.4 - 11.8$ ‰, $N = 4$; *N. parnelli*: $\delta^{13}\text{C} = -15.2$ ‰, $\delta^{15}\text{N} = 11.5$ ‰, $N = 1$; *T. sphaeronotus*: $\delta^{13}\text{C} = -14.5 - -13.8$ ‰, $\delta^{15}\text{N} = 9.9 - 11.4$ ‰, $N = 2$).

When stable isotope ratios of individual parasite samples and those of the host individual from which they were collected were compared in Tlilapan, no significant differences were found in any $\delta^{13}\text{C}$ host-parasite comparison, but $\delta^{15}\text{N}$ values were higher in most parasites compared to their hosts (Table 2). Mean ^{13}C enrichment factors found in ectoparasite species of *A. geoffroyi* and *L. yerbabuenae* were not significantly different

than reference enrichment factors (Table 1). Mean ^{15}N enrichment factors were significantly different than the reference enrichment factor only in *E. clovisi* and *Pe. vargasi* (Table 1). When individual enrichment factors were examined, all parasite species presented positive and negative values, deviating from ^{13}C and ^{15}N reference enrichment factors in several cases (Fig. 1B-C). Based in the Bayesian model, all major parasite species derived nutrients virtually entirely from the host species on which they were found (Fig. 4)

DISCUSSION

Individual host switching

As expected, we found larger mean isotopic ED with respect to hosts in flies than in mites collected from *C. mexicana*. When we examined individual hosts in which both flies and mites were collected, ED was lower in mites than in flies in most cases. We interpreted this isotopic pattern as indicative of higher movement rate of fly ectoparasites among host individuals. In bats, flies move easily and quickly among host groups whereas mites require physical contact to be transmitted from one individual to another and this occurs primarily via vertical transmission (ter Hofstede and Fenton 2005). Molecular work comparing coexisting ectoparasites has also revealed an association between dispersal ability among individuals and parasite natural traits. For example, rates of gene flow among avian hosts (*Buteo galapagoensis* (Gould, 1837)) were low in vertically-transmitted amblyceran lice, intermediate in horizontally-transmitted ischnoceran lice, and high in horizontally-transmitted hippoboscids (Whiteman et al. 2007). When individual enrichment factors in our study were examined, most values did not match the difference expected according to reference enrichment factors derived for blood-eating ectoparasites (Schmidt et al. 2011). This pattern indicates that host switching occurred in both fly and mite individuals, although the largest mismatch between expected and observed enrichment factors were found in fly species, supporting the pattern found with isotopic

ED. Frequent host switching has been inferred in flies (Levin and Parker 2014) and mites (Bruyndonckx et al. 2009) using nuclear and mitochondrial DNA markers. Our isotopic reconstruction indicates that fly and mite ectoparasites switch individual hosts and that this movement might be more frequent in fly species. Fly (Insecta) and mite (Arachnida) species compared in our study belong to different classes which might affect the way in which they incorporate host's C and N into their tissues. Isotopic discrimination depends primarily on food characteristics (Florin et al. 2011) and, since both parasite groups feed on bat's blood, we assume that they have similar enrichment factors. Species specific discrimination factors would probably improve our reconstruction quantitatively but we would not expect major qualitative changes in our interpretations. Our study assumes that the isotopic value of parasites reflected the isotopic composition of ingested food, but the composition of recently hatched individuals might reproduce the isotopic value of the mother from which they developed. Because both streblids and spinturnincids start feeding on bat's blood shortly after they are born, we consider that SIA of parasites most likely mirror the isotopic nature of their food.

Host specialization

We found distinct composition of major ectoparasite species in the two coexisting nectarivores: two fly species (*N. coxata* and *T. sphaeronotus*) and one mite species (*Pe. paracaligus*) in *L. yerbabuenae*, and two fly species (*A. modestini* and *E. clovisi*) and one mite species (*Pe. vargasi*) in *A. geoffroyi*. These ectoparasite species are known to occur on these bat species or in congeneric species (Guerrero and Morales-Malacara 1996; Morales-Malacara 2001; Cole and Wilson 2006; Muñoz-Romo et al. 2011). Both host-parasite systems were significantly different in their stable isotope composition, particularly with respect to their $\delta^{13}\text{C}$ values: *L. yerbabuenae* and its ectoparasites had values characteristic of the CAM food chain, whereas *A. geoffroyi* and its ectoparasites had values representative of the C_3 food chain. In most cases, mean ^{13}C and ^{15}N enrichment

