Molecular Phylogenetics and Evolution xxx (2012) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

Evolutionary history of nematodes associated with sweat bees

Quinn S. McFrederick *, Douglas R. Taylor

Department of Biology, University of Virginia, Charlottesville, VA 22904-4328, United States

ARTICLE INFO

Article history:
Received 20 March 2012
Revised 6 November 2012
Accepted 7 November 2012
Available online xxxx

Keywords:
Cospeciation
Eusocial
Sexual transmission
Host specificity
Symbiont diversity

ABSTRACT

Organisms that live in close association with other organisms make up a large part of the world's diversity. One driver of this diversity is the evolution of host-species specificity, which can occur via reproductive isolation following a host-switch or, given the correct circumstances, via cospeciation. In this study, we explored the diversity and evolutionary history of *Acrostichus* nematodes that are associated with halictid bees in North America. First, we conducted surveys of bees in Virginia, and found six halictid species that host *Acrostichus*. To test the hypothesis of cospeciation, we constructed phylogenetic hypotheses of *Acrostichus* based on three genes. We found *Acrostichus puri* and *Acrostichus halicti* to be species complexes comprising cryptic, host-specific species. Although several nodes in the host and symbiont phylogenies were congruent and tests for cospeciation were significant, the host's biogeography, the apparent patchiness of the association across the host's phylogeny, and the amount of evolution in the nematode sequence suggested a mixture of cospeciation, host switching, and extinction events instead of strict cospeciation. Cospeciation can explain the relationships between *Ac. puri* and its augochlorine hosts, but colonization of *Halictus* hosts is more likely than cospeciation. The nematodes are vertically transmitted, but sexual transmission is also likely. Both of these transmission modes may explain host-species specificity and congruent bee and nematode phylogenies. Additionally, all halictid hosts come from eusocial or socially polymorphic lineages, suggesting that sociality may be a factor in the suitability of hosts for *Acrostichus*.

1. Introduction

Of the vast diversity of life, one of the most common lifestyles is to live in close association with another organism. For example, Price (1980) estimated that nearly half of the animals are parasitic insects. Although soil bacteria represent a huge chunk of diversity, many bacterial lineages are comprised of parasites, commensals or mutualists (Sachs et al., 2011). One of the major goals of biology has been to understand the genesis of this diversity. For parasites, commensals, and mutualists, which we refer to collectively as symbionts, several mechanisms may be at play.

One mechanism that generates symbiont diversity is cospeciation. For obligate, vertically transmitted endosymbionts, cospeciation is an important driver of diversity (Brooks and McLennan, 1993; Moran and Baumann, 1994; Thompson, 2005). Although hosts and mutualistic endosymbionts do not always cospeciate (e.g. Lim-Fong et al., 2008; Nelson and Fisher, 2000; Won et al., 2008), the endosymbiont literature is rich with examples of cospeciation (e.g. Baumann and Baumann, 2005; Clark et al., 2001, 2000; Degnan et al., 2004; Hosokawa et al., 2006; Lo et al., 2003; Peek et al., 1998; Sauer et al., 2000; Thao and Baumann, 2004). Cospeciation, however, is not limited to mutualistic endosymbionts; host-parasite coevolution can also result in patterns of cospeciation (e.g. Clayton et al., 2003; Hafner et al., 1994; Light and Hafner, 2007), although a lack of cospeciation seems to be more common for parasites than mutualistic endosymbionts (e.g. Banks et al., 2006; Desdevises et al., 2002; Roy, 2001). Finally, cospeciation can occur in the absence of reciprocal selection (coevolution). Commensal organisms, by definition, do not impose selective pressure on their hosts, although commensals can adapt to selective pressure imposed by their relationship with a specific host. An empirical example of host and commensal cospeciation is myzostomids that exhibit phylogenetic cocladogenesis with their crinoid hosts (Lanterbecq et al., 2010).

Although cospeciation can drive host specificity and diversification of symbionts, symbiont diversification can also be driven by host specialization in the absence of cospeciation. For example, theory suggests that generalist parasites can evolve host-specificity if the parasites exhibit genetic variation in host preference (Kawecki, 1998). Indeed, the evolution of host specificity and local adaptation of parasites enjoys empirical support in copepods, ticks, lice and trematodes (reviewed in Poulin (2008)). It is important to

* Corresponding author. Present address: Patterson Laboratories, University of Texas at Austin, 1 University Station #C0930, Austin, TX 78712, United States. Fax: +1 512 471 3878.

E-mail addresses: quinnmcfrederick@gmail.com (Q.S. McFrederick), drt3b@virginia.edu (D.R. Taylor).

1055-7903/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.ympev.2012.11.007

note, however, that evolution of parasite host specificity may not be directional; there are also examples of evolution towards generalization (Poulin, 2008).

Studies of host/symbiont associations therefore aim to tease apart the importance of cospeciation from other factors that can drive symbiont diversification. For example, reconciliation analyses or cophylogenetic mapping aims to determine the number of cospeciation events, host switches, duplication events (symbiont speciation within a host species), and sorting events (symbiont loss from a host lineage via symbiont extinction or "missing the boat", which occurs when a host diverges but only one of the new host species continues to associate with the symbiont) (Charleston, 2002; Charleston and Perkins, 2006). When cophylogenetic mapping is coupled with tests of associations between the genetic distance matrices of hosts and symbionts (such as Parafit, Legendre et al. (2002)) and, if possible, associations between the branch lengths or divergence dates of hosts and symbionts, the role of cospeciation versus other evolutionary events can be teased apart. This approach, for example, has been used to determine that the phylogenetic concordance between heteromyid rodents and sucking lice is due to cospeciation (Light and Hafner, 2008).

One relatively unexplored symbiotic relationship is between diplogastrid nematodes and halictid bees. The nematodes may represent a radiation of symbionts, but little data exist (Kanzaki et al., 2010b). Presently, three *Acrostichus* species are described from halictid hosts: *Ac. puri* (Kanzaki et al., 2010b), *Ac. megaloptae* (Kanzaki et al., 2010a), and *Ac. halicti* (Giblin and Kaya, 1984a). These nematodes are phoretic with their hosts, and *Ac. halicti* and *Ac. puri* exhibit reproductive isolation (Giblin-Davis et al., 1990; Kanzaki et al., 2010b), but the distribution and evolutionary history of the nematodes is presently unknown. The natural history of the association indicates that opportunities for both vertical and sexual transmission exist (Giblin-Davis et al., 1990), which suggests that transmission between host species may be rare or absent. If vertical and sexual transmission are the only forms of transmission of these nematodes host specificity and cospeciation are both possible.

Here, we explore the association between bees and *Acrostichus* species in the Southeastern United States. First, we determine how many bee species host *Acrostichus*. Next, we address the hypotheses of host specificity and cospeciation through phylogenetic analyses of the nematodes and their bee hosts. We find evidence for host specificity, cospeciation and at least one ancestral host-switching event. Additionally, we find that many possible hosts do not associate with nematodes, and we discuss possible limitations to the distribution of the nematodes.

2. Methods

2.1. Study organisms

Acrostichus (formerly *Aduncospiculum*) *halicti* (Nematoda: Diplogastridae) and *Ac. puri* are nematodes that associate with bees from several genera in the family Halictidae (Giblin and Kaya, 1984b; Kanzaki et al., 2010b). Giblin and Kaya (1984b) found dauer (non-feeding, transport stage) *Ac. halicti* in the glands and genitalia of *Halictus farinosus*, *Halictus rubicundus*, and *Halictus ligatus*. Giblin-Davis et al. (1990) later reported *Ac. halicti* in association with the halictids *Augochlora pura mosieri* and *Augochlorella gratiosa*. Recently, *Ac. megaloptae*, which was isolated from the Neotropical halictids *Megalopta genalis* and *Megalopta centralis* (formerly *ecuadoria*) was described (Kanzaki et al., 2010a), and the nematodes isolated from *Au. pura* have been described as a separate species, *Ac. puri* (Kanzaki et al., 2010b). In all of these associations, the nematode's life history is tightly aligned with the life cycle of the host bees. In female bees, nematodes are transmitted vertically as dauers into the brood cells along with the Dufour's gland secretion that the bee uses to create the brood cell lining (Giblin and Kaya, 1984b). The presence of nematodes in the penis valves of male halictid bees strongly supports sexual transmission (Giblin-Davis et al., 1990), although environmental transmission has not been experimentally excluded. Once in the brood cell, the nematodes feed on yeast and bacteria, develop into adults, and reproduce sexually. This cycle continues, with several nematode generations occurring entirely within the bee cell, until just before the adult bee emerges from the pupa (Giblin and Kaya, 1984b). At this time, juvenile nematodes molt into the non-feeding dauer stage and enter the Dufour's gland (if the host is female), or penis valves (if the host is male) as the adult bee ecloses. The nematodes remain in the adult bee as the bee leaves its natal cell.

