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COPHYLOGENY OF *NOSEMA* (MICROSPORIDIA: NOSEMATIDAE) AND BEES (HYMENOPTERA: APIDAE) SUGGESTS BOTH COSPECIATION AND A HOST-SWITCH

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ABSTRACT: Some microsporidian parasites belonging to the genus *Nosema* infect bees. Previous phylogenies of these parasites have produced alternative, conflicting relationships. We analyzed separately, and in combination, large and small subunit ribosomal DNA sequences of *Nosema* species infecting bees under neighbor-joining, maximum parsimony, maximum likelihood, and Bayesian frameworks. We observed a sister relationship between *Nosema ceranae* and *Nosema bombi*, with *Nosema apis* as a basal member to this group. When compared to their respective hosts (*Apis cerana*, *Bombus* spp., and *A. mellifera*), 2 plausible evolutionary scenarios emerged. The first hypothesis involves a common ancestor of *N. bombi* host-switching from a historical *Bombus* lineage to *A. cerana*. The second suggests an ancestral *N. ceranae* host-switching to a species of *Bombus*. The reported events offer insight into the evolutionary history of these organisms and may explain host specificity and virulence of *Nosema* in these economically important insects.

Cophylogenetic studies investigate the evolutionary history of interacting organisms (Paterson and Gray, 1997; Johnson and Clayton, 2004). Cophylogenetic studies may suggest a hypothesis of congruence between host-parasite phylogenies as a result of cospeciation. However, in many cases congruence is imperfect because of macroevolutionary processes, including host-switching (a parasite evolves viability on/in a new host species), duplication (a parasite speciates while the host remains a single species), and sorting (a parasite becomes extinct) (Paterson and Gray, 1997; Johnson and Clayton, 2004). The evolutionary history of many host and parasite taxa remains unclear; therefore, resolution of their phylogenies can provide insight into the macroevolutionary events that shaped present-day interactions among these taxa.

Because of their economic importance, microsporidian parasites associated with bees have received considerable attention. *Nosema bombi* infects multiple bumble bee (*Bombus*) species (Tay et al., 2005), and *Nosema apis* and *Nosema ceranae* are known from western (*Apis mellifera*) (Fries, 1993) and Asian honey bees (*Apis cerana*) (Fries et al., 1996), respectively. Recent evidence suggests that *N. ceranae* made a host switch to the western honey bee over 10 yr ago (Klee et al., 2007; Paxton et al., 2007; Chen et al., 2008), displacing *N. apis* as the most common form of *Nosema* disease in many regions of the world (Klee et al., 2007; Chen et al., 2008; Williams, Sampson, et al., 2008; Williams, Shafer, et al., 2008). Symptoms of *Nosema* disease vary among host-parasite systems. *Nosema bombi* may cause sluggish behavior and early death (Schmid-Hempel, 1998), whereas *N. apis* can be virulent to western honey bees in temperate climates (Moeller, 1972). Pathology of *N. ceranae* infection is less studied, but in western honey bees it has been implicated in reduced honey production and increased winter mortality, and it is highly pathogenic when experimentally inoculated into adults (Higes et al., 2006, 2007; Paxton et al., 2007). *Nosema ceranae* may also be contributing to the large number of recent honey bee deaths in Europe (Higes et al., 2005, 2007; Martin-Hernandez et al., 2007) and the United States (Cox-Foster et al., 2007; Oldroyd, 2007).

Although the phylogenetic relationship of *Bombus* spp., *A. cerana*, and *A. mellifera* is unambiguous (e.g., Whitfield and Cameron, 1998; Arias and Sheppard, 2005), the phylogeny of their respective *Nosema* parasites is less clear. Different analyses using almost identical GenBank submissions have yielded conflicting results, placing *N. apis* closer to *N. ceranae* (Slamovits et al., 2004), *N. apis* closer to *N. bombi* (Vossbrinck and Debrunner-Vossbrinck, 2005), or *N. bombi* closer to *N. ceranae* (Fries et al., 2001; Wang et al., 2006). Furthermore, each of these studies used only a single sequence as representative of each taxon and, therefore, may not have accounted for different strains, i.e., haplotypes, within each species. The first objective of the present study was to reanalyze evolutionary relationships among microsporidian species infecting *Bombus* spp., *A. mellifera*, and *A. cerana* (Hymenoptera: Apidae), using multiple sequence data sets. The second objective was to assess congruence between the parasite phylogeny and that of their corresponding hosts. Our results provide insight into the coevolutionary history of these taxa and may help to explain host specificity and pathogenicity of *Nosema* in these economically important insects.

