1 Cophylogenetics and biogeography reveal a coevolved relationship between sloths
2 and their symbiont algae

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9 Abstract

10 Specialized species, like arboreal folivores, often develop beneficial relationships with symbionts
11 to exploit ecologically constrained lifestyles. Although coevolution can drive speciation by
12 specialization of a symbiont to a host, a symbiotic relationship is not indicative of coevolution
13 between host and symbiont. We tested for coevolved relationships between highly specialized
14 two- and three-toed sloths (*Choloepus* spp. and *Bradypus* spp., respectively) and their symbiotic
15 algae using cophylogenies and phylogeography. Our phylogeographic analysis showed a
16 biogeographic pattern for the sloth distribution that was not found in the algal phylogeny. We
17 found support for congruence between the sloth and algae phylogenies, implying cospeciation,
18 only in the *Bradypus* lineage. Algae host-switching occurred from *Bradypus* spp. to *Choloepus*
19 spp. Our results support a previously hypothesized symbiotic relationship between sloths and the
20 algae in their fur and indicate that coevolution may have played a role in algae diversification.
21 More broadly, convergent evolution may facilitate host switching between deeply diverged host
22 lineages.

1. Introduction

Coevolutionary relationships define many interactions in biological communities from predator and prey (e. g. acorn barnacle and gastropods, Lively 1986), to parasites and their hosts (e. g. birds and lice, Clayton and Moore 1987) and plants and their pollinators (e. g. figs and wasps, Herre and West 1997), and can be antagonistic, commensal or mutualistic. Reciprocal selection pressure between two interacting species can result in inherited co-adaptive patterns (Wade, 2007). The frequency and strength of the interaction between individuals defines the degree of reciprocal evolution. A diffuse interaction involves general adaptations for interactions with many populations or a functional group and a tight interaction involves highly specialized adaptations with particular species (Wade, 2007).

Host-symbiont interactions are predicted to become more specialized as environmental quality lowers (i.e. constraints on host productivity) and symbiotic partners may evolve highly specific traits to maintain the interaction (Thrall et al., 2007). Specialized species often evolve unique morphological and behavioral characteristics to survive in novel environments or maintain monophagy (Benkman and Lindholm, 1991; Björnhag and Snipes, 1999; Tieleman and Williams, 2000). Many specialized organisms invest in symbiotic relationships to enhance survival in unique environmental conditions. For example, coral reefs foster a diverse symbiotic community to help protect from variations in sea temperature, turbidity and light levels (Abrego et al., 2008; Baird et al., 2007; Baker, 2003; Maynard et al., 2008). Similar to coral reefs, the highly specialized tree sloths, two- and three-toed sloths (*Choloepus* and *Bradypus*), can be considered individual ecosystems harboring a diverse array of interacting symbionts (Durden, 1987; Gilmore et al., 2001). Pauli et al. (2014) suggested that mutualistic partnerships among sloths, the algae in their fur, and coprophagous pyralid moths on their bodies are the drivers of the risky and unusual behavior of sloths defecating on the ground. The algal growth in the sloth fur may also provide an additional source of nutrition, supplementing a nutritionally poor diet

48 and the moths provide a source of nitrogen for the algae as they die and decompose on the sloth
49 (Pauli et al. 2014). Although the nutritionally restricted lifestyle of sloths would assume a
50 coevolved mutualism between host and symbiont, ecological co-adaptation does not necessarily
51 mean the relationship is coevolved (Janzen, 1980).

52 While mutualisms play a fundamental role in ecosystem function and these coevolved
53 relationships are ubiquitous in nature, they are considered an evolutionary and ecological
54 paradox (Herre et al., 1999; Jandér and Herre, 2010). Mutualistic relationships seem to contradict
55 the theory that selection should favor individuals that receive benefits from an interaction
56 without paying the cost of reciprocal services. Therefore, the persistence of mutualism requires
57 processes that reduce the likelihood of cheating, such as host sanctioning (Pellmyr and Huth,
58 1994). Maintaining a mutualistic association over time depends upon the benefits to the host and
59 symbiont aligning, vertical transmission of symbionts from parent to offspring, genotypic
60 uniformity of symbionts within a host, repeated interactions between host and symbiont and
61 limited opportunity of symbionts to host switch (Herre et al., 1999).

