Interactions between phoretic mites and bark beetles associated with degrading pine habitat

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Abstract

Mites associated with bark beetles feed and reproduce in subcortical habitats engineered by their vectors. These mites lack the ability to disperse independently, but have evolved behaviors that facilitate using beetles for transport between patchy resources. This research addresses interactions between communities of phoretic mites and their bark beetle hosts, with emphasis on *Ips* spp. in Wisconsin. I first determined the major mite species, and quantified their frequencies of association, with beetle hosts. Nearly 78% of *I. pini* in Wisconsin red pine stands carried phoretic mites, and the three predominant species included one each from separate feeding guilds. Mites varied in diversity across geographic regions, and members were more abundant on beetles captured later in their flight season. I expanded this analysis to incorporate a landscape scale and additional vector species. Approximately 21 mite species were associated with 36 beetle species in *Pinus* stands in southern Wisconsin, northern Arizona, and northern Georgia. While host beetles carries largely similar mite species across regions, there was high variation in species composition among host beetle species within each region. I evaluated potential impacts of mites on beetle fitness, using a two-part approach. On field collected *Ips grandicollis* collected in baited traps, there was a positive relationship between beetle emergence and several mite species. In laboratory experiments where the abundance of mites on beetles was manipulated, however, there was no relationship between colonization rates or total emergence of beetles and mite prevelance. This suggests a correlative rather than causal link between beetle reproductive success and mite prevalence. I conducted a series of experiments to characterize the behaviors of mites commonly associated with *Ips*, with an emphasis on the poorly understood, non-

dispersal life stages. Mites were found to be highly specific in the body parts on which they attach to beetles. Further, they detached from beetles in response to cues associated with both beetle and tree hosts, and discern among living, dead, and injured beetles. Within plant tissue, movement by mites can be elicited by phloem volatiles. Fungal symbionts of beetles, but not opportunistic fungal invaders, provide a food resource that benefits mite fitness.

Contents

Introduction

Phoresy is an ecological interaction, usually commensal, in which one organism (phoretic) attaches to another (host) to gain transport to a resource (Farish and Axtell 1971, Houck and OConnor 1991). Phoretic interactions, often considered a form of migration (Kennedy 1961, Binns 1982), are driven largely by the necessity and difficulty of acquiring patchy ephemeral resources. Phoresy often arises in organisms with limited dispersal abilities and has evolved multiple times across a diverse range of taxa (Faasch 1967) including Arthropoda, Nematoda, and some groups of microorganisms (Campos-Herrera et al. 2006). Phoretic hosts likewise occur across a wide range of taxa.

Within Arthropoda, phoresy is exhibited most commonly among Acari (Binns 1982). Examples range from necter-feeding mites, transported by nectivorous insects or birds (Baker and Yunker 1964), to those feeding primarily on decomposing organisms (Perotti and Braig 2009). Acari are often restricted in their ability to transport themselves between patches of resources due their small size, poor motility and narrow range of tolerated environmental conditions (Mitchell 1970). These attributes increase the probability of developing phoretic dispersal behaviors over evolutionary time. Phoresy is thought to have evolved independently within the major mite groups Mesostigmata, Prostimata and Astigmata (Cross and Bohart 1969).

The Acari life cycle, generally consisting of an egg, one to three nymphal instars (proto-, deuto-, and tritonymph) and an adult stage, varies greatly among the six orders (Binns 1982). The deutonymph stage, often termed the hypopus, is most often associated with phoresy, although adults are occasionally phoretic (Binns 1982). The phoretic stage is often induced by a deficient or degrading habitat (Cutcher and Woodring 1969),

usually signaled by moisture availability (Wallace 1960), food quality (Corente and Knülle 2003), or overcrowding (Houck and OConnor 1991). The mechanisms behind attachment vary widely. For example, some *Elattoma* sp. use structures that serve other purposes, such as chelicerae or pre tarsal claws, while some Uropodidae use highly specialized structures such as hyaline anal pedicels (Houck and OConnor 1991). The spatial distribution of attachment to hosts is often non-random (Cross and Bohart 1969). The free living stage of many phoretic mites is unknown (Houck and OConnor 1991).

Phoretic relationships are usually commensal. In some cases phoretic species may impart indirect and direct feedbacks that mediate the ecology of their host (Lindquist 1969). Such feedbacks are variable, and intermediate between strictly phoretic and parasitic classifications. Mites may indirectly affect their hosts, such as by vectoring fungi throughout bark beetle reproductive galleries.

Strategies of phoresy range from generalist to highly specialized. For example, generalist Chironomidae larvae (Insecta: Diptera) occur on aquatic organisms worldwide (Henriques-Oliveira 2011). Alternatively, *Poecilochirus carabi* Canestrini mites specialize on *Nicrophorus* carrion beetles (Brown and Wilson 1992). Host selection strategies arise from developmental requirements and feeding preferences of phoretic individuals. Those with generalist feeding behaviors tend to be euryxenic (less selective when choosing host species), increasing their available transport and ephemeral habitat opportunities. The generalist feeder *Histiogaster arborsignis* Woodring has been found to be phoretic on over 40 host species (OConnor 1982). Individuals with specialist feeding behaviors trend towards stenoxenic (more selective when choosing host species) attachment behaviors (Houck and OConnor 1991), requiring a more specific food source

or narrow range of environmental conditions. Eroiphyoid mites, for example, are obligately phytophagous (Lindquist and Oldfield 1996) and most are highly specialized in both phoretic host and food source (Skoracka 2006). Reproductive strategies range from relatively k-selected species, often mating prior to phoretic events and traveling in small numbers, to r-selected species, traveling in groups and mating after the phoretic event (Binns 1982).

Ecological theory behind the evolution of phoretic interactions has received substantial attention (Houck and OConnor 1991). Likewise, many studies have documented phoretic interactions and physical mechanisms of attachment. Few studies, however, have examined the cues that phoretic mites use to trigger attachment onto and detachment from hosts. For example, phoretic mites become attracted to southern pine beetle just prior to adult beetle emergence (Roton 1978), but the mechanisms driving this behavior are not known. The cues utilized by phoretic organisms for attachment and detachment likely differ based on the organism's relative specialist or generalist strategy. Generalists may rely more heavily on cues such as mechanical stimuli for attachment. For example, mites grouping around bark beetle exit holes (Roton 1978) are likely to attach to beetles when they experience vibrations in their forelimbs (similar to the questing behavior of ticks). Specialist mites may respond to more specific aspects of host chemistry during attachment. Examples include *Polyphagotarsonemus latus* (Banks), which utilizes the cuticular waxes of the whitefly *Bemisia tabaci* (Gennadius) as a cue for attachment (Soroker et al. 2003). Both generalist and specialist phoretic species may use food source chemistry or behavioral changes in their phoretic host to stimulate detachment. Most likely, phoretic mites use a combination of mechanical and chemical

stimuli to elicit attachment and detachment. Studies of these mechanisms and chemical cues need further exploration.

Bark beetles colonize weakened, dying, and in some cases healthy trees. This consequently converts trees into a suitable but ephemeral food and habitat resource for many organisms. The general life cycle of bark beetles is well characterized (Safranyik and Carroll 2007). Adults land on a tree, select it based on chemical and tactile stimuli, and bore through the bark and into the phloem. They attract mates with pheromones, copulate, and excavate galleries along which they oviposit. As beetles mine their galleries, they introduce several species of fungi that colonize the phloem and vascular tissue (Krokene and Solheim 1997, Klepzig and Six 2004). The larvae hatch, feed on the phloem and fungi, and develop in the phloem as they construct galleries that terminate in pupal chambers. Adults emerge and seek new hosts. Conifers are equipped with sophisticated constitutive and induced physical, chemical, and histological defenses against bark beetles (Bohlmann et al. 2000, Franceschi et al. 2005, Raffa et al. 2005). Bark beetles have unique features that allow them to overcome these defenses. They may exhaust tree defenses by coordinated mass attack, in which aggregation pheromones congregate thousands of beetles within just a few days (Wood 1982, Raffa and Berryman 1983). Symbiotic fungi may also interfere with tree defenses (DiGuistini et al. 2007, Hammerbacher et al. 2011). Some bark beetles have specialized organs, called mycangia, for transporting symbiotic fungi species that contribute to beetle nutrition (Bridges 1983, Six 2003, Bleiker and Six 2007).

From an ecological perspective, bark beetles are important to ecosystem functioning as food for birds, and as agents of canopy thinning and forest succession

(Murphy and Lehnhausen 1998, Lynch et al. 2006). From a socioeconomic perspective they have substantial impacts on human well being (Safranyik and Carroll 2007), through extensive tree mortality that harms the forest products industry and reduces timber supplies. In recent years, warming conditions and homogenization of forested landscapes have resulted in the increased frequency and extent of bark beetle outbreaks (Logan et al. 2003, Aukema et al. 2006). For example, the mountain pine beetle, *Dendroctonous ponderosae* Hopkins, has recently extended north of its historical range, and for the first time invaded the zone in Alberta where its major host, lodgepole pine, *Pinus contorta* Douglas, hybridizes with jack pine, *Pinus banksiana* Lamb. As jack pine is widely spread through the central US, this has created an unprecedented link to, and potential for future mortality within, the Great Lakes region. Recent outbreaks in the west have been so extensive as to convert large segments of coniferous forests from carbon sinks to carbon sources, thus exacerbating this damaging global feedback cycle (Kurz and Apps 2008).

Mites phoretic on some species of bark beetles have been well described, including those of European elm bark beetle, *Scolytus multistriatus* (Marsham) (Moser et al. 2005), European spruce beetle, *Ips typographus* Linnaeus (Takov et al. 2010), fir bark beetle, *Pityokteines* spp. Fuchs (Pernek et al. 2007), southern pine beetle, *Dendroctonus frontalis* Zimmermann (Moser and Roton 1971, Moser et al. 1974), and spruce beetle, *Dendroctonus rufipennis* Kirby (Cardoza et al. 2008). Although usually considered commensal, bark beetle associated phoretic mites often have direct and indirect feedbacks that mediate the ecology of their hosts. *Iponemus confusus* parasitize beetle eggs (Lindquist 1969), reducing beetle reproductive success. Mites may also indirectly affect their hosts by vectoring fungi throughout the gallery environment. Phoretic *Tarsonemus*

mites sometimes transport bluestain fungus *Ceratocystis* spp. via sporothecae (Moser 1985). Bridges and Moser (1986) found a significant correlation between the bluestain fungus *Ceratocystis minor* (Hedgcock) and the number of *Tarsonemus krantzi* Smiley mites in southern pine beetle outbreaks in Texas and Louisiana. Another mite species, *Tarsonemus ips* Lindquist, can indirectly inhibit beetle reproductive success through interactions with the antagonistic bluestain fungus *Ophiostoma minus* (Lombardero et al. 2003). These feedbacks from phoretic mite interactions with fungi can be influenced by seasonal factors (Hofstetter, Mahfouz, et al. 2005). Mites can strongly affect the composition of fungi in bark beetle galleries, and likewise the population dynamics of the beetles (Moser and Macias-Samano 2000, Hofstetter, Cronin, et al. 2005). Many species of mites feed on fungi, and some possess specialized structures, termed sporathecae, for transporting spores (Moser 1985). The relative abundance and species composition of mites strongly affects whether mutualistic or antagonistic fungi predominate, and hence the reproductive output of southern pine beetles (Lombardero et al. 2003). In addition to fungivorous mites, some mite species associated with bark beetles are larval and egg predators (Moser 1975).

The most important bark beetle affecting mature pine plantation trees in Wisconsin is the pine engraver, *Ips pini* (Say) (Klepzig et al. 1991). This insect tends to cause chronic losses, rather than the highly episodic outbreaks of the mountain pine beetle. Commercial plantations, while increasing the productivity and management efficiency of pines, also create conditions that favor insects and pathogens. For example, root feeding beetles and their associated fungi colonize plantation pines, thereby reducing tree defenses against the pine engraver (Klepzig et al. 1996, Erbilgin and Raffa 2002). In

western forests, the pine engraver plays a beneficial role, as it is an important competitor of the mountain pine beetle (Boone et al. 2008, Rankin and Borden 2011). Thus, knowledge of phoretic mite impacts on pine engraver can be applied both to reduce its pest role in Wisconsin, and to increase its beneficial role in decreasing effects of potentially newly arriving pest species.