factors found in ectoparasite species of *A. geoffroyi* and *L. yerbabuenae* were not significantly different than enrichment values measured in blood-feeding ectoparasites (Schmidt et al. 2011), which indicates that ectoparasites fed exclusively from the host species on which they were collected. However, individual enrichment factors varied widely in all parasite species and in several instances they were different than expected enrichment factors. This pattern indicates movements of parasites among individual hosts as found in this study in ectoparasites of *C. mexicana* and in other birds and bats (Bruyndonckx et al. 2009, Levin and Parker 2014). Exclusive use of their host species was also supported by the dietary reconstruction with the Bayesian model for all ectoparasite species supporting our hypothesis that parasites would be monoxenous. A few exceptions to this pattern were the presence of *A. modestini* and *E. clovisi* in single individuals of *L. yerbabuenae*, and of *N. coxata* in two *A. geoffroyi*. However, our SIA supports the monoxeny of *A. modestini* and *N. coxata* as their $\delta^{13}\text{C}$ values clearly indicate that their presence on the alternative host was either accidental or temporary. In contrast, the $\delta^{13}\text{C}$ value of *E. clovisi* closely matched that of its host suggesting that this parasite is an oligoxenous species. The presence of *E. clovisi* on *L. yerbabuenae* most likely occurred via the transfer from *A. geoffroyi* in Tlilapan because it was not recorded previously in Zapotitlán. In contrast to the clear isotopic evidence for monoxeny of fly species in vampire and fruit-eating phyllostomid bats (Voigt and Kelm 2006), our study with two coexisting nectarivores shows that host specialization seems to be the rule but use of an alternative host might occur. Recent work with Neotropical bats showed that fly species were either monoxenous or stenoxenous, and that deviations from this pattern most likely resulted from contamination during bat processing (ter Hofstede et al. 2004; Dick and Gettinger 2005). Our study unequivocally indicates that *E. clovisi* is an oligoxenous parasite because *L. yerbabuenae* was the only host species from which it could derive its ^{13}C -enriched

composition. This wingless fly species is mainly associated to *A. geoffroyi* although it has also been reported in *Leptonycteris* (Graciolli and Barros de Carvalho 2001).

Concluding remarks

Tracking host use by blood-eating ectoparasites is an exciting new venue in the study of host-parasite interactions made possible by the incorporation of novel intrinsic methods (Waite 2014). Use of SIA to unveil the relationship between hosts and parasites dates back to nearly one decade but it is a promising approach to ultimately include parasites in food web reconstructions (Lafferty et al. 2008). Even though our study is limited by methodological constraints that are inherent to the application of SIA, it unveils aspects of parasite ecology that are not easily tracked by other means. In particular, our study supports the assumption that streblids move more easily among host individuals than spincturnicids, and challenges the assumption that parasites found in non-typical host are the result of transient associations or contamination. We hope that our study contributes to the understanding of how ectoparasites disperse among host, particularly considering the renovated interest in the study of emerging diseases.

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Figure legends

Fig. 1. Individual enrichment factors (the difference in isotopic values between each individual ectoparasite and its host) for several host-parasite systems. Enrichment factors for ^{13}C ($\delta^{13}\text{C}_{\text{parasite}} - \delta^{13}\text{C}_{\text{host}}$) and ^{15}N ($\delta^{15}\text{N}_{\text{parasite}} - \delta^{15}\text{N}_{\text{host}}$) were estimated for ectoparasite species collected from *Choeronycteris mexicana* (A), *Leptonycteris yerbabuenae* (B), and *Anoura geoffroyi* (C). Lines on figures represent enrichment factors experimentally derived for blood-eating ectoparasites (Schmidt et. al. 2011): 0.7 and -0.1 ‰ for ^{13}C , and 3.85 ‰ (the average of 3.8 and 3.9 ‰) for ^{15}N .

Fig. 2. Isotopic distances of fly (*Trichobius dugesii* and *Paratrachobius longicrus*) and mite species (*Periglischrus leptosternus*) to host individuals of *Choeronycteris mexicana*.

Isotopic distances were estimated as the Euclidian distance of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of parasites and host individuals. Higher distances at the population level (mean $\pm$ SD) were found in fly than mite species (A), whereas mites in bat individuals with both types of parasites had $\leq$ distances than flies (B). Similar letters above bars indicate no significant differences after one-way ANOVA followed by Tukey's HSD test for unequal sizes.

Numbers above bars are sample sizes. Circles = *Pe. leptosternus*; diamonds = *Pa.*

longicrus; triangles = *T. dugesii*.

Fig. 3. Mean ($\pm$ SD) $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of bat hosts (A, B) and their ectoparasites (C, D). Similar letters above bars indicate no significant differences after one-way ANOVA followed by Tukey's HSD test for unequal sizes. Numbers above bars are sample sizes.

AM = *Anastrebla modestini*; EC = *Exastinion clovisi*; PP = *Periglischrus paracaligus*; PV = *Periglischrus vargasi*; NC = *Nycterophilina coxata*; TS = *Trichobius sphaeronotus*.

