J. Parasitol., 99(5), 2013, pp. 827–834

COPHYLOGENY OF QUILL MITES FROM THE GENUS *SYRINGOPHILOPSIS* (ACARI: SYRINGOPHILIDAE) AND THEIR NORTH AMERICAN PASSERINE HOSTS

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ABSTRACT: Species of *Syringophilopsis* quill mites are found in the flight feathers of passerine birds. A phylogeny of species from this genus infecting North American passerines was inferred from the mitochondrial cytochrome oxidase I gene and the nuclear 28S ribosomal RNA gene. Based on the large genetic distance among lineages, the genus appears to be composed of several cryptic species. A reconciliation analysis of these mites and their avian hosts indicates a limited, but significant, degree of cophylogeny. However, strict cospeciation is not found to be occurring in this system.

Little is known about the diversification and evolutionary relationships of the *Syringophilopsis* quill mites. Currently, there are 33 species in this genus, 9 of which are found in North America (Kethley, 1970; Bochkov and Galloway, 2001, 2004; Skoracki et al., 2009). Primarily associated with passeriform birds, these mites live, feed, and reproduce within the confines of the hollow portion of primary and secondary wing feathers (Kethley, 1970). Quill mites must enter through the open superior umbilicus of a developing feather before the feather matures and the umbilicus becomes plugged (Casto, 1974). This suggests that they are transmitted vertically from parent to nestling or during the host molting period (Atyeo and Gaud, 1979; Proctor, 2003). One study found that each species has its own preferred niche within the primary, secondary, and tertiary feathers, which is due to the feeding limitations presented by quill wall thickness and to quill volume limiting the number of dispersants produced (Skoracki et al., 2005). Consequently, limited mobility and dispersal capabilities, in addition to the high degree of host specificity, make it plausible that quill mites may have coevolved with their avian hosts (Proctor and Owens, 2000).

Parasite evolution is greatly influenced by host evolution, and close congruence of host and parasite phylogenies can indicate cospeciation (Fahrenholz, 1913). This has been observed in several cophylogenetic studies of the relationship between mites and their avian hosts. Studies of avenzoariid feather mites and charadriiform birds (Dabert et al., 2001), freyanid feather mites and procellariiform, pelecaniform, anseriform, gruiform, and charadriiform birds (Ehrnsberger et al., 2001), and rhinonyssida-nasal mites and passeriform birds (Morelli and Spicer, 2007) all produced strong statistical evidence of cospeciation. Species of *Pectinophygus*, *Dennyus*, and *Columbicola* and their respective avian hosts have received extensive cophylogenetic scrutiny, with similar findings (Clayton, Bush, Goates, and Johnson, 2003; Johnson and Clayton, 2003; Hughes et al., 2007).

In the present paper, we examine whether cospeciation has occurred between *Syringophilopsis* spp. and their North American hosts or if other ecological factors are driving the evolution of this diverse group of mites. To accomplish this goal, a well-resolved molecular phylogeny of *Syringophilopsis* species from North American passerines was produced using sequences of mitochondrial cytochrome oxidase I (COI) and nuclear DNA 28S ribosomal RNA (28S) genes. Host and parasite trees were then compared and evaluated for patterns of cophylogeny and strict cospeciation. The results of this study provide insight into the evolutionary history of these mites and the relationships to their hosts.

MATERIALS AND METHODS

Specimen collection

Individual birds were collected in California, Texas, and Arizona from 1999 to 2008. All samples were collected under the approval of the Federal Fish and Wildlife Migratory Bird Permit MB092876. Specimens were frozen at –20 C until they were examined. Host identities were made using the American Ornithologists' Union *Checklist of North American Birds*, seventh edition (American Ornithologists' Union, 1998). Remiges and rectrices feathers were extracted from the birds and examined using a dissecting microscope. Mites were visualized by the breakdown of the interior of the calmus and the attendant discoloration. The presence of quill mites was recorded for primary, secondary, and retrice feathers.

Morphological analysis

In the presence of mites, the calmus was opened using a sterile scalpel with the aid of a dissecting microscope. Representative mite specimens were placed in lactophenol and heated at 55 C for 12–24 hr for clearing. Mites were then mounted in Hoyer's media and dried for several days, and the cover slip was sealed with glyptol. Slide-mounted specimens were examined using a Leica DM5000B microscope (Leica Microsystems, Wetzlar, Germany) (10–60×) with phase contrast and identified to genus (Bochkov and Mironov, 1998; Skoracki, 2005; Skoracki and Sikora, 2005; Skoracki et al., 2009). All measurements were made in microns using a Leica reticule. To identify the samples to species, morphological comparisons were made relative to those in the published literature (Bochkov and Mironov, 1998; Skoracki, 2005; Skoracki and Sikora, 2005; Skoracki et al., 2009) for all quill mite species described to date from passerine hosts. Due to limited sampling, some specimens could not be accurately assigned to existing species based entirely on their morphological characters. Those specimens appearing to represent undescribed species (or cryptic species) were inferred by assessing phylogenetic lineages and species boundary threshold limits of the mtDNA COI region (as described below).

