Canadian Journal of Zoology

Research Article

Factors influencing emerald ash borer ecological interactions

Nicole D. Borsato[a], S. Eryn McFarlane[a], Nina R. Garrett[a], Alejandro José Biganzoli-Rangel[a], Daniel Marquina[a], Dirk Steinke[b], Robin Floyd[b], and Elizabeth L. Clare[a]

[a]Department of Biology, York University, Toronto, ON, Canada; [b]Center for Biodiversity Genomics, University of Guelph, Guelph, ON, Canada

Corresponding author: **Elizabeth Clare** (email: eclare@yorku.ca)

Abstract

Emerald ash borer beetles (*Agrilus planipennis* Fairmaire, 1888) in North America are a destructive invasive species that increase tree mortality continent-wide, resulting in major ecological and economic impacts. Trees that are infested experience mortality rates which can exceed 99%, disrupting ecological communities and threatening the $218 billion forestry industry in North America. We use DNA metabarcoding from multiple markers to analyze the fungal, parasitic, and microbial interactions of these beetles, and assess the relative importance of life stage (e.g., larvae, pupae, and adults), collection location, habitat, and date on the detection of ecological interactions. We detected 30 different taxonomic orders including several likely agents of biocontrol, such as the known commercially available *Beauveria* fungus, and several potential parasites and parasitoids including *Wolbachia* and ichneumonid wasps. A random forest model suggests the detection of interactions is best predicted by collection date and life stage, with interactions more likely to be detected in pupal samples which may be the ideal target for future analysis, where cost and time constraints prevent the more thorough analysis of all life stages.

Key words: *Agrilus planipennis* Fairmaire, 1888, Emerald ash borer, pest control, forestry, *Fraxinus* spp.

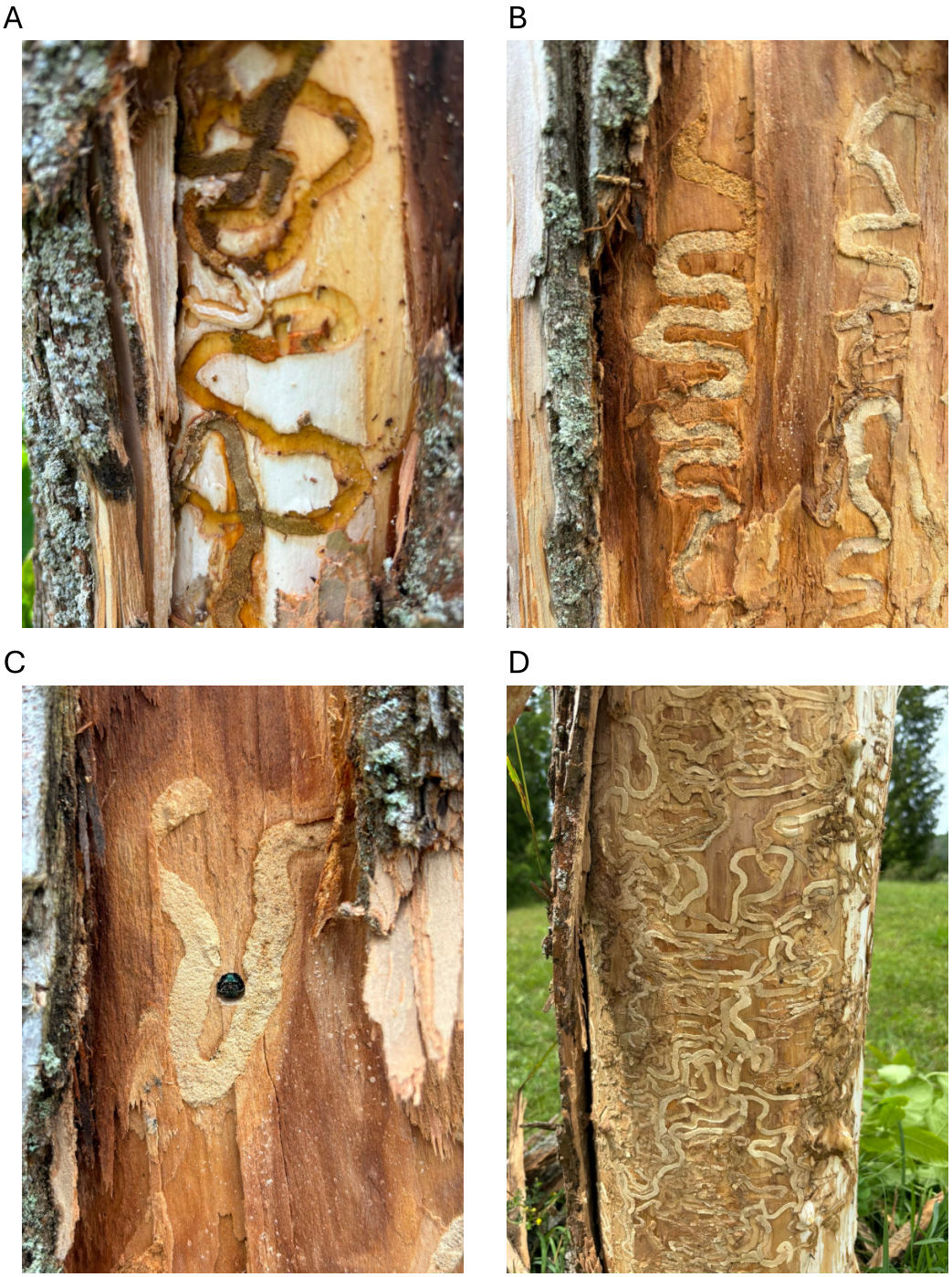
Introduction

The invasive emerald ash borer (*Agrilus planipennis* Fairmaire, 1888) is a wood-boring beetle native to northeastern Asia and was likely introduced to North America from the Tianjin City/Hebei region of China (Bray et al. 2011). It was first detected in North America near Detroit, Michigan in summer 2002, although it is believed that the first individuals were introduced to the area in the 1990s and remained undetected (Cappaert et al. 2005; Siegert et al. 2014). Since its detection in 2002, the beetle has spread to at least five provinces in southeastern Canada (Ontario, Quebec, Manitoba, Nova Scotia, and New Brunswick) and 36 states in the United States of America, the majority of which are in northeastern regions adjacent to Canada (Natural Resources Canada 2013; United States Department of Agriculture 2023).

These forest pests primarily target stressed ash (*Fraxinus* spp. L.) trees (e.g., trees that are girdled, wounded, or growing in unfavourable conditions), although healthy trees are susceptible to beetle colonization as well (Wei et al. 2004; Cappaert et al. 2005; McCullough et al. 2009*a*, 2009*b*; Wang et al. 2010). The larvae consume the phloem and cambium of the ash trees they inhabit, leaving behind characteristic S-shaped galleries in their wake (Fig. 1) that disrupt the transportation of nutrients throughout the tree. Many of these galleries also score the outer sapwood (xylem), further disrupting the flow of water and nutrients in affected trees (Cappaert et al. 2005; Wang et al. 2010). Emerald ash borer infestations are associated with severe ecological and economic impacts. Infestations lead to extensive ash tree canopy loss, which results in tree mortality rates (including those of mature, healthy trees) often exceeding 99% within the first decade of emerald ash borers arriving in an area (Knight et al. 2013; Natural Resources Canada 2013; Klooster et al. 2014) and, as a result, six North American ash species are now included in the International Union for Conservation of Nature's (IUCN) Red List of Threatened Species (International Union for Conservation of Nature 2022).

Increased ash tree mortality reduces the availability of food and shelter for organisms that rely on those trees (e.g., eastern ash bark beetle (*Hylesinus aculeatus* Say, 1826), black-headed ash sawfly (*Tethida barda* Ross, 1938), etc.), alters the structure of ecological communities (e.g., canopy dieback creates gaps that allow light to reach subcanopy levels, encouraging the growth of understory species, such as *Acer* spp L., over infested ash trees, altering the distribution and abundance of taxa (Canham 1988; Gandhi and Herms 2010; Flower et al. 2013)), and threatens the $218 billion gross domestic product forestry industry in Canada and the United States of America (Aukema et al. 2011; United States Department of Agriculture 2021; Natural Resources Canada 2022). Since their introduction in the 1990s, emerald ash borers have

Fig. 1. Extensive damage caused by emerald ash borers (*Agrilus planipennis*). (A) a larva in situ cutting a trail just under the bark, (B) characteristic "S" shaped paths cutting off fluid and nutrient transport, (C) an adult in a recessed tunnel extending through the bark, and (D) extensive damage just under otherwise normal looking bark of an ash tree which had lost >90% of its canopy and was scheduled for removal. All images taken on the Bruce Peninsula, Ontario Canada, 20 July 2024.



killed tens of millions of trees (Cappaert et al. 2005) and are estimated to be the costliest forest pest in North America, responsible for approximately $850 million annually in local government expenditures (i.e., tree removal, replacement, and treatment) and approximately $380 million annually in lost residential property values in the USA alone (Aukema et al. 2011).