62 Molecular phylogenetics has been central in distinguishing coevolved from co-adaptive
63 relationships, and has uncovered cryptic relationships between multiple species (Herre et al.,
64 1999; Wade, 2007). In tightly coevolved relationships, both host and symbionts can lose or gain
65 key phenotypic characters found in their closest relatives making them difficult to place using
66 traditional taxonomic approaches (Herre et al., 1999). The mutualistic relationship between reef
67 building corals and their symbiont dinoflagellates were once thought to be pairwise relationships,
68 but genetic analyses revealed a high diversity of dinoflagellate species associated with corals
69 (Rowan, 1998). Molecular markers also allow for quantifying the degree of phylogenetic patterns
70 in mutualistic relationships and how closely mutualistic partners track speciation events.

We use a molecular phylogenetic approach to test if the relationship between the two-toed sloths (*Choloepus* spp.) and the three-toed sloths (*Bradypus* spp.), their algae, and pyralid moths represents a coevolved system. This study is the first co-phylogenetic analysis of sloths and their algae and we reveal insights into the unique complex of symbiotic relationships found in the living ecosystem of a sloth. Moreover, we hypothesize that mutualisms may benefit host-symbiont relationships in highly specialized species and that convergent evolution plays a role in facilitating host switching in deeply diverged species.

2. Methods

We tested for coevolution between sloths and their algae using cophylogenetic and biogeographic approaches. We explored the relationships with phoretic moths too, but unresolved phylogenetic trees prevented us from testing coevolution between sloths and moths; see Supplementary Data S1. We combined sequence data from field data and GenBank to build the sloth and algae phylogeny. For sloths, we built three alignments for analyses. The first alignment, “extant”, included the following extant species *B. variegatus* (Costa Rica, Colombia, Peru and Brazil), *B. pygmaeus* (Panama), *B. tridactylus* (French Guiana and Brazil), *B. torquatus* (Brazil), *C. hoffmanni* (Costa Rica and Panama), and *C. didactylus* (French Guiana and Brazil). This alignment was used to calibrate the phylogeny using previously published divergence times. The second alignment, “full”, included all extant sloths plus the extinct sloth *Mylodon darwini* (Höss et al., 1996); we included an alignment with the extinct sloth to provide an additional fossil calibration date. The third alignment, “algae match”, included only sloths from localities that matched the localities of the algal sequences for cophylogenetic analysis. See Table S3 for sequence information. We used a Bayesian multi-locus coalescent approach for two mitochondrial genes in sloths, 12S and 16S, and in algae we amplified the ribosomal gene 18S. We tested for coevolution using patristic distance and event costs methods.

2.1 Field and sample collection

Our fieldwork was conducted approximately 85 km northeast of San José, Costa Rica (10.32° N, 83.59° W). We captured previously marked adult *B. variegatus* ($n = 8$) and *C. hoffmanni* ($n = 8$) using standard procedures and collected tissue from the ear of sloths for DNA extraction (Pauli and Peery 2012; Peery and Pauli 2012). We collected (0.1 g) of hair from each sloth, and followed established procedures to extract the algae from the hair (Pauli et al. 2014).

2.2 DNA extraction

We extracted genomic DNA from sloths, algae and moths using a Qiagen DNeasy Blood and Tissue Kit™ (59506, Qiagen) following the manufacturer's protocol for tissue samples with a modification to the last step: two separate, final elutions of each 65 µL were conducted. We extracted DNA from eight ear punches of *C. hoffmanni* and *B. variegatus*, and algae from a single hair sample from each sloth.

2.3 PCR amplification and sequencing

2.3.1 Sloths

We PCR amplified genomic DNA for the two mitochondrial genes, 16S rRNA and 12S rRNA, for both *C. hoffmanni* and *B. variegatus*. A 553 base pair (bp) fragment of the 16S rRNA was amplified using the primers 16sa L02510 and 16sb H03063 and a 573 bp fragment of the 12S rRNA was amplified using the primers 12sb H01478 for 12S (Höss et al., 1996). Each PCR amplification was conducted using *Taq* PCR Core kit (201225, Qiagen). All primer details, PCR reaction mixtures and PCR parameters for 16S and 12S are in Supplementary Data S2, Tables S2.1, S2.2 and S2.4. Prior to sequencing, we cleaned the amplified fragments using ExoSap IT (78200, Affymetrix). We pair-end sequenced the fragments on an ABI 3730xl DNA Analyzer at the Biotechnology Center, University of Wisconsin-Madison.

