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Biogeography Explains Cophylogenetic Patterns in Toucan Chewing Lice

JASON D. WECKSTEIN[1]

Department of Biological Sciences and Museum of Natural Science, 119 Foster Hall, Louisiana State University, Baton Rouge, Louisiana 70803-3216, USA; E-mail: jweckst@inhs.uiuc.edu

Abstract.—Historically, comparisons of host and parasite phylogenies have concentrated on cospeciation. However, many of these comparisons have demonstrated that the phylogenies of hosts and parasites are seldom completely congruent, suggesting that phenomena other than cospeciation play an important role in the evolution of host–parasite assemblages. Other coevolutionary phenomena, such as host switching, parasite duplication (speciation on the host), sorting (extinction), and failure to speciate can also influence host–parasite assemblages. Using mitochondrial and nuclear protein-coding DNA sequences, I reconstructed the phylogeny of ectoparasitic toucan chewing lice in the *Austrophilopterus cancellosus* subspecies complex and compared this phylogeny with the phylogeny of the hosts, the *Ramphastos* toucans, to reconstruct the history of coevolutionary events in this host–parasite assemblage. Three salient findings emerged. First, reconstructions of host and louse phylogenies indicate that they do not branch in parallel, and their cophylogenetic history shows little or no significant cospeciation. Second, members of monophyletic *Austrophilopterus* toucan louse lineages are not necessarily restricted to monophyletic host lineages. Often, closely related lice are found on more distantly related but sympatric toucan hosts. Third, the geographic distribution of the hosts apparently plays a role in the speciation of these lice. These results suggest that for some louse lineages biogeography may be more important than host associations in structuring louse populations and species, particularly when host life history (e.g., hole nesting) or parasite life history (e.g., phoresis) might promote frequent host switching events between syntopic host species. These findings highlight the importance of integrating biogeographic information into cophylogenetic studies. [*Austrophilopterus*; biogeography; cophylogeny; Phthiraptera; Ramphastidae.]

Historically, biologists assumed that because of the tight associations between many hosts and parasites, cospeciation was the most important factor structuring host–parasite assemblages (Hoberg et al., 1997). Chewing lice are an extreme example of this tight association because they spend their entire life cycle on the host, have limited dispersal abilities, and cannot survive for long off of the host (Kellogg, 1913; Marshall, 1981). As a result of this apparent host specificity, cospeciation has been favored as the main mechanism influencing parasite evolution (Hoberg et al., 1997). Moreover, parasites such as chewing lice were often used to infer host phylogenies (Page, 2003). As the number of comparisons of host and parasite phylogenies has increased, so has the number of demonstrations that the phylogenies of hosts and parasites are seldom completely congruent (Barker, 1991; Johnson et al., 2001, 2003; Page, 2003). These findings have led to the realization that other coevolutionary phenomena, such as host switching, parasite duplication (speciation on the host), sorting (e.g., extinction), and failure to speciate, can be just as important as cospeciation in influencing the structure of host–parasite assemblages (Barker, 1991; Johnson and Clayton, 2003; Johnson et al., 2003). By studying different parasite and host groups with varying life history characteristics, we can observe a wide range of cophylogenetic patterns. Additional studies, particularly those involving multiple parasite lineages on the same hosts (Page et al., 1996; Johnson and Clayton, 2003), should be particularly effective at shedding light on the effects that different life histories and ecologies of hosts and parasites have on cophylogenetic history.

In several cases, chewing lice have a geographical distribution that is not closely tied to a host distribution (Clay, 1964; Barker and Close, 1990; Clayton, 1990). For example, at least two species of chewing lice from the genus *Pectinopygus* are found on both the brown booby (*Sula leucogaster*) and the red-footed booby (*S. sula*). Rather than being host specific, each chewing louse species is found on both host species, but only within a limited geographic region (Clay, 1964). Apparently, chewing lice are transferred between these two overlapping host species, causing them to share the same chewing louse species. In this case, major biogeographic barriers limit dispersal of the hosts and therefore limit the distribution of their chewing lice, structuring louse species geographically. Where parasites lack host specificity and host and louse phylogenies show little evidence of cospeciation, analyses of louse biogeographic patterns using louse phylogenies have the potential to identify cases in which biogeography is more important than host association in structuring phylogenetic history of lice.

Here, I compared the phylogeny of the *Ramphastos* toucans with the phylogeny of *Austrophilopterus* chewing lice to reconstruct their coevolutionary history. The *Ramphastos* toucans are large hole-nesting birds in the order Piciformes (woodpeckers and allies) that range from Mexico south to Argentina. *Ramphastos* toucans have traditionally been divided into two groups based on bill shape and vocalizations: the channel-keel-billed toucans with croaking vocalizations and smaller bodies (except *R. toco*, which has a large body), and the smooth-billed toucans with yelping vocalizations and relatively larger

[1]Present address: Illinois Natural History Survey, Center for Biodiversity, 607 E. Peabody Drive, Champaign, Illinois 61820-6970.