Management of emerald ash borer populations

There have been multiple unsuccessful attempts to eradicate emerald ash borer populations in North America since their detection in 2002. This lack of success is partly attributed to infestations being difficult to detect until the population density increases and the host begins exhibiting external symptoms of an infes-

tation (e.g., crown dieback, bark splits, etc. (Cappaert et al. 2005)). Early suppression efforts included quarantine areas (where populations were established; discontinued by the United States Department of Agriculture—Animal and Plant Health Inspection Service (USDA-APHIS) in 2021 (Removal of Emerald Ash Borer Domestic Quarantine Regulations 2020; Hope et al. 2021)) and the regulation of the transportation of wood products from infested quarantine areas both in North America and beyond (Government of Canada 2013*a*; Removal of Emerald Ash Borer Domestic Quarantine Regulations 2020; Hope et al. 2021).

Other suppression efforts make use of a combination of physical, chemical, and biological controls. However, these methods have not substantially controlled the spread and culling of trees has been common once an infestation is identified. Physical controls include creating ash-free firebreak zones and the removal of infested trees. However, adult beetles can fly far enough to cross firebreak zones or can be established outside the zone prior to creation without being noticed (Taylor et al. 2010; Herms and McCullough 2014) which makes firebreak zones ineffective. Additionally, the removal of infested trees is dependent on the infestations having visible external effects on the host tree. Chemical controls include the application of insecticides (e.g., imidacloprid, dinotefuran, azadirachtin, and emamectin benzoate; Herms and McCullough 2014; McCullough 2020) that may have unintended consequences on the environment, which can limit the areas they can be applied to and their utility. Biological controls include the release of natural predators and parasitoids of emerald ash borers (i.e., the parasitoid wasps *Tetrastichus planipennisi* (Yang, 2006), *Spathius agrili* (Yang, 2005), *Spathius galinae* (Belokobylskij and Strazanac, 2012), and *Oobius agrili* (Zhang and Huang, 2005)) that were imported from China and Russia (Government of Canada 2013*b*; Duan et al. 2018). Species native to North America, such as woodpeckers, parasitoid wasps (e.g., *Atanycolus cappaerti* (Marsh and Strazanac, 2009)), and the fungus *Beauveria bassiana* ((Balsamo) Vuillemin, 1912) (now commercially available as FraxiProtec), have also been linked to decreased infestation levels (Cappaert et al. 2005; Lindell et al. 2008; Cappaert and McCullough 2009; Srei et al. 2020), but the effectiveness of these biological controls varies, and it is unclear whether potential agents are functional in the wild. Woodpecker predation, for instance, is dependent on pest population density and the condition of the ash trees (Lindell et al. 2008; Jennings et al. 2013). Similarly, the effectiveness and horizontal transmission of *Beauveria* may depend on the density of infested trees and pest populations (Srei et al. 2020). Additionally, one of the introduced parasitoids (*S. agrili*) was found to be unable to establish self-sustaining populations in regions north of 40°N latitude and has yet to establish well in any release sites south of that latitude. However, the other three (*T. planipennisi*, *S. galinae*, and *O. agrili*) were successful in doing so beyond their initial release sites (Government of Canada 2013*b*; Duan et al. 2023) and tracking their natural dispersal is key to understanding their effectiveness.

Ecological interactions in biological controls

Trophic interactions play an important role in regulating emerald ash borer populations (Cappaert et al. 2005; Lindell et al. 2008; Cappaert and McCullough 2009; Srei et al. 2020). Borsato et al. (2025) compared DNA metabarcoding techniques to generate data on potential interactions from molecular traces across a variety of insect taxa. In this approach, a "host" organism is subjected to DNA extraction and PCR of target molecular markers to amplify co-occurring DNA signatures (i.e., bacteria, fungi, parasites, etc.) that interact with an individual of interest (e.g., by residing in or on a host organism). Interactions may leave behind a genetic trace signature that can be detected forensically, allowing a metabarcoding approach to be used. In this case a detection of new taxa is interpreted as a real or potential ecological interaction, though this can also be a result of simple co-occurrence through trace contact DNA (see discussion).

Borsato et al. (2025) generated a large data set of identified co-occurring taxonomic signals from emerald ash borers across various size classifications as part of the development of methods for generalized detections of ecological interactions. This previous paper considered the technical implications of gene region choice and how to use different sized source individuals (e.g., eggs, larva of different sizes, adults) from a number of different taxonomic groups. However, this work did not consider any ecological pattern of co-occurring and interacting taxa. The objective of this study is to fill this gap and use the same data generated for emerald ash borers to classify the life history and ecological factors that influence our ability to detect potential ecological interactions. In particular, we consider all co-occurring taxa as potential interactions and assess the impact of (1) life stages (e.g., larvae, pupae, and adults), and (2) ecological correlates (e.g., collection date, location, and habitat) on the detection of potential ecological interactions of emerald ash borer hosts. We also look specifically for potential agents of biological control (i.e., fungi, parasites, etc.) or particularly vulnerable pest life stages to highlight any approaches to suppress or better target this invasive species with minimum impact on ecosystems.

Materials and methods

The data used in this study are described in Borsato et al. (2025). In brief, emerald ash borer samples were hand collected in 2022 from ash trees found in Brockville ($n = 185$), Ennotville ($n = 41$), Guelph ($n = 41$), and Puslinch ($n = 10$) in Ontario, Canada by the collection unit of the Canadian Center for Biodiversity Genomics (see Supplemental Material Tables S1 - S4). Samples collected included larvae (which were divided into 3 size classes to optimize DNA extraction; $n = 54$ "size 1" < 1.3 cm, $n = 68$ "size 2" 1.3–2.5 cm, $n = 41$ "size 3" > 2.5 cm), pupae ($n = 53$), and adults ($n = 60$). All specimens were placed in ethanol and frozen for analysis. For molecular work, the specimens were crushed inside a tube using a pestle and DNA was extracted using the Qiagen blood and tissue kit following the manufacturer's guidelines with a decreased final DNA elution volume of 150 μL of Buffer AE to in-

crease DNA yields. To account for differences in biomass, the DNA was extracted from the posterior half of the abdomen for adult specimens, the posterior third for pupa specimens, a 6 mm fragment of the posterior end of size 2 (larva2) and 3 larva (larva3) specimens, and the whole size 1 larva (larva1).

To confirm larva ID and identify potential insect parasites and parasitoids, a partial COI barcode was amplified using the ZBJ-ArtF1c/ZBJ-ArtR2c primers (Zeale et al. 2011). The internal transcribed spacer (ITS) can be used to amplify both fungi and plants, but different primer sets show differential amplification. Because host plant was known (ash trees), the ITS3/ITS4 primers (White et al. 1990), which have a better recovery for fungi, were used to amplify potential symbionts. The 799 F-mod3/1115R primers targeting 16S (Reysenbach and Pace 1995; Hanshew et al. 2013) were used to amplify microbial taxa. All PCR products were sequenced on the Illumina MiSeq v3 using a 600-cycle run at the Barts and the London Genome Center at Queen Mary University of London, Blizzard Institute (UK). To target parasites and parasitoids, DNA extracts were sent to the University of Guelph where a long-read 18S region was amplified using SSU_F_07/SSU_R_26 primers (Floyd et al. 2002; Carta and Li 2018) and sequenced using the Oxford Nanopore MinION platform on a Flongle flowcell. All PCR primers, reaction mix, thermocycling conditions, and positive and negative controls are detailed in Borsato et al. (2025). Bioinformatics processing was performed using DADA 2 (ITS), mBRAVE (COI), QIIME2 (16S), and a purpose built platform for MinION data (18S; Hebert et al. 2023). Full analysis details are given in Borsato et al. (2025).

In addition to larval size and developmental stage (larva, pupa, adult) which may influence ecological interactions (e.g., due to mobility and exposure to the environment) and were included in the methods developed in Borsato et al. (2025), we have added ecological variables from the point of collection. These added variables include spatial factors (region and sector), habitat characteristics (habitat and sub-habitat), and temporal factors (collection date). Spatial variables may correspond to local range variation in ecological interactions, and include "region" which refers to geo-political boundaries within Ontario (i.e., cities) and "sector", a finer scale division of geography within the city (e.g., a specific park within the city). Habitat variables may influence the presence or absence of co-occurring taxa and we classified habitat using a controlled vocabulary, following IUCN habitat classification as closely as possible. We include "habitat", defined as either artificial (which is highly human modified) or woodland (a more natural setting), and "sub-habitat", which is a finer division of urban, arable, and forested land. Temporal variables reflect seasonal variance in co-occurring taxa and life stage and include collection date (day of the year). See Supplemental Material for specimen counts and variable divisions.