2.2. Bee and nematode sampling

During the summers of 2005 through 2009, we conducted surveys of bees and their phoretic nematodes in three localities in northern and central Virginia: Blandy Experimental Farm, Sky Meadows State Park, and Ivy Creek Natural Area. Beginning in 2006, we also sampled bees at several locations in Charlottesville, Virginia: the grounds of the University of Virginia, Azalea Park, and a private residence with a native plant garden. During 2008, we made additional collections at several locations in Colorado and Utah. We dissected the Dufour's glands, oviducts, and genitalia of the bees in order to assay the presence of nematodes. We then either cultured the nematodes on wild bacteria cultured on tryptic soy broth agar plates or stored the nematode specimens at −80 °C until we could perform DNA extractions. In 2005, we sampled common bees at Blandy Experimental Farm in order to determine how many bee species hosted nematodes. We identified the bee either to species or morpho-species. Specimens that were difficult to identify to species, such as *Lasioglossum (Dialictus)* or *Andrena*, were assigned to morpho-species. We identified all remaining host bees to species using Mitchell (1960), and the online key to bees at discoverlife.org. When we discovered a host bee at Blandy Experimental Farm, we targeted that bee for further sampling at the other locations. After 2005, we concentrated collections on host bees, all of which belong to the family Halictidae, while still making collections of other halictids in an effort to identify more hosts. To test for differences in prevalence of infection among species, sex, and year we performed a three-way analysis of variance using the aov function in the program R (R Core Development and Team, 2009). To assess the assumption of homogeneity of variances, we plotted residuals against group means. As the spread of residuals were similar, we performed the test on the raw data (Quinn and Keough, 2002).

2.3. Molecular genetic methods

2.3.1. Nematodes

In order to provide enough nematode DNA to perform reliable PCR, we combined 10 nematodes isolated from a single host into one extraction, but used fewer than 10 nematodes when 10 were not available. We added 50 μl of worm lysis buffer and followed standard nematode DNA extraction techniques (Williams et al., 1992). We amplified three genes: 512 bases of mitochondrial cytochrome oxidase 1 (COI), 649 bases of the D2D3 region of the 28S large subunit ribosomal RNA gene, and 1383 bases of the 18S small subunit ribosomal RNA gene. We deposited the sequences on the genetic sequence database at the National Center for Biotechnical Information (NCBI GenBank IDs: HQ130133 to HQ130276). To amplify COI and 28S, we used previously published primers (COIF1, COIR2, D2A, D3B Kanzaki and Futai (2002) and Ye et al. (2007)). We designed primers for 18S (18SF CTGCGAACGGCTCATTAAAAC,

Please cite this article in press as: McFrederick, Q.S., Taylor, D.R. Evolutionary history of nematodes associated with sweat bees. Mol. Phylogenet. Evol. (2012), http://dx.doi.org/10.1016/j.ympev.2012.11.007

18SR GAGCTGATGACTCACACTTAC). We used 35 cycles for all PCRs, and we conducted the 28S and 18S PCRs with a 55 °C annealing temperature while we used a 51 °C annealing temperature for the COI reactions. We purified the resulting PCR product with exonuclease and shrimp alkaline phosphatase before performing cycle sequencing reactions with Big Dye version 3.1 (Applied Biosystems, Carlsbad, CA, USA), using the same primers as in the PCR amplifications and a set of internal primers for 18S (18S1F CGGTAATTCCAGCTCTCATG, 18S2F GATTAGATACCGCCCTAGTTC, 18S1R CATGAGAGCTGGAATTACCG, 18S2R GAACTAGGGCGGTATCTAATC). To purify the cycle sequencing reactions, we used Sephadex column chromatography, and then ran the samples on an ABI 3130xl sequencer (Applied Biosystems, Carlsbad, CA, USA). We compared the forward and reverse strands from each sample to resolve any ambiguous base calls using the program Sequencher 4.1 (Gene Codes Corporation, Ann Arbor, Michigan, USA), then aligned the entire dataset using ClustalW and manual adjustment in the program Mesquite (Maddison and Maddison, 2010).

2.3.2. Bees

We conducted DNA extraction, sequencing and phylogenetic analyses on host bee species only. To extract bee DNA we froze the bees in liquid nitrogen, ground the head and thorax of the bees, and then suspended the homogenate in CTAB buffer. Next, we performed standard chloroform/isoamylalcohol/phenol extractions (Danforth, 1999; Sambrook et al., 1989). In hopes of comparing the rates of molecular evolution in the host and symbiont, we used mitochondrial COI as a molecular marker in both the nematodes and the bees. We used only one marker in the host, as the phylogeny of the host bees has been well studied previously (Brady et al., 2006; Coelho, 2004; Danforth et al., 1999). We initially used five previously published primers to amplify and sequence COI in bees (Soucy and Danforth, 2002). From the resulting sequences, we then developed species and genus specific primers (*A.pura*F CAGGTGGAGGAGACCCAGTA, *A.pura*R TCAGAGTAACGTCGTGGTATCC, *Augochlorella*F TGGGGGAGACCCTGTTCTAT, *Augochlorella*R ATGCGTCTGGGTAATCTGAA, *H.ligatus*F TTTTGACCCTTCAGGAGGTG, *H.ligatus*R TTTCAACAATAATAGGCATCAGGA, *H.parallelus*F CGGAGGAGGTGACCCTATTC, *H.parallelus*R CTGAGTATCGTCGTGGTATTCCT, *H.rubicundus*F ACCCTTCTGGAGGAGGAGAC, *H.rubicundus*R GCATCTGGGTAATCTGAATATCG). We used a 55 °C annealing temperature and 35 cycles for PCR, and followed the same sequencing protocols as outlined above for the nematodes.

2.4. Phylogenetic analyses

We used two different analyses to build phylogenetic trees of the nematodes: maximum likelihood (ML) analysis with the program Garli 2.0 (Zwickl, 2006) and Bayesian analysis with the program Mr.Bayes 3.1.2 (Ronquist and Huelsenbeck, 2003). We performed both analyses on a concatenated data set containing COI, 28S and 18S. In addition, we performed maximum likelihood analyses on each gene separately in the program Garli. As an out-group, we used four *Acrostichus rhynchophori* samples isolated from palm weevils (Kanzaki et al., 2009), as *Ac. rhynchophori* is the closest known relative to the bee-associated *Acrostichus* (Kanzaki et al., 2010a). In addition, we included publicly available sequences from *Ac. megaloptae* (Kanzaki et al., 2010a). *Megalopta* is a genus of augochlorine bees (Wcislo and Gonzalez, 2006) that is related to the clade that includes *Augochlora* and *Augochlorella*. We deposited our alignments and trees on TreeBASE (http://purl.org/phylo/treebase/phylows/study/TB2:S13579).

To parameterize the ML analysis we used PAUP* 4b10 (Swofford, 2001) and Modeltest 3.7 (Posada and Crandall, 1998) to infer the most likely model of evolution for the entire dataset, each gene separately, and for each codon position in the COI sequence. We then ran four Garli analyses with genthresholdtopoterm set to 1,000,000, parameters unlinked across genes and codon positions, and parameter values being estimated during the runs. We conducted four search replicates for each analysis, with the respective models as suggested by Modeltest: (1) with GTR+G as the model for the entire concatenated data set, (2) with models for each gene: HKY+I+G (COI), GTR+I (18S) and GTR+G (28S), (3) with separate models for the rDNA genes and with one model for the 1st and 2nd codon positions together and a separate model for 3rd codon positions in COI (TRN, K81uf+G, respectively), (4) with models for the rDNA genes and with separate models for the three codon positions in COI (TRN, K81uf, and K81uf+G, respectively). We then used the −ln likelihood from the best scoring search replicate and the sum of the number of parameters in each model to compute the corrected Akaike Information Criterion (AICc). The AICc supported separate models for each codon position of COI and separate models for 18S and 28S. All four search replicates using these models returned the same topology, so to determine support for this topology we conducted 100 bootstrap pseudoreplicates with the same, unlinked model settings and with otherwise default Garli settings.