MATERIALS AND METHODS

Parasite and host sequence data

GenBank was the source of all DNA sequence data. Large and small subunit ribosomal RNA (LS and SS rRNA) data were gathered for all microsporidian species known to infect members of the Apidae, as well as for 2 related parasite outgroups, *Nosema bombycis* and *Nosema spodopterae*, of the silkworm (*Bombyx mori*) and cutworm (*Spodoptera litura*), respectively. For each corresponding host, cytochrome *b* gene sequence data were gathered. Targeted GenBank sequences were >1,200 bp and >400 bp for parasites and hosts, respectively, to incorporate as many data as possible. Ribosomal RNA gene sequences can vary widely among taxa; therefore, parasite sequence data were aligned using the program SOAP (Loytnoja and Milinkovitch, 2001). SOAP employs the standard CLUSTALW algorithm (Thompson et al., 1994) and provides an objective means for identifying unstable sites, i.e., alignments not supported by all weighting schemes, to be excluded prior to phylogenetic and cophylogenetic analyses (all alignments are available from the corresponding author). Host sequence data were aligned using CLUSTALW available in BioEdit v. 7.0.5.3. (Hall, 1999) and verified by eye.

Phylogenetic analyses

Neighbor-joining and maximum parsimony analyses: Neighbor-joining (NJ) analysis with pairwise deletion and maximum parsimony (MP) analysis were used to calculate phylogenetic positions among parasites for LS and SS rRNA sequence data, and among hosts for cytochrome *b* sequence data. For parasite and host sequences, NJ analysis construct-

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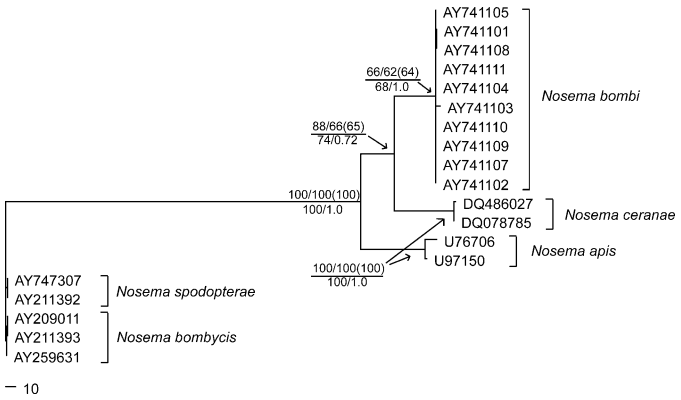

FIGURE 1. Most parsimonious large subunit ribosomal RNA (2145 bp) tree for *Nosema*. Support values for each phylogenenetic method are indicated as follows: *above node,* neighbor-joining/maximum parsimony (maximum parsimony including gaps as fifth character); *below node,* maximum likelihood/Bayesian posterior probability. Bootstrap values for neighbor-joining and maximum parsimony methods obtained from 1,000 replicates. Maximum likelihood support assessed by 100 bootstrap replicates. Bayesian support calculated from 5,000,000 generations. Branch lengths are proportional to the numbers of nucleotide substitutions and are measured by scale bars.

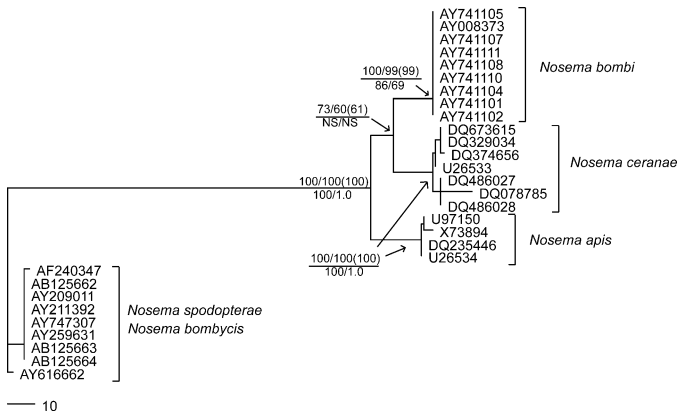

FIGURE 2. Most parsimonious small subunit ribosomal RNA (1058 bp) tree for *Nosema*. Support values for each phylogenenetic method are indicated as follows: *above node,* neighbor-joining/maximum parsimony (maximum parsimony including gaps as fifth character); *below node,* maximum likelihood/Bayesian posterior probability. Bootstrap values for neighbor-joining and maximum parsimony methods obtained from 1,000 replicates. Maximum likelihood support assessed by 100 bootstrap replicates. Bayesian support calculated from 5,000,000 generations. NS means not supported. Branch lengths are proportional to the numbers of nucleotide substitutions and are measured by scale bars.