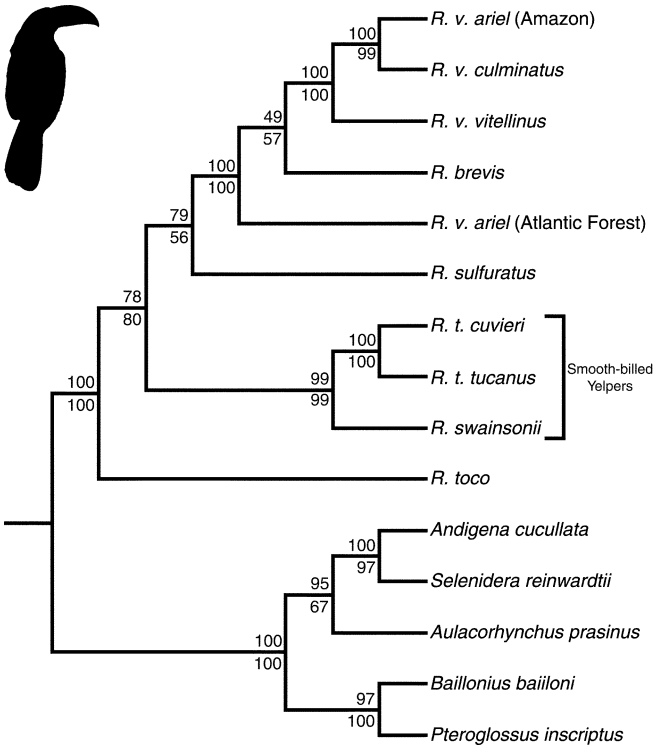

FIGURE 1. Pruned phylogeny of *Ramphastos* toucans from Weckstein (2003), based on maximum parsimony MP and ML analyses of 2,493 base pairs of mitochondrial DNA. For analyses in this study, the tree was pruned to include only a single individual of each taxon from which lice were sequenced. Numbers above the line are ML bootstrap values based on 100 bootstrap replicates using the model TVM+I+G (A ↔ C = 1.3537; A ↔ G = 24.0063; A ↔ T = 1.9397; C ↔ G = 0.5697; C ↔ T = 24.0063; G ↔ T = 1.00), unequal base frequencies (A = 0.2931, C = 0.3925, G = 0.1050, T = 0.2094), rate heterogeneity according to a gamma distribution (shape parameter = 1.2302), and proportion of invariant sites of 0.5388. Numbers below the line are based on 1,000 equally weighted parsimony bootstrap replicates. The bracket identifies the smooth-billed yelping clade. All other *Ramphastos* are channel-keel-billed croakers.

bodies (Haffer, 1974). The smooth-billed yelping *Ramphastos* are monophyletic in phylogenies estimated using equally weighted parsimony and maximum likelihood (ML) analyses, whereas the channel-keel-billed croaking group are monophyletic to the exclusion of *R. toco* (Fig. 1; Weckstein, 2003). At most lowland Neotropical locations, two species of *Ramphastos*, usually one channel-keel-billed croaker and one smooth-billed yelper, are sympatric and even syntopic, which is a situation that might facilitate host switching for their parasites.

Austrophilopterus lice are members of the phthirapteran suborder Ischnocera and are restricted to toucans. Ischnoceran lice are often extreme habitat specialists, spending almost their entire life in a specific niche on the host's body and feeding almost exclusively on feathers and dermal debris (Marshall, 1981). Members of the genus *Austrophilopterus* have a short, round body with a large triangular head and specialize on the shorter, narrower feathers of the host's head and neck (Clay, 1949). Ischnoceran lice, such as *Austrophilopterus*, are relatively short legged and highly sedentary (Marshall, 1981) and apparently do not leave their host readily under their own power, even when the host dies (Keirans, 1975; Marshall, 1981). Lice may transfer from one host to another via (1) vertical transmission from parents to offspring, (2) horizontal transmission between mates, or (3) horizontal transmission without physical contact among hosts. Horizontal transmission without host physical contact can occur via mechanisms such as nest hole takeovers, in which one host takes over the nest hole of another host, or phoresis, which is a short-lived association between two parasite species in which one attaches itself to the other solely for the purpose of transport.

Austrophilopterus lice might disperse between toucan species via nest hole takeovers or phoresis. For example, *Austrophilopterus* lice have been recorded in phoretic association with larger, winged hippoboscid flies (Diptera: Hippoboscidae) that fly between hosts (Kierans, 1975; Marshall, 1981), which may offer a means of dispersal between syntopic host species. Furthermore, there are numerous cases of pairs of different toucan species nesting in close proximity, and interspecific takeovers of nests by other toucan species have also been recorded (Short and Horne, 2002).

To reconstruct cophylogenetic patterns in toucan lice, I compared DNA sequences to estimate phylogenies for hosts and parasites. The apparent host specificity and specialization of *Austrophilopterus* on their toucan hosts implies that *Austrophilopterus* should track the speciation patterns of their hosts. However, if the hole-nesting behavior of the hosts and the ability of *Austrophilopterus* to disperse via phoresis on hippoboscid flies facilitates host switching, then the phylogenies of *Austrophilopterus* and *Ramphastos* should show little parallel speciation and substantial host switching. The cophylogenetic comparisons in this study shed light on the prevalence of the five types of cophylogenetic processes (cospeciation, host switching, parasite duplication, sorting, and failure to speciate) that are possible in this system, which may ultimately help to explain how life history characteristics of the hosts and parasites, such as hole nesting and phoretic associations, affect cophylogenetic interactions.

METHODS

Samples, PCR, and DNA Sequencing

Lice were collected from freshly killed host specimens using the postmortem ethyl acetate fumigation and ruffling method (Clayton et al., 1992; Clayton and Drown, 2001). Individual hosts were immediately isolated in plastic bags upon collection, and working surfaces were thoroughly cleaned between host fumigation and ruffling to insure that louse samples were not contaminated. Louse specimens were stored either frozen at −70°C or in 95–100% ethanol, and DNA was extracted using a Dneasy extraction kit (Qiagen, Valencia, CA) and the voucher extraction method of Johnson et al. (2003). With each set of louse extractions, I included a negative control to test for contamination of extraction kit solutions.

TABLE 1. Collecting localities, voucher numbers, and host associations of louse specimens used in this study.