For the Bayesian phylogenetic analyses we used MrModeltest to infer the best fitting models for the analyses (Nylander, 2004). We conducted three analyses, each with parameters estimated during the runs and unlinked across genes (for analyses 2 and 3): (1) GTR+G for the entire, concatenated dataset, (2) HKY+I+G for COI, GTR+I for 18S, and GTR+G for 28S, and (3) separate GTR models for COI 1st and 2nd codon positions, GTR+G for COI 3rd codon positions, GTR+I for 18S and GTR+G for 28S. For each analysis, we ran two separate runs with 4 Markov chains each. We ran all analyses for 20,000,000 generations, sampling the chain every 100th generation. We examined the parameter files in the program Tracer v1.5 (Rambaut and Drummond, 2009), and discarded the first quarter of the samples from each analysis as burn-in. We determined that the runs had converged by examining the standard deviation of split frequencies (<0.01 for post burn-in samples) and using the split and compare functions in AWTY (Wilgenbush et al., 2004). We then used Bayes factors based on the harmonic means of the likelihoods from each analysis to select the preferred analysis (Kass and Raftery, 1995).

To test whether monophyletic clades of nematodes isolated from a single host species represent cryptic species, we conducted formal species delimitation tests using the splits package in R (Ezard et al., 2009). We first used the APE package in R (Paradis et al., 2004) to resolve multichotomies with the multi2di command, and then ultrametricized the tree by executing the chronopl command with a lambda parameter of two. To delimit species, we conducted a general mixed Yule coalescent (GMYC) analyses. GMYC analysis uses a maximum likelihood framework to determine the threshold between diversification and coalescent events (Pons et al., 2006). We used both the single threshold version and the multiple threshold versions, and executed the compare command to select the best fitting version.

2.5. Cospeciation analyses

We employed distance-based and topology-based tests of cospeciation. For distance-based analyses, we used COI sequence from one North American halictid specimen per species (GenBank accession numbers JX546143–JX546148), along with closely related halictids for which COI sequence data were publicly available (Fig. S1). For *Acrostichus* species, we pruned the COI maximum likelihood phylogeny described above to one randomly sampled representative sequence per putative nematode species (see the nematode tree in Fig. 3). For halictid COI, we used Modeltest 3.7 (Posada and Crandall, 1998) to select the most likely model of sequence evolution (GTR+I+G). We ran a likelihood analyses in PAUP*

Please cite this article in press as: McFrederick, Q.S., Taylor, D.R. Evolutionary history of nematodes associated with sweat bees. Mol. Phylogenet. Evol. (2012), http://dx.doi.org/10.1016/j.ympev.2012.11.007

4b10 for 100 random sequence addition replicates. In agreement with Lin and Danforth (2004), we found that the bee COI dataset exhibited limited phylogenetic utility. We therefore constrained the topology of the bee tree to match published phylogenetic relationships of the Halictidae (Brady et al., 2006; Coelho, 2004; Danforth et al., 1999), and used the bee COI sequence to estimate host branch lengths in PAUP* 4b10, using the likelihood criterion and the GTR+I+G model of evolution as calculated in Modeltest 3.7.

Distance-based tests of cospeciation are commonly implemented by first computing the principal coordinates of genetic or patristic distance matrices from hosts and symbionts and then testing for associations between the principal coordinates in the program Parafit (Legendre et al., 2002). We exported patristic distance matrices from the maximum likelihood host and symbiont trees in the program Mesquite (Maddison and Maddison, 2010). We then used the program Parafit (Legendre et al., 2002) as implemented in the APE library in the program R (Paradis et al., 2004). To test for a significant signal of cospeciation, we used 10,000 permutations and the lingoes correction for negative eigenvalues in the host principal coordinate matrix.

To test for a topological signal of cospeciation in the phylogenies of *Acrostichus* species and their sweat bee hosts, we used the programs TreeMap 3.0 (Charleston and Page, 2002) and Treefitter (Ronquist, 2003). TreeMap returns optimal reconstructions of host and symbiont evolutionary histories that minimize the cost of the reconciliation, based on user-defined costs for host switches, duplication events and sorting events. To test if cospeciation differs from a random expectation, the number of cospeciation events can be tested against a distribution of randomly generated parasite trees. For our TreeMap analysis, we used a tree of the host species based on the their published phylogentic relationships (see Fig. S2, relationships based on Brady et al., 2006; Coelho, 2004; Danforth et al., 1999), along with the maximum likelihood nematode phylogeny based on COI (same topology as Fig. 2, also see Fig. S2), pruned to one randomly selected sequence per putative nematode species. We kept the default weights of zero for a cospeciation event and one for a host switching, duplication or sorting event. We also tried several permutations of the cost weighting scheme, to determine if our weighting scheme had a large impact on the results. We randomly permuted the symbiont tree 1000 times to create a probability distribution of cospeciation events. To include halictids closely related to our hosts and to determine if we had missed possible scenarios, we repeated these topological tests in the program TreeFitter (Ronquist, 2003). We used the same nematode tree as in the TreeMap analysis (Fig. S2), along with the halictid phylogeny shown in Fig. 3 (topology based on Brady et al., 2006; Coelho, 2004; Danforth et al., 1999). We used the estimate command to explore cost optimization and the fit command to explore cost scenarios that were found to be significant at $P < 0.05$ from 10,000 random permutations of the host and parasite trees.

Congruence between host and symbiont trees can be caused by factors other than cospeciation, such as resource tracking, where the symbiont evolves in response to a host trait with a phylogenetic signal (Brooks and McLennan, 1993). To confirm cospeciation, it is therefore desirable to determine if host and symbiont divergences are contemporaneous. Unfortunately, the nematode fossil record is sparse (Poinar, 1983), and nematode fossils to calibrate the *Acrostichus* phylogeny are lacking. If DNA sequence data conform to a molecular clock, branch lengths represent divergence times and can therefore be compared between hosts and symbionts by correlation analysis (Page, 1991). We used PAUP* 4b10 to test for the molecular clock with the same nematode isolate and host specimen datasets and maximum likelihood model settings as described above. We performed separate likelihood runs with 10 random sequence addition replicates with both nematode and bee datasets and the molecular clock enforced or relaxed. To test the molecular clock, we performed likelihood ratio tests on the resulting likelihood scores.

3. Results

3.1. Prevalence of infection

We collected and dissected a total of 3581 specimens representing 106 species and morpho-species of bees. Of these 106 species, we found only six that were infected by *Acrostichus* species. These hosts were all in the family Halictidae, and represented two tribes (Augochlorini and Halictini) and three genera (*Augochlora*, *Augochlorella*, and *Halictus* (*Nealictus*, *Odontalictus*, and *Protohalictus*)). We sampled 2489 specimens of these six host species, and found that the prevalence of infection varied greatly across host species (Fig. 1, $F_{5,30} = 33.56$, $P < 0.0001$), but not across year ($F_{1,30} = 1.75$, $P = 0.20$) or sex ($F_{1,30} = 0.01$, $P = 0.9$). The mean infection rate was 33.4%, ranging from 100% in *H. parallelus* females to 11.6% in *H. rubicundus* males (Fig. 1). We examined a total of 30 species or morpho-species of halictid bees in Virginia, Utah and Colorado that did not harbor *Acrostichus* species (Table 1). We sampled from all of the common Halictinae genera found in the United States (Michener et al., 1994).

3.2. Nematode phylogenetics

Nematodes in the *Ac. halicti* and *Ac. puri* species complexes cluster by host species instead of geography (Fig. 2). The ML and Bayesian analyses returned similar topologies, however, the Bayesian topology exhibited fewer polytomies and differed in the placement of *Ac. megaloptae* when compared to the ML topology (see online supplemental nexus file or TreeBASE study S13579 for trees from all of our analyses). In the Bayesian analysis, *Ac. megaloptae* was positioned as sister to both *Ac. puri* and *Ac. halicti*, while in the ML analysis *Ac. megaloptae* was positioned as sister to only *Ac. puri*. Both phylogenetic hypotheses suggested that *Ac. puri* and *Ac. halicti* are species complexes made up of host specific nematode species that associate with closely related host species. Our species delimitation test confirmed that *Ac. puri* and *Ac. halicti* are comprised of host-specific coalescent groups ($P = 0.0001$), and additionally suggested that *Ac. megaloptae* and *Ac. rhynchophori* are species complexes (Fig. S3). The single threshold GMYC model was not a significantly better fit to the data than the multiple threshold model ($P = 0.36$), so we present the single threshold model in Fig. S3. The single gene ML analyses all resulted in similar tree topologies, with the exception of their placement of *Ac. megaloptae*: *Ac. megaloptae* was placed as sister to both *Ac. halicti* and *Ac. puri* species complexes in the COI tree while *Ac. megaloptae* was placed as sister to the *Ac. puri* species complex in the 18S and 28S trees.