We visualized the sequence chromatograms for 16S sequences ($n = 16$) and 12S sequences ($n = 16$), using FinchTV 1.4 (Geospiza) and we manually aligned the forward and reverse sequences in Mega 6.06 (Tamura et al., 2013). After alignment, we BLAST (Altschul et al., 1990) searched the sequences to determine identity. We downloaded all available sloth 16S and 12S sequences from GenBank to include in the phylogenetic analyses (Table S3). In Mega 6.06, we built three sequence datasets for 16S and 12S then we aligned each using WebPRANK (Loytynoja and Goldman, 2010) under the default settings.

2.3.2 Algae

We amplified a ~1.2 kb fragment of the ribosomal gene 18S with either the universal primers UNI7F and UNI1534R (Moon-van der Staay et al., 2001) or 501F and 1700R (Suutari et al., 2010; see Supplementary Data S2, Tables S2.1, S2.3 and S2.5 for primer and PCR details). All positive algae samples were cloned using pGEM®-T Easy Vector System II- cloning kit (A1380, Promega) following the manufacturer's protocol. Five positive colonies were picked with a sterile pipette tip, dipped into the PCR reaction mixture and PCR amplified using the vector primers T7 and SP6 (Supplementary Data S2, Table S2.1). We cleaned and sequenced all positive reactions as above.

We visualized and confirmed the sequence chromatograms for 18S algae clones ($n = 55$) following the protocol that we used for the sloths. We removed the vector sequence and then reduced the clone sequences to singletons. All sequences at a 95% or higher identity match (Table S3) and all 18S clones obtained from sloth hair by Suutari et al (2010) were downloaded from GenBank. We collapsed any downloaded sequences into a singleton if five or less bp were different. We built two alignments for 18S in Mega 6.06 then we aligned each using WebPRANK. The first alignment, here on referred to as "all sloths" consisted of the algae singletons from this study and those from Suutari et al. (2010) and the second alignment, here on

referred to as “sloth match” consisted of algae sequences that matched localities of the sloths sequences.

2.4 Phylogenetic analysis

For Bayesian phylogenetic analyses to find the best fit evolutionary model we used the Akaike information criterion corrected for sample size (AICc) with jModeltest 2.1.1 (Darriba et al., 2012). Best fit models for each gene are listed in Table 1. We performed Bayesian analyses using a multilocus coalescent approach for species tree inference in *BEAST (Heled and Drummond, 2010) as implemented in BEAST 1.8.2 (Drummond et al., 2012).

2.4.1 Sloths

We conducted Bayesian analyses on the 16S and 12S sequences using the three data sets: “extant,” “full” and “algae match”. In the *BEAST runs for each alignment, 16S was analyzed under a strict molecular clock and 12S was analyzed under an uncorrelated lognormal relaxed clock. The gene trees were considered linked as 16S and 12S are both mitochondrial genes. The speciation tree was analyzed with a Yule process tree prior. The default of a hierarchical prior for effective population size was assumed as described by Heled and Drummond (2010). Two independent runs of 70 million generations were conducted, sampling every 3000 generations. The tree was calibrated using previously published speciation dates (Gibb et al. 2015) . The root height was constrained at the estimated time of a split between Megalonychidae (two-toed) and Bradypodidae (three-toed) using a normal distribution with a mean 29.9 and standard deviation 5.0. A second calibration was used by enforcing monophyly in *Bradypus* and constraining the group using a normal distribution with a mean 19.0 and standard deviation 4.0. Additionally, for the analysis of the “full” alignment a calibration of 13,000 years for tip date was placed on the *M. darwinii* sequence. Convergence and effective sample size of each parameter was assessed in Tracer 1.6 (Rambaut et al., 2014) and the samples from the two runs were pooled using Log

Combiner 1.8.2. A maximum clade credibility tree was compiled in TreeAnnotator 1.8.2 after discarding the initial 10% burn-in.