Louse species	Voucher no.	Host species	Locality
Austrophilopterus cancellosus 1	3.13.01.1	*Ramphastos t. tucanus*	Brazil: Pará
A. cancellosus 1	3.13.01.4	*Ramphastos t. cuvieri*	Brazil: Amazonas
A. cancellosus 1	5.30.01.1	*Ramphastos t. cuvieri*	Brazil: Mato Grosso
A. cancellosus 1	5.30.01.2	*Ramphastos t. cuvieri*	Brazil: Pará
A. cancellosus 1	5.30.01.3	*Ramphastos t. cuvieri*	Peru
A. cancellosus 1	1.4.03.1	*Ramphastos t. cuvieri*	Peru
A. cancellosus 1	1.4.03.3	*Ramphastos t. cuvieri*	Peru
A. cancellosus 1	3.13.01.2	*Ramphastos toco*	Bolivia
A. cancellosus 1	4.1.01.2	*Ramphastos v. ariel* (Amazon)	Brazil: Pará
A. cancellosus 1	4.1.01.6	*Ramphastos v. culminatus* > *ariel*	Brazil: Mato Grosso
A. cancellosus 1	3.13.01.3	*Ramphastos v. culminatus*	Brazil: Amazonas
A. cancellosus 1	1.4.03.5	*Ramphastos v. culminatus*	Peru
A. cancellosus 1	1.4.03.6	*Ramphastos v. culminatus*	Peru
A. cancellosus 2	1.27.1999.1	*Pteroglossus torquatus*	Mexico
A. cancellosus 2	5.30.01.5	*Pteroglossus beauharnaesii*	Brazil: Mato Grosso
A. cancellosus 2	5.30.01.8	*Pteroglossus i. humboldti*	Brazil: Amazonas
A. cancellosus 2	5.30.01.12	*Pteroglossus flavirostris mariae*	Peru
A. cancellosus 3	5.30.01.4	*Pteroglossus i. inscriptus*	Brazil:Mato Grosso
A. cancellosus 3	5.30.01.6	*Pteroglossus bitorquatus*	Brazil: Mato Grosso
A. cancellosus 3	5.30.01.7	*Pteroglossus aracari*	Brazil: Pará
A. cancellosus 4	1.27.1999.12	*Ramphastos sulfuratus*	Mexico
A. cancellosus 4	1.17.2000.6	*Ramphastos brevis*	Ecuador
A. cancellosus 4	4.1.01.3	*Ramphastos swainsonii*	Panama
A. cancellosus 4	4.1.01.4	*Ramphastos sulfuratus*	Mexico
A. cancellosus 5	4.1.01.1	*Ramphastos v. ariel* (SE Brazil)	Brazil: Sao Paulo
A. cancellosus 6	4.1.01.5	*Ramphastos v. vitellinus*	Brazil: Pará
Outgroup			
A. spinosus 1	1.17.2000.9	*Aulacorhynchus derbianus*	Peru
A. spinosus 1	5.30.01.9	*Aulacorhynchus derbianus*	Peru
A. spinosus 1	5.30.01.10	*Aulacorhynchus prasinus*	Peru
A. spinosus 2	1.4.03.7	*Aulacorhynchus coeruleicinctus*	Bolivia
A. andigenae	1.13.03.2	*Andigena hypoglauca*	Peru
A. pacificus	1.17.2000.8	*Andigena nigrirostris*	Peru
A. sp. 1	1.17.2000.7	*Selenidera gouldii*	Brazil
A. sp. 2	5.30.01.13	*Selenidera reinwardtii*	Peru
Picicola snodgrassi	10.5.1999.8	*Melenerpes carolinensis*	Louisiana
P. porisma	10.17.2000.5	*Colaptes auratus*	New Mexico
P. capitatus	2.3.1999.10	*Dendropicos fuscescens*	South Africa
P. sp.	4.11.2000.9	*Mesopicos pyrrhogaster*	Ghana
P. sp.	1.17.2000.1	*Nystalus chacuru*	Bolivia
P. sp	1.17.2000.3	*Monasa nigrifrons*	Bolivia
P. sp	1.17.2000.10	*Galbula albirostris*	Brazil
P. sp	1.17.2000.12	*Chelidoptera tenebrosa*	Brazil
Degeeriella carruthi	9.8.1999.7	*Falco sparverius*	Utah

I amplified and sequenced DNA from 26 *Austrophilopterus* lice collected from 10 *Ramphastos* toucans and 7 araçaris, which are small toucans in the genus *Pteroglossus* (see Table 1 for host associations, voucher numbers, and collecting localities). For the phylogenetic reconstructions of the *Austrophilopterus cancellosus* group, I also included *Austrophilopterus* lice from the host genus *Pteroglossus* because previous studies have shown that they are closely related to the *A. cancellosus* found on *Ramphastos* (Johnson et al., 2002). When possible for the ingroup, I used multiple individual lice from each host species but from different individual hosts and as many localities as possible. Based on the findings of Johnson et al. (2002), I chose outgroup taxa consisting of members of *Austrophilopterus*, *Picicola*, and *Degeeriella* (Table 1).

I followed the species level taxonomy of Carriker (1950, 1967) and Carriker and Diaz-Ungria (1961). However, mitochondrial DNA (mtDNA) and nuclear gene sequences revealed divergent monophyletic groups within morphological species of lice. The uncorrected mtDNA sequence divergences between these clades ranged from 9.5% to 18.7%. However, within these monophyletic groups uncorrected mtDNA sequence divergence was relatively low, ranging from 0% to 3.9%. I did not assign names to these monophyletic groups because taxonomic revision was not the goal of this study. However, within each morphological species, I designated each monophyletic group with a number (e.g., *A. cancellosus* 1) and used these numbered groups as terminals for the cospeciation analyses (see Johnson et al., 2003, for rationale).

For all louse specimens, I amplified and sequenced 379 base pairs (bp) of the mitochondrial gene cytochrome oxidase I (COI) using primers L6625 and H7005 (Hafner et al., 1994) and 347 bp of the nuclear protein-coding

gene elongation factor 1α (EF-1α) using primers EF1-For 3 and EF1-Cho10 (Danforth and Ji, 1998). For PCR, I used the same amplification temperature regime as Johnson et al. (2002) and purified PCR products with a QIAquick PCR purification kit (Qiagen). I used the ABI Big Dye kit (version 2, Applied Biosystems, Foster City, CA) and approximately 75 ng of purified PCR product to perform cycle sequencing reactions. Unincorporated dyes were removed from sequencing reaction products using Centrisep columns (Princeton Separations, Adelphia, NJ) repacked with Sephadex G-50, and these sequencing reaction products were run on an ABI 377 DNA automated sequencer (Applied Biosystems). I used Sequencher (version 3.1, GeneCodes Co., Ann Arbor, MI) to reconcile double-stranded sequences and to align sequences for phylogenetic analyses. All sequences used in this study and their associated voucher number, host species, host voucher number, and collecting locality data are deposited in GenBank (AY430425–AY430482, AF447184–AF447188, AF447196, AF447201–AF447208, AF444846–AF444850, AF444860, AF444866–AF444873).

Phylogenetic and Cophylogenetic Analyses

The host phylogenetic trees used for cophylogenetic analysis were taken from Weckstein (2003). I constructed louse phylogenetic trees using maximum parsimony (MP), ML, and Bayesian analyses as implemented in PAUP* (version 4.0b10; Swofford, 2001) and MrBayes (version 2.01; Huelsenbeck and Ronquist, 2001). Uncorrected pairwise p-distances were calculated using PAUP*. I used the partition homogeneity test (Farris et al., 1994, 1995) as implemented in PAUP* to compare phylogenetic signal and test for incongruence between the COI and EF-1α data sets. When one partition experiences multiple substitutions or contains random information (Dolphin et al., 2000; Barker and Lutzoni, 2002) or when evolutionary rates of partitions are different (Darlu and Lecointre, 2002), the partition homogeneity test can produce erroneous significant results. However, this is not a problem when the phylogenetic trees produced from separate partitions are completely compatible. Therefore, I also ran separate MP analyses for each data partition to assess the compatibility of these trees.