3.3. Cospeciation analyses

Several nodes in the nematode phylogeny match the branching patterns in the host phylogeny (Fig. 3). The placement of *Ac. megaloptae* as sister to the rest of the bee-associated *Acrostichus* species in the Bayesian phylogeny, however, is not congruent with the host's phylogeny. Additionally, at least one host switch and multiple extinctions are necessary to fit the nematode phylogeny to the bee phylogeny.

3.3.1. Distance based test of cospeciation

We used the program Parafit (Legendre et al., 2002) to test for a signal of cospeciation in the patristic distance matrices of the bee hosts and their nematode symbionts. The host and symbiont patristic distance matrices exhibited a significant signal of cospeci-

Please cite this article in press as: McFrederick, Q.S., Taylor, D.R. Evolutionary history of nematodes associated with sweat bees. Mol. Phylogenet. Evol. (2012), http://dx.doi.org/10.1016/j.ympev.2012.11.007

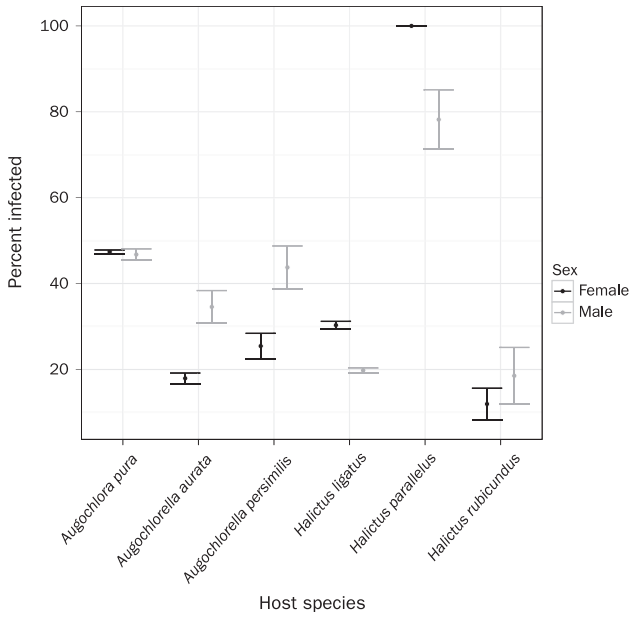

Fig. 1. Mean prevalence of *Acrostichus* infection of six halictid species in Virginia, from 2005 to 2009, with 95% confidence intervals. Black represents female hosts, while gray represents male hosts.

ation globally ($P = 0.006$), and at several individual host-symbiont links. The individual symbiont links with *Ag. aurata* ($P = 0.007$), *Ag. persimilis* ($P = 0.005$), *Au. pura* ($P = 0.008$), and *H. ligatus* ($P = 0.04$) were significant, while the *H. parallelus* ($P = 0.09$) and *H. rubicundus* ($P = 0.07$) associations were marginally significant and the *M. genalis* association ($P = 0.74$) was not significant.

3.3.2. Topology based tests of cospeciation

For topology-based tests of cospeciation, we used TreeMap 3.0 and Treefitter to compare the bee and nematode phylogenies. The TreeMap analysis returned two optimal solutions, each with five cospeciation events and two speciation events within a single host species. The solutions differed in that one involved an ancestral host switch, while the other involved three symbiont losses. Both solutions exhibited a significant signal of cospeciation ($P = 0.002$ for the host switch solution and $P = 0.005$ for the three losses solution). The Treefitter analysis found 16 cost setting scenarios where the optimized sequence of historical events was significantly better than patterns arising by chance in 10,000 random permutations of the host and parasite trees ($P = 0.01$ to $P = 0.001$). When the cost of cospeciation events was set prohibitively high (10), the randomization tests were no longer significant, indicating the importance of cospeciation in the fit between the bee and nematode phylogenies. Two unique models arose from the significant cost setting scenarios. In the first model, which also exhibited the lowest costs (10–11), there were four cospeciation events, five extinction events, and two switching events. In this model, several host switches were possible, but all of them targeted *Halictus* species as the new host. The switches either arose from the Augochlorini (nodes between *Megalopta* and *A. pura*) or from *H. ligatus* or the ancestor to *H. rubicundus*, *H. parallelus*, and *H. farinosus*. The new hosts in these switches were either *H. ligatus* or the ancestor to *H. rubicundus*, *H. parallelus*, and *H. farinosus*. The second model was associated with higher costs (11.5–18), and included five cospeciation events, eight extinction events, and one host switch. The host switch in this second model arose from either of two ancestral nodes in the Augochlorini clade of the host tree and the ancestor of *Halictus* (*Nealictus*, *Odontalictus*, and *Protohalictus*) was colonized.

3.3.3. Nematode genetic divergence

Due to a lack of nematode fossils, estimating divergence dates was not possible. Additionally, our sequence data did not conform to a molecular clock, as we found that both bee ($P < 0.001$) and nematode ($P < 0.005$) phylogenies had significantly better likelihood scores without the molecular clock enforced. We are therefore unable to compare branch lengths between our bee and nematode trees (Light and Hafner, 2008). As an uncalibrated estimate of nematode divergence, we found that the *Ag. aurata*-associated nematodes had 0.772 substitutions per site from the tip of the branch to the common ancestor with the *Ac. halicti* species complex, while the *H. parallelus*-associated nematodes had 0.493 substitutions per site for the same distance. The divergence between Augochlorini (which contains *Augochlorella* and *Augochlora*) and Halictini (which contains *Halictus*) occurred around 65 million years ago (Brady et al., 2006), indicating that if their common ancestor was infected with *Acrostichus*, the *Acrostichus* COI substitution rate would be around 0.009 substitutions per site per million years. On the other hand, if there was a host switch after the divergence of *Megalopta* and the common ancestor of *Augochlorella* and *Augochlora*, which occurred around 30 million years ago (Brady et al., 2006), the *Acrostichus* substitution rate would be around 0.021 substitutions per site per million years.

4. Discussion

Our phylogenetic hypotheses and species delimitation tests suggest that North American bee-associated *Acrostichus* are actually species complexes comprised of cryptic, host-specific nematode species. In addition, several nodes in the bee and nematode phylogenies are congruent, and both distance and topology based tests reveal a significant history of cospeciation. It appears, however, that cospeciation is not the only evolutionary force at play. Several lines of evidence suggest that a series of cospeciation, host-switching, and extinction events are required to understand the history of this association.

4.1. Cospeciation, host switching and extinction events

First, the placement of *Ac. megaloptae* as sister to the North American nematodes supports the occurrence of one or more host switches. One of the two optimal TreeMap models and both of the significant Treefitter models invoke host-switching events. The other optimal TreeMap reconciliation suggests that the common ancestor to Halictini and Augochlorini was infected with *Acrostichus* and involves multiple extinction or "missing the boat" events and no host switching. Several lines of evidence suggest that this reconciliation is not plausible. First, the "missing the boat" hypothesis requires extinction of nematodes in clades where the nematodes were already extinct. The "missing the boat" hypothesis additionally requires a substitution rate for *Acrostichus* COI that is about two orders of magnitude slower than measured nematode mitochondrial mutation rates (Denver et al., 2000; Molnar et al., 2011). Although rate heterogeneity and differences between mutation rates measured in the lab versus substitution rates in nature suggest that comparisons should be viewed cautiously, the relationship between *Acrostichus* and halictids may not be 60 million years old, as required by the "missing the boat" hypothesis. Instead, a more likely interpretation may be that the relationship arose no more than 30 million years ago, and that the substitution rate is more along the lines of 0.021 substitutions per site per million years.