Associate, predator, and competitor beetles such as *Ips grandicollis* (Eichhoff), *Dendroctonus valens* LeConte, *Thanasimus dubius* (Fabricius) and *Monochamous* spp. also play important roles in this system. Phoretic mites inhabiting *P. resinosa* may utilize all, some subset of, or only one or two of these species as hosts. This makes it important to study the interactions within phoretic mite and bark beetle communities within their ephemeral resources.

This dissertation explores the interactions within the communities of phoretic mites on bark beetles associated with trees in the genus *Pinus*. Chapter one describes the mites associated with *I. pini* in Wisconsin red pine stands. Chapter two assesses the more expansive interactions between the communities of bark beetles and phoretic mites associated with pine habitat on a larger scale at stands in southern Wisconsin, northern Arizona and northern Georgia. Chapter three investigates the effect of phoretic mites on the reproductive success of *I. grandicollis*. Chapter four describes a series of experiments that highlight several important behavioral aspects of phoretic mite-bark beetle interactions during primarily non-dispersal life stages.

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Pfammatter, Moser and Raffa: Survey of phoretic mites on *Ips pini* in Wisconsin

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Chapter 1: Mites Phoretic on *Ips pini* (Say) (Coleoptera: Curculionidae: Scolytinae) in Wisconsin Red Pine Stands

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Abstract

We sampled the bark beetle *Ips pini* in Wisconsin red pine stands in order to describe its phoretic mite community. Nearly 78% of adult beetles carried phoretic mites, with an average of 12.78 ± 0.76 individuals and 1.69 ± 0.05 species per beetle. Phoretic mites from flying beetles represented over 13 families. Five species of mites showed relatively close associations, being present on at least 10% of adult beetles. The most common mite species included one from each of the major guilds on bark beetles, the scavenger / fungivore *Histiostoma* spp. (41.5% of beetles), the egg predator *Iponemus confusus* (41.1% of beetles) and the nematode predator *Dendrolaelaps quadrisetus* (32.8% of beetles). Beetles in northern Wisconsin had more diverse phoretic mite communities than those in central or southern Wisconsin. Beetles collected late in the season (July-Aug) carried more individuals and species of mites than those collected early in the season (May-Jun). The method we used to collect live bark beetles may be useful for other studies requiring fine-scale quantification of symbionts, such as bacteria, fungi, and nematodes.

Introduction

Phoresy is an ecological interaction in which one organism (phoretic) attaches to a host to gain transport to a resource (Farish and Axtell 1971, Houck and O'Connor 1991). Phoretic interactions, often considered a form of migration (Kennedy 1961, Binns 1982), are driven largely by the necessity and difficulty of acquiring patchy ephemeral resources. Phoresy often arises in organisms with limited dispersal abilities and has evolved multiple times across a diverse range of taxa including Arthropoda, Nematoda (Campos-Herrera et al. 2006), and some groups of microorganisms. Phoretic hosts likewise occur across a wide range of taxa. Phoretic relationships are usually commensal, but in some cases may influence the ecology of the host (Lindquist 1969). Such feedbacks are variable, and intermediate between strictly phoretic and parasitic classifications.

Within Arthropoda, phoresy is exhibited chiefly among the Acari (Binns 1982). Examples range from nectar-feeding mites on nectivorous insects or birds (Baker and Yunker 1964), to those feeding primarily on decomposing organisms (Perotti and Braig 2009). Acari often have poor ability to transport themselves between patches of resources due to their small size, low motility and narrow range of tolerated environmental conditions (Mitchell 1970). Phoresy is thought to have evolved independently within the major mite groups Mesostigmata, Prostigmata and Astigmata (Cross and Bohart 1969). The deutonymph (DN) stage, often termed the hypopus, is most often associated with phoresy (Binns 1982). The phoretic stage is often induced by a deficient or degrading habitat (Cutcher and Woodring 1969), usually signaled by moisture availability (Wallace

1960), food quality (Corente and Knulle 2003), or overcrowding (Houck and O'Connor 1991).

Strategies of phoresy range from generalist to highly specialized. Phoretic mite attachment mechanisms vary widely. For example, *Elattoma* spp. use structures that serve other purposes, such as chelicerae or pre tarsal claws, while some Uropodidae use specialized structures such as hyaline anal pedicels (Houck and O'Connor 1991). The spatial distribution of attachment to hosts is often non-random (Cross and Bohart 1969). The free-living stage of many phoretic mites is unknown (Houck and O'Connor 1991).

Host selection strategies of phoretic mites arise from their developmental requirements and feeding preferences. For example, the generalist *Histiogaster arborsignis* Woodring has been found phoretic on over 40 species (O'Connor 1990). Specialist Eroiphyoid mites are obligatorily phytophagous (Lindquist and Oldfield 1996) and most are extremely specialized in both phoretic host and food source (Skoracka 2006).

Bark beetles (Coleoptera: Curculionidae, Scolytinae) colonize weakened, dying, and in some cases healthy trees, converting them into a suitable but ephemeral food and habitat resource for many organisms. The general life cycle of bark beetles is well characterized (Safranyik and Carroll 2006). Adults land on a tree, selecting it based on chemical and other stimuli, and then bore through the bark and into the phloem. They attract mates with pheromones, copulate, and excavate galleries along which they oviposit. As beetles mine their galleries, they introduce several species of fungi that colonize the phloem and vascular tissue (Krokene and Solheim 1998, Klepzig and Six

2004). The larvae hatch, feed and develop in the phloem, constructing galleries that terminate in pupal chambers.

Phoretic mites have been documented on several species of bark beetles, including *Scolytus multistriatus* (Marsham) (Moser et al. 2005), *Ips typographus* (L.) (Takov et al. 2009), *Pityokteines* spp. Fuchs (Pernek et al. 2008), *Dendroctonus frontalis* Zimmermann (Moser and Roton 1971, Moser et al. 1974), and *Dendroctonus rufipennis* Kirby (Cardoza et al. 2008). Some have direct and indirect feedbacks that mediate the ecology of their hosts. *Iponemus confusus* Lindquist parasitize beetle eggs (Lindquist 1969), and other species are larval and egg predators (Moser 1975). Mites may indirectly affect their hosts by vectoring fungi throughout the gallery environment (Moser 1985). Many species feed on fungi, and some possess specialized structures, termed sporathecae, for transporting spores (Moser 1985, Hofstetter et al. 2006). Bridges and Moser (1986) found a positive relationship between the occurrence of bluestain fungus and *Tarsonemus krantzi* Smiley and Moser mites in *D. frontalis* outbreaks. *Tarsonemus ips* Lindquist indirectly inhibits beetle reproductive success through interactions with the antagonistic bluestain fungi *Ophiostoma minus* (Hedgcock) H. and P. Sydow (Lombardero et al. 2003). These feedbacks can be influenced by seasonality (Hofstetter et al. 2006). Mites can strongly affect the composition of fungi in bark beetle galleries, and hence the population dynamics of the beetles (Moser and Macias-Samano 2000, Lombardero et al. 2003, Hofstetter et al. 2005).

The most important pest of mature red pine plantation trees in Wisconsin is the pine engraver, *Ips pini* (Say) (Klepzig et al. 1991). This bark beetle tends to cause chronic losses, often following colonization by root feeding beetles and their associated fungi

(Klepzig et al. 1996, Erbilgin and Raffa 2002), rather than large-scale outbreaks. The objectives of our study were to characterize the mites phoretic on pine engraver in Wisconsin, and identify potential sources of variation in mite community composition within the state.

Materials and Methods

Ips pini were sampled for phoretic mites at eight red pine, *Pinus resinosa* Ait, stands throughout Wisconsin (Table 1). Selected stands were >500 ha in continuous area and 35-55 years old. Age determination records were provided by the Wisconsin Department of Natural Resources, Plum Creek Timber Company, and private land owners; records were validated by counting rings on six representative trees from each site in 2009. Sites were selected to provide three latitudinal regions (northern: 45-46^{o} N, central: 44^{o} N, and southern: 43^{o} N). Temperature and precipitation records for each field site represent nearest weather station historical averages from Climatography of the United States (1971-2001, serially complete daily data) provided by the National Oceanic and Atmospheric Administration (www.ncdc.noaa.gov).

Individual trapping of live beetles allowed for analysis of phoretic mite communities on a per beetle basis, without the disturbance associated with host death. Five 8-funnel flight traps (Lindgren 1983) were hung with dry collection cups, suspended from a wire 1.5 m above the ground tied between two *P. resinosa* trees (approximately 1.5 m apart). Individual traps were placed 50 m apart and arranged in a cross formation. All traps were at least 25 m from the edge of the pine stand. Traps were baited with 40 mg 50^{+}/50^{-} racemic ipsdienol and 4 mg lanierone slow release bubble cap lures (Contech, BC, Canada). Fresh pheromone lures were cut open and the first 20 beetles to arrive at

freshly baited funnel traps were immediately placed individually in 1.5 ml micro centrifuge vials (Eppendorf, Hamburg, Germany) containing 70% ethanol. No trapping event lasted longer than three hours. Beetles were sampled twice per summer. Early season collection took place from 6/23 thru 7/8 in 2009, and 5/17 thru 6/3 in 2010. Late season collection took place from 7/23 thru 8/10 in 2009, and 7/19 thru 7/28 in 2010. The initial sample period was determined based on activity of *Ips* spp. at sentinel baited flight traps located in Mazomanie, WI.

Beetles in ethanol were removed from their micro centrifuge vials and placed individually into 25×75×6 mm deep-well slides (16 mm diameter well). Vials were checked for remaining phoretic mites and rinsed again if necessary. Slides with beetles were then placed in a drying oven at 80^{o}C for 15 min to allow ethanol to evaporate. Dorsally oriented beetles were measured for pronotal width and total length using a 15.2 cm digital calipers (Control Company, Friendswood, TX) accurate to 0.03 mm. Sex was determined based on elytral spine characteristics as outlined in Wood (1982). Specimen clearing fluid (#6373A, Bioquip, Rancho Dominguez, CA) was added to the deep well slides, followed by a cover slip, and slides were placed in the drying oven at 80^{o}C for a minimum of 24 hours for lipid digestion. Lipid-digested beetles were cleared of phoretic mites by gently rubbing them with forceps and a probe, and removed from the well slides. Mites were removed from the remaining solution in order of size (largest to smallest) using micro-tools (flat filed #2 insect pin stuck into a paintbrush handle), mounted on 75×25 mm glass microscope slides (Corning Glass Works, Corning, NY) with PVA mounting media (#6371A, Bioquip) and secured with an 18×18 mm No. 1

coverslip. Prepared slides were then placed at 80^{o} C for 24 h to allow PVA mounting media to set.

Phoretic mites were counted and classified by Jesse Pfammatter. John Moser confirmed representative sample identifications. Voucher specimens were deposited in the University of Wisconsin-Madison Insect Research Collection. In cases where the number of mites exceeded the capacity of the 30 available micro-tools (often multiple mites could be acquired with each micro-tool) the remaining individuals in the well slide were left unmounted. The unmounted individuals always consisted of the smallest species of phoretic mites and were assumed during analysis to consist of *Elattoma* spp., *Histiostoma* spp., *I. confusus*, and *Tarsonemus* spp. in equal proportion to those mites that had been mounted.

Analyses were performed using R statistical software version 2.14.0 for Mac OS X (R Development Core Team 2011) (T-tests, regression, diversity indices, rarefaction, non-linear multi-dimensional scaling, and analysis of similarities) and Primer 6 (Primer-E, Plymouth, UK) (similarity percentages).

Rarefaction curves were developed to determine if sampling intensity was adequate to identify the majority of phoretic mite species on *I. pini* in our study region (Heck et al. 1975), and to compare expected species richness between measured factors. Rarefaction curves were calculated using individual beetles as the unit of replication, and partitioned by beetle sex, collection period (early, late season), study region (northern, central, southern) and year (function: specaccum, package: vegan, method: rarefaction, permutations = 300).

T-tests were used to determine the potential effects of beetle sex, collection period, and year on total phoretic mite load (function: t.test). Regression analysis was used to determine the significance of beetle size regressed on log+1 transformed total phoretic mite load (function: lm).

Shannon and Simpson diversity indices were calculated (function: diversity, package: vegan, index = "shannon", "simpson") on phoretic mite species community data mean aggregated (function: aggregate, FUN = "mean") by beetle sex, region, year, and collection period factors.