2.4.2 Algae

In the *BEAST Bayesian analyses for the two algae alignments: all sloths and sloth match, 18S was analyzed under an uncorrelated lognormal relaxed clock. *BEAST was chosen as a multispecies coalescent approach where individual sloths were considered to harbor a “population” of algae and all variable algal sequences obtained from a single sloth were included in the analyses. Therefore, the dataset was treated as having several sequences from within and amongst populations and to consist of multiple species. All other tree priors were the same as those in the sloth analyses except 100 million generations were conducted, sampling every 4000 and *BEAST determined the placement of the tree root. Convergence and effective sample size were assessed and the maximum clade credibility trees were built as stated above for the sloths.

2.5 Coevolutionary analyses

For cophylogenetic analyses, we used the maximum clade credibility trees from the sloth “algae match” and the algae “sloth match” Bayesian analysis. In the algae tree, we collapsed any branch split with less than 0.65 posterior probability resulting in 29 algae branch tips included in the cophylogenetic analysis. For the sloth tree, we used the species tree with seven host species for cophylogenetic analysis. We constructed a tanglegram using TreeMap 3.0 (Charleston and Robertson, 2002) to indicate the association of the sloths and their algae (Figure 1). In Jane 4.0 (Conow et al., 2010) we conducted a tree-reconciliation cophylogenetic analysis under two parameter profiles: 1) default analysis using the evolutionary event cost parameters of zero for codivergence, one for duplication, loss and failure to diverge and two for a host switch and 2) an analysis using the same values for cospeciation, loss and failure but two for duplication and one for a host switch. The significance values were calculated by comparing actual costs with

expected distribution of costs by performing randomization tests of 1000 generated symbiont trees.

We conducted a distance-based cophylogenetic analysis using PACo (Balbuena et al., 2013) implemented in R (R Core Team, 2013). PACo uses a Procrustes approach in which a principal coordinates analyses is performed on the host patristic distance matrix (sloth) followed by a Procrustes rotation of the parasite distance matrix (algae). A residual sum of squares is performed to test the fit of the parasite phylogeny onto the host phylogeny and a goodness-of-fit statistic is provided. The significance of the congruence was determined using 100,000 random permutations. We also tested the contribution of individual host-parasite associations in PACo using jackknife method in which host-parasite links that contribute very little to the squared residuals are likely to represent coevolutionary links. To determine if geographic structuring influenced the tree structure, we constructed geophylogenies in GenGIS 2.4.1 (Parks et al., 2009) from the sloth and algae maximum clade credibility trees against a land cover relief map obtained from the GenGIS companion program, MapMaker.

3. Results

All sloth *BEAST analyses parameters had high ESS values for all criteria in all datasets (> 491), except for three speciesTree.split.population parameters in the “algae match” analysis where ESS was >107. The likelihood means for extant, full and algae match were -3295.57, -3564.73, and -3136.91. The maximum clade credibility trees for the sloth extant, full and algae match showed strong support for species divergences except for the divergence between *B. tridactylus* from Brazil and French Guiana and *B. variegatus* from Brazil (Figures 2 and S4). The median divergence between Bradypodidae (*Bradypus*) and Megalonychidae (*Choloepus*) ranged between 25.81 (17.92-33.71) and 27.05 (19.05-34.73) mya, extant and full, respectively. Overall, the topology of the trees and the estimated timing of deeper divergences are comparable to those

in Gibb et al. (2015) and Delsuc et al. (2012) . The divergence between *B. tridactylus* and *B. variegatus* (7.73 extant and 8.09 full mya) and the most recent common ancestor between *C. hoffmanni* and *C. didactylus* (3.87 extant and 4.04 full mya) were both placed later than the divergence times estimated in Gibb et al. 2015. In the sloth full maximum clade credibility tree, *Myiodon darwini* speciated from the rest of *Choloepus* 15.18 (9.01-21.86) mya (Figure S4).

The majority of the algal clones matched sequences published in Suutari et al. 2010 (Figure S5). For the *BEAST analysis, all ESS values were >489 and the likelihood mean was -9085.18. Most major clades in the maximum clade credibility trees for algae were strongly supported (Figures 1 and 3) and the topology was comparable to that in Suutari et al. (2010).