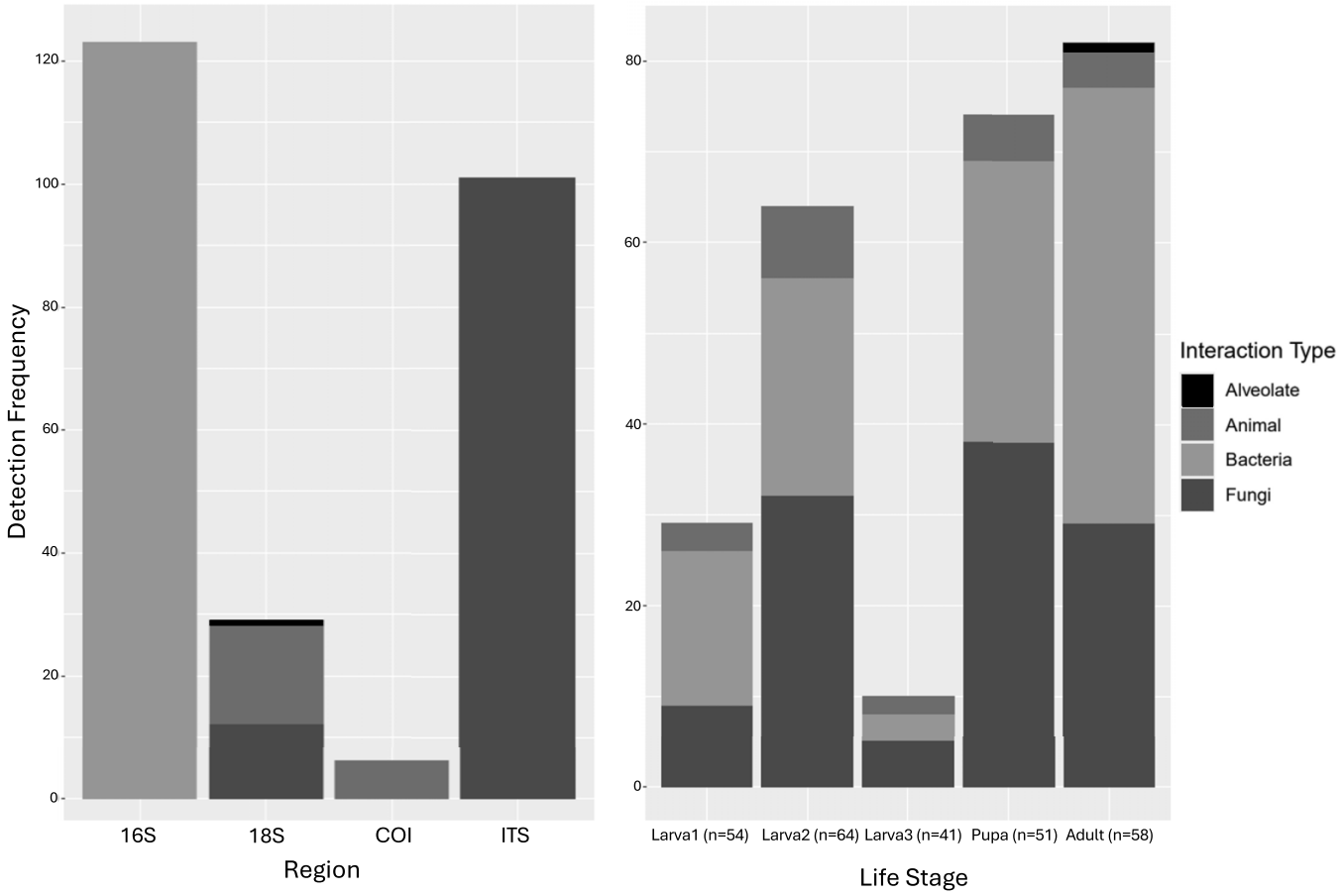
To assess the relative influence of age, location (i.e., region and sector), habitat, sub-habitat, and collection time (day of the year) we constructed a random forest classification model (Breiman 2001) where the presence or absence of potential order-level interactions was classed as a response variable, and specimen age, collection region, collection sector, collection habitat, collection sub-habitat, and collection date were treated as predictors (Supplemental Material Tables S1 to S4, and Code for Models). We then considered fungi, microbes, and parasites/parasitoids as separate taxonomic groups and repeated the analysis using these taxa as separate response variables. The random forest models were generated using the *caret* package (Kuhn 2008) in R Studio (R Core Team 2023). A random forest model was selected over other models due to its robust nature (e.g., ability to handle both continuous and categorical variables and outliers), high accuracy, and ability to evaluate variable importance (i.e., which variables most influence the accuracy of the random forest model predictions; Breiman 2001).

To ensure reproducibility, the seed was initialized to 42 using the set.seed function prior to generating the models. Data was then randomly split into "training" (70%) and "testing" (30%) sets, with the former used to train the random forest model. Repeated k-fold cross validation ($k = 10$, repeats $= 5$) was included in each model to estimate the performance (accuracy) of the models, and to improve model accuracy by allowing more data to be used when training the model. The number of variables tried in each split (mtry) was determined using the tuneRF function in the *randomForest* package (Liaw and Wiener 2002), where mtry $= 2$ was found to be optimal for the animal and overall models, while mtry $= 1$ and mtry $= 4$ were found to be optimal for the bacteria and fungi models, respectively. The testing set was used to evaluate model accuracy by generating a confusion matrix using the *caret* package (Kuhn 2008). Rather than using the default threshold value of 0.5, which was biased toward predicting the majority class ("absent") and prevented "present" cases from being accurately detected, an optimized threshold value for each model was determined using the *pROC* package (Robin et al. 2011). The optimized threshold for each model was identified by maximizing Youden's Index from the receiver operating characteristic (ROC) curve, which balanced sensitivity and specificity, and improved the detection of the minority class ("present" possible interactions) while maintaining the overall performance of the models. Variable importance (i.e., the mean decrease in the Gini coefficient) was then determined using the *caret* package (Kuhn 2008). Accumulated local effects (ALE) plots were also generated from the training data using the *ilm* package (Molnar et al. 2018) to determine the impact each variable has on the presence or absence of potential interactions. A correlation matrix was also generated using the *stats* (R Core Team 2023) and *ggcorrplot* packages (Kassambara 2023) to determine the correlation between predictor variables.

Results

The potential species interaction data used here are fully described by Borsato et al. (2025) in Tables S5–S8 of that publication. In summary, after host data was excluded, we retained 65 identifications (61 unique taxa) from 30 taxonomic orders across the four gene regions analyzed (ITS $= 23$; 16S $= 21$; COI $= 4$; 18S $= 17$). Using the COI target region four arthropod orders were detected. Due to strict quality filtering protocols, no ASVs were retained from the larva1 samples (see discussion). However, two orders were identified in the larva2

Fig. 2. Interactions by target region and in life stages of emerald ash borers (*Agrilus planipennis*). The recovery of various targets by target amplified region (left) shows high variability in taxonomic richness. The profile of taxonomic recovery of co-amplified taxa is highly variable between life stages (right).