Phoretic mite community assemblage trends were visualized using non-linear multi-dimensional scaling (NMS) (function: nmds, package: ecodist, 300 runs, random start configuration) (Shepard, 1962, Kruskal, 1964) and labeled by collection period. Statistical trends in community assemblage data for geographic region, collection year, and collection period were calculated by analysis of similarities (ANOSIM) (function: anosim, package: vegan) (Clarke 1993). Ordination analyses were performed on presence/absence transformed, additively aggregated (function: aggregate, constraints: region, collection period, site and collection period) community data resembled to a Bray-Curtis dissimilarity matrix (function: distance, package: ecodist). Aggregated samples with fewer than five total species and species appearing in less than five samples were removed from the data matrix for NMS analyses. Significant ($P < 0.05$) species correlations vectors (Jongman et al. 1995) (function: vf, package: ecodist) were overlaid on the NMS.

To better describe the differences in phoretic mite communities between collection year and period we used similarity percentages (SIMPER) (Clarke 1993) in the

PRIMER v6 software package (Clarke and Gorley 2006). SIMPER was calculated using the same Bray-Curtis dissimilarity matrix used in the NMS.

Results

We collected over 8,000 phoretic mites, representing 13 families, from a sample of 674 *I. pini* adults (191 males, 483 females) (Table 2). Five hundred seventeen (76.7%) beetles had at least one phoretic mite, and 287 (42.6%) of these beetles had 10 or fewer mites (Fig. 1). Each beetles carried an average of 12.78 ± 0.76 mites, and 1.69 ± 0.05 species per beetle. Beetle size ($R^2 = 0.0003$, $F = 0.1524$, df = 1, 600, $P = 0.6964$) and sex ($t = 0.4389$, df = 334, $P = 0.661$) did not affect the total phoretic mite load.

Ips pini most often carried the scavengers *Histiostoma* spp. (41.5% of beetles), followed by the egg predator *I. confusus* (41.1% of beetles), and the nematode predator *Dendrolaelaps quadrisetus* (Berlese) (32.8% of beetles). *Histiostoma* spp. and *I. confusus* were the most prevalent mite species, comprising approximately 77% of all individuals captured (Table 2).

A rarefaction curve of all beetles obtained indicates sufficient sampling effort to characterize the phoretic mite community on *I. pini* in Wisconsin (Fig. 2A). The total species richness of phoretic mites on beetles obtained in the northern study region was greater than that of the central or southern regions (Fig. 2B). Diversity indices indicated higher phoretic mite diversity in the central region than in the northern or southern regions (Table 3). Composition of the phoretic mite community was similar among these three regions (ANOSIM R = -0.005, $P = 0.487$). Individual phoretic mite species abundances varied among regions (Table 2). For example, we found 244 *Elattoma* spp.

on 35 beetles in the central region while 15 mites on nine beetles in the northern region and 79 mites on 13 beetles in the southern region.

Beetles collected late in the season (Jul-Aug) had an increased mite load (17.9 ± 0.91) and species richness (Fig. 2C) compared to those captured early in the season (8.0 ± 1.19) (May-Jun) (t = 6.570, df = 624, P < 0.001). Early season beetles had a more diverse phoretic mite community than late season beetles (Table 3). The phoretic mite community varied with early and late collection period (ANOSIM R = 0.2305, P = 0.001), with most phoretic mite species occurring more frequently on late season beetles (Fig. 3). *Iponemus confusus* (22.05%), *Histiostoma* spp. (18.71%) and *D. quadrisetus* (14.39%) contributed most substantially to the average dissimilarity between early and late collection periods.

Beetles collected in 2009 and 2010 had similar species richness (Fig. 2D), but overall mite community diversity was higher in 2009 (Table 3). Beetles carried more mites on average in 2009 (15.72 ± 1.23) than in 2010 (10.25 ± 0.94) (t = 3.5356, df = 606, P < 0.001). The community composition of phoretic mites differed between 2009 and 2010 (ANOSIM R = 0.1277, P = 0.008). *Histiostoma* spp. (21.20%), *I. confusus* (20.75%) and *D. quadrisetus* (13.82%) contributed most significantly to the average dissimilarity between 2009 and 2010.

Images of many phoretic mite species are provided in Figure 4.

Discussion

Ips pini in Wisconsin have a robust community of phoretic mites. Over 78% of beetles carried mites, representing a total of over 8000 individual and 20 species of phoretic mites. By comparison, 75% of *D. rufipennis* carry phoretic mites (Cardoza et al.

2008), while only 11% *I. typographus* carry mites (Takov et al. 2009). Reports of species richness range from 57 phoretic mite species associated with *D. frontalis* in central America and Mexico (Moser et al. 1974) to 18 species of phoretic mites in the genus *Pityokteines* (Pernek et al. 2008; Pernek et al. 2012). While there is high variation in species diversity and abundance of phoretic mites among bark beetle systems, there is a substantial overlap in the mite species found within these systems. For example, *D. quadrisetus* was the most abundant mite found on *I. typographus* (Takov et al. 2009) and *H. arborsignis* the most abundant on *D. rufipennis* (Cardoza et al. 2008), both species were found commonly in our study.

Five species of mites (*D. quadrisetus, Ereynetes propescutulis, Histiostoma spp.*, *I. confusus, Trichouropoda australis*) showed relatively close associations with *I. pini*, being present on at least 10% of adult beetles (Table 2). The top three most abundant mite species included one representative from each of the three primary feeding guilds associated with bark beetles. *Histiostoma* spp. (41.5%) are scavengers; *I. confusus* (41.1%) are beetle egg predators; and *D. quadrisetus* (32.8%) are nematode predators. The underlying processes that drive niche differentiation and species partitioning based on feeding guilds remains unstudied in phoretic mite systems.

We did not observe vast differences in phoretic mite community composition between regions despite habitat differences that include precipitation, temperature and soil type (Table 1). This may reflect the relative consistency of the bark beetle/phoretic mite within-tree microhabitat. The slight increase in species richness in the northern region (Fig. 2B) may be due to the higher forest acreage (Table 1), conifer diversity, and forest fragmentation as compared with the central and southern regions of the state. The

seasonal increase in incidence of most phoretic mite species (Fig. 3) likely reflects the multi-voltine life history of *I. pini* as well as increased temperature during the course of the season. For example, *I. confusus*, a predator of beetle eggs, increased its incidence on beetles 1.5 times and its total abundance by 3.6 times within season (years pooled).

Distribution of phoretic mite species on *I. pini* was non-random. Smaller species such as *I. confusus* and *Tarsonemus* spp. clustered in body crevices (elytral declivity, crevices at leg attachment points, etc.), presumably to avoid mechanical perturbation during beetle tunneling. *Dendrolaelaps quadrisetus* was often found underneath elytra. This may provide protection from predators and mechanical removal during beetle tunneling and flight; it likely may foster feeding on the abundant nematodes that occur under the beetle elytra. *Elattoma* spp. were most often found on the ventral side of the beetle, attached to setae on the thorax near the head capsule. It is possible that these mites may make their way deep into crevices of the head capsule. *Histiogaster arborsignis* is normally found in the elytral declivity or on the exterior of the beetle exoskeleton. Some small phoretic mite species (*I. confusus* and *Tarsonemus* spp.) have been found hyper-phoretic on *D. quadrisetus*.

Our trapping method provides a useful technique for beetle collection when studying phoretic mites. Live trapping avoids confounding of mite count data due to movement between beetles (including non-host beetles) within collection cups during long trapping periods and reduces mite detachment prior to analyses. This technique could also be applied to studies of insect associated fungi and bacteria, in which conspecific or non-target insect contact may contaminate samples (Aukema et al. 2005).

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Table 1. Locations and descriptions of sites (Wisconsin) in which *I. pini* were sampled for phoretic mites in 2009 and 2010. Precipitation and temperature data were obtained from the National Oceanic and Atmospheric Administration (www.ncdc.noaa.gov). Soil type data were obtained from the Wisconsin Geological Survey (http://wisconsingeologicalsurvey.org/soil.htm). Pine stand age represents the range of tree ring counts from three representative trees at each site in 2009. Forest acreage provided by http://www4.uwsp.edu/cnr/landcenter/forestplanning/pdf%20files/forest_cover.pdf.

County	Latitude	Wisconsin Region	Mean Annual Precipitation (cm)	Mean Annual Temperature (^{o}C)	Soil Type*	Percent Forest Acreage (County)	Weather station ID
Dane	43^{o} 12’ 51.5”	southern	33.48	44.6	silty	13	471416
Sauk	43^{o} 10’ 48.7”	southern	33.79	43.4	silty	32	470516
Waushara	44^{o} 15’ 34.3”	central	31.62	43.4	sandy	43	473405
Adams	44^{o} 14’ 10.4”	central	32.34	45.8	sandy	65	475786
Jackson	44^{o} 14’ 50.7”	central	33.00	44.7	sandy	58	477997
Washburn	45^{o} 46’ 53.3”	northern	30.06	42.9	sandy/loamy	68	478027
Vilas	45^{o} 59’ 47.7”	northern	29.59	40.1	sandy/loamy	88	472314
Vilas	46^{o} 09’ 03.7”	northern	32.64	38.4	sandy/loamy	88	477092

*Sites were categorized as the most common soil type within a 25 km radius.

Table 2. Phoretic mites obtained from 674 live caught *Ips pini* in Wisconsin red pine plantations (2009-2010) using racemic ipsdienol and lanierone lures. Total phoretic mite load is partitioned by region (northern, central, southern) and season (early and late). Each cell represents the number of *I. pini* hosting a phoretic mite species (1st number) followed by the total number of phoretic mites isolated from all beetles (2nd number, in parentheses).

				Northern		Central		Southern		Total Beetles Carrying	Percentage of Total Beetles Carrying
Family	Species	Feeding Guild[2]	Phoretic Lifestage	early n=132	late n=109	early n=112	late n=164	early n=100	late n=101	n=674	
Digamasellidae[P]	*Dendrolaelaps quadrisetus* (Berlese)	Predatory	DN	36 (119)	44(124)	34(90)	33(104)	33(114)	41(147)	221(698)	32.8
Digamasellidae[P]	*Dendrolaelaps neodisetus* (Hurlbutt)	Predatory	DN	0(0)	1(1)	1(1)	2(2)	2(2)	0(0)	6(6)	0.9
Pyemotidae[A]	*Elattoma* spp.*	Mycetophagous	♀	7(10)	2(5)	15(83)	20(161)	3(8)	10(71)	57(338)	8.5
Ereynetidae[A]	*Ereynetes propescutulis* Hunter	Predatory	♀♂	10(16)	23(48)	4(6)	36(56)	5(12)	14(28)	82(162)	12.2
Acaridae[A]	*Histiogaster arborsignis* Woodring	Scavenger	DN	0(0)	3(7)	2(2)	7(22)	3(9)	1(1)	16(41)	2.4
Histiostomatidae[A]	*Histiostoma* spp.*[1]	Scavenger	DN	44(583)	54(597)	47(534)	54(535)	29(287)	52(929)	280(3465)	41.5
Tarsonemidae[A]	*Iponemus confusus* Lindquist*	Egg Predator	♀	39(371)	49(463)	34(173)	25(815)	17(49)	63(887)	277(2758)	41.1
Cheyletidae[A]	*Mexecheles virginiensis* (Baker)	Predatory	♀	0(0)	1(1)	0(0)	0(0)	0(0)	0(0)	1(1)	0.1
Pyemotidae[A]	*Paracarophaenax* spp.	Egg Parasitoid	♀	9(24)	4(11)	0(0)	1(3)	0(0)	1(1)	15(39)	2.2
Ascidae[P]	*Proctolaelaps* spp.	Generalist	♀	2(7)	4(10)	0(0)	2(2)	1(1)	2(3)	11(23)	1.6
Cymbaeremaeidae[A]	*Scapheremaeus palustris* Sellnick	Mycetophagous	♀♂	1(1)	1(1)	0(0)	1(1)	0(0)	0(0)	3(3)	0.5
Acaridae[A]	*Schwebia* spp.	Generalist	DN	1(1)	0(0)	1(1)	0(0)	2(3)	1(1)	5(6)	0.7
Tarsonemidae[A]	*Tarsonemus* spp.*[2]	Mycetophagous	♀	5(5)	9(1)	7(12)	12(62)	3(6)	9(124)	45(219)	6.7

				Northern		Central		Southern		Total Beetles Carrying	Percentage of Total Beetles Carrying
Family	Species	Feeding Guild[2]	Phoretic Lifestage	early n=132	late n=109	early n=112	late n=164	early n=100	late n=101	n=674	
Uropodidae[P]	*Trichouropoda australis* Hirschmann	Generalist	DN	15(29)	22(29)	22(52)	16(32)	17(32)	23(84)	115(258)	17.1
Scutacaridae[A]	*Scutacarus* spp.	Mycetophagous	♀?	0(0)	0(0)	0(0)	0(0)	0(0)	2(3)	2(3)	0.3
Laelapidae[P]	unknown (u2)	Predatory		0(0)	1(1)	0(0)	0(0)	0(0)	0(0)	1(1)	0.1
Tetranychidae[A]	*Tetranychidae* spp.	Phytophagous		0(0)	0(0)	1(1)	1(1)	0(0)	0(0)	2(2)	0.3
Acaridae[A]	*Histiogaster anops* Griffiths	Mycetophagous		2(2)	0(0)	0(0)	0(0)	0(0)	0(0)	2(2)	0.3
Acaridae[A]	*Tyrophagus putrescentiae* (Schrank)	Mycetophagous		0(0)	1(1)	0(0)	0(0)	0(0)	1(1)	1(1)	0.2

* In cases where not all mites from a beetle could be slide mounted for final identification, the remaining portion of unmounted mites consisted of only the smallest four species, *Elattoma* spp., *Histiostoma* spp., *I. confusus*, and *T. ips*, and were added to the final species counts in equal relative proportion to those same species that were represented in the slide mount.