ed distance matrices using p-distance, Jukes-Cantor, and Kimura's 2-parameter models. Trees accompanying NJ analyses were constructed using the software package MEGA 3 (Kumar et al., 2004). In the MP analysis, the software package PAUP v. 4.0b10 (Swofford, 2002) was used to implement a full heuristic search with all characters unweighted and unordered. Gaps were treated both as missing data and a fifth character. All searches included 100 stepwise random addition replicates with tree bisection-reconnection branch (TBR) swapping and MAXTREES set to 100. Confidence in all NJ and MP analyses was assessed using 1,000 bootstrap replicates.

Maximum likelihood and Bayesian analyses: The appropriate model of nucleotide substitution for the maximum-likelihood (ML) analysis was determined using Modeltest v. 3.06 (Posada and Crandall, 1998). This program selected a GTR+G model using hLRT criteria for the LS rRNA sequences and a HKY+G model of nucleotide evolution for the SS rRNA sequences. Because ML analysis is computationally taxing, a subset of host sequence data (n = 15; 4 for bumble bees, 4 for the Asian honey bee, 4 for the western honey bee, and 3 for the silkworm) were used in the analysis, and, from these data, the GTR+I+G model of substitution was selected. All samples were added to the tree using 10 random addition replicates and the TBR branch-swapping algorithm. The number of trees saved per replicate was reduced to 1, and confidence of the ML tree topology was tested with 100 bootstrap replicates.

Because not all models of evolution available in Modeltest can be run using the software program MrBayes v. 3.0. (Huelsenbeck and Ronquist, 2001), Mr Modeltest 2.2 (Nylander, 2004) was used to select the most appropriate available model. Based on hLRT criteria, the best-fit nucleotide substitution model available in Mr Modeltest was GTR+G for LS rRNA and cytochrome *b* data, and HKY+G for SS rRNA data. Applying the selected substitution model in MrBayes v. 3.0, the Markov chain Monte Carlo algorithm was run for all sequence data sets with 4 chains until the average standard deviation of split frequencies stabilized at <0.01. In total, 5,000,000 generations were run with sampling every 100 generations. Using these data the first 1,250,000 (25%) generations were discarded as burn-in, and the remaining 37,500 trees were used to construct the phylogenetic tree.

Combined parasite data analyses: Consensus sequences were created for the LS and SS rRNA sequences of each parasite that was monophyletic (Figs. 1, 2). To generate a more robust phylogeny, the LS and SS rRNA consensus sequences of each parasite species were combined into a single data set and further analyzed. To test for homogeneity among data sets, we conducted the incongruence length difference (ILD) test (Farris et al., 1994) implemented in PAUP v.4.0b10 (Swofford, 2002) under a flat-weighting scheme with uninformative characters removed. One hundred permutations were then run under the specified character partitions. Unequal size between data sets can lead to increased (albeit faulty) measures of congruence (Dowton and Austin, 2002); therefore, we ran a weighting scheme of equal-parsimony informative weights. NJ and MP analyses for these new parasite data were performed using the aforementioned criteria, and ML analysis was run using a GTR+G model of substitution as selected by Modeltest. Because MrBayes v. 3.0. allows for the decoupling of parameters in an evolutionary model (Jansa and Voss, 2005), combined LS and SS rRNA data were analyzed using a joint GTR+G and HKY+G substitution model for the respective data sets.