DNA amplification and sequencing

In total, 32 mite taxa were sequenced, as seen in Table I. One to 6 quill mites from an individual host were placed into 0.5-ml tubes containing 14% Chelex (Bio-Rad Laboratories, Hercules, California) for DNA extraction. Once the mites were in the Chelex solution, the solution was boiled at 100 C. Samples were frozen in the Chelex solution until PCR use. The mitochondrial cytochrome oxidase subunit I (COI) gene and the nuclear 28S ribosomal RNA (domain 8 through domain 11) gene (28S) were amplified and sequenced for taxa listed in Table I. The universal primers LCO1490 and HCO2198 (Folmer et al., 1994) were used to amplify approximately 500 base pairs (bp) of the mtDNA COI region of all samples except for specimens from the brown creeper (*Certhia americana*) host. Redesigned interior primers 2198BRCR (5′-CCA GGT TCA TTA CTA GAA AAC GA-3′) and 1490BRCR (5′-ACA TAG TAA TTG CTC CGG CAA G-3′) were used to amplify and sequence a slightly

Received 27 November 2009; revised 15 March 2013; accepted 15 April 2013.

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DOI: 10.1645/GE-2400.1

TABLE I. Host and parasite taxa included in the phylogenetic and cophylogenetic analyses. Passerine nomenclature follows the *Checklist of North American Birds*, seventh edition (American Ornithologists' Union, 1998). S. = *Syringophilopsis* and T. = *Torotrogla*.

Mite species	Host species	Specimen number	Collection locality
S. dendroicae A	Grace's Warbler, *Dendroica graciae*	GSS#1593F	Jeff Davis County, Texas
S. dendroicae A	Yellow-rumped Warbler, *Dendroica coronata*	GSS#2220F	Riverside County, California
S. dendroicae A	Yellow-rumped Warbler, *Dendroica coronata*	GSS#1379F	Johnson County, Texas
S. dendroicae B	Grace's Warbler, *Dendroica graciae*	GSS#1614F	Jeff Davis County, Texas
S. elongatus	Brewer's Blackbird, *Euphagus cyanocephalus*	GSS#1574F	Shasta County, California
S. empidonax	Gray Flycatcher, *Empidonax wrightii*	GSS#1326F	Jeff Davis County, Texas
S. empidonax	Hammond's Flycatcher, *Empidonax hammondii*	GSS#1424F	Jeff Davis County, Texas
S. empidonax	Hammond's Flycatcher, *Empidonax hammondii*	GSS#1612F	Jeff Davis County, Texas
S. passerinae	Indigo Bunting, *Passerina cyanea*	GSS#2513F	Trinity County, Texas
S. passerinae	Indigo Bunting, *Passerina cyanea*	GSS#2518F	Trinity County, Texas
S. passerinae	Painted Bunting, *Passerina ciris*	GSS#2551F	Tarrant County, Texas
S. polioptilus	Blue-gray Gnatcatcher, *Polioptila caerulea*	GSS#1354F	Johnson County, Texas
S. polioptilus	Blue-gray Gnatcatcher, *Polioptila caerulea*	GSS#1346F	Johnson County, Texas
S. sialiae	Western Bluebird, *Sialia mexicana*	GSS#1617F	Jeff Davis County, Texas
S. turdus	American Robin, *Turdus migratorius*	GSS#35F	Sierra County, California
S. turdus	American Robin, *Turdus migratorius*	GSS#34F	Sierra County, California
S. tyranni A	Western Kingbird, *Tyrannus verticalis*	GSS#1582F	Lake County, California
S. tyranni A	Western Kingbird, *Tyrannus verticalis*	GSS#1583F	Lake County, California
S. tyranni A	Western Kingbird, *Tyrannus verticalis*	GSS#2156F	Mendocino County, California
S. tyranni A	Western Kingbird, *Tyrannus verticalis*	GSS#2239F	Mono County, California
S. tyranni A	Scissor-tailed Flycatcher, *Tyrannus forficatus*	GSS#2017F	Johnson County, Texas
S. tyranni B	Ash-throated Flycatcher, *Myiarchus cinerascens*	GSS#1342	Jeff Davis County, Texas
S. tyranni B	Ash-throated Flycatcher, *Myiarchus cinerascens*	GSS#2200F	Jeff Davis County, Texas
S. tyranni B	Great-crested Flycatcher, *Myiarchus crinitus*	GSS#1672F	Erath County, Texas
S. sittae	White-breasted Nuthatch, *Sitta carolinensis*	GSS#2579F	Mendocino County, California
S. certhiae	Brown Creeper, *Certhia americana*	GSS#98F	Kern County, California
S. sp. C	Black-headed Grosbeak, *Pheucticus melanocephalus*	GSS#2241F	Mono County, California
S. sp. D	Chipping Sparrow, *Spizella passerina*	GSS#1629F	Jeff Davis County, Texas
S. sp. E	Yellow-headed Blackbird, *Xanthocephalus xanthocephalus*	GSS#1980F	La Paz County, Arizona
S. sp. F	Bullock's Oriole, *Icterus bullockii*	GSS#2118F	Riverside County, California
T. aphelocoma	Western Scrub-jay, *Aphelocoma californica*	GSS#1344F	Jeff Davis County, Texas
T. piranga	Western Tanager, *Piranga ludoviciana*	GSS#1497	Sierra County, California