For the MP analysis, all characters were treated as unordered and weighted equally and trees were built using a heuristic search with tree bisection–reconnection (TBR) branch swapping and 100 random addition replicates. I also ran an MP bootstrap analysis using 1,000 heuristic search replicates with TBR branch swapping and 10 random additions per replicate (Felsenstein, 1985).

For ML analyses, I selected the simplest model of sequence evolution that could not be rejected in favor of a more complex model by using the general procedure described by Cunningham et al. (1998) and Huelsenbeck and Crandall (1997), which is implemented in the program Modeltest (version 3.06; Posada and Crandall, 1998). I also used Modeltest to obtain ML model parameters, including empirical base frequencies, rate substitution parameters and the gamma distribution shape parameter. To evaluate the statistical support for branches in the likelihood tree, I used 100 bootstrap replicates with TBR branch swapping and one random addition per replicate.

For Bayesian analysis, I used a site-specific gamma model with six data partitions consisting of the three codon positions for both COI and EF-1α. I did not define model parameter values a priori; instead, they were estimated as part of the analysis. I initiated the Bayesian analysis with random starting trees and ran each analysis for 4.0×10^6 generations with four incrementally heated Markov chains and the default heating values. Trees were sampled from the Markov chains every 1,000 generations. I performed a second independent Bayesian analysis using these same settings to confirm that the initial analysis had adequate convergence and mixing after burn-in. For both analyses, log-likelihood scores of all sampled trees were plotted against generation time to determine when log-likelihood values reached a stable equilibrium (Huelsenbeck and Ronquist, 2001). All trees sampled prior to this equilibrium point were discarded as burn-in (Leaché and Reeder, 2002).

I performed reconciliation analysis, as implemented in TreeMap 1.0 (Page, 1995), to compare host and parasite trees (Page, 1990a). Reconciliation analysis provides a visualization of the relationship between host and parasite trees. In a case of perfect cospeciation, the parasite tree perfectly tracks the host tree. Incongruence between host and parasite trees can be explained and visualized by postulated sorting or duplication events. I also randomized each parasite tree 10,000 times with respect to each host tree to determine whether cospeciation was reconstructed more than expected by chance alone (Page, 1990b). However, reconciliation analysis does not allow for host switching, so I used TreeFitter 1.0 (Ronquist, 1998), an event-based parsimony method that infers host–parasite associations by searching for minimum-cost or maximum-benefit reconstructions and estimates the number of cospeciation (codivergence), duplication, sorting, and host-switching events to explore other potential reconstructions for these cophylogenetic events. Using TreeFitter 1.0, I permuted the parasite trees on the host trees to test whether the number of these cophylogenetic events was greater or less than what would be expected by chance. For TreeFitter analyses, I set cost of cospeciation to 0 and costs of duplication and sorting to 1 and varied the cost of host switching from 1 to 10 (Johnson et al., 2003). For both TreeMap and TreeFitter, host and parasite phylogenies need to be fully resolved. Therefore, I performed these cophylogenetic analyses using one host tree topology (MP and ML optimal topologies were the same) and two parasite tree topologies (MP, ML), for a total of two tree comparisons.

I used MacClade (version 3.07; Maddison and Maddison, 1992) to map and reconstruct biogeographic distributions onto louse phylogenies and to perform Maddison and Slatkin's (1991) randomization procedure to test whether biogeography contains significant phylogenetic signal. Biogeographic areas used in this analysis

were based on those identified by Cracraft (1985), including Central America + Chocó, Atlantic forest, and four quadrants of the Amazon Basin: Napo, Inambari, Guyana, and Rondônia + Pará, which are divided by major riverine barriers (Amazon/Solimões, Madeira, and Negro rivers) (Haffer, 1992, 1997). I combined a few of Cracraft's (1985) areas of endemism because divergences among toucan chewing lice from these areas were extremely low. For the Maddison and Slatkin test, I randomized biogeographic region 1,000 times on each of five *Austrophilopterus* louse phylogenetic topologies and compared the number of changes in biogeographic region with a random distribution of changes in biogeographic region on each of these topologies. The five topologies used for this test included parsimony, parsimony bootstrap, ML, ML bootstrap, and Bayesian trees. For these biogeographic analyses, I pruned taxa from the complete louse phylogenetic trees to include only one louse from one individual of each host species from each biogeographic region. This pruning prevented multiple sampling of lice from the same host species within each region, which would bias the test toward rejecting the null hypothesis.

RESULTS

Phylogenetic Analyses

None of the 26 *A. cancellosus* specimens sequenced had identical sequences for both COI and EF-1α. Therefore, all individuals were included in phylogenetic tree reconstructions. Six relatively divergent clades were found among the sequences (Figs. 2, 3) and were used as terminal taxa in cophylogenetic comparisons with the host phylogeny. Two of these clades occurred only on *Pteroglossus* araçaris, and four were found only on *Ramphastos* toucans. Between lice in the ingroup clades, uncorrected sequence divergence (p-distance) ranged from 0% to 18.7% for COI and 0% to 1.2% for EF1α. The average uncorrected sequence divergence within each of these louse clades ranged from 0.7% to 2% for COI and 0% to 0.3% for EF1α. However, uncorrected p-distance (for COI) between host species associated with each louse clade was much higher, ranging from 4.8% to 7.7%. These relatively high divergences between hosts associated with single clades of lice show that relatively distantly related hosts are carrying either the same or closely related lice.

The partition homogeneity test between COI and EF-1α from *Austrophilopterus* indicated that these data partitions were not in significant conflict ($P = 1.00$), and MP trees produced from separate analyses of COI and EF-1α were completely compatible. Therefore, I combined COI and EF-1α data sets for all phylogenetic analyses. The aligned matrix of 726 bp of DNA sequence for 43 lice (17 outgroup, 26 ingroup) provided a total of 245 variable characters, of which 211 were potentially parsimony-informative. Of these potentially parsimony-informative characters, 169 were from COI and 42 were from EF-1α. MP analysis of this combined *Austrophilopterus* data set produced 16 equally parsimonious trees. Most clades shown in a strict consensus of the 16 most-parsimonious trees are also supported by >50% of the bootstrap replicates (Fig. 2). In all 16 most-parsimonious trees, *Austrophilopterus* from *Ramphastos* toucans and *Austrophilopterus* from *Pteroglossus* araçaris were reciprocally monophyletic. However, bootstrapping suggested that the reciprocal monophyly of *A. cancellosus* from *Ramphastos* and *Pteroglossus* is somewhat equivocal. Monophyly of the *A. cancellosus* from *Pteroglossus* was only supported by 52% of parsimony bootstrap replicates. The monophyly of the entire *A. cancellosus* group is supported by 100% of the parsimony bootstrap replicates, consistent with the species-level taxonomy of (Carriker, 1950, 1967; Carriker and Diaz-Ungria, 1967) in which nearly all lice from *Ramphastos* and *Pteroglossus* are assigned to the species *A. cancellosus*. However, the molecular phylogeny is not consistent with