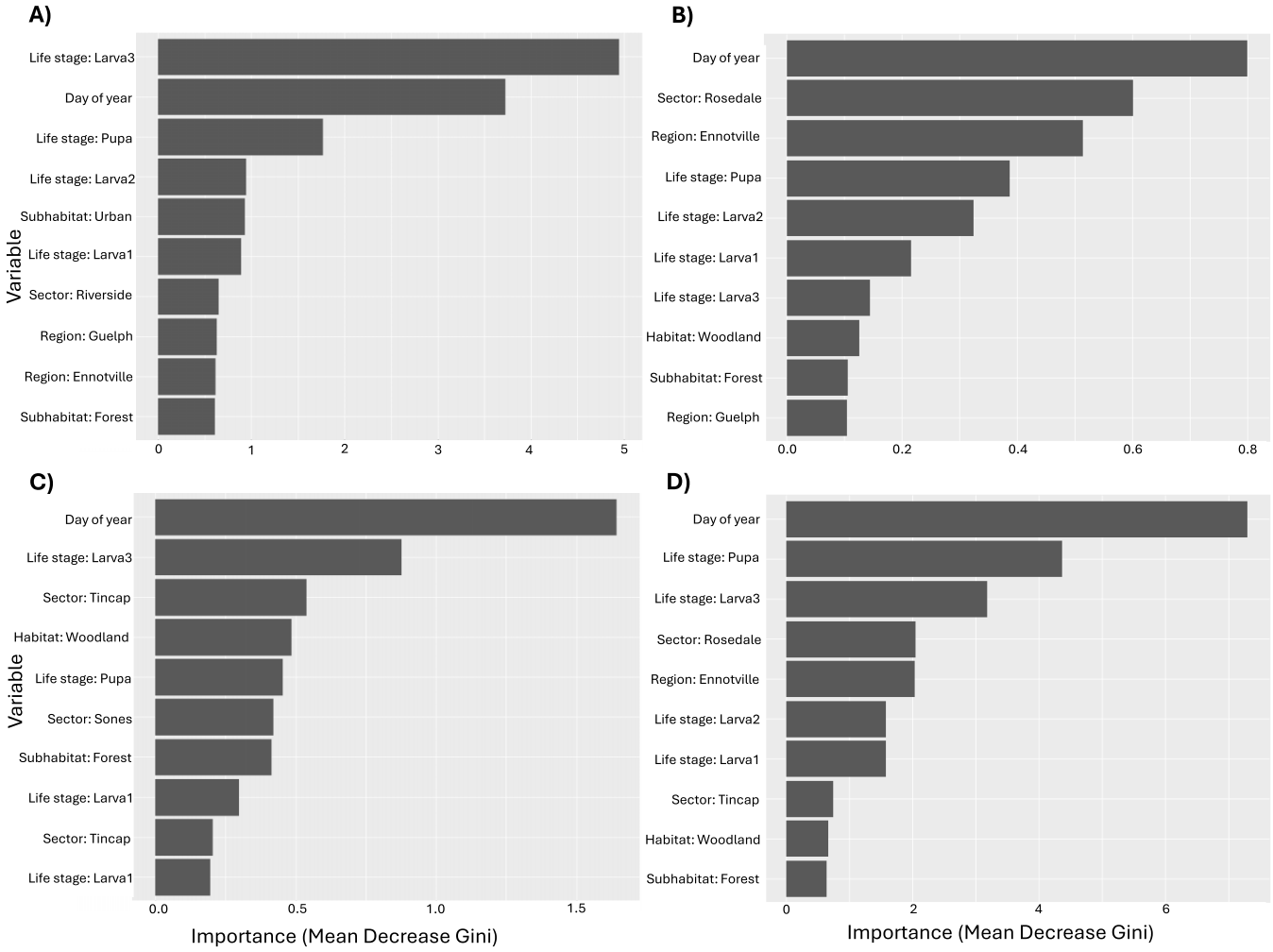


samples, one in the larva3 and pupae samples, and two in the adult samples. These identifications included a family of parasitoid wasps (Ichneumonidae, order Hymenoptera). We detected 13 orders of bacteria using the 16S marker, with larva1 specimens hosting a microbial richness of five orders, larva2 having three orders, larva3 having two orders, pupae having eight orders, and adults having nine orders. Notably, the insect pathogen *Wolbachia* sp. (Hertig, 1936) (order Rickettsiales) was detected in only one sample. The ITS region allowed the detection of 11 fungal orders, with five orders detected in larva1, seven orders in larva2, three in larva3, six in pupae, and eight in adults. Notably, *Beauveria* sp. (order Hypocreales), a fungal biocontrol agent, was detected in two samples (with 14 633 and 13 007 reads, respectively). Although *Beauveria* sp. was also detected in one negative control (37 reads) and one positive control (1456 reads), the identification was ultimately kept due to the very low read abundance in these controls compared to the read abundance in the emerald ash borer samples, and there being no lab-based source of contamination. The detection of *Beauveria* sp. is also supported by the 18S data, where it was also detected at order-level in the same samples. We detected nine orders using the 18S region, with six of the nine representing fungi (overlapping with those identified using ITS) and the remaining three representing nematodes, insects, and a parasitic alveolate protist (*Cryptosporidium* spp. (Tyzzer, 1907), order Cryptosporida). We identified three orders in the larva1 samples, five in larva2, one in larva3, four in pupae, and two in adults.

These identifications account for 259 potential order-level interactions across emerald ash borer life stages (Fig. 2). In total, we retained 29 potential order level interactions in the larva1 samples (three animal, 17 bacteria, and nine fungi), 64 in the larva2 samples (eight animal, 24 bacteria, and 32 fungi), ten in the larva3 samples (two animal, three bacteria, and five fungi), 74 in the pupal life stage (five animal, 31 bacteria, and 38 fungi), and 82 in the adult life stage (four animal, 48 bacteria, 29 fungi, and one parasitic alveolate). Overall, the adult life stage had the most potential interactions detected, and the majority of potential interactions detected occurred between emerald ash borers and fungi.

The classification accuracy of the total potential interaction random forest model was 68.92%, with an out-of-bag (OOB) error estimate of 31.18%. The sensitivity of the model was 79.07% while the specificity was 68.57%, based on potential interactions present in 146 samples and absent in 118. The model was found to correctly classify the testing data 74.36% of the time. The three most important features (Fig.

Fig. 3. Top ten most important variables in random forest models. The ten variables with the highest importance (mean decrease Gini) are ordered top to bottom from most important to least important in classifying the presence or absence of interactions in (A) the overall interaction model, (B) the animal interaction model, (C) the bacteria interaction model, and (D) the fungi interaction model.

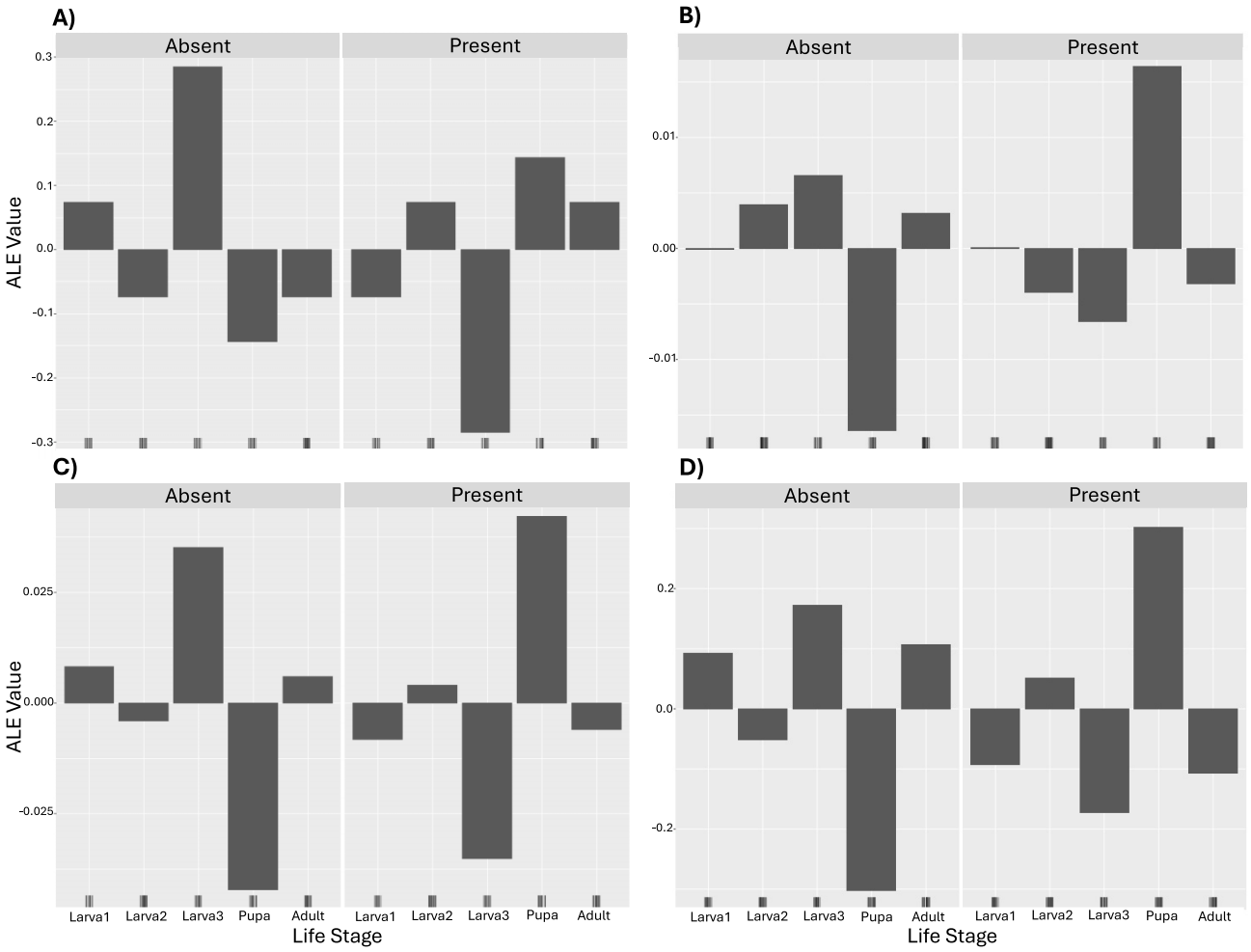


3), determined by the mean decrease in the Gini coefficient (MDG), were larva3 life stage (MDG = 4.94), collection date (represented as day of the year; MDG = 3.72), and pupa life stage (MDG = 1.76). The ALE plots indicated that potential interactions were more likely to be present in the pupal life stage (Fig. 4), in samples collected prior to 14 July (195th day of the year), and in samples collected from the Ennotville region, Rosedale sector, woodland habitat, and forest sub-habitat (Figs. S1–S5).

For potential bacterial interactions, the estimated classification accuracy of the random forest model was 68.28%. The model had an OOB error estimate of 31.72%, a sensitivity of 58.33%, and a specificity of 68.52%, based on potential interactions present in 83 samples and absent in 181 samples. The model was found to correctly classify the testing data 65.38% of the time. The three most important features (Fig. 3) were collection date (MDG = 1.64), larva3 life stage (MDG = 0.17), and Tincap sector (MDG = 0.54). The ALE plots indicated that potential interactions with bacteria were more likely to be present in the pupal life stage (Fig. 4), in samples collected prior to 22 June (173rd day of the year), and in samples collected from the Guelph region, Sones sector, woodland habitat, and forest sub-habitat (Figs. S1–S5).

The potential fungal interactions random forest model had an estimated classification accuracy of 68.81% and an OOB error estimate of 31.18%. The model had a sensitivity of 59.26% and a specificity of 86.27%, based on potential interactions present in 93 samples and absent in 171. The fungi random forest correctly classified the testing data 76.92% of the time, and the three most important variables (Fig. 3) were collection date (MDG = 7.28), pupa life stage (MDG = 4.36), and larva3 life stage (MDG = 3.17). The ALE plots indicated that potential interactions with fungi were more likely to be present in the pupal life stage (Fig. 4), in samples collected prior to 6 July (187th day of the year), and in samples collected from the Ennotville region, Rosedale sector, artificial habitat, and urban sub-habitat (Figs. S1–S5).

Fig. 4. Effect of emerald ash borer (*Agrilus planipennis*) life stage on the detection of interactions. Accumulated local effects (ALE) plots portray the effect life stage had on the presence or absence of (A) overall interactions, (B) animal interactions, (C) bacteria interactions, and (D) fungi interactions.