Tests of alternative topological hypotheses: We also conducted the nonparametric Shimodaira-Hasegawa (SH) (Shimodaira-Hasegawa, 1999) and parametric SOWH (Swofford et al., 1996; Goldman et al., 2000) bootstrap tests on all 3 data sets. First, ML trees were constructed in PAUP v. 4.0b10 (Swofford, 2002) using heuristic searches with random sequence addition and tree-bisection-reconnection. Analyses were run under the previously specified model of evolution (parameters estimated) with constraints according to the possible 2 alternative topologies [1: ((*N. apis*)(*N. ceranae*)) and 2: ((*N. bombi*)(*N. apis*))]. Under the SH test, 1,000 bootstrap replicates of each ML tree [our ML tree ((*N. ceranae*)(*N. bombi*)) and each of the 2 alternate topologies] were resampled using the re-estimated log likelihoods on ML trees corresponding to each topology. Any topology with $P < 0.05$ in the 1-tailed *t*-test was considered incongruent. For the SOWH test, the test statistic (δ) was calculated by subtracting the likelihood of 1 of the 2 alternate topologies, i.e., H_A, from the maximum likelihood tree, i.e., H_O. One hundred replicate data sets were then simulated in Seq-Gen v. 1.5.3 (Rambaut and Grassly, 1997) under the H_A with the previously selected model and parameters fixed to be the ML estimates for the alternate ML topology with no polytomies. For each simulated data set, we then calculated ML estimates of the H_A and H_O under a GTR+G model, but with parameters re-estimated (see Goldman et al., 2000). The $\delta^{(100)}$ was then calculated from the simulated data ML estimates, giving a null distribution of the test statistic. A 1-tailed test was conducted to assess whether the observed δ fell below 95% of the ranked list of $\delta^{(100)}$.

Cophylogenetic analyses

Congruence between parasite and host phylogenies (excluding outgroups) constructed from ML analysis of the consensus sequences was assessed using TreeMap 1.0. (Page, 1994) and 2.0β (Charleston and Page, 2001), and TreeFitter (Ronquist, 2001). In TreeMap 1.0 a parsimony-based reconciliation argument was used to assess congruence by

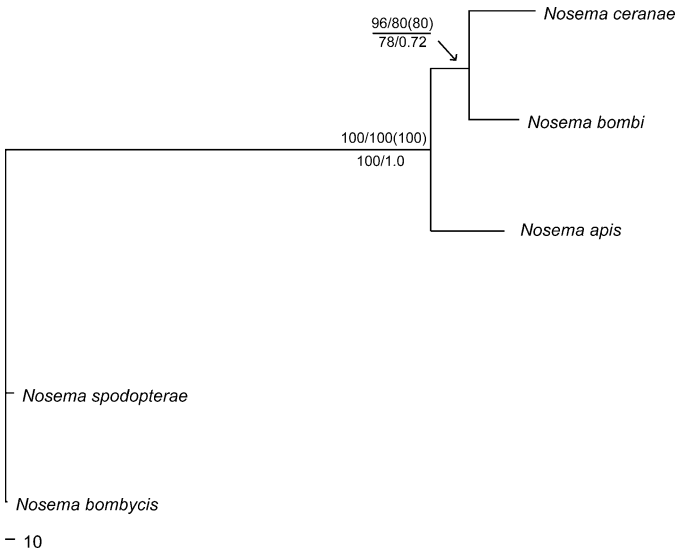

FIGURE 3. Most parsimonious tree for *Nosema* using a combined data set of large and small subunit ribosomal RNA (3203 bp). Support values for each phylogenenetic method are indicated as follows: *above node,* neighbor-joining/maximum parsimony (maximum parsimony including gaps as fifth character); *below node,* maximum likelihood/Bayesian posterior probability. Bootstrap values for neighbor-joining and maximum parsimony methods obtained from 1,000 replicates. Maximum likelihood support assessed by 100 bootstrap replicates. Bayesian support calculated from 5,000,000 generations. Branch lengths are proportional to the numbers of nucleotide substitutions and are measured by scale bars.

estimating the number of cospeciation (C), host-switching (H), duplication (D), and sorting (S) events. A second analysis to assess the cophylogenetic relationship between hosts and parasites was performed using Treemap 2.0β, which employs an exhaustive search using the Jungles algorithm (Charleston, 1998). This program allows for costs to be assigned to each of the 4 possible macroevolutionary events (C, H, D, S). Costs for each event consisted of the default setting (C = 0, H = 1, D = 1, S = 1). In addition, the event-based parsimony program TreeFitter estimated the number of macroevolutionary events. Using 10,000 permutational steps of both the host and parasite trees, we ran the analysis using a variety of event cost combinations for the 4 possible macroevolutionary events (C, H, D, S) (Ricklefs et al., 2004).