Another line of evidence supporting the host switch hypothesis involves the biogeography of the Halictidae. Augochlorini is restricted to the Americas (Coelho, 2004), while it is thought that

Please cite this article in press as: McFrederick, Q.S., Taylor, D.R. Evolutionary history of nematodes associated with sweat bees. Mol. Phylogenet. Evol. (2012), http://dx.doi.org/10.1016/j.ympev.2012.11.007

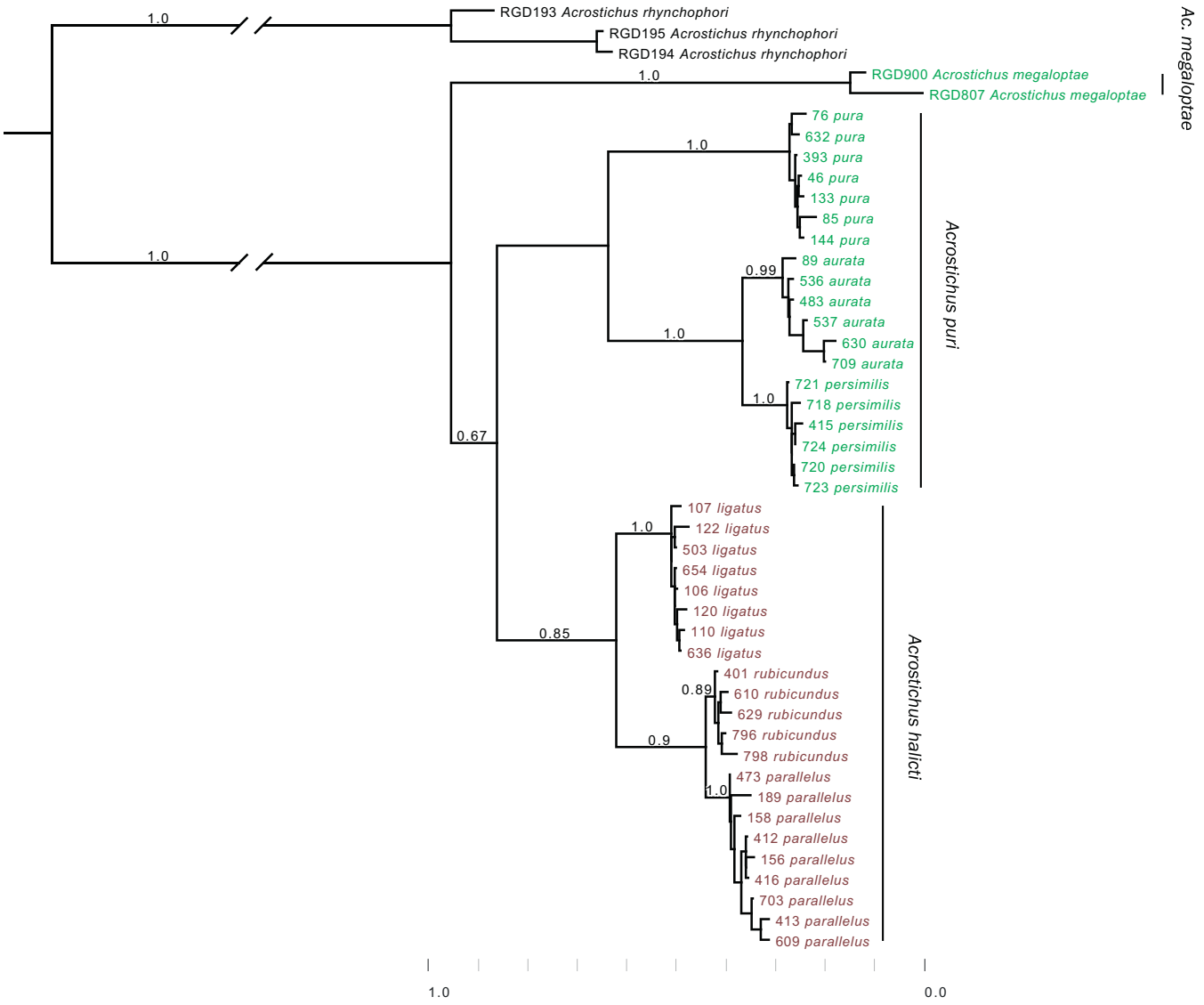

Fig. 2. Phylogram of bee-associated *Acrostichus* spp., based on a three-gene, five-model Bayesian analysis. Node labels represent posterior probability distributions. Names include the isolate number (first) and the specific epithet of the host from which the nematode was isolated (second).

Halictus (*Nealictus*, *Odontalictus*, and *Protohalictus*) species colonized North America from the old world (Danforth et al., 1999). Furthermore, it is thought that North America was colonized by *Halictus* (*Odontalictus* and *Nealictus*) species twice, once by the common ancestor of *H. ligatus* and *H. poeyi* and once by the lineage that includes *H. parallelus* and *H. rubicundus* (Danforth et al., 1999; Michener, 1979). Bee-associated *Acrostichus*, however, have not been reported from Afro-Eurasia. In a large scale survey of bee-associated nematodes in Turkey, Hazir et al. (2010) recorded 14 different halictids that hosted nematodes in the genus *Bursaphelenchus*, but no *Acrostichus*. While cospeciation between *Acrostichus* and augochlorine hosts is possible, and even likely according to our data, the limitation of *Acrostichus* to the Americas and the diversification of *Halictus* in Afro-Eurasia support the TreeMap and Treefitter models that include one or more host switches. The Treefitter model with the lowest associated cost involves two host switches: one arising from the augochlorine hosts and one within *Halictus*, with *H. ligatus* and the *H. rubicundus, H. parallelus,* and *H. farinosus* ancestor as targets. These host switches reconcile the biogeographic histories of North American *Halictus* species with the congruent nodes in the *Halictus* and *Ac. halicti* phylogenies.

4.2. Patchiness of the Acrostichus/halictid association

Our study is not the first to find that a mixture of cospeciation and other evolutionary processes can shape a symbiont's evolutionary history (e.g. Hughes et al., 2007; Kawakita et al., 2004; Light and Hafner, 2008; Page, 1996). The *Acrostichus* and halictid association differs from the above examples, however, by the patchy distribution of *Acrostichus* across the host tree. Although our study was not designed to determine why the distribution of halictid-associated *Acrostichus* spp. is so patchy, the natural history of the association suggests several possible explanations. First, the combination of vertical and likely sexual transmission may explain the evolution of host specificity and cophylogeny. Vertical transmission can be a major driver of cospeciation (Herre et al., 1999; Moran and Baumann, 1994), but sexual transmission also limits opportunities for horizontal transmission between host species, as most mating occurs within species (Antonovics et al., 2011; Lockhart et al., 1996). So far, there is no evidence suggesting that other methods of transmission exist for bee-associated *Acrostichus* species, so perhaps it is not surprising that each host species harbors its own nematode species and that there is evidence for some cospeciation. However, this begs the question of how the host

Please cite this article in press as: McFrederick, Q.S., Taylor, D.R. Evolutionary history of nematodes associated with sweat bees. Mol. Phylogenet. Evol. (2012), http://dx.doi.org/10.1016/j.ympev.2012.11.007