The estimated classification accuracy of the potential animal interactions random forest model was 92.49%. The OOB error estimate for the model was 7.57%, sensitivity was 50%, and specificity was 79.45%, based on potential interactions present in 20 samples and absent in 244 samples. It was found to correctly classify the testing data 77.22% of the time. The three most important variables (Fig. 3) were collection date (MDG = 0.80), Rosedale sector (MDG = 0.60), and Ennotville region (MDG = 0.51). The ALE plots indicated that potential interactions with animals (i.e., insects, nematodes, etc.) were more likely to be present in the pupa life stage (Fig. 4), in samples collected after 21 July (202nd day of the year), and in samples collected from the Ennotville region, Rosedale sector, artificial habitat, and arable sub-habitat (Figs. S1–S5). The correlation between most of the predictor variables was low, although there was high correlation detected between some variables (e.g., the correlation between the Rosedale collection sector and Ennotville collection region was high, which was unsurprising given Rosedale is a sublocation of Ennotville; Fig. S6).

Discussion

Ecological interactions play an important role in the evolution and survival of a species (Thompson 1999; Barraclough 2015). In the case of emerald ash borers, interactions with biological controls (e.g., woodpeckers, *Beauveria*, etc.) may lead to population control of the pest. We applied data generated by metabarcoding of four genetic markers to assess the factors which influence the potential ecological interactions across the tree of life of the destructive (in North America) emerald ash borers. Our objectives were to determine whether different life stages and ecological correlates can predict the detection of non-target taxa within a sample. Our model suggests that life stage targeted, collection date, and location (region and sector) can predict potential interactions, while habitat and sub-habitat play minor roles (Fig. 3). We identified potential interactions with a variety of taxa (e.g., bacteria, mites, fungi, nematodes, etc.), including interesting potential agents of biocontrol (e.g., *Beauveria* sp., parasitoid wasps) in several samples of emerald ash borer.

Taxonomic identifications

A total of 259 potential order-level interactions were detected across emerald ash borer life stages. These potential interactions represent 61 unique taxa from 30 taxonomic orders across the four markers used in this analysis (COI, ITS, 16S, and 18S).

Excluding host data from the COI data, we identified four orders of arthropods, which included one family of gall midge (Cecidomyiidae, order Diptera), two families of mites (Oppiidae, order Sarcoptiformes, and Eupodidae, order Trombidiformes), and notably, ichneumonid parasitoid wasps (order Hymenoptera, see biological control discussion below). We reported no data for larva1 samples, however it should be noted that this was not a result of there being no data recovered. The data recovered from these samples did not reach the criteria required for retention that we set out *a priori*, specifically because of negative control filtering. As in the rest of the samples, much of the data matched the specimens themselves (this is expected as the primers readily amplify *Agrilus planipennis*). This means we are looking for traces of other taxa among the dominant host DNA. A small amount of data was found in ASVs in very low copy number, which was removed during negative control filtering. However, some high copy number ASVs also corresponded to groups reported above (e.g., the same groups of mites reported in other stages), but a case of contamination in a negative control (3985 reads) caused a large portion of the COI data from all samples to be removed, and all data was lost from larva1 specifically. If we ignore this control, the next highest read count in a negative (1426 reads) does not substantially retain more data. For comparison, the highest read count in a positive control was only 2686 reads (note: below the first negative control read count), but among our reported data the ichneumonid wasp was represented by more than 15 000 reads and the two mites by more than 33 000 or 16 000 reads, respectively. Read counts are very tricky to interpret. In general, they should not be used to interpret biomass or abundance as they can be influenced by many factors (e.g., primer binding, DNA extraction efficiency, natural degradation processes of biological material, etc.). However, in a contradictory manner they are routinely used as filtering thresholds to interpret negative and positive controls. While it seems reasonable to report the wasps and mites which are potentially interesting biocontrol agents, our low reporting for COI data are more a reflection of a problematic control and process of filtering than actual biology. Our suggestion for future work is to use either longer COI fragments to generate better quality data or a marker less sensitive to *Agrilus planipennis* so that more of the data represents other taxa.

The 16S marker was used to detect potential bacterial interactions, although the majority of the bacteria associated with our samples are ubiquitous throughout the environment and can be associated with soil (e.g., *Terriglobus* sp. Eichorst et al., 2007) or the rhizosphere (e.g., *Neorhizobium* sp. Mousavi et al., 2015). Many of these detections are thus likely co-occurring rather than direct interactions (co-occurring and co-evolving), characteristic of the symbiome. Notably, we did detect the intracellular parasite *Wolbachia* sp. (order Rickettsiales) in one sample (see biological control discussion below). Similar to the bacteria detected by 16S, the majority of the fungi sequenced using the ITS marker are associated with soil (e.g., *Fusarium* sp. Link, 1809), decaying plant matter (e.g., *Yamadazyma* sp. Billon-Grand, 1989), lichens (e.g., *Candelaria* sp. Massal.), or plant pathogens (e.g., *Diaporthe* sp. Nitschke, 1870), and not the emerald ash borers themselves (though note that some of these e.g., *Fusarium* are found in a wide variety of habitats and members of these may include insect pathogens). It is likely that these are fungi associated with the host tree or surrounding environment, and would be classed as co-occurring rather than direct interactions. However, the fungus *Beauveria* sp. (order Hypocreales) was detected, and likely represents a true ecological interaction (see biological control discussion below). The 18S sequence data contained the greatest diversity of interaction types, including animals (i.e., nematodes and arthropods), a parasitic alveolate, and fungi. The fungal orders detected using the ITS marker overlapped with those identified using the 18S marker, even though the taxonomic resolution was the same or worse (i.e., taxa identified in both regions such as *Diaporthe* sp. (Nitschke, 1870) and *Niesslia* sp. (Auerswald, 1869) were only resolved at a higher taxonomic level in 18S) likely due to a lack of sequence variability at lower taxonomic levels in very conserved marker regions. We also detected non-host arthropods belonging to the order Hymenoptera with the 18S marker. Detected interactions with nematodes (order Rhabditida) included Neotylenchidae (whose members can be parasitic, e.g., *Deladenus proximus* (Bedding, 1974) (Zieman et al. 2015)), *Panagrellus* sp. (Thorne, 1938) (free-living nematodes associated with many habitats, including insect frass; Ferris 2009; Srinivasan et al. 2013), and *Rhabditolaimus* sp. (Fuchs, 1914) (bacteriophagous and commensal with other beetles, e.g., *Scolytus multistriatus* (Marsham, 1802); Ryss and Polyanina 2022). The parasitic alveolate (*Cryptosporidium* sp.) that was detected has been reported to infect humans and animals (including insects; Helmy and Hafez 2022), thus this may be a true parasitic interaction.

Detection of biological controls

Several potential agents of biocontrol were identified across all markers used. Ichneumonidae parasitoid wasps (order Hymenoptera) were detected in larva2, larva3, and pupae samples using the COI marker. Ichneumonids have previously been observed to parasitize emerald ash borers (Duan et al. 2009), so these are likely cases of true parasitism. Additionally, the entomopathogenic fungus *Beauveria* sp. (order Hypocreales), which is known to successfully infect and neutralize emerald ash borers, was identified in two samples (one size 2 larva and one adult beetle) using the ITS marker. Although naturally occurring in the environment (e.g., in soil; Rehner et al. 2011), biocontrol products containing *Beauveria* such as FraxiProtec (GDG Environment, Canada), are commercially available and are able to significantly reduce emerald ash borer populations (Srei et al. 2020).

Using the 16S region, we identified the intracellular parasite *Wolbachia* sp. (order Rickettsiales) in one pupal sample. *Wolbachia* can infect many insects, and infections can be ver-

tically and horizontally transmitted between hosts (Werren 1997). Infection is associated with reproductive abnormalities, such as the feminization of genetic males, parthenogenesis induction, and reproductive incompatibility (i.e., through cytoplasmic incompatibility between hosts infected by different strains or between uninfected/infected hosts; reviewed in Werren (1997)). However, it may also have a protective benefit in some viral infections (Pimentel et al. 2020; Cogni et al. 2021). The detection of *Wolbachia* is notable as it may have potential biocontrol applications (Werren 1997; Zabalou et al. 2004).

The parasitic alveolate and nematodes identified using the 18S marker may potentially act as biocontrols as well. The alveolate (*Cryptosporidium* sp.) is known to infect insects (Helmy and Hafez 2022), and can therefore be considered a true parasitic interaction. Similarly, members of the Neotylenchidae family (order Rhabditida) of nematodes detected can be parasitic (e.g., *Deladenus proximus*; Zieman et al. 2015), and thus this may also represent a true parasitic interaction that may have potential biocontrol applications. The detection of all these interactions suggests that some natural biocontrol may be occurring in the collection location.

Factors influencing the potential interactions assessed using random forest models

Four random forest models were generated in total: one to predict the presence of any potential interaction at all, and the other three to specifically test predictions about the presence of potential fungi, microbial, or animal interactions. The models were successfully able to predict the number of potential interactions detected using the testing dataset in a range of 65%–77% of the time. Potential animal and fungal interactions were most likely to be classified correctly (~77% of the time), while potential bacteria and total interactions were less likely to be classified correctly (~65% and ~74% of the time, respectively). This means that these models can be used to predict which specimens are likely to have detectable potential interactions based on factors such as life stage, collection date, location, and habitat up to 77% of the time. They can thus be used to identify ideal target specimens when resources limit the more thorough analysis of all specimens. In all models, collection date (represented as day of year) and life stage played the most important roles in correctly classifying the presence or absence of potential interactions, with the exception of the animal and bacteria models, where location (sector and region) also played a major role. It should be noted that this observed variable importance may be a result of the tendency of random forest classification models to be biased towards categorical variables with more levels (i.e., life stage and sector, which each have five levels; Strobl et al. 2007). However, features were not omitted from the models based on variable importance, so this likely does not impact the accuracy of the models used.