RESULTS

Phylogenetic analyses

Using SOAP, 2145 bp and 1058 bp were selected from each parasite's LS and SS rRNA gene sequences (which are nuclear in microsporidia), respectively. The NJ trees generated for the *Nosema* samples were identical regardless of the distance metric used, i.e., p-distances, Jukes-Cantor, or Kimura's 2-parameter model. All subsequent NJ analyses are, therefore, based on p-distances, which require the fewest assumptions (Nei and Kumar, 2000; Malia et al., 2003). All phylogenetic analyses of the LS rRNA sequence data supported *N. bombi* as a sister species to *N. ceranae*, with *N. apis* as a basal member to the group (Fig. 1). Both NJ and MP analysis of the SS rRNA weakly supported the aforementioned relationships (Fig. 2); however, the Bayesian and ML analyses did not produce this relationship, suggesting a relationship between *N. ceranae* and *N. apis*. In the combined analysis, concatenation of data was supported under both weighting schemes using the ILD test ($ps > 0.05$). All phylogenetic approaches supported the sister relationship of *N. bombi* and *N. ceranae* (Fig. 3). For the hosts, 432 bp of cytochrome *b* gene were analyzed. All phylogenetic analyses robustly supported western and Asian honey bees as monophyletic taxa, with all species of bumble bee forming a sister clade (trees not shown; see Fig. 4). All SOWH tests supported the sister clade of *N. bombi* and *N. ceranae* in Table I. Furthermore, δ had the highest rank in the $\delta^{(100)}$ distribution in all tests. The SH test rejected ((*N. ceranae*) (*N. apis*)) as a viable topology given these data. However, the topological alternative ((*N. apis*) (*N. bombi*)) was not rejected using the SH test ($P = 0.06, 0.09$). Finally, these tests were not conducted on the SS rRNA because of a lack of likelihood support (Fig. 2).

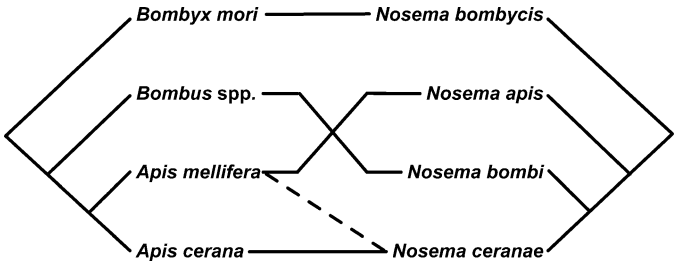

FIGURE 4. Comparisons of maximum likelihood trees of *Nosema* and their corresponding bee hosts. The dashed line represents what may be a recent host switch of *Nosema ceranae* to *Apis mellifera*. Only host *Bombyx mori* and parasite *N. bombycis* are included as outgroup references.

Cophylogenetic analyses

The ML topologies of the combined parasite sequence data set and host data set were used in the cophylogenetic analysis and considered as species trees. This revealed that host and parasite phylogenies were not congruent (Fig. 4). Without introducing host-switching events, TreeMap 1.0 introduced 1 cospeciation (disregarding parasite and host outgroups), 1 duplication, and 3 sorting events. When host switches were permitted

TABLE I. Topology tests comparing our proposed sister relationship (*N. bombi, N. ceranae*) and 2 alternatives using SH and SOWH tests.

Topology in this study	Alternative topologies	LSrRNA	SSrRNA†	Concatenated data set
(*N. ceranae, N. bombi*)	(*N. apis, N. ceranae*)	SH: $p = 0.03$*	SH: NA	SH: $p = 0.02$*
		SOWH: $p < 0.01$*	SOWH: NA	SOWH: $p < 0.01$*
	(*N. apis, N. bombi*)	SH: $p = 0.09$	SH: NA	SH: $p = 0.06$
		SOWH: $p < 0.01$*	SOWH: NA	SOWH: $p < 0.01$*

*Denotes significant *p* value.
†NA = tests are not applicable given the alternate maximum likelihood topology (see Fig. 2).