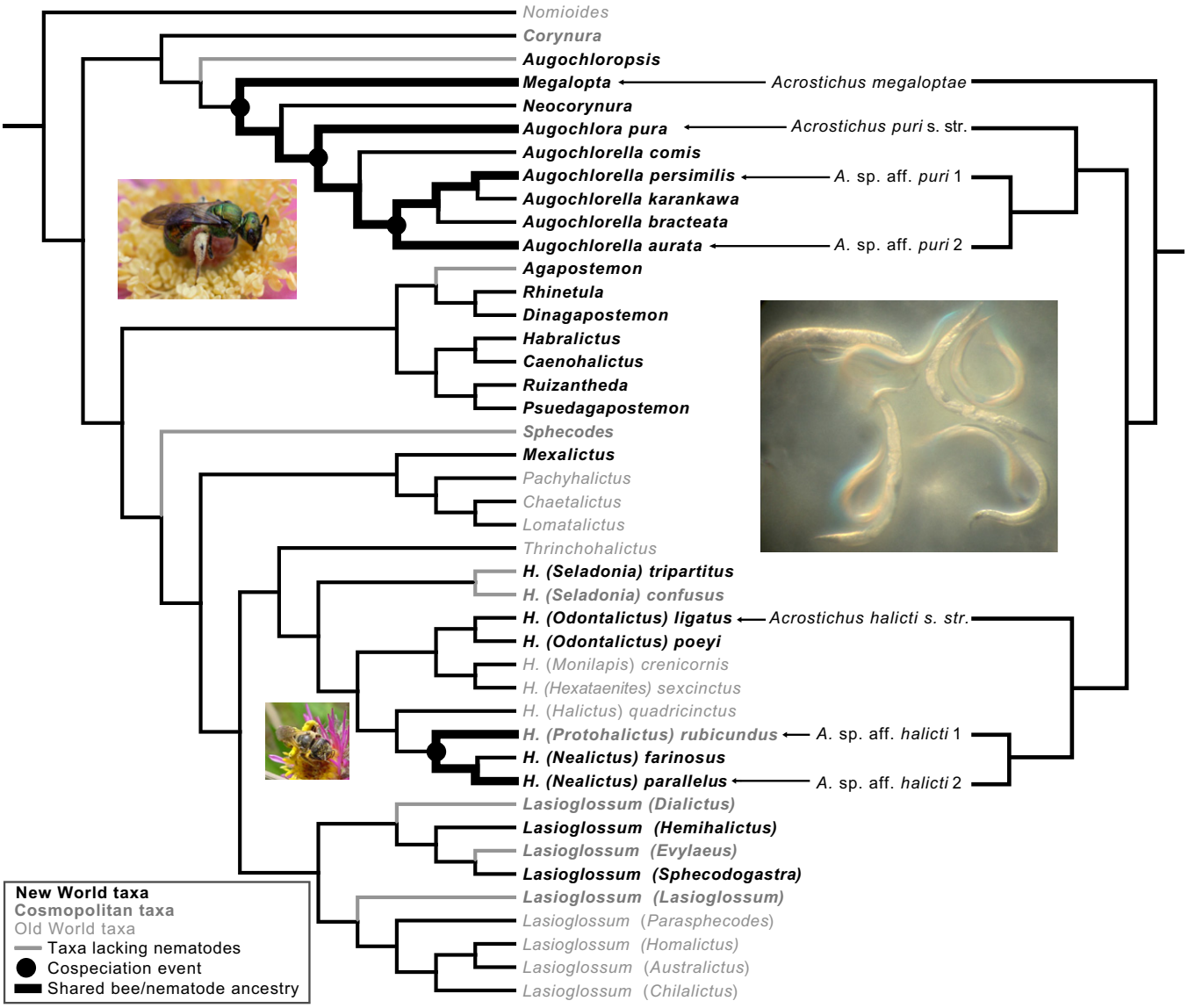

Fig. 3. The evolutionary history of the *Acrostichus*/halictid association. The *Acrostichus* phylogeny is portrayed on the right, with arrows connecting nematodes to their hosts in the halictid phylogeny on the left. Circles on nodes in the halictid phylogeny represent cospeciation events as inferred in the optimal Treefitter analysis. Bold edges in the host phylogeny indicate paths of shared ancestry between *Acrostichus* and their halictid hosts. Gray branches in the host phylogeny represent groups that we surveyed and did not associate with *Acrostichus*. Biogeography is represented in the terminal names on the host phylogeny: bold, black names represent Neartic or Neotropical groups, gray names represent cosmopolitan groups and light gray names represent Afro-Eurasian or Australian groups. The relationships in the host phylogeny are based off of several phylogenetic studies (Brady et al., 2006; Coelho, 2004; Danforth et al., 1999), while the *Acrostichus* phylogeny is a simplified version of Fig. 2.

switches from the Augochlorini to *Halictus* occurred. Rare sexual transmission between lineages via confamilial mating attempts, transmission at multi-species nest aggregations, or reuse of infected nests by different species are all possible explanations for inter-specific transmission events.

A separate, but not mutually exclusive hypothesis involves the social behavior of the host. Although *Au. pura* is solitary, the solitary *Augochlora* species are thought to represent a reversal to solitary nesting (Danforth and Eickwort, 1997; Schwarz et al., 2007). Therefore, all of the halictids presently known to associate with *Acrostichus* species come from eusocial or socially polymorphic lineages, although not all social halictids associate with *Acrostichus* (Fig. 4). Social structure may therefore be a factor that determines the suitability of a host, and the persistence of the association with *Au. pura* may be a relict of its social ancestry. Why social halictids appear to be better hosts for *Acrostichus* is another open question. Contacts within a social nest may facilitate transmission and help maintain the association. Uninfected workers may become infected via sexual transmission, and subsequently transmit nematodes to their sisters when building brood cells. The association between social structure and nematode infection merits further research.

Table 1
Number of specimens examined for halictid genera or subgenera that did not harbor nematodes.

Halictid genera	Species or morphospecies	Dissected
Agapostemon	2	29
Augochloropsis	1	10
Halictus (Seladonia)	3	90
Lasioglossum (Dialictus)	12	106
Lasioglossum (Evylaeus)	2	21
Lasioglossum (s.str.)	10	55
Sphecodes	2	10
Total	32	321

Please cite this article in press as: McFrederick, Q.S., Taylor, D.R. Evolutionary history of nematodes associated with sweat bees. Mol. Phylogenet. Evol. (2012), http://dx.doi.org/10.1016/j.ympev.2012.11.007

Fig. 4. Halictid phylogeny indicating eusocial or socially polymorphic groups with black edges, and solitary or communal groups with gray edges. Names in bold represent groups known to host *Acrostichus* while names in regular font are not known to associate with *Acrostichus*. The phylogenetic relationships are the same as in Fig. 3, while social data are from Brady et al. (2006) and Wcislo and Gonzalez (2006).

An additional hypothesis relates to the mode of reproduction of these nematodes. Bee-associated *Acrostichus* species are gonochoristic (separate males and females, Giblin-Davis et al., 1990), and it has been suggested that gonochoristic nematodes may be less likely to invade new habitats than hermaphroditic nematodes (Herrmann et al., 2010). Perhaps the gonochoristic nature of *Acostichus* species helps maintain host specificity and limits the distribution of the nematodes.

4.3. Future work

Our data suggests promising future research. *H. farinosus*, *H. poeyi,* and *Ag. gratiosa* also host *Acrostichus* nematodes (Giblin-Davis et al., 1990), but as of yet no genetic data exist for these nematodes and their phylogenetic relationships remain unexplored. Studies utilizing molecular barcodes and mating crosses (Kiontke et al., 2011) or molecular barcodes, morphometric analyses, and mating crosses (Herrmann et al., 2006a,b) have been employed to explore the diversity of the insect-associated nematodes in the genera *Caenorhabditis* and *Pristionchus*, respectively. A similar approach was used to distinguish *Ac. puri, Ac. halicti,* and *Ac. megaloptae* (Giblin-Davis et al., 1990; Kanzaki et al., 2010a,b), and our findings suggest that finer scale mating crosses and morphometric studies of bee-associated *Acrostichus* species, including those isolated from hosts such as *H. farinosus*, *H. poeyi,* and *Ag. gratiosa*, would add to our understanding of the diversity of this genus.

Please cite this article in press as: McFrederick, Q.S., Taylor, D.R. Evolutionary history of nematodes associated with sweat bees. Mol. Phylogenet. Evol. (2012), http://dx.doi.org/10.1016/j.ympev.2012.11.007

Acknowledgments

The authors would like to acknowledge the following for their assistance with this research: Robin Giblin-Davis for providing training in nematology and Natsumi Kanzaki for providing sequences for *Ac. megaloptae* and *Ac. rhyncophori*; T'ai Roulston for both support and assistance; Lindsay Parsons, Jessica Wignall, Tara Adiesheshan, and Aine O'Sullivan for assistance in the laboratory and in the field; the Taylor and Antonovics lab groups for comments on a previous version of this manuscript; Bryan Danforth for conversation that benefitted the manuscript, and three anonymous reviewers for their helpful comments. This material is based upon work supported by the National Science Foundation under Doctoral Dissertation Improvement Grant 0808454.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ympev.2012.11.007.