shorter segment of the same gene fragment from the brown creeper quill mites. Reactions were carried out in a 25-µl reaction mixture containing 1 µl of genomic DNA, 10.5 µl of 25 mM PCR water, 3.5 µl of 25 mM MgCl, 2 µl of 10 mM dNTP, 1.25 µl of 25 mM of each primer, 5 µl of Expand High Fidelity^{PLUS} buffer without MgCl, 0.5 µl of bis(trimethylsilyl) acetamide (BSA), and 0.15 µl of Expand High Fidelity^{PLUS} Taq Polymerase (Roche Applied Science, Indianapolis, Indiana). PCR parameters were as follows: initial denature at 94 C for 2 min followed by 35 cycles of 94 C for 15 sec, 48 C for 1 min, and 68 C for 2 min, followed by a final extension of 68 C for 7 min. Domains 8 through 11 of the ribosomal 28S gene were amplified and sequenced in 2 segments. These primers are redesigned from Hillis and Dixon (1991). The primers for domain 8 to domain 10 were D8Anm (5′-GTT CTG TAA CTT TGG GAG AAG GGA TGG CTC-3′) and D10AR (5′-AGA GTA GTG GTA TTT CA-3′). The primers for domain 9 to domain 11 were D9A (5′-CGG CGG GAG TAA CTA TGA CTC TCT T-3′) and D11AR (5′-CAC GAC GGT CTA AAC CCA GCT CAC-3′). Reactions were carried out in a 25-µl reaction mixture containing 1 µl of genomic DNA, 9.85 µl of 25 mM PCR water, 4 µl of 25 mM MgCl, 2 µl of 10 mM dNTP, 1.25 µl of 25 mM of each primer, 5 µl of Expand High Fidelity^{PLUS} buffer without MgCl, 0.5 µl of BSA, and 0.5 µl Expand High Fidelity^{PLUS} Taq Polymerase (Roche Applied Science). PCR parameters were as follows: initial denature at 94 C for 2 min followed by 35 cycles of 94 C for 30 sec, 65 C for 30 sec, and 72 C for 1 min, followed by a final extension of 72 C for 7 min.

After DNA amplification, PCR products were screened on 1.2% agarose gels, stained with ethidium bromide, and visualized with a UV light source. The PCR products were cleaned using ExoSAP-IT ® (USB Corporation, Cleveland, Ohio). The manufacturer's protocol was slightly modified, i.e., 1 µl of ExoSAP-IT was used per 5 µl of product.

Cycle sequencing was then completed to add fluorescent dyes to the amplified DNA fragment. The cycle sequencing reactions consisted of 5–8 µl of PCR water, 2.2 µl of 5× Buffer, 0.5 µl of one of the original PCR primers, 0.5 µl of Big Dye Terminator v3.1 (Applied Biosystems Incorporation, Foster City, California), and 1–4 µl of cleaned PCR product. Cycle sequence parameters were as follows: initial denature at 94 C for 30 sec followed by 25 cycles of 94 C for 30 sec, 55 C for 30 sec, and 60 C for 4 min. Cycle sequencing products were cleaned by isopropanol precipitation. Cleaned cycle sequence products were resuspended in 15 µl of Hi-Di™ Formamide (Applied Biosystems), denatured at 85 C for 2 min, snap chilled on ice for 5 min, and then loaded into a 96-well plate. Each sample was sequenced in each direction on an ABI Prism 3100 automated sequencer (Applied Biosystems).

Mite phylogenetic analysis

The sequences were initially analyzed using Sequencer 4.8 (GeneCodes Corporation, Ann Arbor, Michigan). ClustalX 2.0.9 (Larkin et al., 2007) was used to complete a preliminary alignment of the sequences. The COI alignment was further tested by translating the sequences into amino acids in MacClade 4.08 OS X (Maddison and Maddison, 2005) and checking for inappropriately placed stop codons. The alignment for the 28S sequences was furthered checked by eye in MacClade.

Phylogenetic analyses were performed using PAUP*Ver.4.0b10 (Swofford, 2001) to infer relationships. Uncorrected pairwise distances (p-distance) were calculated in PAUP* to determine percent difference among and within taxa. Two species from the closely related genus

Torotrogla were chosen as the outgroup taxa (Table I; Johnston and Kethley, 1973).

Parsimony analysis was executed using a heuristic search of 1,000 replicates and the random stepwise addition option with unordered changes of equal weight. If searches produced multiple trees, a strict consensus was performed to summarize data analyses (Rohlf, 1982). Bootstrap analysis was performed using 1,000 replicates of the closest stepwise addition to assess the robustness of the branching pattern of the parsimony trees (Felsenstein, 1985). To test for conflict between different gene data sets, the partition homogeneity test (Incongruence Length Difference [ILD] test; Farris et al., 1995; Cunningham, 1997) was performed using 1,000 replicates in PAUP*.