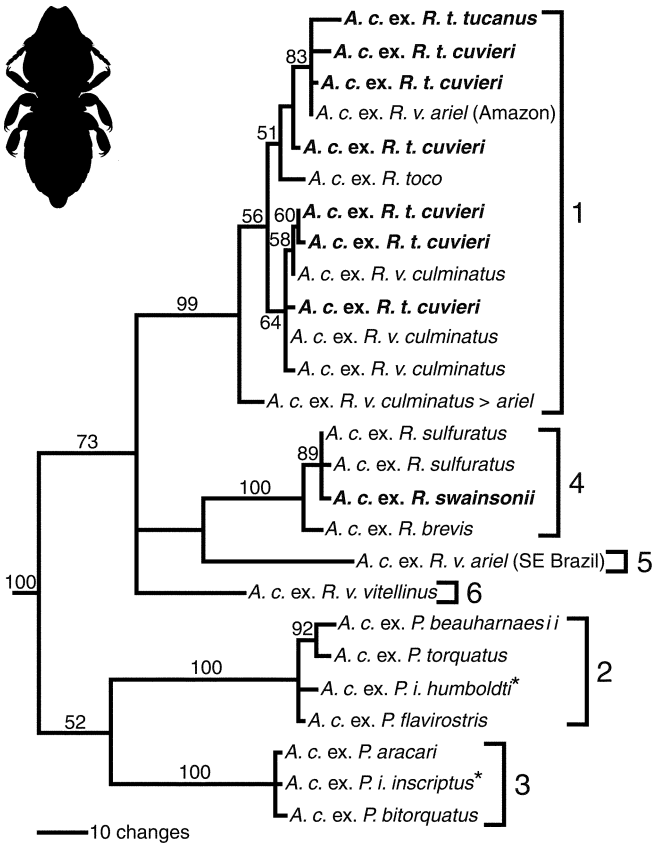

FIGURE 2. Phylogram of strict consensus tree of the 16 most-parsimonious trees (length = 1,000; rescaled consistency index = 0.303) for *Austrophilopterus cancellosus*. Numbers above and below branches indicate support from 1,000 bootstrap replicates. Bootstrap values for unresolved nodes and those values <50% are not shown. Outgroup taxa are not shown. Numbers to the right of brackets refer to arbitrarily numbered clades within *A. cancellosus* defined by their monophyly and relatively high genetic divergences (ranging from 5% to 10%) from other lineages within this morphologically defined louse species. Taxon names in bold identify lice collected from a monophyletic group of smooth-billed yelping *Ramphastos* toucans. The asterisks identify *A. cancellosus* collected from two closely related araçaris, which are smaller toucans in the genus *Pteroglossus*.

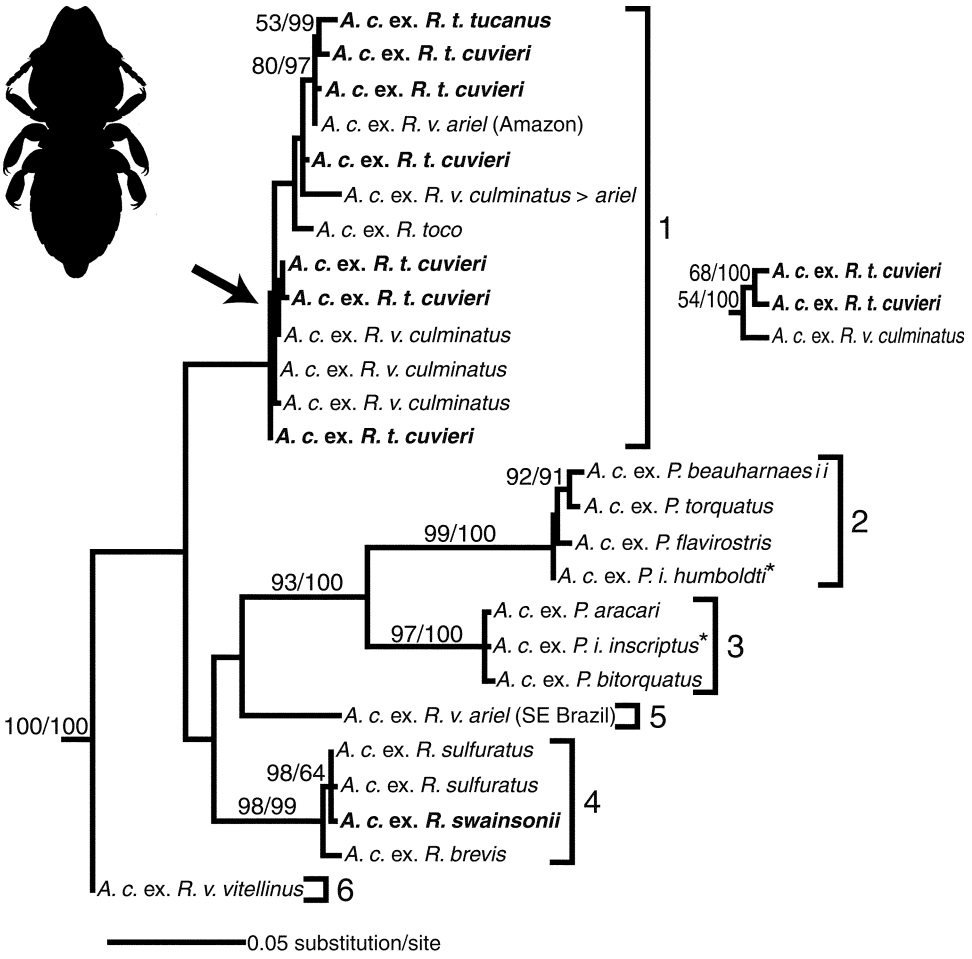

FIGURE 3. ML tree (log-likelihood = 5159.67) for *Austrophilopterus cancellosus* built using the model TVMef+I+G, which includes general time reversible substitutions (A ↔ C = 0.3844; A ↔ G = 8.5383; A ↔ T = 2.9153; C ↔ G = 0.7544; C ↔ T = 8.5383; G ↔ T = 1.00), equal base frequencies, invariant sites (0.5387), and rate heterogeneity according to a gamma distribution (shape parameter = 0.5139). Numbers on the left indicate support from 100 ML bootstrap replicates, and those at the right are Bayesian posterior probabilities. Values are only shown when ML bootstrap values are >50% or Bayesian posterior probabilities are >90%. See clade to the right of bracket 1 for support values from the compressed region of the tree marked by the arrow. Numbers, brackets, and taxon labeling are the same as in Figure 2.