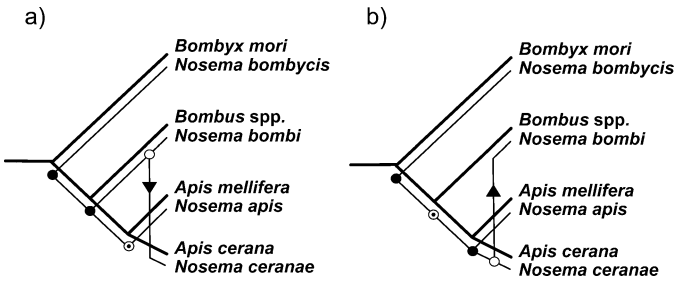

FIGURE 5. Reconstructed coevolutionary histories between *Nosema* spp. and their hosts consisting of 2 cospeciation events (black circles), a sorting event (dotted circle), and a host switch (white circle) from (a) *Bombus* spp. to *A. cerana* or (b) *A. cerana* to *Bombus*.

TABLE II. Range in the number of events required by TreeFitter to reconcile the host and parasite phylogenies under different event cost settings.

Event costs*	Cospeciation	Duplication	Sorting	Host-switching
0, 0, 1, 2	1	0	0	1
1, 1, 1, 1	0–1	0	0	1–2
0, 1, 1, 1	1	0	0	1–2
1, 0, 1, 1	0–1	0	0	1–2
1, 1, 0, 1	0–1	0–2	0–5	0–2
1, 1, 1, 0	0	0	0	2

*Event costs are as follows: cospeciation, duplication, sorting, host-switching.

(using an exact search), the analysis yielded 2 optimal scenarios, each with 1 cospeciation (disregarding parasite and host outgroups), 1 host switch, and 1 sorting event (Figs. 5a, b). Percentage of cospeciating nodes was 50%. These results were not significant ($P > 0.05$). When the costs of macroevolutionary events were left at default values in TreeMap 2.0β, the optimal solution (excluding results for outgroups) was 1 cospeciation, 1 host switch, and 1 sorting event (Figs. 5a, b). Furthermore, when the cost of host-switching was set to 10, the same reconstructions were optimal, but scored lower than a reconstruction (not shown) consisting of 2 cospeciations, 2 duplications, and 3 sorting events. TreeFitter postulated a number of different scenarios for the different cost structures (Table II); however, in all penalty schemes, host-switching and a single cospeciation event were observed (less 1 scheme for the latter), thus supporting the hypothesized scenarios in Figure 5.

DISCUSSION

Our resolved phylogeny of microsporidians associated with bees reveals that *N. ceranae* is a sister species to *N. bombi* and that *N. apis* is the basal member of the clade. Although bootstrap values varied considerably, 13 of the 15 analyses conducted support this relationship. Bootstrap values are generally considered conservative (e.g., Wilcox et al., 2002), so we are confident in this placement. Support from the SH and SOWH add additional confidence to the purported topology, as they strongly rejected the weak ML SS rRNA topology of ((*N. ceranae*)(*N. apis*)). This is the first study to use LS rRNA, and combined LS rRNA and SS rRNA, to resolve the phylogeny of *Nosema* species that naturally infect bees.

Previous studies using SS rRNA have suggested a number of different relationships among *N. bombi*, *N. ceranae*, and *N. apis* (e.g., Fries et al., 2001; Slamovits et al., 2004; Vossbrinck and Debrunner-Vossbrinck, 2005; Wang et al., 2006). Much of the discrepancy may be explained by the treatment of indels (insertions or deletions). Hierarchical model-based analyses such as ML and those employed by MrBayes do not consider gaps and likely explain the poor resolution observed with the SS rRNA data set. For consistency with the ML and Bayesian analyses, we excluded all gaps from the reported topologies (Figs. 1–3). When coding gaps as a fifth character, the problem of order, number (multiple site insertion or deletion), and weighting of indels occurs; however, both treatments can be equally reliable (e.g., Ogden and Rosenberg, 2007). Differing phylogenetic topologies may also be a result of alternative alignments. When rRNA data across species and genera are analyzed, removal of ambiguously aligned sites improves phylogenetic certainty; removal occurred in only 1 (i.e., Slamovits et al., 2004) of the aforementioned studies. A final consideration is the presence of multiple copies of rRNA genes. Both *N. bombi* and *N. apis* contain multiple rRNA copies (Gatehouse and Malone, 1998; Tay et al., 2005) that may represent a case of concerted evolution (Dover and Coen, 1981; Dover, 1982; Hillis and Dixon, 1991). However, O'Mahony et al. (2007) have recently documented multiple rRNA variants of *N. bombi* failing to homogenize, raising into question the phylogenetic utility of microsporidian rRNA genes. Nevertheless, given our multiple rRNA markers, combined data set, and limited within-species sequence variation and observed monophyly of each group, we are confident in the reported phylogeny. Furthermore, some morphological and biological data are consistent with this relationship, because spore length and width of *N. bombi* and *N. ceranae* are more similar to each other than either is to *N. apis* (Fries et al., 1996; Li et al., 2005) and, unlike *N. apis*, both *N. ceranae* and *N. bombi* parasitize multiple host tissue types (J. Chen, pers. comm.).