For maximum likelihood (ML) analyses, ModelTest version 3.7 (Posada and Crandall, 1998) was used to evaluate the most appropriate nucleotide substitution model for the hierarchical likelihood ratio tests (hLTRs) and the Akaike Information Criterion (AIC; Huelsenbeck and Crandall, 1997). The neighbor-joining algorithm was used to generate a Jukes-Cantor tree to obtain initial maximum likelihood model parameter estimates. Upon finding a more likely tree topology, parameters were re-estimated until all model parameters converged on the same maximum likelihood tree. The Shimodaira-Hasegawa test (Shimodaira and Hasegawa, 1999) was used to test for significant differences between genes, and was implemented in PAUP*. The test was completed using the resampling estimated log-likelihood (RELL; Kishino et al., 1990) method with 1,000 bootstrap replicates. The results were evaluated as a one-tailed test. ML bootstrap analyses (Felsenstein, 1985) were performed using the neighbor-joining tree as the starting tree for a heuristic search consisting of 300 replicates. Bayesian analyses, which were performed with MrBayes v3.1.2 (Ronquist and Huelsenbeck, 2003), were also used to evaluate the robustness of the results. The Metropolis-coupled MCMC (MCMCMC) methods were implemented using the GTR+I+G model. Two parallel searches were run for 2 million generations with the chains sampled every 2,000 generations. The parameters were defined by the default settings in MrBayes. Trees sampled before reaching a split deviation frequency of 0.02 were discarded as the burn-in. The remaining trees were used to calculate the Bayesian Posterior Probabilities (BPP).

Host-parasite cophylogeny

Tree-based and data-based methods were used to examine the presence and amount of congruence between the host and parasite phylogenies. Currently, there is no molecular phylogeny available for the hosts based on the genes used in this study. Therefore, distance-based analyses, such as ParaFit (Legendre et al., 2002), were not appropriate. Existing phylogenies were used to produce the most likely phylogenetic tree for the host taxa, which included 9 families, 15 genera, and 19 species. Phylogenetic relationships among the families come from Klicka et al. (2000), Barker et al. (2002, 2004), Ericson and Johansson (2003), and Spicer and Dunipace (2003). Relationships among genera from the family Tyrannidae come from Ericson et al. (2006), Tello and Bates (2007), Barber and Rice (2007), and Ohlson et al. (2008). For the Icteridae, which includes orioles and blackbirds, the phylogenies of Johnson and Sorenson (1999) and Eaton (2006) were used.

Tree-based methods were used to determine whether the number of cospeciation events was greater than would be expected by chance (Page, 1990, 1994). The level of statistical significance of cospeciation was determined using reconciliation analyses implemented in the program JANE 4.0 (Conow et al., 2010). As recommended by Conow et al. (2010) , the genetic algorithm parameters were set at the default values for selection strength and mutation rate, and number of generations (G) was set as 2 times greater than the population size (S), which in this case was G = 100 and S = 50. Event cost values used to test for cophylogeny were cospeciation = −1, duplication = 0, duplication and host switch = 0, loss = 0, failure to diverge = 0, and with no host switching. With these values, the minimizing of total costs results in the maximizing of cospeciation events. A total of 1,000 random replicates was used, implemented with random tip mapping. A search was also performed that allowed host switching. In this case, the event cost values used were cospeciation = 0, duplication = 1, duplication and host switch = 2, loss = 1, failure to diverge = 1. TreeMap 3.0b (Page, 1995; Charleston, 1998, 2013) was used to produce a tanglegram, which shows the mite phylogeny relative to the bird phylogeny.

Data-based methods were used to statistically test for strict cospeciation. Specifically, the Shimodaira-Hasegawa test (Shimodaira and Hasegawa, 1999) was used to test for significant differences in tree lengths, and was implemented in PAUP*. The test was completed using the resampling estimated log-likelihood (RELL; Kishino et al., 1990) method with 1,000 bootstrap replicates. The results were evaluated as a 1-tailed test.

RESULTS

Specimen and DNA sequence collection

In total, 32 mite samples representing 18 mite taxa were successfully sequenced. For the COI gene (GenBank accession numbers HQ156243–HQ156275), a total of 30 samples was sequenced, and the 28S rRNA gene (GenBank accession numbers HQ174218–HQ174242) included 27 sequences. We were unable to obtain COI sequences for *Syringophilopsis polioptilus* Skoracki, Flannery & Spicer, which was represented in the final data set by only 28S. All mites were found in the quills of the primary and secondary flight feathers.

Mite phylogenetic analysis

The final combined data set consisted of 2,556 aligned base pairs (bp) of mitochondrial COI and nuclear 28S. The COI alignment consisted of 560 bp, which included no gap (indels) positions. For the COI positions, 339 sites (61%) were variable, and 171 sites (31%) were parsimony informative, including the outgroup taxa. The 28S alignment consisted of 1,996 bp, which included 590 gap (indels) positions. For the 28S positions, 689 sites (35%) were variable, and 498 sites (25%) were parsimony informative, including the outgroup taxa. The sequence data set was analyzed as both combined and separate.