Tree reconciliation analysis under default parameters, found six equally parsimonious cost scenarios with a total of 37 cost events (Figure 4). The event-cost solutions had 4 cospeciation events, 18 duplications, 6 host switches, 2 sorting events and 5 instances where the algae failed to diverge with the sloth hosts (Figure 4). After 1000 random permutations of the algal tree, none of the sampled scenarios (mean = 55, standard deviation = 5) were less than or equal to the original cost events (Figure S6) suggesting that the sloth and algal trees are not randomly associated. Under parameter set two (cospeciation 0, duplication 2, host switch loss 1 and failure to diverge 1) six equally parsimonious cost scenarios with a total of 42 cost events were found (Figure S7). The event-cost solution had 3 cospeciation events, 12 duplications, 13 host switches, 0 sorting events and 5 failures to diverge. After the random permutation test, 21.1% of the samples scenarios (mean = 49.3, standard deviation = 8.2) were less than or equal to the original suggesting that the sloth and algal trees were randomly associated (Figure S8). For the distance based analysis, PACo showed co-diversification in the sloth and algae was significant at the alpha = 0.05 ($p < 0.002$); therefore, rejecting the null hypothesis that the sloth and algal genetic distance correlations evolved by chance. Seven links between *Bradypus* hosts

and their algae largely contributed to the co-diversification results; however, 12 of the 34 sloth-algae links showed some degree of association (Figure 5).

A Monte Carlo permutation test on the sloth geophylogeny supported that the phylogenetic structure was likely influenced by biogeographic location rather than random ($p = 0.04$, Figures 2 and S9). The algae geophylogeny did not support any biogeographic structure ($p = 0.118$, Figures 3 and S10).

4. Discussion

We detected a strong pattern of codivergence between sloths and their algae based on patristic distance and the first event-cost analyses. Codivergence was no longer supported when we reduced the cost of host-switches in the event-cost analyses, but host-switching events occurred between species separated by large geographic distances. As these host-switches were unlikely due to the genetic biogeographic structuring and given the poor dispersal capabilities of sloths; the second event-cost analysis seems less plausible than the analysis supporting codivergence in sloths and algae. Regardless of the cost for host-switching and duplication, cospeciation in the *Bradypus* lineage and an early host-switching event from *Bradypus* to *Choloepus* were supported in both analyses.

Our findings also suggest that duplication has played a more important role than cospeciation in the speciation of algae. Although cospeciation is often considered as one of the strongest lines of evidence for of coevolution, cospeciation and coevolution are not synonymous (Smith et al., 2008). Codivergence has historically been viewed as strong evidence for coevolution (Page, 2003); however, coevolution requires reciprocal evolutionary change which cannot be proven through codivergence alone (Janzen, 1980). Incongruent cophylogenies are often detected between hosts and symbionts, as we have found in our sloth and algal trees. Patterns of cophylogenetic associations can be driven by other processes, such as host switching,

duplication, lineage sorting (loss) and failure to speciate (Johnson et al., 2003; Page, 1994; Paterson and Gray, 1997; Paterson et al., 1999).

Our analysis of geophylogenies supports a biogeographic pattern in sloths but not algae. The lack of biogeographic signature suggests algal distribution may be more reliant on environmental conditions rather than geographic structuring. Suutari et al. (2010) noticed similar eukaryotic diversity in the sloth fur samples analyzed across Central and South America and suggested similar sloth habitat and not geography influenced diversity. The divergence dating of sloths are consistent with the dates previously published (Delsuc et al., 2004; Gibb et al., 2016). The calibrated tree consisted of well-supported nodes for deeper divergences; although, some divergences between *B. variegatus* and *B. tridactylus* were not supported. These unsupported clades may be the result of mis-identification or a product of hybridization (de Moraes Barros et al., 2011; Gibb et al., 2016). Using only mtDNA for phylogeographic studies has known limitations such as the inability to determine introgression and hybridization and possibly incorrect delimitation of recently radiated species (Edwards and Bensch, 2009). However, gene trees derived from sequence-based markers are still useful in describing phylogeographic pattern (Brito and Edwards, 2009) and the speciation events within the deeper nodes of the sloth phylogenetic tree (~2-25 mya) suggest reciprocal monophyly within the nuclear genes has occurred. We recommend future studies using a multilocus approach that includes nuclear DNA or SNPs to elucidate the finer details of phylogeographic patterns.