References

Antonovics, J., Boots, M., Abbate, J., Baker, C., McFrederick, Q., Panjeti, V., 2011. Biology and evolution of sexual transmission. Ann. N. Y. Acad. Sci. 1230, 12–24.
Banks, J.C., Palma, R.L., Paterson, A.M., 2006. Cophylogenetic relationships between penguins and their chewing lice. J. Evol. Biol. 19, 156–166.
Baumann, L., Baumann, P., 2005. Cospeciation between the primary endosymbionts of mealybugs and their hosts. Curr. Microbiol. 50, 84–87.
Brady, S.G., Sipes, S., Pearson, A., Danforth, B.N., 2006. Recent and simultaneous origins of eusociality in halictid bees. Proc. Roy. Soc. B – Biol. Sci. 273, 1643–1649.
Brooks, D., McLennan, D., 1993. Parascript: Parasites and the Language of Evolution. Smithsonian Institution Press, Washington and London.
Charleston, M., 2002. Principles of cophylogenetic maps. In: Lassig, M., Valleriani, A. (Eds.), Biological Evolution and Statistical Physics. Springer, pp. 122–147.
Charleston, M.A., Page, R.D.M., 2002. TreeMap (v3.0). Application for the Apple Macintosh. University of Oxford, Oxford, UK.
Charleston, M.A., Perkins, S.L., 2006. Traversing the tangle: algorithms and applications for cophylogenetic studies. J. Biomed. Inform. 39, 62–71.
Clark, M.A., Moran, N.A., Baumann, P., Wernegreen, J.J., 2000. Cospeciation between bacterial endosymbionts (*Buchnera*) and a recent radiation of aphids (*Uroleucon*) and pitfalls of testing for phylogenetic congruence. Evolution 54, 517–525.
Clark, J.W., Hossain, S., Burnside, C.A., Kambhampati, S., 2001. Coevolution between a cockroach and its bacterial endosymbiont: a biogeographical perspective. Proc. Roy. Soc. Lond. Ser. B – Biol. Sci. 268, 393–398.
Clayton, D.H., Bush, S.E., Goates, B.M., Johnson, K.P., 2003. Host defense reinforces host-parasite cospeciation. Proc. Natl. Acad. Sci. USA 100, 15694–15699.
Coelho, B.W.T., 2004. A review of the bee genus *Augochlorella* (Hymenoptera: Halictidae: Augochlorini). Syst. Entomol. 29, 282–323.
Danforth, B.N., 1999. Phylogeny of the bee genus *Lasioglossum* (Hymenoptera: Halictidae) based on mitochondrial COI sequence data. Syst. Entomol. 24, 377–393.
Danforth, B.N., Eickwort, G.C., 1997. The evolution of social behavior in the augochlorine sweat bees (Hymenoptera: Halictidae) based on a phylogenetic analysis of the genera. In: Choe, J.C., Crespi, B.J. (Eds.), The Evolution of Social Behavior in Insects and Arachnids. Cambridge University Press, Cambridge, UK, pp. 270–292.
Danforth, B.N., Sauquet, H., Packer, L., 1999. Phylogeny of the bee genus *Halictus* (Hymenoptera: Halictidae) based on parsimony and likelihood analyses of nuclear EF-1 alpha sequence data. Mol. Phylogenet. Evol. 13, 605–618.
Degnan, P.H., Lazarus, A.B., Brock, C.D., Wernegreen, J.J., 2004. Host-symbiont stability and fast evolutionary rates in an ant-bacterium association: Cospeciation of *Camponotus* species and their endosymbionts, *Candidatus Blochmannia*. Syst. Biol. 53, 95–110.
Denver, D.R., Morris, K., Lynch, M., Vassilieva, L.L., Thomas, W.K., 2000. High direct estimate of the mutation rate in the mitochondrial genome of *Caenorhabditis elegans*. Science 289, 2342–2344.
Desdevises, Y., Morand, S., Jousson, O., Legendre, P., 2002. Coevolution between *Lamellodiscus* (Monogenea: Diplectanidae) and Sparidae (Teleostei): The study of a complex host-parasite system. Evolution 56, 2459–2471.
Ezard, T., Fujisawa, T., Barraclough, T., 2009. SPecies LImits by Threshold Statistics. <http://R-Forge.R-project.org/projects/splits/>.
Giblin, R., Kaya, H., 1984a. Aduncospiculum halicti n. gen., n. sp. (Diplogasterida: Diplogasteroididae), an associate of bees in the genus Halictus (Hymenoptera: Halictidae). Rev. Nematol. 7, 189–197.
Giblin, R.M., Kaya, H.K., 1984b. Associations of halictid bees with the nematodes, *Aduncospiculum halicti* (Diplogastrida, Diplogasteroididae) and *Bursaphelenchus kevini* (Aphelenchida, Aphelenchoididae). J. Kansas Entomol. Soc. 57, 92–99.
Giblin-Davis, R.M., Norden, B.B., Batra, S.W.T., Eickwort, G.C., 1990. Commensal nematodes in the glands, genitalia, and brood cells of bees (Apoidea). J. Nematol. 22, 150–161.
Hafner, M.S., Sudman, P.D., Villablanca, F.X., Spradling, T.A., Demastes, J.W., Nadler, S.A., 1994. Disparate rates of molecular evolution in cospeciating hosts and parasites. Science 265, 1087–1090.
Hazir, C., Giblin-Davis, R.M., Keskin, N., Ye, W., Hazir, S., Scheuhl, E., Thomas, W.K., 2010. Diversity and distribution of nematodes associated with wild bees in Turkey. Nematology 12, 65–80.
Herre, E.A., Knowlton, N., Mueller, U.G., Rehner, S.A., 1999. The evolution of mutualisms: exploring the paths between conflict and cooperation. Trends Ecol. Evol. 14, 49–53.
Herrmann, M., Mayer, W.E., Sommer, R.J., 2006a. Nematodes of the genus *Pristionchus* are closely associated with scarab beetles and the Colorado potato beetle in Western Europe. Zoology 109, 96–108.
Herrmann, M., Mayer, W.E., Sommer, R.J., 2006b. Sex, bugs and Haldane's rule: the nematode genus *Pristionchus* in the United States. Front. Zool. 3, 14.
Herrmann, M., Kienle, S., Rochat, J., Mayer, W.E., Sommer, R.J., 2010. Haplotype diversity of the nematode *Pristionchus pacificus* on Reunion in the Indian Ocean suggests multiple independent invasions. Biol. J. Linn. Soc. 100, 170–179.
Hosokawa, T., Kikuchi, Y., Nikoh, N., Shimada, M., Fukatsu, T., 2006. Strict host-symbiont cospeciation and reductive genome evolution in insect gut bacteria. PLoS Biol. 4, 1841–1851.
Hughes, J., Kennedy, M., Johnson, K.P., Palma, R.L., Page, R.D.M., 2007. Multiple cophylogenetic analyses reveal frequent cospeciation between pelecaniform birds and *Pectinopygus* lice. Syst. Biol. 56, 232–251.
Kanzaki, N., Futai, K., 2002. A PCR primer set for determination of phylogenetic relationships of *Bursaphelenchus* species within the xylophilus group. Nematology 4, 35–41.
Kanzaki, N., Giblin-Davis, R.M., Zeng, Y.S., Ye, W.M., Center, B.J., 2009. *Acrostichus rhynchophori* n. sp (Rhabditida: Diplogastridae): a phoretic associate of *Rhynchophorus cruentatus* Fabricius and *R. palmarum* L. (Coleoptera: Curculionidae) in the Americas. Nematology 11, 669–688.
Kanzaki, N., Giblin-Davis, R.M., Wcislo, W.T., Zeng, Y., Ye, W., Center, B.J., Esquivel, A., Thomas, K.W., 2010a. *Acrostichus megaloptae* n. sp. (Nematoda: Diplogastridae), a phoretic associate of *Megalopta* spp. (Hymenoptera: Halictidae) in Central America. Nematology 12, 453–468.
Kanzaki, N., Giblin-Davis, R.M., Zeng, Y., Ye, W., Center, B.J., Thomas, W.K., 2010b. *Acrostichus puri* n. sp. (Nematoda: Diplogastridae), a phoretic associate of *Augochlora pura mosieri* Cockerell (Hymenoptera: Halictidae). Nematology 12, 49–64.
Kass, R.E., Raftery, A.E., 1995. Bayes factors. J. Am. Stat. Assoc. 90, 773–795.
Kawakita, A., Takimura, A., Terachi, T., Sota, T., Kato, M., 2004. Cospeciation analysis of an obligate pollination mutualism: Have *Glochidion* trees (Euphorbiaceae) and pollinating *Epicephala* moths (Gracillariidae) diversified in parallel? Evolution 58, 2201–2214.
Kawecki, T.J., 1998. Red Queen meets Santa Rosalia: arms races and the evolution of host specialization in organisms with parasitic lifestyles. Am. Nat. 125, 635–651.
Kiontke, K.C., Felix, M.A., Ailion, M., Rockman, M.V., Braendle, C., Penigault, J.B., Fitch, D.H.A., 2011. A phylogeny and molecular barcodes for *Caenorhabditis*, with numerous new species from rotting fruits. BMC Evol. Biol. 11, 339.
Lanterbecq, D., Rouse, G.W., Eeckhaut, I., 2010. Evidence for cospeciation events in the host-symbiont system involving crinoids (Echinodermata) and their obligate associates, the myzostomids (Myzostomida, Annelida). Mol. Phylogenet. Evol. 52, 357–371.
Legendre, P., Desdevises, Y., Bazin, E., 2002. A statistical test for host-parasite coevolution. Syst. Biol. 51, 217–234.
Light, J.E., Hafner, M.S., 2007. Cophylogeny and disparate rates of evolution in sympatric lineages of chewing lice on pocket gophers. Mol. Phylogenet. Evol. 45, 997–1013.
Light, J.E., Hafner, M.S., 2008. Codivergence in heteromyid rodents (Rodentia: Heteromyidae) and their sucking lice of the genus *Fahrenholzia* (Phthiraptera: Anoplura). Syst. Biol. 57, 449–465.
Lim-Fong, G.E., Regali, L.A., Haygood, M.G., 2008. Evolutionary relationships of "*Candidatus Endobugula*" bacterial symbionts and their *Bugula* bryozoan hosts. Appl. Environ. Microbiol. 74, 3605–3609.
Lin, C.P., Danforth, B.N., 2004. How do insect nuclear and mitochondrial gene substitution patterns differ? Insights from Bayesian analyses of combined datasets. Mol. Phylogenet. Evol. 30, 686–702.
Lo, N., Bandi, C., Watanabe, H., Nalepa, C., Beninati, T., 2003. Evidence for cocladogenesis between diverse dictyopteran lineages and their intracellular endosymbionts. Mol. Biol. Evol. 20, 907–913.
Lockhart, A.B., Thrall, P.H., Antonovics, J., 1996. Sexually transmitted diseases in animals: ecological and evolutionary implications. Biol. Rev. Camb. Philos. Soc. 71, 415–471.
Maddison, W.P., Maddison, D.R., 2010. Mesquite: A Modular System for Evolutionary Analysis. Version 2.74. <http://mesuiteproject.org>.
Michener, C.D., 1979. Biogeography of the bees. Ann. Mo. Bot. Gard. 66, 277–347.
Michener, C.D., McGinley, R.J., Danforth, B.N., 1994. The Bee Genera of North and Central America. Smithsonian Institution Press, Washington and London.
Mitchell, T.B., 1960. Bees of the Eastern United States. North Carolina Agricultural Experiment Station, Raleigh, North Carolina.