[A] and [P] represent superorder classification for Acariformes and Parasitiformes respectively.

[1] Most of the *Histiostoma* spp. sampled were *Histiostoma varia* Woodring and Moser, but small structural features make it difficult to confirm all are *H. varia*.

[2] The majority of the *Tarsonemus* spp. were *T. ips*, although *Tarsonemus fusari* Cooreman were identified in a few samples. Small structural features make it difficult to confirm all are *T. fusari.*

Table 3. Species diversity indices calculated for beetle sex, collection region, year, and collection period from *I. pini* in Wisconsin.

		Diversity Indices	
Grouping		**Shannon-Wiener**	**Simpson**
Beetle Sex	male beetle	1.46	0.70
	female beetle	1.41	0.68
Region	northern region	1.36	0.66
	central region	1.48	0.70
	southern region	1.33	0.66
Year	2009	1.52	0.71
	2010	1.27	0.64
Collection Period	early season	1.46	0.68
	late season	1.38	0.66

Figure Legends

Figure 1. Phoretic mite loads of *Ips pini* captured in Wisconsin (2009 and 2010).

Figure 2. Rarefaction curves (95% CI) for phoretic mite species obtained from live *Ips pini* in Wisconsin (2009 and 2010) as accumulated by A) all captures combined, B) region, C) early and late season, D) year, and E) beetle sex.

Figure 3. Nonmetric Multi-Dimensional Scaling (NMS) plot of the phoretic mite community composition on *Ips pini* obtained in Wisconsin (2009 and 2010). Bray-Curtis resembled data are labeled by collection period (early and late). Significant ($P<0.05$) species correlations are overlaid.

Figure 4. Microscope images of mites phoretic on *Ips pini* in Wisconsin. A) *Dendrolaelaps quadrisetus* DN, B) *Dendrolaelaps neodisetus* DN, C) *Elattoma* sp. ♀, D) *Ereynetes propescutulis* ♂, E) *Histiogaster arborsignis* DN , F) *Histiostoma varia* DN, G) *Iponemus confusus* ♀ , H) *Mexecheles virginiensis* ♀, I) *Paracarophaenax* spp. ♀, J) *Proctolaelaps* sp. ♀, K) *Histiogaster anops* DN, L) *Schwebia* sp. DN, M) *Tarsonemus ips* ♀, N) *Trichouropoda australis* DN, O) *Scapheremaeus palustris* ♀ (image from individual found under elytra of *D. valens*) ,P) ♀ *Elattoma* spp. attached to beetle setae. DN = deutonymph. Images provided in part by the U.S. Forest Service, Southern Research Station, Unit 4552.

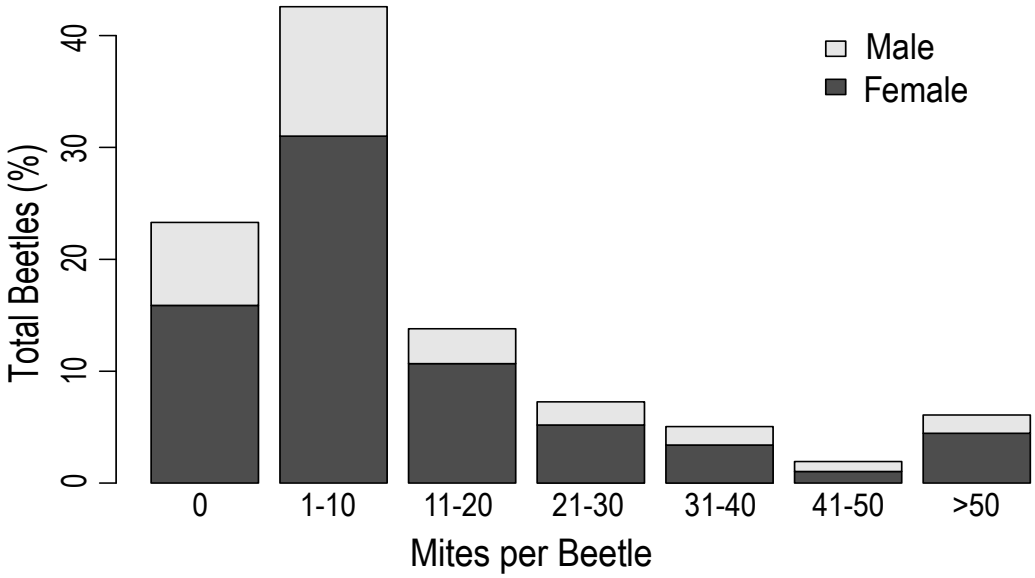
Male
Female
Total Beetles (%)
0
10
20
30
40
0
1-10
11-20
21-30
31-40
41-50
>50
Mites per Beetle

Figure 1.

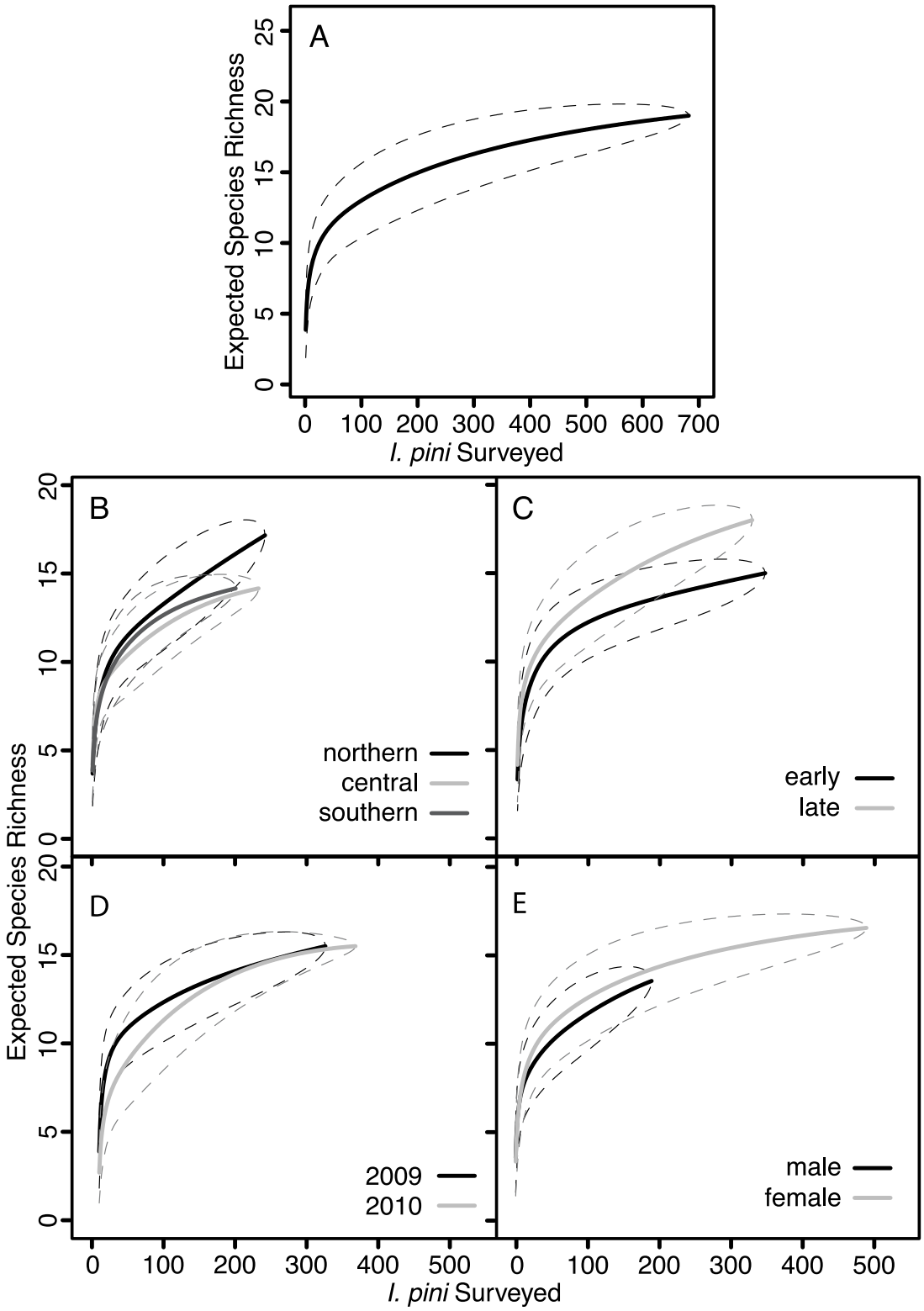
A
Expected Species Richness
0
5
10
15
20
25
0
100
200
300
400
500
600
700
I. pini Surveyed
B
C
D
E
northern
central
southern
early
late
2009
2010
male
female
Expected Species Richness
0
5
10
15
20
0
100
200
300
400
500
I. pini Surveyed

Figure 2.

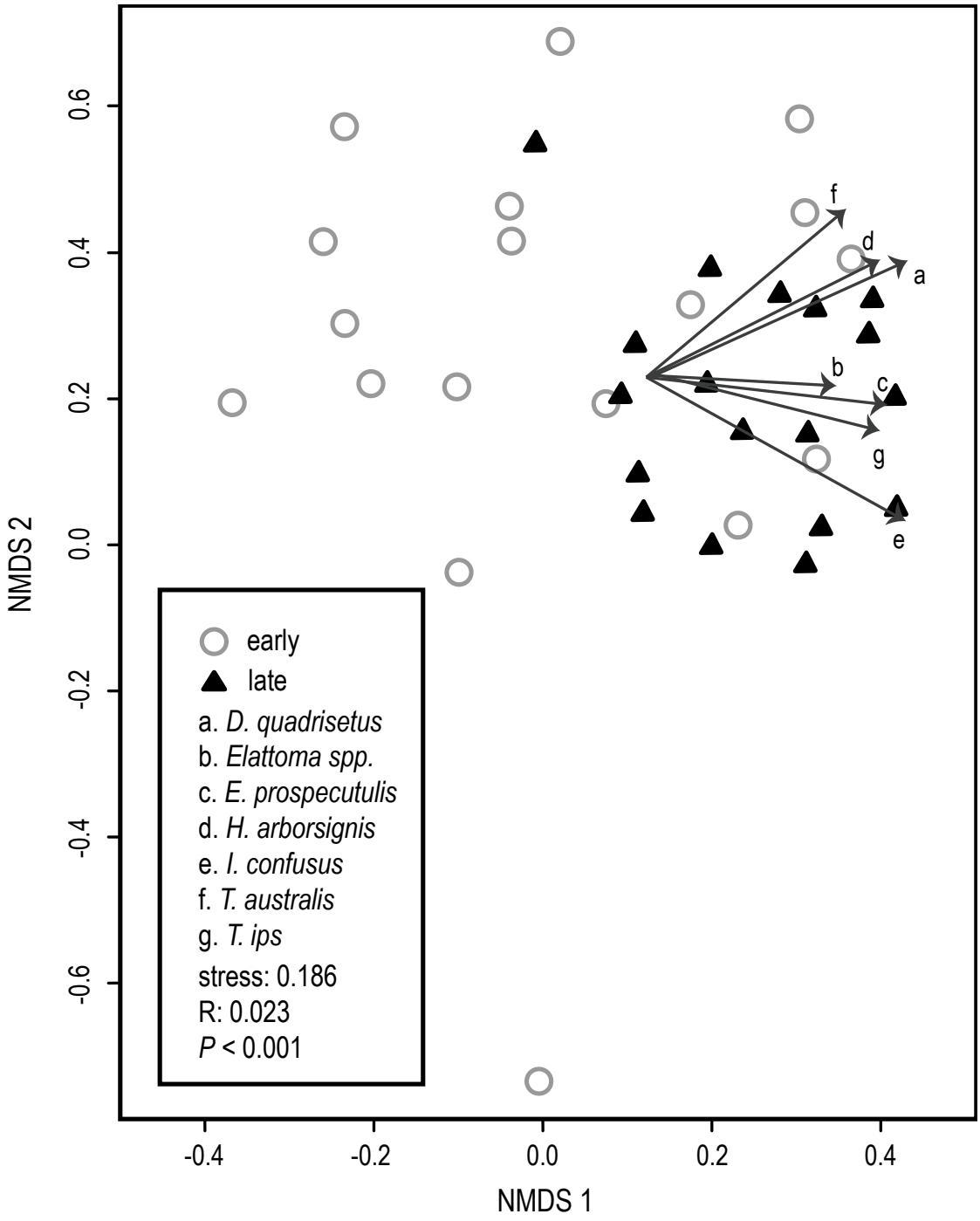
NMDS 2
NMDS 1
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.4
-0.2
0.0
0.2
0.4
f
d
a
b
c
g
e
early
late
a. D. quadrisetus
b. Elattoma spp.
c. E. prospecutulis
d. H. arborsignis
e. I. confusus
f. T. australis
g. T. ips
stress: 0.186
R: 0.023
P < 0.001