Without a calibrated algal tree (due to a depauperate fossil record for green algae and no known mutation rate for algae 18S gene), it is difficult to definitively verify cospeciation. In the absence of a calibrated algal phylogeny, we tested the event-cost analysis under two parameter conditions that differed in the cost of duplication and host-switch. We detected three or four cospeciation events depending on the selected cost of host switching. Three of the same cospeciation events were identified regardless of the host switching costs: a cospeciation event

for *B. torquatus*, followed by a cospeciation event for *B. tridactylus* and a third cospeciation event between *B. variegatus* and *B. pygmaeus*. The early cospeciation event of *B. torquatus* and its algae is supported by the early divergence of *B. torquatus* (19 mya) from the other *Bradypus* species. Additionally, the small geographic range of *B. torquatus* (Wetzel and de Avila-Pires, 1980) suggests that host-switching events are less likely due to fewer encounters with other sloth species. The patristic distance analysis in PACo showed that the codivergence between the sloths and their algae is heavily influenced by some of the *B. torquatus*-algae links, further supporting cospeciation. The strong influence of the *torquatus*-algae links on the co-phylogenetic structure may be the result of the restricted distribution and early divergence of *B. torquatus*.

Cospeciation is more likely to occur when hosts have a patchy distribution and the symbiont has poor dispersal ability (Hafner et al., 2003). In the case of sloths, both the host and symbiont (algae) would be considered poor dispersers, suggesting that cospeciation should be a common method of algae speciation. The rarity of cospeciation events may be the result of several sloth species having broad geographic ranges and co-occurring in the same areas. For example, at our study site in Costa Rica, two- and three- toed sloths are found inhabiting the same small area. The rarity in cospeciation events we observed between sloth and algae is typical of cophylogenetic studies as only 7% of cospeciation studies show true cospeciation (de Vienne et al., 2013). In the 7% of studies that identified cospeciation, the event is usually found in cases of mutualism and vertical transmission of a symbiont. The algae in sloth fur is considered to be vertical transmission from parent to offspring (Suutari et al., 2010) and Pauli et al. (2014) suggested that the sloth-algae relationship is mutualistic. Cospeciation was an a priori expectation as the dominate method of algae speciation, but we did not observe this pattern in our study.

The cospeciation events only occurred within the *Bradypus* lineage. The algae in sloth fur are more abundant in *Bradypus* compared to *Choloepus* (Pauli et al., 2014). The benefit of algae

to sloths is likely to be reduced predation from enhanced crypsis while occupying tree canopies (Chiarello, 2008). Also, this algae is highly digestible and has high carbohydrate and lipid content and could be a source of nutrition for the highly specialized *Bradypus* (Pauli et al., 2014). In contrast, the two-toed sloths are more vagile than three-toed sloths and utilize a greater range of food resources (Mendoza et al., 2015), thus may not be strictly dependent on algae for either nutrition or camouflage. The dependency of *Bradypus* on algae may have maintained the cospeciation events in three-toed sloths and explain why no cospeciation events were found in *Choloepus*. The hair structure of *Bradypus* and *Choloepus* differ, with the former having transverse cracks which allows for absorption of rain water and promotes the growth of algae (Gilmore et al., 2001). Although the fur of *Choloepus* does not facilitate the growth of algae, the hairs have longitudinal ridges which may allow algae to rest in the grooves (Aiello, 1985). The host-switching of algae from *Bradypus* to *Choloepus* may be facilitated by convergent evolution of unique fur characteristics that allow for algae to become embedded in the hairs. Convergent evolution is considered the mechanism for other similar traits in both lineages, e. g. inner ear structure (Billet et al., 2013), suspensory posture and locomotion (Nyakatura, 2012), pectoral girdle (Nyakatura and Fischer, 2011).