Amounts of genetic variation differ between the genes and among the taxonomic level examined. The COI gene is probably the most useful for comparisons, since so many other taxa have been examined (Hebert et al., 2003). For COI intraspecific comparisons based on morphological species, the genetic variation is generally low: 0–1.4% for *Syringophilopsis passerinae* (Clark) (3 sequences), 0.2% for *Syringophilopsis turdus* (Fritsch) (2 sequences), 0.5–0.9% for *Syringophilopsis empidonax* Skoracki, Flannery & Spicer (3 sequences), and 1.3–3.8% for *Syringophilopsis dendroicae* Bochkov & Galloway (3 sequences). A more extreme example is the comparison for *Syringophilopsis tyranni* Bochkov & Galloway, which shows a 4.1–5.0% pairwise difference. However, if this morphological species is examined relative to its host relationships, it becomes more like the other comparisons. The variation for the samples from the flycatcher genus *Myiarchus* is 1.0–1.6% (3 sequences), while the variation for samples from the flycatcher genus *Tyrannus* is 0–0.7% (5 sequences). Another instance concerns the morphological species *Syringophilopsis dendroicae*, which has a divergence of 1.3–10.2% among 4 sequenced samples, although 1 sample has a divergence of 8.8–10.2% from the other 3 samples, which only have a 1.3–3.8% divergence among each other. Consequently, these have been treated as separate lineages in the cophylogeny analyses (as A and B). Examination of interspecific variation among closely related morphological species reveals greater levels of divergence. For example, a comparison among *Syringophilopsis sittae* Skoracki, Hendricks & Spicer versus *Syringophilopsis certhiae* Skoracki, Hendricks & Spicer versus *S. dendroicae* A reveals a divergence of 5.2–6.8%. Likewise, a comparison of *S. turdus* versus *Syringophilopsis sialiae* versus *S.*

empidonax shows divergence levels of 12.1–13.8%. Consequently, divergences greater than 4% were considered as separate lineages for the cophylogeny analyses. This means that interspecific pairwise distances in COI varied from 4.4 to 17% among species. The ribosomal 28S pairwise distances ranged from 0 to 0.6% within species, and from 0.05 to 17% between species.

The parsimony analysis for COI produced 18 trees with a length of 580. ModelTest using AIC selected HKY+I+G as the appropriate substitution model. The ML tree resulting from this analysis produced 2 trees with a score of 3,279.045. The trees (not shown) produced between these 2 analyses differ little, with only a rearrangement in position between *S. passerinae* and *S.* sp. D. The parsimony analysis of 28S produced 45 trees with a length of 1,526. ModelTest using AIC selected HKY+I+G as the appropriate substitution model. The ML analysis produced 1 tree with a score of 9,005.431. A comparison of the trees (not shown) between the 2 analyses showed they differed only in the placement of *S. polioptilus*; in the parsimony consensus tree, it is considered as part of the clade including *S. dendroicae*, *S. sittae*, *S. certhiae*, and *S.* sp. D., while in the ML tree, it is sister to this group. To determine the effects of the placement of insertion/deletion (indels) positions in the alignment on the analyses of the 28S sequence, 2 separate analyses were performed on the aligned data set. The first set of analyses, which have already been mentioned, included the gapped (indels) positions. The other set of analyses excluded gapped (indels) positions. In this instance, the 28S data set excluding gapped (indels) positions produced 1 tree with a length of 531, which is perfectly congruent with the parsimony analysis including gapped (indels) positions. ModelTest using AIC selected HKY+I+G for 28S excluding gapped (indels) positions. The ML tree score for this tree is 4,478.836, which is identical in topology to the tree including gapped (indels) positions. Consequently, the full 28S aligned data set was used for all subsequent analyses.

Tests were performed to determine if the mitochondrial (COI) and nuclear (28S) genes could be concatenated for a combined analyses. Since *S. polioptilus* was not sequenced for COI, it was excluded from the 28S data set for the following analyses. A partition homogeneity (ILD) test was performed and revealed that the COI and 28S regions were congruent ($P = 0.104$). In addition, the Shimodaira-Hasegawa test was performed comparing the phylogeny of COI to 28S, and this revealed no statistical difference (L1 = 3,279.41966 – L2 = 3,297.47836, diff = 18.058, $P = 0.173$). Therefore, for further analyses, the COI and 28S regions were combined to create 1 data set.

The parsimony analysis of the combined COI and 28S data set resulted in 108 trees with tree length of 2,118. A strict consensus tree was generated, which resulted in a tree compatible with the ML tree (Fig. 1), except that the parsimony tree had *S. turdus* basal to *S. sialiae* and *S. empidonax*, but without any bootstrap support. ModelTest selected 2 different substitution models depending on the technique. The AIC procedure selected the TIM+I+G substitution model, while the hLTRs procedure selected GTR+I+G as the appropriate substitution model. Both ML models and the Bayesian analysis (using GTR+I+G) produced trees with the same topology (Fig. 1). The GTR+I+Γ model produced a score of 12,815.39944; this model permits rate variation in all 6 base substitution types (AC: 1.09888, AG: 2.05513, AT: 1.30689, CG: 1.39984, CT: 3.83385, and GT: 1.00000), for unequal base composition (A = 0.304616, C = 0.139890, G = 0.183463, T = 0.372031), among site rate variation ($\alpha = 1.772982$), and invariable sites (I = 0.570791). The Bayesian run attained a split deviation frequency of 0.02 after 83,000 generations (prior trees were discarded as burn-in) and finished with a value of 0.004, so it reached convergence.