Figure 3.

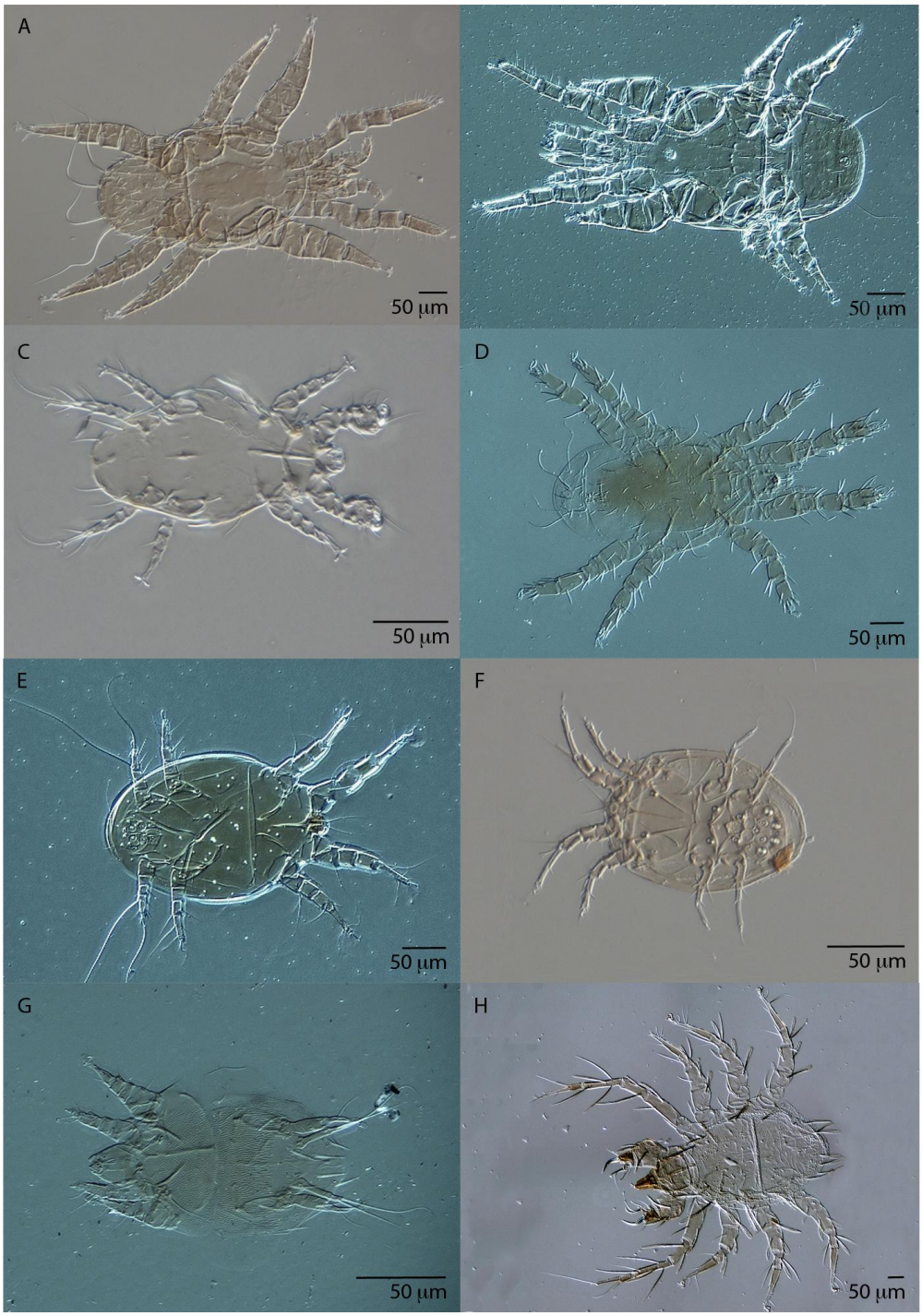

Figure 4.

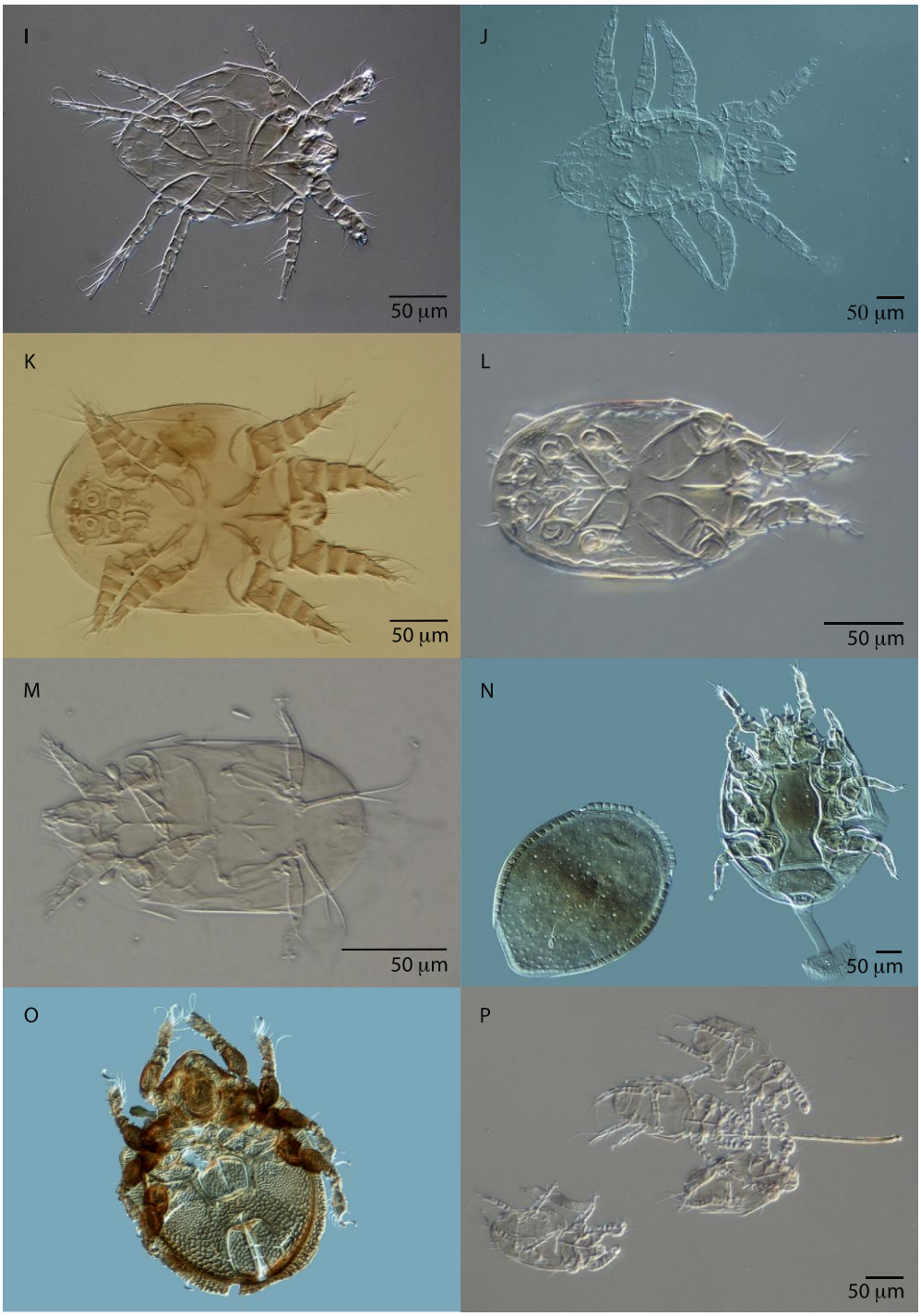

Figure 4. (cont.)

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Chapter 2: Structure of phoretic mite communities across bark beetle species at local and regional scales

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Abstract

Mites phoretically associated with bark beetles feed and reproduce within subcortical habitats engineered by tree-killing herbivores. Mites lack the ability to independently disperse among these island-like habitats, and thus have evolved behaviors and morphologies that facilitate using insects to achieve transport between patchy resources. Studies of associations between phoretic mites and bark beetles have often been approached from the beetle's perspective, whereby an assemblage of mite species is characterized on a particular beetle species. However, the available evidence suggests there may be substantial overlap among phoretic mite species on various species of bark beetles associated with similar host tree habitats. Here we provide a holistic view of the interactions between the communities of beetles and mites across pine landscapes in order to better characterize mite dispersal and the formation of mite-beetle phoretic pairings. We assessed the phoretic mite communities of multiple bark beetle species attracted to baited funnel traps in *Pinus* stands from three regions: southern Wisconsin, northern Arizona, and northern Georgia. We identified approximately 21 mite species totaling 10,575 individuals on 36 beetle species totaling 983 beetles. Of all phoretic mites collected, 97% were represented by eight species. Phoretic mite communities were heavily overlapping across bark beetle species, likely due to these beetles' common association with trees in the genus *Pinus*. Most species of mites were found on at least three beetle species, and *Histiostoma* spp., *Iponemus confusus* Lindquist, *Histiogaster arborsignis* Woodring and *Trichouropoda australis* Hirschmann were each found on at least seven species of beetle. While host beetles had largely the same phoretic mite membership, we observed a large amount of variation in mite community composition among beetle host species within each sampling region. Phoretic mite communities also varied within beetle host species between regions, notably for *Ips pini* (Say) and *Ips*

grandicollis (Eichhoff). The apparent stochasticity in patterns of phoretic mite-beetle associations are likely driven at the scale of habitat, i.e., tree-to-tree variation.

Introduction

Numerous organisms exploit the degrading subcortical environment engineered by bark beetles and their symbionts when they mass attack and kill trees. Mites (Acari) readily feed and reproduce within tree phloem, and have a diverse array of life history characteristics and resource requirements. These life histories include nematode predators such as *Dendrolaelaps quadrisetus* (Berlese) (Kinn 1984), egg parasitoids of beetles such as *Iponemus confusus* (Lindquist) (Lindquist 1969), generalist bacterial filter feeders such as *Histiostoma varia* Stone and Simpson (OConnor 1984) and fungivores such as *Tarsonemus ips* Lindquist (Moser and Roton 1971). Mites range from relatively specialized feeders to broad feeding generalists that include scavengers and facultative predatory or fungal feeders. Influences on mite reproductive success are largely unknown, but are likely driven by multiple factors including habitat availability, micro climate (Hofstetter et al. 2007), fungal populations (Lombardero et al. 2003), and natural enemy prevalence. The high variation in phoretic mite morphologies and life histories is likely related to the large number of ecological niches available within the beetle-engineered habitat.