Molnar, R.I., Bartelmes, G., Dinkelacker, I., Witte, H., Sommer, R.J., 2011. Mutation rates and intraspecific divergence of the mitochondrial genome of *Pristionchus pacificus*. Mol. Biol. Evol. 28, 2317–2326.

Moran, N.A., Baumann, P., 1994. Phylogenetics of cytoplasmically inherited microorganisms of arthropods. Trends Ecol. Evol. 9, 15–20.

Nelson, K., Fisher, C.R., 2000. Absence of cospeciation in deep-sea vestimentiferan tube worms and their bacterial endosymbionts. Symbiosis 28, 1–15.

Nylander, J., 2004. MrModeltest. Program Distributed by the Author, Evolutionary Biology Center. Uppsala University.

Page, R.D.M., 1991. Clocks, clades and cospeciation- comparing rates of evolution and timing of cospeciation events in host-parasite assemblages. Syst. Zool. 40, 188–198.

Page, R.D.M., 1996. Temporal congruence revisited: comparison of mitochondrial DNA sequence divergence in cospeciating pocket gophers and their chewing lice. Syst. Biol. 45, 151–167.

Paradis, E., Claude, J., Strimmer, K., 2004. APE: analyses of phylogenetics and evolution in R language. Bioinformatics 20, 289–290.

Peek, A.S., Feldman, R.A., Lutz, R.A., Vrijenhoek, R.C., 1998. Cospeciation of chemoautotrophic bacteria and deep sea clams. Proc. Natl. Acad. Sci. USA 95, 9962–9966.

Poinar, G.O., 1983. The Natural History of Nematodes. Prentice-Hall Englewood Cliffs, NJ.

Pons, J., Barraclough, T.G., Gomez-Zurita, J., Cardoso, A., Duran, D.P., Hazell, S., Kamoun, S., Sumlin, W.D., Vogler, A.P., 2006. Sequence-based species delimitation for the DNA taxonomy of undescribed insects. Syst. Biol. 55, 595–609.

Posada, D., Crandall, K.A., 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14, 817–818.

Poulin, R., 2008. Evolutionary Ecology of Parasites. Princeton Univ Press, Princeton, New Jersey.

Price, P.W., 1980. Evolutionary Biology of Parasites. Princeton Univ Press, Princeton, New Jersey.

Quinn, G.P., Keough, M.J., 2002. Experimental Design and Data Analysis for Biologists. Cambridge University Press, Cambridge, UK.

R Core Development Team, 2009. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Rambaut, A., Drummond, A.J., 2009. Tracer v1.5.

Ronquist, F., 2003. Parsimony analysis of coevolving species associations. In: Page, R.D.M. (Ed.), Tangled Trees: Phylogeny, Cospeciation and Coevolution. The University of Chicago Press, Chicago, pp. 22–64.

Ronquist, F., Huelsenbeck, J.P., 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572.

Roy, B.A., 2001. Patterns of association between crucifers and their flower-mimic pathogens: host jumps are more common than coevolution or cospeciation. Evolution 55, 41–53.

Sachs, J.L., Skophammer, R.G., Regus, J.U., 2011. Evolutionary transitions in bacterial symbiosis. Proc. Natl. Acad. Sci. 108, 10800–10807.

Sambrook, J., Fritsch, E.F., Maniatis, T., 1989. Molecular Cloning. Cold Spring Harbor Laboratory Press Cold Spring Harbor, NY.

Sauer, C., Stackebrandt, E., Gadau, J., Holldobler, B., Gross, R., 2000. Systematic relationships and cospeciation of bacterial endosymbionts and their carpenter ant host species: proposal of the new taxon *Candidatus Blochmannia* gen. nov. Int. J. Syst. Evol. Microbiol. 50, 1877–1886.

Schwarz, M.P., Richards, M.H., Danforth, B.N., 2007. Changing paradigms in insect social evolution: insights from halictine and allodapine bees. Annu. Rev. Entomol. 52, 127–150.

Soucy, S.L., Danforth, B.N., 2002. Phylogeography of the socially polymorphic sweat bee *Halictus rubicundus* (Hymenoptera: Halictidae). Evolution 56, 330–341.

Swofford, D.L., 2001. PAUP* 4.0 beta 5: Phylogenetic Analysis Using Parsimony (and other methods). Sinauer Associates Sunderland, MA.

Thao, M.L., Baumann, P., 2004. Evolutionary relationships of primary prokaryotic endosymbionts of whiteflies and their hosts. Appl. Environ. Microbiol. 70, 3401–3406.

Thompson, J., 2005. The Geographic Mosaic of Coevolution. The University of Chicago Press, Chicago.

Wcislo, W.T., Gonzalez, V.H., 2006. Social and ecological contexts of trophallaxis in facultatively social sweat bees, *Megalopta genalis* and *M. ecuadoria* (Hymenoptera, Halictidae). Insectes Soc. 53, 220–225.

Wilgenbush, J.C., Warren, D.L., Swofford, D.L., 2004. AWTY: A System for Graphical Exploration of Convergence in Bayesian Phylogenetic Inference.

Williams, B.D., Schrank, B., Huynh, C., Shownkeen, R., Waterston, R.H., 1992. A genetic mapping system in *Caenorhabditis elegans* based on polymorphic sequence-tagged sites. Genetics 131, 609–624.

Won, Y.J., Jones, W.J., Vrijenhoek, R.C., 2008. Absence of cospeciation between deep-sea mytilids and their thiotrophic endosymbionts. J. Shellfish Res. 27, 129–138.

Ye, W., Giblin-Davis, R.M., Braasch, H., Morris, K., Thomas, W.K., 2007. Phylogenetic relationships among *Bursaphelenchus* species (Nematoda: Parasitaphelenchidae) inferred from nuclear ribosomal and mitochondrial DNA sequence data. Mol. Phylogenet. Evol. 43, 1185–1197.

Zwickl, D.J., 2006. Genetic Algorithm Approaches for the Phylogenetic Analysis of Large Biological Sequence Datasets under the Maximum Likelihood Criterion. The University of Texas at Austin.

Please cite this article in press as: McFrederick, Q.S., Taylor, D.R. Evolutionary history of nematodes associated with sweat bees. Mol. Phylogenet. Evol. (2012), http://dx.doi.org/10.1016/j.ympev.2012.11.007