Host-parasite cophylogeny

The analysis using the computer program Jane 4 suggests significant cophylogeny between the mite species of *Syringophilopsis* and their North American passerine hosts. Although the tanglegram produced by TreeMap (Fig. 2) does not show full congruence between the host and parasite topologies, the reconciliation analysis indicates that there are more cospeciation events than expected by chance ($P = 0.021$). Without invoking host switches (horizontal transmission), 9 cospeciation events, 1 duplication event, 31 loss/sorting events, and 4 failure to speciate events were identified to explain the differences between the host and parasite phylogenies. With host switching of the parasite enabled, the analysis resulted in 7,690 optimal reconstructions. These reconciled trees proposed 9 cospeciations, 1 duplication, 5 duplication and host switches, 2 loss/sorting events, and 4 failure to speciate events for a total cost value of 17. The Shimodaira-Hasegawa test revealed that there was a significant difference in the topologies of the parasite tree and the host tree ($P = 0.0001$), which indicates that strict cospeciation should not be considered in this case.

DISCUSSION

Mite phylogenetic analysis

This study presents the first resolved molecular phylogeny of *Syringophilopsis* species. The tree, based on a gene from both the nuclear (28S) and mitochondrial (COI) genome, is generally well resolved and has robust support, making it a reasonable hypothesis for the phylogeny of the genus. Additional genetic information, taxon sampling, and a morphological tree for comparison might further support our findings.

Syringophilopsis is the most diverse genus within the Syringophilidae, yet only 9 North American species have been recorded prior to the present paper (Kethley, 1970; Bochkov and Galloway, 2001, 2004; Skoracki et al., 2009). New species were defined by large pairwise divergence values for their COI sequences, as well as by morphological differences, which added to the known fauna of this group. Excluding possible cryptic species, 6 new species were identified. Given the low rate of prevalence (Malenke et al., 2009; 14%, G. Spicer and S. Hendricks, unpubl. obs.) and the great diversity of North American passerines, there are likely many more species of *Syringophilopsis* to be discovered. For this reason, it is important to continue to study quill mites for a better understanding and estimate of the biodiversity of the family.

Two *Syringophilopsis* species, *S. dendroicae* and *S. tyranni*, appear to be cryptic species. Their COI sequences revealed relatively high levels of genetic divergence, which ranged from 8.8 to 9.9% and 4.4 to 5%, respectively. The *S. tyranni* complex was found on different genera of hosts, which further supports the hypothesis of cryptic speciation (Malenke et al., 2009). The cryptic species designated as *S. tyranni* A was found on hosts