While mites can be highly successful at utilizing available resources within the subcortical environment of beetle-killed trees, dispersal poses a significant challenge. Mites are limited in their ability to transport between resources due to their small size, poor motility and narrow range of tolerated environmental conditions (Mitchell 1970). Recently killed trees are patchy, ephemeral, and dictated in abundance and distribution by bark beetle populations, which themselves are quite variable, across the landscape. In response to these pressures, many mites

have evolved mechanisms that facilitate phoretic attachment to beetles and dispersal between resource islands (Farish and Axtell 1971). These adaptations include behavioral specializations in attachment location (Pfammatter 2015) and morphological structures that facilitate attachment to specific host structures. Some examples of behavioral modifications include selective alignment of *D. quadrisetus* underneath elytra of host beetles (Moser and Bogenschuütz 1984, Pernek et al. 2007), and attachment of *I. confusus* inside the relatively disturbance-free elytral declivity (Lindquist 1969). Examples of morphological adaptations include a series of suckers on the anal plate of *Histiostoma* spp. (Binns 1982), haustoria stalks on *Trichouropoda australis* Hirschmann (Faasch 1967, Binns 1982), and modified claw-like forelegs *Elattoma* sp. (Binns 1982).

Patterns of phoretic mite associations with bark beetles have most often been studied from the perspective of single host beetle species. Examples of bark beetle species that have been examined for phoretic mites include the pine engraver (Pfammatter et al. 2013), European elm bark beetle (Moser et al. 2005), European spruce beetle (Takov et al. 2010), fir bark beetle (Pernek et al. 2007), spruce beetle (Cardoza et al. 2008), and southern pine beetle (Moser and Roton 1971, Moser et al. 1974). These studies have provided invaluable insight into our understanding of paired beetle-mite associations, but we have less understanding of mite community structure from a regional or landscape perspective. One influence on our lack of understanding may be attributed to the fact that mites are often driven by bottom-up ecological processes and so may be more likely to be habitat- than vector-specific (Moser 1995). That is, they may exploit, with variable levels of behavioral specificity, the diverse array of subcortical beetles associated with degrading tree habitats and these habitats may vary between regions.

The processes that influence the patterns phoretic pairings observed between entire communities of beetles and mites remain largely unknown. Given that mites have the potential to impact bark beetles and the community structure of beetle-engineered habitat (Lombardero et al. 2003), it is important investigate the structure of these phoretic pairings and how they differ between within and between beetle species and across regions and landscape scales.

Conservatively, North America has over 475 species of bark and ambrosia beetles (Wood 1982). Many of these beetles, including all the native North American irruptive species, are associated with conifers and use aggregation pheromones to mass attack trees (Coulson 1979, Wood 1982). These conifer-associated bark beetles have varying behaviors, strategies and mechanisms for colonizing host trees (Lindgren and Raffa 2013), jointly engineering an environment suitable for their brood production. This transformed environment is suitable for co-habitation by a large diversity of organisms such as nematodes, fungi, bacteria, and other arthropods, in addition to phoretic mites. We know little about community characteristics such as fidelity, redundancy, and substitutability of mites within these systems, both within their tree host and on their host carrier beetles. The characteristics that define these interacting communities of beetles, mites and other microorganisms likely all contribute to the apparent relationships and phoretic pairings between beetles and mites. In addition, as these characteristics vary across landscapes, our understanding of variation in beetle-mite pairings will likely benefit from landscape scale studies.

The purpose of this research is to quantify the extent to which phoretic mite communities vary among potential bark beetle vectors within and across regions. We currently do not know whether each host insect is characterized by a mite community that is consistent in its species composition and proportions across geographic regions or if variation in these communities is

related more closely to specific regions than of particular bark beetles. This information will provide insight into the extent to which these symbioses are driven by factors unique to each interspecific relationship, local abiotic and tree-species factors, and general features of the bark beetle engineered habitat.

Materials and Methods

We characterized the phoretic mite communities of pine-associated bark beetles in Wisconsin, Arizona, and Georgia. We compared communities among beetle species within and across regions, and evaluated whether the feeding breath of mite species influences the relative degree of overlap in the communities.

Beetle Sampling

Beetles were trapped live at three mixed pine stands (sites) in each of the three sample regions (southern Wisconsin, northern Arizona, and northern Georgia) in 2013. Sites consisted primarily of red pine plantation with sparse white pine, and various oak and maple species in Wisconsin; ponderosa pine with sparse gambel oak and Arizona locust in Arizona; and planted loblolly pine with hardwood components including sweetgum, tulip poplar, and various oak species in Georgia. Beetles were also sampled in *Pinus resinosa* Ait sites near Arkdale and Mazomanie, Wisconsin in 2011 (Table 1). Each of the three multiple-funnel traps (Lindgren 1983) at each site were baited with one of three lures deemed to be most attractive to the predominant bark beetle species in each region. We used: α-pinene and EtOH ultra high release lures, Ipsdienol $3^{+}/97^{-}$ 40 *mg* bubble caps or western pine beetle lure (exo-brevicomin, frontalin and myrcene) in Arizona sites, and α-pinene and EtOH ultra high release lures, Ipsdienol $50^{+}/50^{-}$ 40 *mg* and 4 *mg* bubble caps, or Ipsenol $50^{+}/50^{-}$ 40 *mg* bubble caps in Georgia and Wisconsin. All lures were purchased from Contech Enterprises Inc. (BC, Canada). Traps at each site were

suspended from a wire between two trees or from a metal pole 1.5m above the ground. All beetles were collected live from dry collection cups during eight hour trapping sessions in which fresh lures were cut open to release high volumes of attractants. Collection cups were partially filled with Kimwipes (Kimberly-Clark, Irving, TX) to provide beetles protection from predation during trapping events. Live trapping allows for the analysis of phoretic mite communities on a per beetle basis without the disturbances associated with host insect death (Pfammatter et al. 2013).

Beetles were sampled on four occasions, 25 - 27 Jun, 9 - 10 Jul, 25 - 27 Jul, and 6 - 8 Aug at each site. Collected beetles were placed in individual gel capsules that were placed on ice immediately and frozen within the same day. Beetle identifications were confirmed using a combination of the following resources: Yanega (1996), Arango and Young (2012), Lingafelter (2007), Wood (1982), Dorshorst and Young (2009), Arnett and Thomas (2000), and Arnett et al. (2010).

Additionally, *Dendroctonus valens* LeConte were excavated from newly cut *P. resinosa* tree stumps in 2011. These were subsequently handled identically to the other beetle samples.

Phoretic Mite Sampling

Mites were removed from each beetle using a size no. 1 insect pin affixed to a pasteur pipette, and mounted on a 75 × 25 *mm* glass microscope slide (Corning Glass Works, Corning, NY) with specimen clearing fluid (\#6373A, Bioquip, Rancho Dominguez, CA). Microscope slides with mites were placed in a drying oven at 80 ^{o}C for a minimum of 24 *h* in conjunction with clearing fluid to allow for rapid lipid digestion of mite internal organs. After lipid digestion, only chitinous products remained, facilitating identification based on mite exoskeleton

morphology. Phoretic mites from beetles in all regions were counted and identified, and representative samples were confirmed by Dr. John Moser.

Statistical Analyses

All data were analyzed using R statistical software v3.0.2 (R Core Team 2014). Rarefaction curves were generated to determine the effectiveness of sampling intensity (Heck et al. 1975) in each region. Rarefaction curves were generated using code from Chao et al. (2014) where q = 0, 0.5, 1, and 2 (described in the next section).

The diversities of phoretic mite communities from beetle species with at least 35 representative specimens from each capture region were compared using the rarefied "Hill numbers" procedure, as described in Chao et al. (2014). Hill numbers, expressed as q values, provide a method for unifying community diversity indexes (Hill 1973). Hill's equations (at any value for q) generate a value for effective species richness that is interpretable as species richness at q = 0, the exponential Shannon's index at q = 1, and the inverse Simpson's index at q = 2 (Hill 1973). We present Hill numbers for q = 0, 1, and 2 rarefied over total number of beetles for each region. We also present a rarefied Hill index at q = 0.5, which weights the integration of species evenness towards the least common species in the community (Chao et al. 2014). Calculation of rarefied Hill numbers and rarefaction curves were performed for species groupings as defined in Table 2. We hereafter refer to expected species richness at q values of 0, 0.5, 1, and 2 by superscript annotation (i.e. expected species richness^{0}, richness$^{0.5}$, etc.).

Phoretic mite communities were visualized using two- and three-dimensional Nonmetric Multidimensional Scaling (2D, 3D NMS) (function: nmds, package: ecodist, 150 runs, random start configuration) (Shepard 1962, Kruskal 1964) and labeled by region and beetle species. Analysis of Similarities (ANOSIM) (function: anosim, package: vegan, 999 permutations) was

used to test for significance of separation for both region and beetle species. Significant ($P < 0.05$) correlation vectors (Jongman et al. 1995) (function: vf, package: ecodist) of mite species were overlaid on the 2D NMS visualization. Ordination (NMS, ANOSIM and vector correlations) analyses were performed on additively aggregated (function: aggregate, constraints: beetle species, and collection region) square root transformed, Wisconsin double-standardized (function: wisconsin) (Bray and Curtis 1957) phoretic mite community abundance data resembled to a Bray-Curtis dissimilarity matrix (function: distance, package: ecodist). Aggregated samples with no phoretic mites or unidentified beetle genera were removed from the data matrix prior to dissimilarity resemblance.

We individually assessed regional variation in the community of relatively abundant phoretic mites on *I. pini* and *I. grandicollis* with a series of generalized linear models fit with a Poisson distribution. Post-hoc Tukey tests were performed to compare individual mite abundances between regions in a pairwise manner.

We calculated Pearson correlations and associated *P*-values for pairs of phoretic mite species on beetle species with at least 35 representative individuals in a region. Resultant matrices were visualized (function: corrplot, package: 'corrplot') with non-significant correlation values marked with an ‘X’. Mite species that occurred less than 10 times across the group of beetles selected for this analyses were removed from analysis.

Results

We sampled 983 individual beetles, representing 36 species from nine families (Table 2). Sixteen of these 36 beetle species carried at least one phoretic mite. All of the beetle species on which no phoretic mites were observed were captured in relatively low abundances. Species that carried mites averaged just under five mite species per host beetle. Overall we found

approximately 21 mite species totaling 10,575 individuals. The eight most abundant phoretic mite species represented 97% of all mites (based on 2013 data). Mites on six individual beetles in 2013 were unidentifiable, and we found approximately seven unidentified phoretic mite species, mostly in association with *Thanasimus dubius* (F.), on beetles in 2011. Rarefaction of the phoretic mite communities on pooled beetle samples indicated adequate sampling effort (Fig. 1). Expected mite species richness for Wisconsin, Arizona, and Georgia were similar, although estimates of Georgia samples effective species richness may be slightly lower than the other two regions (Fig. 1). Projections indicate a potential for higher species richness for phoretic mite communities on beetles in Arizona given a stronger sampling effort (Fig. 1).

The prevalence and abundance of various mite species on each beetle species × region × year combination is presented in Table 2. The average proportion for each of the identified phoretic mite species on beetles species that carried mites (pooled by region and year) are presented in a network map in Fig. 2. Across all beetle species, we found more *Elattoma* sp. (6,561) than any other species of mite. We also obtained high abundances of *I. confusus* (1,923), *Histiostoma* spp. (659), *Histiogaster arborsignis* Woodring (296), *T. australis* (268), *Tarsonemus* spp. (258), and *D. quadrisetus* (238). All other phoretic mite species were represented by less than 125 individuals each. *Histiostoma* spp. had the widest breadth of phoretic association, occurring on 12 beetles species (Table 2). *Iponemus confusus* and *H. arborsignis* were found on nine, *Tarsonemus* spp. and *Elattoma* sp. were found on seven, and *D. quadrisetus* and *Proctolaelaps* sp. were each found on six beetle species (Table 2). *Mexecheles virginiensis* (Baker) and *Schwebia* sp. had the narrowest breadth of host beetle species association, only being found on one and two beetle species respectively (Table 2).