Fig 4. Relative contribution of two coexisting bat species to the diet of their ectoparasites. Contributions were calculated using a Bayesian model (Parnell et al. 2010) on measured $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of parasites found on (A) *Leptonycteris yerbabuenae* and (B) *Anoura geoffroyi*. Proportion values are probability densities at 95, 75 and 25% credibility intervals.

Fig. 1
190x142mm (300 x 300 DPI)

Fig. 2
254x338mm (300 x 300 DPI)

Fig. 3
190x142mm (300 x 300 DPI)

Fig. 4
190x142mm (300 x 300 DPI)

Table 1.- Mean ($\pm$ SD) C ($\Delta^{13}\text{C}$) and N ($\Delta^{15}\text{N}$) isotopic enrichment factors (the difference in isotopic values between ectoparasites and their hosts) in several host-parasite systems. Enrichment factors were compared with enrichment factors experimentally derived for blood-eating ectoparasites ($\Delta^{13}\text{C}_1 = 0.7\text{‰}$; $\Delta^{13}\text{C}_2 = -0.1\text{‰}$; $\Delta^{15}\text{N}_3 = 3.85\text{‰}$; Schmidt et. al. 2011) using one-sample *t*-tests. * $P < 0.05$; ** $P < 0.01$. Numbers in parenthesis are samples analyzed for each parasite species and for each species host. Each parasite species sample represents parasites collected in a single host individual; in some cases, samples from more than one parasite species were collected in the same host individual.

Host/parasite	$\Delta^{13}\text{C}$ (‰)	$t \Delta^{13}\text{C}_1$	$t \Delta^{13}\text{C}_2$	$\Delta^{15}\text{N}$ (‰)	$t \Delta^{15}\text{N}_3$
<i>Choeronycteris mexicana</i> (17)					
<i>Periglischrus leptosternus</i> (8)	1.3 ± 1.4	1.2	2.8*	3.3 ± 0.8	1.4
<i>Paratrichobius longicrus</i> (8)	-1.3 ± 4.1	1.3	0.8	2.9 ± 2.3	1
<i>Trichobius dugesii</i> (12)	-0.1 ± 3.9	0.7	0.04	1.9 ± 2.1	2.9*
<i>Anoura geoffroyi</i> (9)					
<i>Anastrebla modestini</i> (6)	0.1 ± 1.2	0.1	0.5	2.7 ± 1.6	1.5
<i>Exastinion clovisi</i> (8)	0.1 ± 1.2	1.4	0.4	2.7 ± 0.8	3.5**
<i>Periglischrus vargasi</i> (6)	1.3 ± 1.5	1	2.2	0.9 ± 1.5	4.7**

<i>Leptonycteris yerbabuenae</i> (37)					
<i>Nycterophilia coxata</i> (33)	0.4 ± 2.1	0.7	1.4	3.2 ± 2.1	1.5
<i>Trichobius sphaeronotus</i> (22)	-0.9 ± 2.5	2.9	1.4	3.4 ± 1.4	1.1
<i>Paratrichobius paracaligus</i> (5)	-0.3 ± 1.2	1.9	0.5	2.6 ± 1.7	1.5

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Table 2.- Mean ($\pm$ SD) $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of parasites and those of the host individuals from which they were collected.

$\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ parasite and host values were compared using paired t -tests. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Host/parasite	$\delta^{13}\text{C}$ (‰)			$\delta^{15}\text{N}$ (‰)		
	Host	Parasite	t	Host	Parasite	t
<i>Anoura geoffroyi</i>						
<i>Anastrebla modestini</i>	-22.9 $\pm$ 1.5	-22.7 $\pm$ 0.5	0.3	8.8 $\pm$ 1.9	11.5 $\pm$ 0.9	4.1**
<i>Exastinion clovisi</i>	-22.9 $\pm$ 1.3	-22.8 $\pm$ 0.4	0.2	9.3 $\pm$ 1.8	12.1 $\pm$ 1.2	8.9***
<i>Periglischrus vargasi</i>	22.9 $\pm$ 1.5	-21.6 $\pm$ 0.4	2.1	9.1 $\pm$ 2.1	10.1 $\pm$ 0.8	1.5
<i>Leptonycteris yerbabuenae</i>						
<i>Nycterophilia coxata</i>	-15.4 $\pm$ 2.3	-15.1 $\pm$ 0.9	1.6	7.9 $\pm$ 1.3	11.1 $\pm$ 1.3	8.9***
<i>Trichobius sphaeronotus</i>	-15.1 $\pm$ 2.4	-16.1 $\pm$ 1.2	1.6	7.9 $\pm$ 1.5	11.4 $\pm$ 0.8	11.1***
<i>Periglischrus vargasi</i>	-15.7 $\pm$ 1.9	-16.1 $\pm$ 1.5	0.7	7.5 $\pm$ 1.8	10.2 $\pm$ 0.7	3.5*