Carriker's subspecific taxonomy in which each host has its own subspecies of louse.

All significant nodes identified by the two independent Bayesian runs were either identical or within two percentage points of one another. Therefore, the posterior probabilities presented here are averages of those produced by these two independent runs. ML and Bayesian topologies are slightly different from the parsimony topology (Fig. 3). In the Bayesian and ML topologies, *A. cancellosus* from *Pteroglossus* might fall within *A. cancellosus* from *Ramphastos*, although the statistical support for this relationship is not strong (ML bootstraps <50%, Bayesian posterior probability <95%). However, parsimony analysis places *A. cancellosus* from the *Pteroglossus* basal to all other *A. cancellosus* from *Ramphastos*. In addition, although parsimony does not strongly support the monophyly of lice from *Pteroglossus*, Bayesian posterior probability (100%) and ML bootstrap replicates (93%) indicate strong support for monophyly of this group.

Two key findings are common to MP, ML, and Bayesian phylogenetic analyses: (1) the monophyly of the *A. cancellosus* group is strongly supported, and (2) lice from closely related hosts (see Figs. 2, 3), such as the monophyletic smooth-billed yelping *Ramphastos* or the closely related araçaris *P. i. inscriptus* and *P. i. humboldti*, are often not closely related.

Cophylogenetic Analyses

Using TreeMap 1.0, I performed two reconciliation analyses (Page, 1990a) between the optimal host tree topology and two parasite tree topologies (MP and ML). In both comparisons, tanglegrams of host and parasite associations are a tangled web, indicating a lack of cospeciation (Fig. 4). Both analyses identified one potential cospeciation event and required three duplications and either 17 or 22 sorting events, depending on whether the MP (17) or ML (22) parasite tree topology was used. For both TreeMap comparisons, the one reconstructed

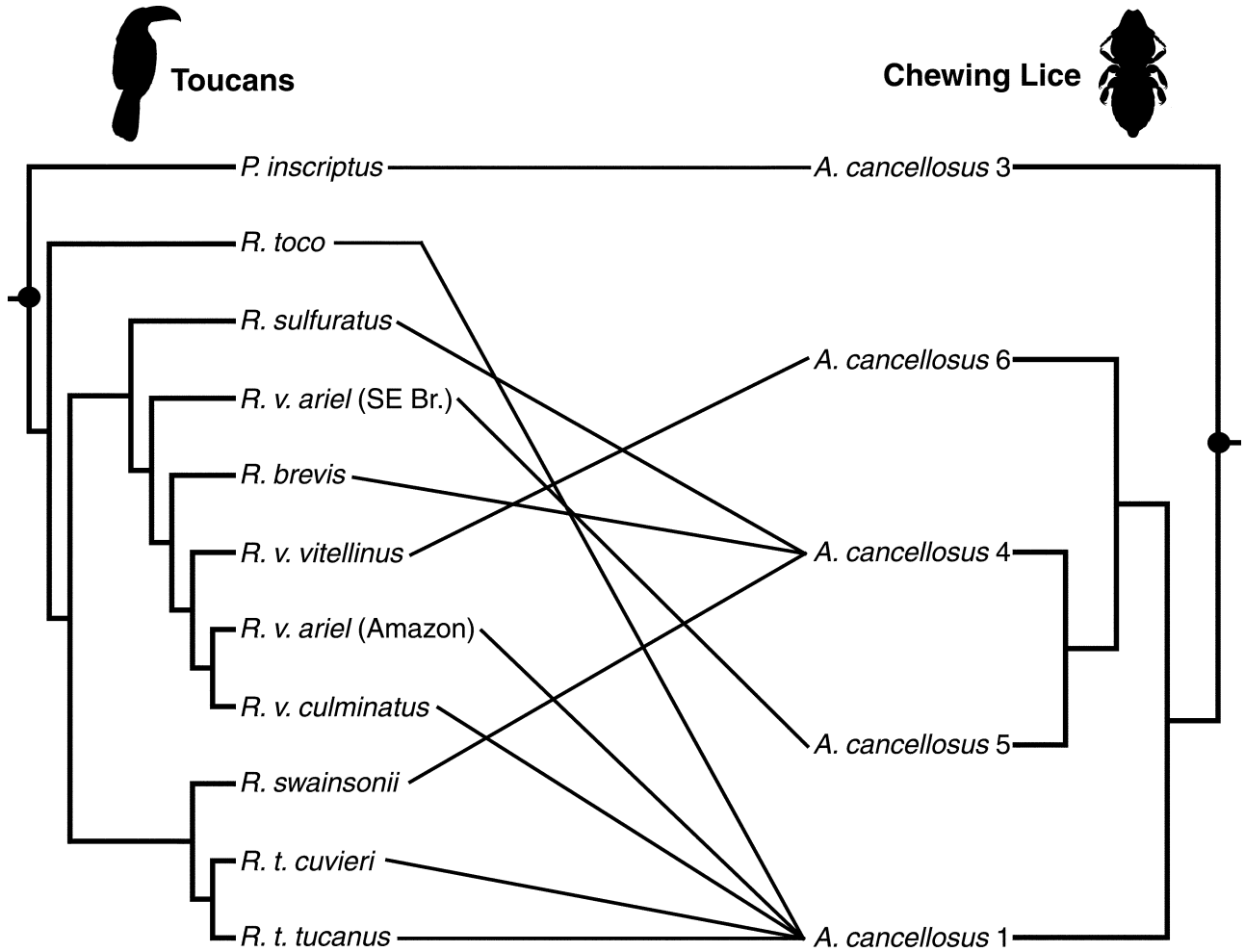

FIGURE 4. A tanglegram comparison (constructed using TreeMap 1.0) of the MP/ML tree topology for toucan hosts (*Ramphastos* and one *Pteroglossus*) and a parsimony topology for *Austrophilopterus* parasites. Lines connecting taxa indicate host–parasite associations. Solid circles on nodes are cospeciation events inferred from reconciliation analysis. The number of cospeciation events (one) was not significantly higher than that expected by chance in 1,000 randomizations of the parasite tree ($P = 0.86$).

cospeciation event is not greater than that expected by chance alone (both cases, $P \geq 0.89$). Analyses using TreeMap failed to detect significant cospeciation.