Phoretic mite community assemblages varied among sampling regions (3D NMS Stress = 0.094 , r^2 = 0.927) (ANOSIM R = 0.191, P = 0.053) (Fig. 3A, 2B). Based on the 2D NMS visualization, regional groupings appear distinct for Wisconsin, while samples from Arizona and Georgia appear somewhat overlapping (Fig. 3A). A 3D depiction of phoretic mite community dissimilarity shows that each region fills a unique NMS space (Fig. 3B), and thus beetles from each region host different phoretic mite communities in terms of phoretic mite incidence and abundance.

Beetle species was also a significant factor in predicting phoretic mite community dissimilarity (ANOSIM R = 0.643, P = 0.01) (2D NMS Stress = 0.188, r^2 = 0.819) (Fig. 3). NMS visualization indicates clustering of the phoretic mite communities on samples from *I. grandicollis* and *I. pini* from all three sample regions (Fig. 4A). Beetles in the genus *Ips* were differentially associated with *D. quadrisetus*, *I. confusus*, or *Elattoma* sp. depending on beetle species and sampling region (Fig. 3A). *Dendroctonus brevicomis* LeConte and *Dendroctonus frontalis* Zimmerman from Arizona had very similar phoretic mite communities, where both were characterized by a high abundance of *T. ips* (Fig. 3A). The phoretic mite community on *T. dubius* ordinated opposite that of *Ips* spp. and *Dendroctonus* spp., and were highly associated with *H. arborsignis* and the collective presence of unidentified mite species. Trends in phoretic mite communities ordination indicate an interactive effect between beetle taxonomy and collection region.

Thanasimus dubius in Wisconsin and *Hylastes porculus* Ericsson in Georgia, respectively had the highest and lowest expected phoretic mite species richness^0 of any beetle species at all values of q (Fig. 4A). Expected species richness^0 approaches asymptotes for *I. pini* in Arizona and *H. porculus* in Georgia (Fig. 4A). Projections for *T. dubius* in Arizona, *I. pini* in Georgia, *D.*

frontalis in Arizona, *D. brevicomis* in Arizona and *D. valens* in Wisconsin indicate the potential for increased species richness^{0} given increased sample effort (Fig. 4A). Analysis of effective species richness indices weighted towards rare species (q = 0.5). Specifically, *T. dubius* in Wisconsin, followed by *I. pini* in Arizona, had the highest effective species richness$^{0.5}$ of any species × region combination (Fig. 4B). *Dendroctonus brevicomis* in Arizona, *I. grandicollis* in Georgia and *I. pini* in Georgia had moderate values of effective species richness$^{0.5}$, though projections for *I. pini* in Georgia indicate the potential for higher effective species richness (q = 0.5) at higher sampling efforts (Fig. 4B). *Dendroctonus frontalis* in Arizona, *H. porculus* in Georgia, *D. valens* in Wisconsin and *Ips avulsus* Eichoff in Georgia had the lowest expected species richness$^{0.5}$ values (Fig. 4B). Effective species richness$^{0.5}$ plateaued for *I. avulsus* in Georgia and *I. pini* in Arizona (Fig. 4B). Dramatic relative increases in the relative effective species richness^{2} for *I. grandicollis* in Georgia, *H. porculus* in Georgia and *I. avulsus* in Arizona were observed relative to indices less heavily weighted towards common species (i.e. species richness^{0}) (Fig. 4C). Effective species richness^{2} for *T. dubius* in Wisconsin remained highest, but projections for samples > 100 exceed effective species richness^{2} for all other species × region combinations (Fig. 4C). Data for the beetle community at effective species richness^{1} can be described as intermediate between effective species richness$^{0.5}$ and richness^{2} where trends described as emergent at values of q = 2 begin to diverge from the q = 0.5 values (Fig. 4).

Iponemus confusus occurred on *Ips* spp. more often than on any other beetle genus (Fig. 5A). *Histiogaster arborsignis* occurred in relatively low incidence on *I. pini* in Wisconsin and Arizona, *I. grandicollis* in Georgia, *I. avulsus* in Georgia, *H. porculus* in Georgia, and *D. valens* in Wisconsin, but on over 40% of *T. dubius* in Wisconsin (Fig. 4B). *Tarsonemus ips* occurred relatively frequently on many species (Fig. 5C). *Elattoma* sp. occurred on *I. pini* in Arizona and

Georgia but not in Wisconsin, and on *I. grandicollis* from Wisconsin and Georgia but not Arizona (Fig. 5D). *Elattoma* sp. occurred on approximately 20% of *D. frontalis* and *D. brevicomis* (Fig. 5D). *Histiostoma* spp. occurred on over 20% of *D. valens*, *I. pini*, and *T. dubius* in Wisconsin, and *I. grandicollis* in Georgia and Wisconsin (Fig. 5E). *Trichouropoda australis* occurred on approximately 10% of beetles *I. avulsus* in Georgia, *I. grandicollis* in Arizona and Wisconsin, *I. pini* in Arizona and *T. dubius* in Wisconsin and on 15 - 25% of *I. grandicollis* in Georgia, *I. pini* in Georgia and *I. pini* in Wisconsin (Fig. 5F). *Dendrolaelaps quadrisetus* occurred on approximately 20% of *I. grandicollis* in Arizona and Wisconsin and *I. pini* in Georgia and Wisconsin (Fig. 5G). *Proctolaelaps* sp. occurred on fewer than 10% on *D. valens* in Wisconsin, *Pachylobius picivorus* (German) in Georgia and on less than 5% on *D. frontalis* in Arizona, *I. avulsus* in Georgia and *I. pini* in Georgia and Arizona (Fig. 5H).

Models for regional phoretic mite variation within *I. pini* showed significant differences in incidence rates for *I. confusus*, *H. arborsignis*, *T. ips*, *Elattoma* sp., *Histiostoma* spp., *T. australis* and *D. quadrisetus* (Table 3). Regional phoretic mite variation models for *I. grandicollis* showed significant differences for *I. confusus*, *Elattoma* sp., *Histiostoma* spp., *T. australis*, *D. quadrisetus* (Table 3).

Paired species *T. ips*:*Elattoma* sp., *T. ips*:*T. australis*, *Elattoma* sp.:*D. quadrisetus*, *H. arborsignis*:*Paracarophaenax* sp. and *T. australis*:*D. quadrisetus* were found in positive association on *I. pini* in Arizona (Fig. 6B). *Iponemus confusus*:*T. ips*:*Elattoma* sp. and *Elattoma* sp.:*Ereynetes propescutulis* Hunter were found in positive association on *I. pini* in Georgia (Fig. 6D). *Ips pini* in Wisconsin carried fewer phoretic mite species than other *Ips* spp. (Table 2). The only strong inter-mite associations on *I. pini* in Wisconsin were *T. australis*:*D. quadrisetus* (Fig. 6F). *Histiostoma* spp.:*T. ips* and *T. australis*:*D. quadrisetus* were found in strong positive

correlations on *I. grandicollis* in Wisconsin (Fig. 6E) but not in Arizona or Georgia. *Iponeumus confusus* was found in positive association with *T. ips* and *Elattoma* sp. on *I. grandicollis* in Georgia (Fig. 6C), but not in Arizona or Wisconsin. *Ips grandicollis* in Arizona had no phoretic mite species in significant constant association with one another (Fig. 6A). *Iponemus confusus*, *T. ips* and *Elattoma* sp. showed a strong positive association on *I. avulsus* in Georgia (Fig. 6J). For all pairwise comparisons, no combination of mite species was found to be significantly negatively associated with one another (Fig. 6). *Dendroctonus brevicomis*, *H. porculus*, *P. picivorus* and *T. dubius* had no mite species that showed any degree of association with each other (Fig. 6G, I, K, L).

Discussion

We characterized the phoretic mite communities of 36 bark beetle species across three geographic regions in the US. Our study demonstrates substantial overlap in phoretic mite community membership on bark beetle species, with most phoretic mites being associated with at least three and ranging up to twelve species. Even those mite species that we observed on few host beetle species, such as *M. virginiensis* and *Schwebia* sp., have been previously found on pine-associated beetles (Pfammatter et al. 2013). This overlap is almost surely underestimated in our study, because all of the beetle species with at least five individuals yielded mites. These under-sampled beetle species would likely carry mites from a similar membership pool. The high overlap in phoretic mite-beetle associations suggests that reported ranges in numbers of mite species per beetle species may be as indicative of local community structures of subcortical beetle guilds as of individual host beetle species. For example, some host beetle species (e.g. *Ips typographus* (L.)) have been shown to carry a low number of phoretic associates (Takov et al. 2010), while others (e.g. *I. pini*) have been associated with just over 20 phoretic mite species

(Pfammatter et al. 2013). In extreme, the southern pine beetle has been found in association with over 50 mite species (Moser 1975).

Overall, phoretic associations between beetles and mites were relatively diffuse within and across beetle species and sampling regions. Even mite species such as *I. confusus*, that we would predict to have more specialist mite-carrier relationships, due to its specificity of feeding on bark beetle eggs, was found on nine beetle species. In general, we found little evidence that would indicate close pairwise relationships between specific beetle and mite species. In addition, it is difficult to ascribe regional patterns of beetle-mite pairings in order to account for the variability in these pairings. This may be due to the fact that while regional variation exits, it is likely dampened by the insulating effect of the within-tree microhabitat environment. We also considered that positive or negative relationships between pairs of co-transported mite species might influence patterns of beetle-mite phoretic relationships. That is, competition and synergy between species in both phoretic and within-tree life stages could contribute to variation among beetle species and regions. However, we found little evidence of strongly positive and almost no evidence of negative associations among phoretic mite species co-occurring during phoretic transport. This suggests that most patterns of phoretic mite-beetle pairings are driven largely by within-tree factors, reflecting the roles of these mites as specialists on degrading tree habitat. The processes that drive the within-tree success of phoretic mites are poorly understood.

Mite-bark beetles associations appear to show both differences and similarities with other well-described mite-host relationships, such as that of *Macrocheles saceri* Costa which stays in constant association with one or very few species of dung beetles (Niogret and Lumaret 2009). Other, more generalist *Macrocheles* mites associated with dung beetles are less selective of their carrier hosts, appearing in association with many beetle species -- these mites species are more

selective of habitat quality than phoretic host (Niogret and Lumaret 2009). Most mites in our system may be more aptly described as habitat specialists, requiring the environment engineered by bark beetles rather than a specific mite-carrier relationships or food source and are thus more generalist in their phoretic preferences. Niogret and Lumaret (2009) described a positive relationship between ephemeral resource longevity and phoretic mite-carrier specificity in dung beetle systems. Degrading beetle-attacked trees may provide suitable food and shelter resources for more than five years (personal observation) which may be an important contributor to the patterns of diffuse mite-carrier relationships we see in our system. The relatively high variability in patterns of mite-bark beetle relationships is consistent with strong effects of bottom-up ecological processes related to resource quality and abiotic environmental conditions. Variability in patterns that drive individual tree resource quality may contribute to the lack of strong associations at the site, beetle species, or regional levels. Results of this landscape-scale study are consistent with the variation in phoretic mite communities previously studied on *I. pini* among red pine sites across Wisconsin (Pfammatter et al. 2013).

The limited species richness of phoretic mite communities we observed likely reflects the specificity of the recently-dead pine habitat that is shared among beetle species, and the behavioral specificity of the beetle hosts is this plant genus. We might expect to encounter additional phoretic mites within a region on subcortical beetles associated with other tree species, especially those taxonomically distant from *Pinus*. For example, mite communities associated with the elm-colonizing *Scolytus multistriatus* (Marsham) are somewhat different than those obtained in our study, including species from the genera *Chelacheles* and *Pseudotarsonemoides* (Moser et al. 2005). Even across these widely separated plant genera, however, there are overlapping species such as *Elattoma* sp., *Proctolaelaps* spp. and *Trichouropoda*. Crossover of

these species might occur in mixed hardwood-conifer forests where degrading habitats on hardwoods and pines physically intermingle. Mites such as *Proctolaelaps* spp., move quite rapidly within trees while in their tree-associated stage (personal observation) and could possibly travel between trees that have fallen over one another and are thus physically connected on the forest floor. Some species such as *H. varia* are also found commonly across many beetle and habitat groups (Woodring and Moser 1970, Houck and OConnor 1991). Some between-habitat transport by this mite may occur during non-phoretic life stages via unassisted movement across the forest floor. We find *H. varia* in five percent of duff samples in healthy red pine sites, although rarely as samples containing *H. varia* only had one mite per liter of soil (Pfammatter 2015). It is possible that *H. varia* is successful at feeding and reproducing in these leaf litter habitats rather than only being found there for dispersal reasons. We found no evidence of any of the other mites phoretically associated with beetles outside of degrading tree or host beetle environments.