The ALE plots generated for each potential interaction type were used to determine what effect, if any, each variable has on the presence or absence of potential interactions detected. In our models, all types of potential interactions were more likely to be present at the pupal life stage. Collection date also seemed to play a significant role, with potential interactions more likely to be present in samples collected by the end of July. This differed slightly for specific potential interaction types (e.g., potential bacterial interactions were most likely to be present in samples collected by 22 June 2022), but the latest the date ranges to is 21 July 2022 (for potential animal interactions) before the detection rate decreases. In the case of life stage and collection date, it is possible that this reflects an accumulation of potential interactions and co-occurrences over time, particularly if lethal interactions removed individuals and are thus not detectable. Additionally, pupae may accumulate more DNA due to their immobility. The presence of potential total, fungi, and animal interactions were highest in the Ennotville collection region and Rosedale collection sector, while the presence of potential bacterial interactions was greatest in the Guelph region and Sones sector. Similarly, potential fungi and animal interactions were more likely to be present in artificial habitats, while total and bacterial interactions were more likely to be present in forest and woodland habitats. Finally, we found that potential bacteria and total interactions were more likely to be present in forest subhabitats, potential fungi interactions in urban subhabitats, and potential animal interactions in arable subhabitats. Future studies can therefore target specific life stages, locations, habitats, and collection dates depending on the target interactions of interest (e.g., a study that wishes to focus on fungi-beetle interactions should consider prioritizing pupae samples collected by July from artificial/urban habitats located in Ennotville).

Novel challenges in differentiating interactions versus co-occurrence

One of the main challenges in determining a true interaction from co-occurrence data is the growing understanding that environmental DNA (eDNA) likely contaminates all samples collected from the wild to some degree. eDNA includes all biological material (cells, excreta, etc.) shed by organisms continually, and it accumulates in the environment and can be collected from air (Clare et al. 2021; 2022), water (Uchida et al. 2020), soil (Marquina et al. 2019; Foucher et al. 2020), and surfaces such as leaves (Valentin et al. 2020; Macher et al. 2023) and the bodies of other animals through casual contact. eDNA accumulates and settles on biotic and abiotic surfaces where it then degrades due to environmental conditions. Consequently, eDNA of nearby organisms may be found on the surface of arthropods even if there was no direct interaction between the two. For example, Huszarik et al. (2023), demonstrated that eDNA of an exotic insect could be detected on spiders who occupied the same wet pitfall trap. We must thus expect that a sample or target organisms could potentially become coated in eDNA simply from the environment or during collection and storage (e.g., pitfall traps, ethanol, etc.; Shokralla et al. 2010; Huszarik et al. 2023) and the duration and magnitude of this effect in wild data are only starting to be realized and little quantification of the effect has been provided. Although the ubiquitous nature of eDNA allows for its use in biodiversity and community composition assessments (Clare et al. 2022; Littlefair et

al. 2023; Macher et al. 2023), it can complicate metabarcoding for species interaction quantifications, making it difficult to determine true interactions from co-occurrence. While we might speculate that true interactions leave more DNA which could be quantified by qPCR or similar approaches, this has not been tested, and variation in recovery and amplification due to lab biases (such as primer affinity) might alter these natural occurrences.

The effect is suspected here. For example, most of the fungi detected are ubiquitous throughout the environment and are often found in soil (e.g., *Fusarium* sp.) or plants (e.g., *Alternaria* sp. (Nees, 1816)). The fungus *Candelaria* sp. is known to form lichen complexes (Sanders and Masumoto 2021) but was identified in the emerald ash borer samples despite likely not interacting directly with the specimens. Similarly, beetles and a family of gall midges (Cecidomyiidae, order Diptera) detected in the COI data are unlikely to directly interact, but the DNA was co-occurring in the same samples. Thus, many identifications are thought to reflect simple co-occurrence in the local environment. As a result, we refer to all these data as "potential" interactions in this manuscript, reflecting this uncertainty.

While it is extremely difficult to envision an immediate solution to this problem, there are mitigation steps and data analysis steps which might reduce the effect. First, there is some evidence that specimens can be washed (e.g., in a bleach solution) prior to DNA extraction to remove eDNA (Binetruy et al. 2019; Hausmann et al. 2021; Huszarik et al. 2023), though this would then increase the false negative rate by removing fungi and microbes associated with that specimen's surface ecology. Dissection so that only the gut undergoes DNA extraction would allow direct interactions related to feeding and gut parasitism to be determined, and potentially increase the target DNA concentration relative to host DNA, a problem when using generalist arthropod primers when the host and parasite or parasitoid are both arthropods, but this would limit the scope of the interactions being assessed. However, this is very time-consuming and requires destruction of the specimen, a process objected to when analysis involves mass screening of samples, or museum and collection specimens, respectively. Just as important is ecological assessment of all recovered taxa to determine if they are "reasonable" given known life history. While this will limit discovery of novel interactions, it should be encouraged so that all identifications are not automatically accepted as a true interaction.

Conclusions

In this analysis we describe taxa co-amplified in emerald ash borer samples at different life stages and evaluate whether the detection of these potential ecological interactions can be predicted by collection date, location, life stage, or habitat using random forest models. Our models suggest that life stage is an important predictor of potential interactions with the pupal life stage showing more potential interactions. Future work must consider life stage when targeting interactions, particularly when cost and time constraints limit options for analysis. We also found some support for sampling earlier in the year and regional variation in data, though with smaller sample sizes this should be treated with caution. Our data also highlights the potential environmental contamination of specimens in ecological analyses, and the difficulty in distinguishing true interactions, a challenge only starting to be considered in the vast literature on the use of DNA to identify ecological interactions. The risk of generating false positive "interactions" from contact environmental DNA requires creative solutions to estimate and mitigate the challenge.

Acknowledgements

We would like to thank Sean Prosser at the Centre for Biodiversity Genomics for bioinformatics support and the collection unit who supported this research, particularly Gergin Blagoev, Simonne Clout, Isaiah Dowling, Hillary Hale, Reid Harrop, Paul Hebert, Chris Ho, Nao Ito, Meredith Miller, and Emily Perry. We thank Melissa Mac Leod-Bigley for lab support.

This work was supported by The Natural Sciences and Engineering Research Council of Canada through the Discovery Grants Program, The Government of Canada's New Frontiers in Research Fund (NFRFT-2020-0073) Tracing the Patterns of Life on a Changing Planet, Genome Canada (BIOSCAN Canada) and Ontario Genomics (OGI-208).

Article information

History dates

Received: 7 January 2026
Accepted: 7 June 2026
Accepted manuscript online: 17 July 2026
Version of record online: 21 August 2026

Copyright

Data availability

The sequences are available at GenBank in the SRA BioProject: PRJNA1150198.

Author information

Author ORCIDs

Elizabeth L. Clare https://orcid.org/0000-0002-6563-3365

Author notes

Present address for Daniel Marquina is AllGenetics & Biology SL., Cubelos 21 bajo A2, Perillo (Oleiros) Spain.

Author contributions

Conceptualization: NDB, DM, DS, ELC
Data curation: NDB, NRG, DM, DS
Formal analysis: NDB, SEM, NRG, AJBR, RF
Funding acquisition: ELC
Project administration: ELC
Software: AJBR
Supervision: ELC

Visualization: NDB
Writing – original draft: NDB, ELC
Writing – review & editing: NDB, SEM, DM, DS, RF, ELC

Competing interests

The authors declare no competing interests. DM is employed by AllGenetics & Biology SL.

Funding information

This work was supported by The Natural Sciences and Engineering Research Council of Canada through the Discovery Grants Program, The Government of Canada's New Frontiers in Research Fund (NFRFT-2020-0073), and through Genome Canada and Ontario Genomics (OGI-208). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Supplementary material

Supplementary data are available with the article at https://doi.org/10.1139/cjz-2026-0003.

References

Aukema, J.E., Leung, B., Kovacs, K., Chivers, C., Britton, K.O., Englin, J., et al. 2011. Economic impacts of non-native forest insects in the continental United States. PLoS One, **6**(9): e24587. doi:10.1371/journal.pone.0024587. PMID: 21931766.

Barraclough, T.G. 2015. How do species interactions affect evolutionary dynamics across whole communities? Annu. Rev. Ecol. Evol. Syst. **46**(1): 25–48. doi:10.1146/annurev-ecolsys-112414-054030.

Binetruy, F., Dupraz, M., Buysse, M., and Duron, O. 2019. Surface sterilization methods impact measures of internal microbial diversity in ticks. Parasit. Vectors, **12 (** 1): 268. doi:10.1186/s13071-019-3517-5.

Borsato, N.D., Lunn, K., Garrett, N.R., Biganzoli-Rangel, A.J., Marquina, D., Steinke, D., et al. 2025. Identification of insect ecological interactions using a metabarcoding approach. PeerJ, **13**: e18906. doi:10.7717/peerj.18906. PMID: 39981046.

Bray, A.M., Bauer, L.S., Poland, T.M., Haack, R.A., Cognato, A.I., and Smith, J.J. 2011. Genetic analysis of emerald ash borer (*Agrilus planipennis* Fairmaire) populations in Asia and North America. Biol. Invasions, **13**(12): 2869–2887. doi:10.1007/s10530-011-9970-5.