The results of TreeFitter analyses, using the same topological comparisons, are similar but not identical to the results of the reconciliation analysis. For most host-switching cost settings, few reconstructed cophylogenetic events were significantly different than expected by chance (Table 2). Where significant cospeciation was reconstructed, the number of events was relatively low, ranging from zero to two events, and more than expected by chance. Where significant host switching was reconstructed, the number of events ranged from two to four and was fewer than expected by chance. No sorting or duplication events were significant. However, in cases such as these comparisons between *Austrophilopterus* and *Ramphastos* phylogenies, where individual parasite

TABLE 2. Results of TreeFitter[a] analyses of speciation events in *Austrophilopterus*.

Switching cost	Cospeciation	Duplication	Sorting	Switching
1	0–2[b] (0–1[b])	0 (0)	0–2 (0–1)	2–4[c] (3–4[c])
2	2–3 (1)	0 (0)	2–4 (1)	1–2 (3)
3	3 (1)	0–1 (0)	4–6 (1)	0–1 (3)
4	3 (1–2)	1 (0–1)	6 (1–8)	0 (1–3)
5	3 (2)	1 (1)	6 (8)	0 (1)
6	3 (2)	1 (1)	6 (8)	0 (1)
7	3 (2)	1 (1)	6 (8)	0 (1)
8	3 (2)	1 (1)	6 (8)	0 (1)
9	3 (2)	1 (1)	6 (8)	0 (1)
10	3 (2)	1 (1)	6 (8)	0 (1)

[a]Numbers indicate the number of each event type reconstructed in TreeFitter analysis, with the results from comparison of ML trees in parentheses.

[b]Significantly ($P < 0.05$) more events than expected by chance under given costs.

[c]Significantly ($P < 0.05$) fewer events than expected by chance under given costs.

clades have widespread host distributions, TreeFitter can reconstruct significant cospeciation and few significant host-switching events because the analysis assumes that a widespread parasite is restricted to a single host (Ronquist, 1998). These widespread, nonspecific parasites are therefore constrained to have recent host-switching events, which are not taken into account in the TreeFitter analysis. Therefore, TreeFitter only assesses ancestral and not contemporary host-switching events (Johnson et al., 2003) and may underestimate host-switching events and overestimate cospeciation events. In this analysis, both topological comparisons have six of these terminal host switches/dispersal events, which are not included in the TreeFitter optimal reconstructions.

Geographic Analyses

For all five tree topologies tested, biogeographic region, when mapped onto the phylogeny, is not randomly distributed. In at least four clades, closely related lice are not from closely related hosts but were collected from the same biogeographic region (Fig. 5). *P*-values for the randomization of biogeographic region on each of the five pruned *Austrophilopterus* topologies range from 0.01 to 0.04.

Discussion

Three salient findings emerge when *Ramphastos* toucan and *Austrophilopterus* louse phylogenies are compared. First, the phylogenetic history of *Austrophilopterus* lice does not mirror patterns in the *Ramphastos* toucan phylogenetic history as one would expect if cospeciation were the only process influencing louse diversification (Fig. 4; Page, 2003). Although reconciliation analysis reconstructs a single cospeciation event, that event is not statistically significant ($P \geq 0.83$). The event-based analyses of TreeFitter also reconstructs a low number (zero to two) of cospeciation events. Therefore, there are few if any cospeciation events between *Ramphastos* toucans and their *Austrophilopterus* lice. Second, members of monophyletic louse lineages are not necessarily specific to monophyletic host lineages (Figs. 2, 3). Often, closely related lice are found on sympatric toucan hosts from different toucan clades. For example, lice from the monophyletic smooth-billed yelping toucans do not form a monophyletic group (Figs. 2, 3) as would be predicted if cospeciation were structuring louse diversification; often *Austrophilopterus* from both smooth-billed and channel-keel-billed toucans are members of the same clade (Figs. 2, 3). Third, biogeography apparently plays a role in structuring the *Austrophilopterus* louse phylogeny, indicating that geographic proximity and dispersal are important factors in the speciation of these lice. For example, lice from both smooth-billed toucans and channel-keel-billed toucans collected in western Amazonia south of the Amazon River form a monophyletic group.

These results indicate that some combination of sorting, duplication, failure to speciate, or host switching has contributed to the lack of host specificity within louse clades and the lack of phylogenetic concordance between *Austrophilopterus* and their toucan hosts. In most cases, these alternatives are quite difficult to distinguish. However, several pieces of information suggest that for *Austrophilopterus* lice, host switching is the primary mechanism structuring this host–parasite assemblage. First, TreeFitter identified six terminal (recent) host switching events. For host switching to occur between two different host taxa, they must be sympatric (Barker and Close, 1990) or more specifically syntopic. Therefore, if host switching is common, louse lineages should show geographic structure rather than host-related phylogenetic structure. This lack of host specificity with concomitant biogeographic specificity is expected if host switching is relatively common, because the process of host switching mixes parasites up among hosts within the limits of major biogeographic barriers and keeps the parasites from differentiating on one host species. This pattern has been noted in owls and their lice (*Strigiphilus*), with sympatric owl species sharing one louse species (Clayton, 1990). The *Austrophilopterus* phylogeny with biogeographic region mapped onto it also illustrates this pattern (Fig. 5). Furthermore, for all *Austrophilopterus* phylogenetic topologies tested, biogeographic region shows a nonrandom distribution with significant phylogenetic signal on the phylogeny (*P*-values range from 0.01 to 0.04). Clades of *Austrophilopterus* lice share a common biogeographic region; however, they do not share closely related hosts.