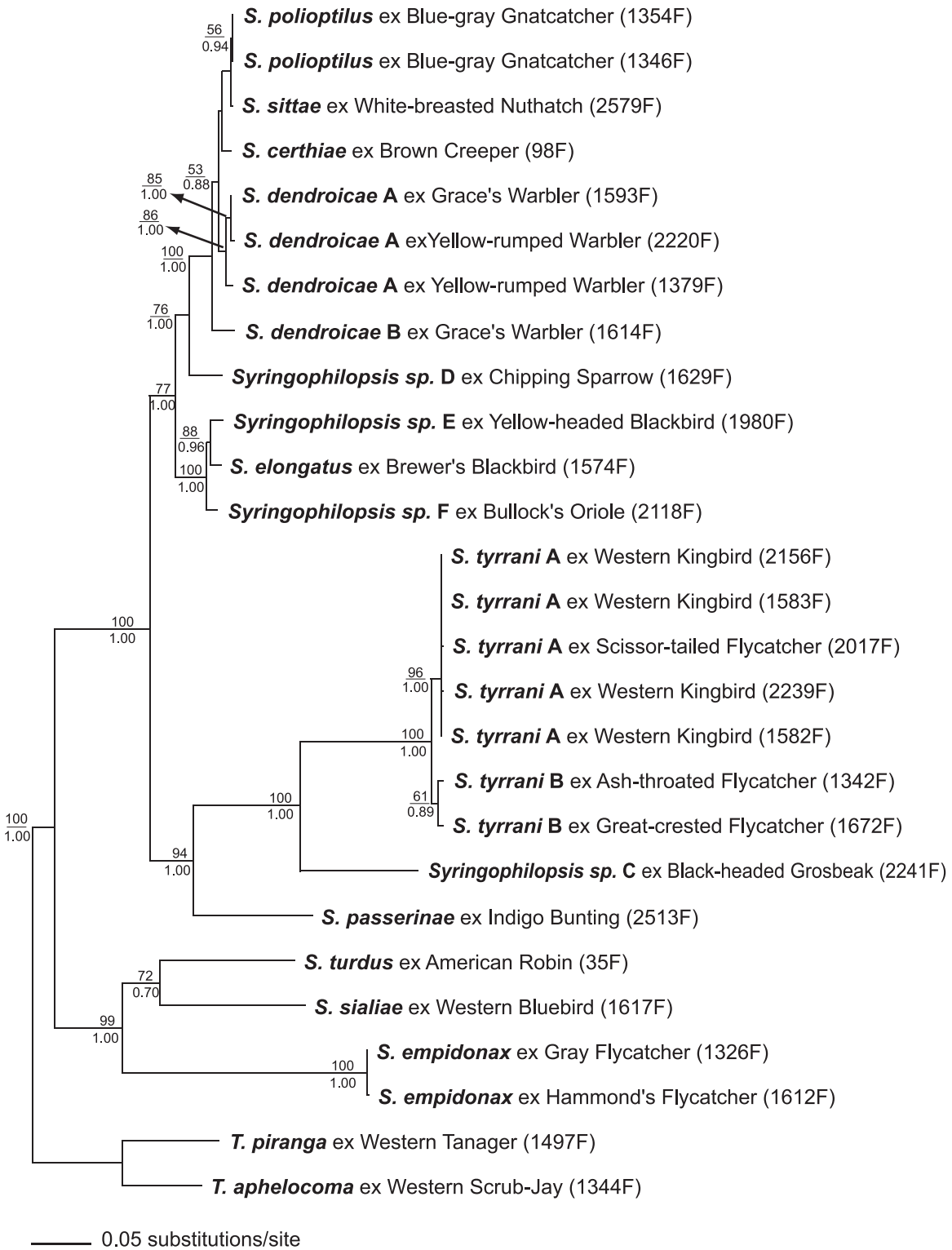

FIGURE 1. Maximum likelihood tree for *Syringophilopsis* spp. using a combined data set of COI and 28S (2,556 bp). Maximum likelihood bootstrap values are above the nodes, and Bayesian Posterior Probability values are below the nodes. Only values >50% are shown.

belonging to species of *Tyrannus*, specifically the Western Kingbird (*Tyrannus verticalis*) and the Scissor-tailed Flycatcher (*Tyrannus forficatus*). In contrast, the cryptic species designated as *S. tyranni* B parasitized species of *Myiarchus*, specifically the Ash-throated Flycatcher (*Myiarchus cinerascens*) and the Great-crested Flycatcher (*Myiarchus crinitus*). More finely detailed morphological examination of these genetically distinct complexes may uncover slight morphological differences.