Duplications and intra-host divergence were the main method in which the algae speciated. For a duplication event to occur, the symbionts found on the host species should be genetically divergent (Banks et al., 2006); therefore, the presence of genetically divergent algae found on the same sloth species support the duplication hypothesis. Our phylogenetic algae tree indicated that genetically divergent algae do reside on the same species. Furthermore, Suutari et al. (2010) found similar results with two classes of green algae, Ulvophyceae and Trebouxiophyceae, present on *B. torquatus* and *B. tridactylus*. When we weighted host-switches as less costly than duplication events, host-switching was as likely as duplication for the pattern of algae speciation. Although sloths do have overlapping geographic ranges, host-switches

between sloths in Costa Rica and French Guiana, as suggested in our second analysis, seems unlikely given the poor dispersal capability of sloths and the low likelihood that sloths separated by large geographic distances would come into contact. Although the distribution of sloths may have been larger in the past, the genetic differentiation and divergence timing of Central and South American sloths suggest that sloths in Costa Rica and French Guiana did not likely come into contact after diverging 7.7 mya. However, the frequency of host-switching events often exceed cospeciation events (de Vienne et al., 2013) as we observed with both cost-event tests for sloths. The host switches detected in the default parameter test mainly occurred between species that are likely to encounter one another, i.e. *C. hoffmanni* and *B. variegatus* in Costa Rica. Despite the lack of calibration dates in the algal tree, results from the default cost analysis suggesting coevolution seems more plausible. Additionally, assigning a higher cost to host-switching, as in the default cost analysis, may be justified as algae do not have a dispersal phase (Desdevises et al., 2002).

Our study supports codivergence between sloths and the algae in their fur and suggests that cospeciation may be an important mechanism of algae diversification in *Bradypus*. The benefits of the algae and the suitable habitat for algal growth in the fur of sloths support a coevolved mutualistic relationship. The relationship between sloths and the pyralid moths and the other phoretic microbiota needs further investigation to determine if the relationship is a coevolved. Sloths harbor a large number of symbionts that not only rely on the sloth as a host but also have their own possible coevolved links. The interactions between the diverse types of symbionts found on a sloth form a community likely with complex ecological relationships. A community approach would be needed to obtain a complete picture of the mutualistic, commensal and antagonistic interactions occurring on a sloth.

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505 Table 1. Best evolutionary model selected using the AICc in JModelTest.

506

Model		
Sloth		
16S “full”	TrN+G	(Tamura and Nei 1993)
12S “full”	TrN+I+G	(Tamura and Nei 1993)
16S “extant”	TrN+G	(Tamura and Nei 1993)
12S “extant”	TrN+G	(Tamura and Nei 1993)
16S “algae match”	TrN+I	(Tamura and Nei 1993)
12S “algae match”	TrN+G	(Tamura and Nei 1993)
Algae		
18S “all sloths”	GTR+G	
18S “sloth match”	GTR+G	

507 Figure 1. Tanglegram between sloths (black) and their fur algal communities (blue). Sloths are
508 labeled by species name. *Hoffmanni* is represented in the sloth tree by two sampling locations,
509 Costa Rica (CR) and Panama (PA) which are listed after the species name. The algae are labeled
510 by the sloth species from which the sequences was obtained. Lines connecting the sloth and
511 algae indicate associations.

512 Figure 2. The species maximum clade credibility tree for extant sloths mapped onto geographic
513 location. The tips of the tree and lines are colored by the geographic, collection location. Stars
514 represent posterior probabilities ≥ 0.99 . The numbers below the branch nodes are mean
515 divergence times in million years ago. The tree suggests a genetic correlation along latitudinal
516 gradient.

517 Figure 3. The 18S maximum clade credibility tree for all sloth algae from our study and Suutari
518 et al. (2010) mapped onto geographic location. Branch tips or internal node for a group
519 consisting of algal sequences from the same sloth species are labeled with the sloth host species.
520 The tips of the tree and lines are colored by collection location. Stars represent posterior
521 probabilities ≥ 0.99 .

522 Figure 4. The result from one of six cost solutions from the default reconciliation analysis
523 between sloths (black lines) and algae (blue lines). The best scenario has a total of 37 event-costs
524 consisting of: 4 cospeciations, 18 duplications, 6 host switches, 2 losses and 5 failures to diverge.

525 Figure 5. Bar chart showing the individual contribution of a sloth to the Procrustes sums of
526 squares. The bars are the jackknifed squared residuals with error bars showing the upper 95%
527 confidence interval. The dashed line indicates the median sums of squares value.

Sloth Bayesian multilocus tree

Algae Bayesian 18S tree

Choloepus hoffmanni

Bradypus variegatus

Algae embedded in
Choloepus hoffmanni hair