Several characteristics of the life histories of these hosts and parasites would seem to facilitate opportunities for host switching. First, toucans are highly social, hole-nesting birds, and nest holes have been implicated in host switching of ischnoceran lice among species of birds (Hopkins, 1939; Eveleigh and Threlfall, 1976; Clayton, 1990). For toucans, there are numerous cases of multiple pairs of different species nesting in close proximity (Short and Horne, 2002), and interspecific nest hole takeovers can be common (Merilä and Wiggins, 1995), with several recorded for toucans (Short and Horne, 2002). Furthermore, live lice have been recovered from bird nests (Nordberg, 1936) and might survive for a short while off of the host (Johnson et al., 2002) owing to the relatively humid environment inside a nest cavity (Moyer et al., 2002). Second, phoresis, a short-lived association between two species in which one attaches itself to the other solely for the purpose of transport, might also be an important mode of host switching for *Austrophilopterus*. Most Ischnocera, and thus potentially *Austrophilopterus*, cannot disperse under their own power because they are relatively short legged and apparently reluctant to leave the host, even upon the host's death (Marshall, 1981). However, ischnoceran lice, including *Austrophilopterus*, are known to have a phoretic association with hippoboscid flies (Keirans, 1975). In this short-lived association, the chewing louse attaches, using its mandibles, to the body of a larger winged hippoboscid fly, which presumably transports the louse from one host to another.

The relative roles of nest hole takeovers and phoresis in host switching of chewing lice should be examined in

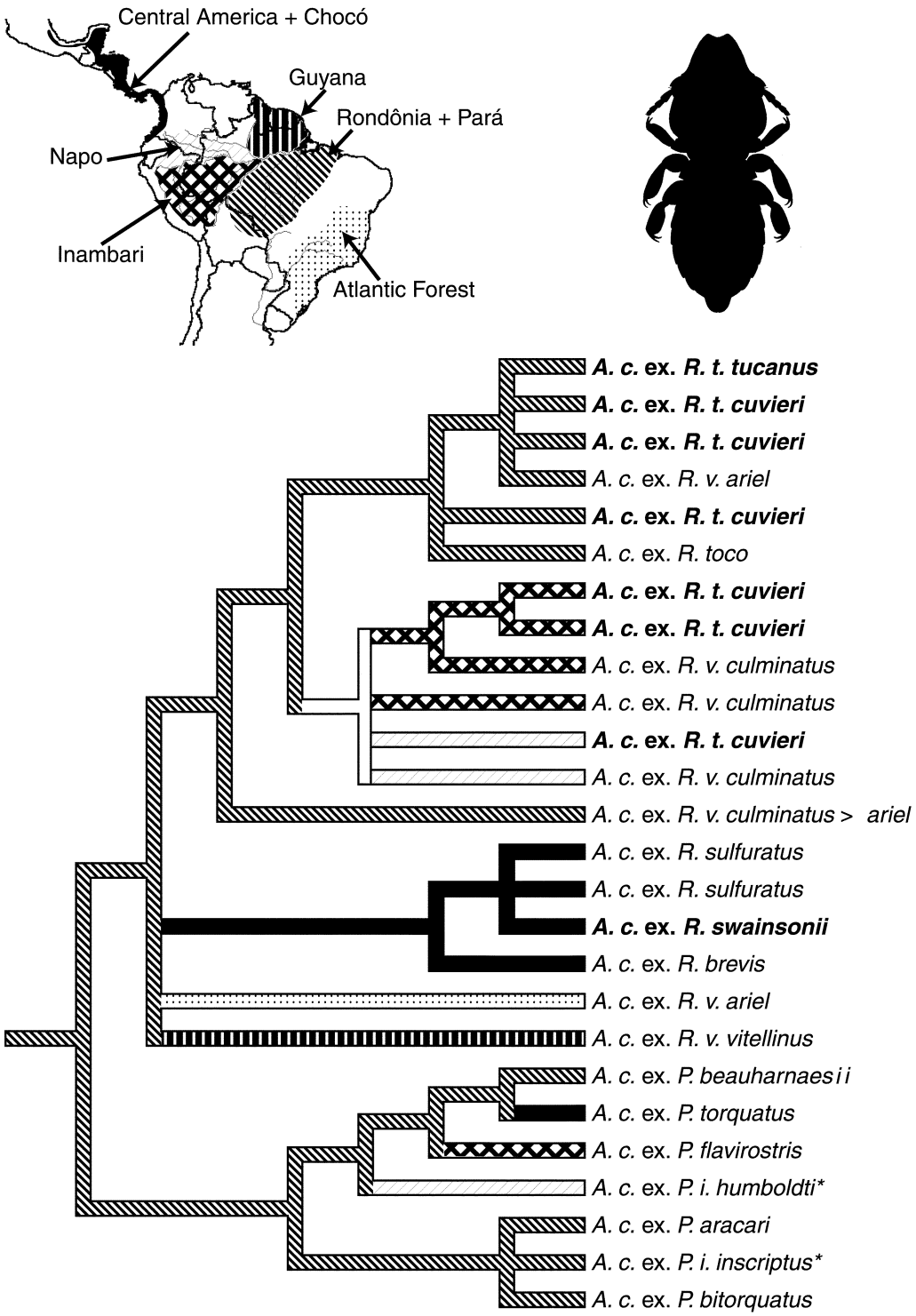

FIGURE 5. Biogeographic regions mapped onto the MP topology. Patterns on branches match approximated biogeographic regions as indicated on the map. These biogeographic regions are based on Cracraft's (1985) areas of endemism, which are combined in some cases. Names in bold identify taxa collected from the smooth-billed yelping *Ramphastos* clade. Asterisks identify *A. cancellosus* from two closely related *Pteroglossus* araçaris.

more detail (Johnson et al., 2002). With the samples used in this study, it is difficult to determine whether nest hole takeovers or phoresis is the predominant mechanism that has facilitated host switching in *Austrophilopterus*. Future comparisons with other toucan louse genera with different dispersal abilities and analyses including lice from host species without sympatric congeners may shed light on these potential modes of host switching. The present study revealed the potential importance of biogeography in structuring the phylogenetic history of lice. In this case, the phylogeny of the host species and the widespread host associations of the parasites led to the conclusion that this system lacks significant cospeciation. The finding that biogeography has a significant effect on the parasite phylogeny indicates that host switching between syntopic hosts is the dominant factor structuring *Austrophilopterus* speciation patterns and cophylogenetic patterns with their hosts. However, the exact method of switching remains unknown. This study underscores the importance of looking at biogeographic patterns in louse phylogenies when these parasites and their hosts lack cospeciation. Through these kinds of comparisons, we can begin to make generalizations about the biological factors that result in cospeciation and to understand how ecology and life history characteristics of hosts and parasites favor other coevolutionary phenomenon such as host switching.

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