Breiman, L. 2001. Random forests. Mach. Learn. **45**(1): 5–32. doi:10.1023/A:1010933404324.

Canham, C.D. 1988. Growth and canopy architecture of shade-tolerant trees: response to canopy gaps. Ecology, **69**(3): 786–795. doi:10.2307/1941027.

Cappaert, D., and McCullough, D.G. 2009. Occurrence and seasonal abundance of *Atanycolus cappaerti* (Hymenoptera: Braconidae) a native parasitoid of emerald ash borer, *Agrilus planipennis* (Coleoptera: Buprestidae). Great Lakes Entomol. **42**(1 & 2): 16–29.

Cappaert, D., McCullough, D.G., Poland, T.M., and Siegert, N.W. 2005. Emerald Ash Borer in North America: a research and regulatory challenge. Am. Entomol. **51**(3): 152–165. doi:10.1093/ae/51.3.152.

Carta, L.K., and Li, S. 2018. Improved 18S small subunit rDNA primers for problematic nematode amplification. J. Nematol. **50**(4): 533–542. doi:10.21307/jofnem-2018-051.

Clare, E.L., Economou, C.K., Bennett, F.J., Dyer, C.E., Adams, K., McRobie, B., et al. 2022. Measuring biodiversity from DNA in the air. Curr. Biol. **32**(3): 693–700.e5. doi:10.1016/j.cub.2021.11.064. PMID: 34995488.

Clare, E.L., Economou, C.K., Faulkes, C.G., Gilbert, J.D., Bennett, F., Drinkwater, R., and Littlefair, J.E. 2021. eDNAir: proof of concept that animal DNA can be collected from air sampling. PeerJ, **9**: e11030. doi:10.7717/peerj.11030. PMID: 33850648.

Cogni, R., Ding, S.D., Pimentel, A.C., Day, J.P., and Jiggins, F.M. 2021. Wolbachia reduces virus infection in a natural population of Drosophila. Commun. Biol. **4**(1): 1–7. doi:10.1038/s42003-021-02838-z.

Duan, J.J., Bauer, L.S., Van Driesche, R.G., and Gould, J.R. 2018. Progress and challenges of protecting North American ash trees from the emerald ash borer using biological control. Forests, **9**(3): Article 3. doi:10.3390/f9030142.

Duan, J.J., Fuester, R.W., Wildonger, J., Taylor, P.B., Barth, S., and Spichiger, S.E. 2009. Parasitoids attacking the emerald ash borer (Coleoptera: Buprestidae) in western Pennsylvania. Fla. Entomolog. **92**(4): 588–592.

Duan, J.J., Gould, J.R., Quinn, N.F., Petrice, T.R., Slager, B.H., Poland, T.M., et al. 2023. Protection of North American ash against emerald ash borer with biological control: ecological premises and progress toward success. BioControl, **68**(2): 87–100. doi:10.1007/s10526-023-10182-w.

Ferris, H. 2009. The beer mat nematode, *Panagrellus redivivus*: a study of the connectedness of scientific discovery. J. Nematode Morphol. System, **12**(1): Article 1.

Flower, C.E., Knight, K.S., and Gonzalez-Meler, M.A. 2013. Impacts of the emerald ash borer (*Agrilus planipennis* Fairmaire) induced ash (*Fraxinus* spp.) mortality on forest carbon cycling and successional dynamics in the eastern United States. Biol. Invasions, **15**(4): 931–944. doi:10.1007/s10530-012-0341-7.

Floyd, R., Abebe, E., Papert, A., and Blaxter, M. 2002. Molecular barcodes for soil nematode identification. Mol. Ecol. **11**(4): 839–850. doi:10.1046/j.1365-294X.2002.01485.x. PMID: 11972769.

Foucher, A., Evrard, O., Ficetola, G.F., Gielly, L., Poulain, J., Giguet-Covex, C., et al. 2020. Persistence of environmental DNA in cultivated soils: implication of this memory effect for reconstructing the dynamics of land use and cover changes. Sci. Rep. **10**(1): 10502. doi:10.1038/s41598-020-67452-1. PMID: 32601368.

Gandhi, K.J.K., and Herms, D.A. 2010. Direct and indirect effects of alien insect herbivores on ecological processes and interactions in forests of eastern North America. Biol. Invasions, **12**(2): 389–405. doi:10.1007/s10530-009-9627-9.

Government of Canada, C. F. I. A. 2013a. RMD-13-01: Regulated Areas for Emerald Ash Borer (EAB) *(Agrilus planipennis* Fairmaire*)* March 2014 (1st revision) [Reference material,policy]. Available from https://inspection.canada.ca/plant-health/invasive-species/directives/pest-risk-management/emerald-ash-borer/eng/1368741925939/1368741926892#item5.2.

Government of Canada, C. F. I. A. 2013b. Questions and Answers: Wasps as biological control agents for Emerald Ash Borers. Available from https://inspection.canada.ca/plant-health/invasive-species/insects/emerald-ash-borer/wasps/eng/1371137262586/1371137530758.

Hanshew, A.S., Mason, C.J., Raffa, K.F., and Currie, C.R. 2013. Minimization of chloroplast contamination in 16S rRNA gene pyrosequencing of insect herbivore bacterial communities. J. Microbiol. Methods, **95**(2): 149–155. doi:10.1016/j.mimet.2013.08.007. PMID: 23968645.

Hausmann, A., Höcherl, A., Niessner, A., Zakharov, E., Dolynskyj, S., and Diller, J. 2021. Accurate decontamination of insects from bulk samples does not affect DNA sequencing success. Spixiana, **44**(1): 71–76.

Hebert, P.D.N., Floyd, R., Jafarpour, S., and Prosser, S.W. 2023. Barcode 100 K specimens. *In* a Single Nanopore Run(p. 2023.11.29.569282). bioRxiv. doi:10.1101/2023.11.29.569282.

Helmy, Y.A., and Hafez, H.M. 2022. Cryptosporidiosis: from prevention to treatment, a narrative review. Microorganisms, **10**(12): 2456. doi:10.3390/microorganisms10122456. PMID: 36557709.

Herms, D.A., and McCullough, D.G. 2014. Emerald Ash Borer Invasion of North America: history, biology, ecology, impacts, and management. Annu. Rev. Entomol. **59**(1): 13–30. doi:10.1146/annurev-ento-011613-162051. PMID: 24112110.

Hope, E.S., McKenney, D.W., Pedlar, J.H., Lawrence, K., and MacDonald, H. 2021. Canadian efforts to slow the spread of Emerald Ash Borer (*Agrilus planipennis* Fairmaire) are economically efficient. Ecol. Econ. **188**: 107126. doi:10.1016/j.ecolecon.2021.107126.

Huszarik, M., Röder, N., Eberhardt, L., Kennedy, S., Krehenwinkel, H., Schwenk, K., and Entling, M.H. 2023. External DNA contamination and efficiency of bleach decontamination for arthropod diet analysis. Environ. DNA, **5**(3): 540–550. doi:10.1002/edn3.410.

International Union for Conservation of Nature. 2022. The IUCN Red List of threatened species. IUCN Red List of Threatened Species. Available from https://www.iucnredlist.org.

Jennings, D.E., Gould, J.R., Vandenberg, J.D., Duan, J.J., and Shrewsbury, P.M. 2013. Quantifying the impact of woodpecker predation on

population dynamics of the emerald ash borer (*Agrilus planipennis*). PLoS One, **8**(12): e83491. doi:10.1371/journal.pone.0083491. PMID: 24349520.

Kassambara, A. 2023. ggcorrplot: Visualization of a Correlation Matrix using "ggplot2" (Version R package version 0.1.4.1, p. 0.1.4.1) [Computer software]. Available from https://CRAN.R-project.org/package=ggcorrplot.

Klooster, W.S., Herms, D.A., Knight, K.S., Herms, C.P., McCullough, D.G., Smith, A., et al. 2014. Ash (*Fraxinus* spp.) mortality, regeneration, and seed bank dynamics in mixed hardwood forests following invasion by emerald ash borer (*Agrilus planipennis*). Biol. Invasions, **16**(4): 859–873. doi:10.1007/s10530-013-0543-7.

Knight, K.S., Brown, J.P., and Long, R.P. 2013. Factors affecting the survival of ash (*Fraxinus* spp.) trees infested by emerald ash borer (*Agrilus planipennis*). Biol. Invasions, **15**(2): 371–383. doi:10.1007/s10530-012-0292-z.

Kuhn, M. 2008. Building predictive models in R using the caret package. J. Stat. Softw. **28**: 1–26. doi:10.18637/jss.v028.i05.

Liaw, A., and Wiener, M. 2002. Classification and regression by randomForest. R News, **2**(3): 18–22.

Lindell, C.A., McCullough, D.G., Cappaert, D., Apostolou, N.M., and Roth, M.B. 2008. Factors influencing *Woodpecker* predation on emerald ash borer. Am. Midl. Nat. **159**(2): 434–444. doi:10.1674/0003-0031(2008)159%5b434:FIWPOE%5d2.0.CO;2.

Littlefair, J.E., Allerton, J.J., Brown, A.S., Butterfield, D.M., Robins, C., Economou, C.K., et al. 2023. Air-quality networks collect environmental DNA with the potential to measure biodiversity at continental scales. Curr. Biol. **33**(11): R426–R428. doi:10.1016/j.cub.2023.04.036. PMID: 37279659.