Two equally parsimonious scenarios emerged for evolutionary relationships between *Nosema* and their corresponding host bees, both involving host switches (each with a sorting event). The first hypothesis involves a common ancestor of *N. bombi* host-switching to an ancestral lineage of the Asian honey bee, giving rise to *N. ceranae* (Fig. 5a). Ancestral stock of *N. ceranae* may also include the precursors to other *Nosema* species such as *Nosema necatrix* and *Nosema oulemae* (Wang et al., 2006). *Nosema bombi* infects a number of different host tissues, which may explain why infection in multiple bumble bee species is observed (Fries et al., 2001). This low host specificity could have facilitated host-switching by ancestral *N. bombi* stock to Asian honey bees. In addition, virtually ubiquitous bumble bee species (~300 in total) (Prys-Jones and Corbet, 1987; Williams, 1998) have undoubtedly had historical contact with Asian honey bees, which could have aided host-switching. The second hypothesis involves an ancestral *N. ceranae* host-switching to an ancestral lineage of bumble bees (Fig. 5b). Similar distribution patterns support this argument, because home ranges of ancestral Asian honey bees and some bumble bee species likely overlapped. Furthermore, the recent host switch by *N. ceranae* to the western honey bee (Higes et al., 2006; Huang et al., 2007; Klee et al., 2007) demonstrates the ability of *Nosema* to switch hosts when suitable conditions occur, e.g.,

sympatry of Asian and western honey bees created by global commercialization of the latter.

Very little is known about effects of the emerging parasite *N. ceranae* on western honey bees, and, surprisingly, even less is known about pathology associated with this parasite in its historical host, the Asian honey bee. However, Fries et al. (2001) hypothesized that impacts of *N. ceranae* on Asian honey bees are similar to those on *N. apis* in western honey bees. Preliminary investigations suggest that *N. ceranae* is highly virulent to western honey bees (Higes et al., 2006, 2007; Paxton et al., 2007), a species whose annual pollination services to agriculture are valued at $14 billion annually in the United States alone (Morse and Calderone, 2001). Although *N. ceranae* is not likely to be the primary cause of recent colony deaths in the United States, it may be a contributing factor (Oldroyd, 2007). Recent work also suggests *N. ceranae* may be the main contributing factor to the bee die-offs in Europe (Higes et al., 2007; Martin-Hernandez et al., 2008). Preliminary data suggest fumagillin, an antibiotic used to control *N. apis*, is effective against *N. ceranae* (Williams, Sampson, et al., 2008). However, because fumagillin was ineffective against *N. bombi* in the bumble bee *Bombus occidentalis* (Whittington and Winston, 2003) and, given the above noted similarities shared with *N. ceranae*, i.e., infection of multiple tissue types, further work is needed.

The most common molecular marker used in microsporidian systematics is ribosomal RNA; however, recent phylogenetic concerns over these genes have been raised (Ironside, 2007). To fully resolve the microsporidian phylogeny and to test the evolutionary scenarios presented within, future studies should use multiple single copy genes, such as RNA polymerase II. Additionally, extensive sampling of all bee-infecting *Nosema* species, particularly in Asia where *N. ceranae* and *N. bombi* may have historically existed in sympatric populations of Asian honey bees and bumble bees, respectively, will aid our understanding of the phylogeny, transmission, and virulence of these globally important parasites. Particular attention should also be paid to bumble bees that, similar to honey bees, are valued at billions of dollars annually to agriculture globally (Velthuis and van Doorn, 2006). Our results showing *N. ceranae* closely related to *N. bombi*, as well as experimental infections showing susceptibility of bumble bees to the more distant *N. apis* (Fantham and Porter, 1913; Showers et al., 1967), suggest that this genus may be vulnerable to *N. ceranae*, especially as other bumble bee species encounter this parasite around the world.

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