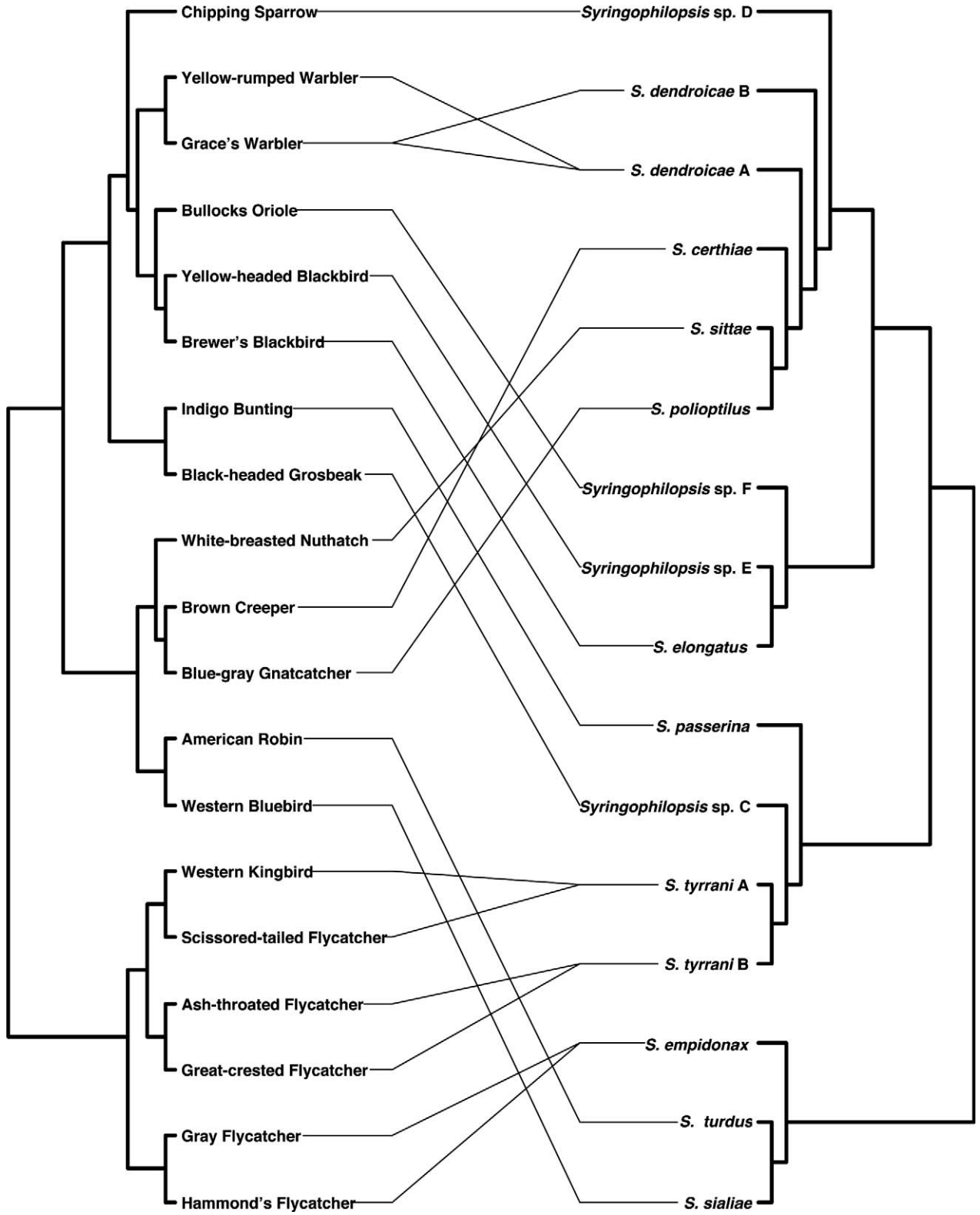

FIGURE 2. A tanglegram of the ML tree of *Syringophilopsis* spp. (right) compared to a composite passerine host tree (left) with lines indicating host-parasite associations.

Host-parasite cophylogeny

The cophylogenetic analysis suggests that species of *Syringophilopsis* quill mites have some degree of congruence with their avian hosts. The reconciliation test indicated that statistically more cospeciation events have occurred than expected by chance. However, as seen in the tanglegram in Figure 2 and the results of the Shimodaira-Hasegawa test, strict cospeciation is not occurring in this particular host-parasite system. These results are similar to many other cophylogenetic studies performed on mite-bird associations (Dabert et al., 2001; Ehrnsberger et al., 2001; Morelli and Spicer, 2007).

Our reconciliation analysis resulted in a large number of possible reconstructions of the evolutionary chronology of *Syringophilopsis* spp. and their avian hosts. With the high cost of host switching in this particular host-parasite system, it is more likely that the evolutionary history would have fewer host switches and more sorting events (Paterson et al., 2003). If we consider that parasites are dependent on their hosts, maximizing the number of cospeciations is likely to lead to the most appropriate reconciled tree (Page, 1994). With so many potential trees, it appears that we need additional information about the timing of host-parasite divergences to determine which among the many reconciliation trees would be the most plausible evolutionary path of these mites and their hosts.

Several reasons may explain the incongruence of the host and parasite phylogenies. Recent host switching, sorting, and duplication events can cause cryptic cospeciation. Multiple-host species can arise when a recent host switch has occurred and the parasite has yet to differentiate sufficiently to be recognized as a new species. Similarly, phylogenetic incongruence can arise when a host has undergone a recent speciation without time for the parasite to speciate. Also, a single mite species parasitizing multiple hosts may be hybridizing between different populations on different host species. *S. empidonax*, for example, has been found on 2 closely related *Empidonax* spp. flycatchers, which have similar ecological needs and life history strategies, making it possible for parasite exchange and hybridization between populations.

The indication of cophylogeny does not automatically imply a history of parallel evolution between parasites and their hosts. 'False' congruence can occur if a parasite switches to several of its host's closest relatives and then undergoes speciation (Brooks and McLennan, 1991; Clayton, Bush, and Johnson, 2003; Banks and Paterson, 2005). In addition to 'false' congruence, low sampling can greatly affect the accuracy of phylogenetic analysis. Increasing the number of taxa sampled can increase the accuracy of phylogenetic estimates (Zwickl and Hillis, 2002). For this particular system, it is difficult to obtain samples, particularly with the low prevalence of quill mite infestation (14%; G. Spicer and S. Hendricks, unpubl. obs.). Sampling error could also have resulted from inadequate gene sampling. More DNA sequences could further resolve the mite phylogeny and increase the strength of the cophylogenetic analyses. DNA sequence data for hosts would have allowed for comparison of the evolutionary rates of host and parasite, which might have supported our findings.

In the present study, the phylogenetic relationships among species of *Syringophilopsis* were inferred from the mitochondrial gene COI and the nuclear gene 28S, with 2 outgroup and 25 ingroup taxa. Our molecular analysis, as well as morphology, supports the finding of 6 new species and potentially 2 cryptic species. The parasite and their host phylogenies exhibit some congruence and are statistically cospeciating. However, strict cospeciation is not occurring. Future work should include a greater sample size of both parasite and host species as well as ecological studies. This would increase our knowledge of the diversity within the genus and further resolve the *Syringophilopsis*–North American passerine cospeciation hypothesis.

ACKNOWLEDGMENTS

We thank A. D. Carmichael, J. Banbury, and J. Steele (San Francisco State University) and John Karges (Texas Nature Conservancy) for field assistance. We are grateful to M. Skoracki for mite identification and verification. Miya Frick assisted with laboratory work. M. Charleston gave us helpful advice for using TreeMap. B. Perry gave beneficial insight into our Bayesian analyses. Funding was provided by the Center for Bay Area Biodiversity, San Francisco State University Biology Instructional Research Award fund, and the National Science Foundation (grant 0728279). We also thank Eric Routman, Ravinder Sehgal, and Tara Roth for comments on this paper.

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