Macher, T.-H., Schütz, R., Hörren, T., Beermann, A.J., and Leese, F. 2023. It's raining species: rainwash eDNA metabarcoding as a minimally invasive method to assess tree canopy invertebrate diversity. Environ. DNA, **5**(1): 3–11. doi:10.1002/edn3.372.

Marquina, D., Esparza-Salas, R., Roslin, T., and Ronquist, F. 2019. Establishing arthropod community composition using metabarcoding: surprising inconsistencies between soil samples and preservative ethanol and homogenate from Malaise trap catches. Mol. Ecol. Resour. **19**(6): 1516–1530. doi:10.1111/1755-0998.13071. PMID: 31379089.

McCullough, D.G. 2020. Challenges, tactics and integrated management of emerald ash borer in North America. Int. J. For. Res. **93**(2): 197–211. doi:10.1093/forestry/cpz049.

McCullough, D.G., Poland, T.M., Anulewicz, A.C., and Cappaert, D. 2009a. Emerald ash borer (Coleoptera: Buprestidae) attraction to stressed or baited ash trees. Environ. Entomol. **38**(6): 1668–1679. doi:10.1603/022.038.0620.

McCullough, D.G., Poland, T.M., and Cappaert, D. 2009b. Attraction of the emerald ash borer to ash trees stressed by girdling, herbicide treatment, or wounding. Can. J. For. Res. **39**(7): 1331–1345. doi:10.1139/X09-057.

Molnar, C., Casalicchio, G., and Bischl, B. 2018. iml: an R package for Interpretable Machine Learning. J. Open Source Software, **3**(26): 786. doi:10.21105/joss.00786.

Natural Resources Canada. 2013. Emerald ash borer. Natural-Resources.Canada.Ca; Natural Resources Canada. Available from https://natural-resources.canada.ca/our-natural-resources/forests/wildland-fires-insects-disturbances/top-forest-insects-and-diseases-canada/emerald-ash-borer/13377.

Natural Resources Canada. 2022. How does the forest sector contribute to Canada's economy? Natural Resources Canada; Natural Resources Canada. Available from https://www.nrcan.gc.ca/our-natural-resources/forests/state-canadas-forests-report/forest-industry-contribute/16517.

Pimentel, A.C., Cesar, C.S., Martins, M., and Cogni, R. 2020. The antiviral effects of the symbiont bacteria wolbachia in insects. Front. Immunol. **11**: 626329. doi:10.3389/fimmu.2020.626329. PMID: 33584729.

R Core Team. 2023. R: a language and environment for statistical computing [Computer software]. R Foundation for Statistical Computing. Available from https://www.R-project.org/.

Rehner, S.A., Minnis, A.M., Sung, G.-H., Luangsa-ard, J.J., Devotto, L., and Humber, R.A. 2011. Phylogeny and systematics of the anamorphic, entomopathogenic genus Beauveria. Mycologia, **103**(5): 1055–1073. doi:10.3852/10-302. PMID: 21482632.

Removal of Emerald Ash Borer Domestic Quarantine Regulations, 85 F.R. 81085. 2020. Available from https://www.federalregister.gov/documents/2020/12/15/2020-26734/removal-of-emerald-ash-borer-domestic-quarantine-regulations.

Reysenbach, A., and Pace, N. 1995. Reliable amplification of hyperthermophilic archaeal 16 s rRNA genes by the polymerase chain reaction. *In* Archaea: a laboratory manual. *Edited by* F. Robb. Cold Spring Harbor Laboratory Press. pp.101–107.

Robin, X., Turck, N., Hainard, A., Tiberti, N., Lisacek, F., Sanchez, J.-C., and Müller, M. 2011. pROC: an open-source package for R and S+ to analyze and compare ROC curves. BMC Bioinf. **12**(1): 77. doi:10.1186/1471-2105-12-77.

Ryss, A.Y., and Polyanina, K.S. 2022. Life cycle and population dynamics of *Rhabditolaimus ulmi* (Nematoda: Rhabditida: Diplogastridae) in vitro. Russ. J. Nematol. **30**(1): Article 1.

Sanders, W.B., and Masumoto, H. 2021. Lichen algae: the photosynthetic partners in lichen symbioses. Lichenologist, **53**(5): 347–393. doi:10.1017/S0024282921000335.

Shokralla, S., Singer, G.A.C., and Hajibabaei, M. 2010. Direct PCR amplification and sequencing of specimens' DNA from preservative ethanol. BioTechniques, **48**(3): 305–306. doi:10.2144/000113362.

Siegert, N.W., McCullough, D.G., Liebhold, A.M., and Telewski, F.W. 2014. Dendrochronological reconstruction of the epicentre and early spread of emerald ash borer in North America. Divers. Distrib. **20**(7): 847–858. doi:10.1111/ddi.12212.

Srei, N., Guertin, C., Lavallée, R., Lajoie, M.-È., Brousseau, C., Bergevin, R., et al. 2020. Microbial control of the emerald ash borer (Coleoptera: Buprestidae) using *Beauveria bassiana* (Hypocreales: Cordycipitaceae) by the means of an autodissemination device. J. Econ. Entomol. **113**(6): 2657–2665. doi:10.1093/jee/toaa226. PMID: 33089312.

Srinivasan, J., Dillman, A.R., Macchietto, M.G., Heikkinen, L., Lakso, M., Fracchia, K.M., et al. 2013. The draft genome and transcriptome of *Panagrellus redivivus* are shaped by the harsh demands of a free-living lifestyle. Genetics, **193**(4): 1279–1295. doi:10.1534/genetics.112.148809. PMID: 23410827.

Strobl, C., Boulesteix, A.-L., Zeileis, A., and Hothorn, T. 2007. Bias in random forest variable importance measures: illustrations, sources and a solution. BMC Bioinf. **8**(1): 25. doi:10.1186/1471-2105-8-25.

Taylor, R.A.J., Bauer, L.S., Poland, T.M., and Windell, K.N. 2010. Flight performance of *Agrilus planipennis* (Coleoptera: Buprestidae) on a Flight mill and in free Flight. J. Insect Behav. **23**(2): 128–148. doi:10.1007/s10905-010-9202-3.

Thompson, J.N. 1999. The evolution of species interactions. Science, **284**(5423): 2116–2118. doi:10.1126/science.284.5423.2116. PMID: 10381869.

Uchida, N., Kubota, K., Aita, S., and Kazama, S. 2020. Aquatic insect community structure revealed by eDNA metabarcoding derives indices for environmental assessment. PeerJ, **8**: e9176. doi:10.7717/peerj.9176. PMID: 32566391.

United States Department of Agriculture. 2021. The State of the Forest. United States Department of Agriculture. Available from https://www.usda.gov/media/blog/2019/04/22/state-forest.

United States Department of Agriculture. 2023. Emerald Ash Borer. Aphis.Usda.Gov. Available from https://www.aphis.usda.gov/aphis/ourfocus/planthealth/plant-pest-and-disease-programs/pests-and-diseases/emerald-ash-borer.

Valentin, R.E., Fonseca, D.M., Gable, S., Kyle, K.E., Hamilton, G.C., Nielsen, A.L., and Lockwood, J.L. 2020. Moving eDNA surveys onto land: strategies for active eDNA aggregation to detect invasive forest insects. Mol. Ecol. Resour.Mol. Ecol. Resour. **20**(3): 746–755. doi:10.1111/1755-0998.13151.

Wang, X.Y., Yang, Z.Q., Gould, J.R., Zhang, Y.N., Liu, G.J., and Liu, E. 2010. The biology and ecology of the emerald ash borer, *Agrilus planipe nnis*, in China. J. Insect Sci. **10**: 128. doi:10.1673/031.010.12801.

Wei, X., Reardon, D., Wu, Y., and Sun, J. 2004. Emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), in China: a review and distribution survey. Acta Entomol. Sin. **47**: 679–685.

Werren, J.H. 1997. Biology of Wolbachia. Annu. Rev. Entomol. **42**(1): 587–609. doi:10.1146/annurev.ento.42.1.587. PMID: 15012323.

White, T.J., Bruns, T., Lee, S., and Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In* PCR protocols: a guide to methods and applications *Edited by* M.A. Innis, D.H. Gelfand, J.J. Sninsky and T.J. White. Academic Press. pp. 315–322. doi:10.1016/B978-0-12-372180-8.50042-1.

Zabalou, S., Riegler, M., Theodorakopoulou, M., Stauffer, C., Savakis, C., and Bourtzis, K. 2004. Wolbachia-induced cytoplasmic incompatibility as a means for insect pest population control. Proc. Natl. Acad. Sci. U.S.A. **101**(42): 15042–15045. doi:10.1073/pnas.0403853101. PMID: 15469918.

Zeale, M.R.K., Butlin, R.K., Barker, G.L.A., Lees, D.C., and Jones, G. 2011. Taxon-specific PCR for DNA barcoding arthropod prey in bat faeces. Mol. Ecol. Resour. **11**(2): 236–244. doi:10.1111/j.1755-0998.2010.02920.x. PMID: 21429129.

Zieman, E.A., Reeve, J.D., Braswell, W.E., and Jiménez, F.A. 2015. Pathology, distribution, morphological, and genetic identity of *Deladenus proximus* (Tylenchida: Neotylenchidae) a parasitic nematode of the woodwasp, *Sirex nigricornis* in the eastern United States. Biol. Control, **87**: 14–22. doi:10.1016/j.biocontrol.2015.04.009.