At least two factors contribute to limiting our understanding of phoretic mite-bark beetle systems and delineate needs for future studies. First, interactions between phoretic mites and bark beetles are most easily studied during the phoretic stage. Studies at the tree habitat scale have provided new insight into the formation of patterns of phoretic mite-bark beetle associations, but more work is necessary to aid in further elucidating of these relationships. Second, several species identifications of mites are extremely difficult due to the relatively understudied nature of this group's taxonomy. A recent study involving molecular analysis of mites phoretically associated with *Nicrophorus* burying beetles found that *Uroobovella nova* (Oudemans) actually represents at least five morphologically cryptic species (Knee et al. 2012).

Further development of molecular identification methods applicable to rapid identification of individual mites could greatly facilitate ecologically-oriented studies.

Overall, our findings suggest that highly specific paired relationships may be relatively uncommon between bark beetle and phoretic mites in pine systems. Perhaps the lack of strong microbe players that mediate and influence interactions between beetles and mites, such as the antagonist fungus *O. minus* that occurs in the southern pine beetle system, reduces selective pressure for strong associations between mites or mite-beetle pairs. Overall, reproductive success of most phoretic mites appears largely driven by conditions that favor reproduction of their host bark beetles, suggesting primarily commensal relationships. Most of those mites likely maximize reproduction and development within tree for as long as favorable breeding conditions persist (Binns 1982), and attach to any number of departing beetle species across a relatively long timescale of beetle-tree interactions. These mites appear to use relatively nonspecific mechanisms such as initiation of attachment via stimulation of leg setae (Schulze 1924). In degrading tree resources where pathways for phoretic transport are numerous in both host beetle species and time, and where resource quality is maintained for multiple years, maintaining diffuse relationships appear advantageous to phoretic mites of bark beetles.

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Table 1. Collection sites of bark beetles sampled for phoretic mites in Wisconsin, Arizona, and Georgia.

Year	Site	Region	County	GPS Coordinates	Major Tree Type
2011	Mazomanie	Wisconsin	Dane	43.210150, -89.792150	*P. resinosa*
2011[a]	near Arkdale	Wisconsin	Adams	NA	*P. resinosa*
2013	sg	Wisconsin	Sauk	43.180194, -90.155444	*P. resinosa*
2013	wr	Wisconsin	Waushara	44.259528, -89.314000	*P. resinosa*
2013	km	Wisconsin	Walworth	42.832414, -88.610179	*P. resinosa*
2013	tm	Georgia	Jackson	34.123996, -83.796720	*P. taeda*
2013	on	Georgia	Greensboro	33.738522, -83.271386	*P. taeda*
2013	os	Georgia	Jasper	33.275289, -83.738976	*P. taeda*
2013	cf	Arizona	Cocoino	35.16865, -111.77169	*P. ponderosa*
2013	bs	Arizona	Cocoino	35.26780, -111.80611	*P. ponderosa*
2013	me	Arizona	Cocoino	35.24715, -111. 63531	*P. ponderosa*

[a]*Dendroctonus valens* were excavated from recently cut *P. resinosa* stumps approximately 5 *km* north northeast or Arkdale, WI.

Table 2. Beetle and phoretic mites obtained in Wisconsin (WI), Arizona (AZ), and Georgia (GA) in 2011 and 2013. Beetles of the same species are presented independently for each region. Cells represent percent of beetles carrying phoretic mites followed (in parenthesis) by the average number of phoretic mites on beetles carrying mites.

Region	Beetle Family	Beetle Species	Total Beetles (Mites)	Total Mite Species	*Iponemus confusus* Lindquist	*Dendrolaelaps neodisetus* (Hurlbutt)	*D. quadrisetus* (Berlese)	*Ereynetes propescutulis* Hunter	*Paracarophaena x* sp.	*Tarsonemus* spp.[a]	*Elattoma* sp.	*Histiogaster arborsignis* Woodring	*Histiostoma* spp.[b]	*Histiostoma anops*	*Trichouropoda australis* Hirschmann	*Proctolaelaps* sp.	Unidentified
GA	Cerambycidae	Monochamus spp.[c]	1 (0)	0	--	--	--	--	--	--	--	--	--	--	--	--	--
WI		Monochamus scutellatus (LeConte)	2 (20)	2	0	0	0	0	0	0	0	0	0	0	0.5 (20)	0	0
Wi[d]		Rhagium inquisitor L.	1 (0)	0	--	--	--	--	--	--	--	--	--	--	--	--	--
WI		*Xylotrechus* sp.	1 (200)	5	0	0	0	1 (1)	0	0	0	1 (92)	0	0	0	0	1 (107)
GA	Cleridae	*Enoclerus ichneumoneus* (F.)	1 (0)	0	--	--	--	--	--	--	--	--	--	--	--	--	--
WI		*Enoclerus nigrifrons* (Say)	4 (1)	2	0	0	0	0	0	0	0	0.25 (1)	0	0	0	0	0
WI4		*Thanasimus dubius*[e] (F.)	21 (91)	14	0.1 (2)	0.14 (3)	0	0.05 (1)	0	0.05 (1)	0	0.57 (3.08)	0.24 (1.8)	0.05(6)	0.5 (1)		0.5 (6)
GA		*Thanasimus dubius* (F.)	1 (0)	0	--	--	--	--	--	--	--	--	--	--	--	--	--
WI		*Thanasimus dubius* (F.)	17 (131)	4	0	0	0	0	0	0	0	0.29 (21)	0.29 (2.8)	0	0.18 (4)	0	0
GA	Colydiinae	*Lasconotus* sp.	3 (0)	0	--	--	--	--	--	--	--	--	--	--	--	--	--
GA		*Cossonus* sp.	1 (0)	0	--	--	--	--	--	--	--	--	--	--	--	--	--
WI	Curculionidae	*Anthonomus* sp. Germar	1 (2)	3	1 (1)	0	0	0	0	0	0	1 (1)	0	0	0	0	0
AZ		*Dendroctonus brevicomis*[f] LeConte	137 (152)	10	0.01 (1)	0	0	0	0.04 (3.6)	0.22 (3.07)	0.05 (2.86)	0	0	0	0.03 (1.5)	0	0.01 (1)
AZ		*Dendroctonus frontalis* Zimmermann	36 (48)	6	0.06 (4.5)	0	0	0	0.03 (1)	0.19 (2)	0.17 (3.33)	0	0	0	0	0.03 (1)	0

Region	Beetle Family	Beetle Species	Total Beetles (Mites)	Total Mite Species	*Iponemus confusus* Lindquist	*Dendrolaelaps neodisetus* (Hurlbutt)	*D. quadrisetus* (Berlese)	*Ereynetes propescutulis* Hunter	*Paracarophaena x* sp.	*Tarsonemus* spp.[a]	*Elattoma* sp.	*Histiogaster arborsignis* Woodring	*Histiostoma* spp.[b]	*Histiostoma anops*	*Trichouropoda australis* Hirschmann	*Proctolaelaps* sp.	Unidentified
AZ		*Dendroctonus* sp.	1 (0)	0	--	--	--	--	--	--	--	--	--	--	--	--	--
AZ		*Dendroctonus valens* LeConte	1 (15)	2	0	0	0	0	0	0	0	0	0	1(15)	0	0	0
GA		*Dendroctonus valens* LeConte	1 (0)	0	--	--	--	--	--	--	--	--	--	--	--	--	--
Wi[c]		*Dendroctonus valens* LeConte	55 (118)	6	0.02 (2)	0	0	0	0	0	0	0.02 (1)	0.35 (4.42)	0	0.04 (3.5)	0.09 (4.4)	0
WI		*Dendroctonus valens* LeConte	1 (2)	3	0	0	1(1)	0	0	0	0	0	0	0	1 (1)	0	0
GA		*Dryocoetes autographus* (Ratzeburg)	3 (0)	0	--	--	--	--	--	--	--	--	--	--	--	--	--
Wi[c]		*Dryopthorus americanus* Bedel	5 (0)	0	--	--	--	--	--	--	--	--	--	--	--	--	--
GA		*Dryopthorus americanus* Bedel	4 (0)	0	--	--	--	--	--	--	--	--	--	--	--	--	--
WI		*Dryopthorus americanus* Bedel	2 (0)	0	--	--	--	--	--	--	--	--	--	--	--	--	--
GA		*Gnathotrichus materarius* Bedel	2 (3)	2	0	0	0.5 (2)	0	0	0	0	0	0	0	0	0	0
AZ		*Hylastes porculus* Erichson	1 (1)	2	0	0	0	0	0	0	1 (1)	0	0	0	0	0	0
GA		*Hylastes porculus* Erichson	47 (16)	6	0	0	0	0	0	0.02 (2)	0	0.04 (1)	0.04 (1)	0	0	0	0.02 (1)
WI[c]		*Hylastes porculus* Erichson	3 (0)	0	--	--	--	--	--	--	--	--	--	--	--	--	--
GA		*Hylobius* sp.	1 (0)	0	--	--	--	--	--	--	--	--	--	--	--	--	--
GA		*Ips avulsus* (Eichhoff)	239 (2886)	9	0.23 (2.57)	0.02 (1)	0	0	0	0.16 (2.03)	0.6 (17.78)	0 (1)	0.02 (4.5)	0	0.13 (1.44)	0 (1)	0

Region	Beetle Family	Beetle Species	Total Beetles (Mites)	Total Mite Species	*Iponemus confusus* Lindquist	*Dendrolaelaps neodisetus* (Hurlbutt)	*D. quadrisetus* (Berlese)	*Ereynetes propescutulis* Hunter	*Paracarophaena x* sp.	*Tarsonemus* spp.[a]	*Elattoma* sp.	*Histiogaster arborsignis* Woodring	*Histiostoma* spp.[b]	*Histiostoma anops*	*Trichouropoda australis* Hirschmann	*Proctolaelaps* sp.	Unidentified
GA		*Ips calligraphus* (Germar)	1 (36)	3	0	0	0	0	0	0	1 (35)	0	1 (1)	0	0	0	0
AZ		*Ips grandicollis* (Eichhoff)	11 (194)	5	0.45 (17.8)	0.18 (3)	0.18 (3)	0	0	0	0.55 (16)	0	0	0	0.09 (2)	0	0
GA		*Ips grandicollis* (Eichhoff)	35 (407)	7	0.29 (2.2)	0	0	0	0	0.06 (2.5)	0.26 (20.44)	0.03 (1)	0.37 (12.92)	0	0.17 (4.5)	0	0
WI		*Ips grandicollis* (Eichhoff)	10 (189)	6	0.8 (4.12)	0	0.2 (3.5)	0	0	0.1 (1)	0	0	0.5 (21.4)	0	0.1 (40)	0	0
AZ		*Ips pini* (Say)	222 (4688)	14	0.44 (14.05)	0.02 (1.75)	0.23 (3.56)	0.07 (1.75)	0.01 (1.33)	0.08 (2.44)	0.65 (19.91)	0.02 (10.8)	0.05 (1.83)	0	0.21 (2.07)	0.01 (1)	0 (1)
GA		*Ips pini* (Say)	51 (901)	11	0.33 (4.76)	0	0 (4)	0.02 0	0	0.14 (2.86)	0.76 (19.95)	0.02 (1)	0.02 (1)	0	0.08 (1)	0.02 (1)	0.04 (1)
WI		*Ips pini* (Say)	20 (203)	7	0.3 (27)	0	0.25 (3.4)	0	0	0	0	0	0.25 (3)	0	0.25 (1.2)	0	0.1 (1.5)
AZ		*Orthotomicus caelatus* (Eichhoff)	1 (0)	0	--	--	--	--	--	--	--	--	--	--	--	--	--
GA		*Pachylobius picivorus* (Germar)	15 (215)	5	0	0	0.07 (4)	0	0	0	0	0	0.07 (1)	0.2 (53.67)	0	0.07 (49)	0
GA		*Pityopthorus* sp.	2 (0)	0													
WI		*Rhyncolus* sp.	1 (0)	0													
GA		*Xylosandrus* sp.	1 (0)	0													
WI	Elateridae	*Ampedus* sp.	1 (0)	0													
WI		*Melanotus* sp.	1 (7)	2	0	0	0	0	0	0	0	0	1 (7)	0	0	0	0
WI[c]		*Platysoma cylindrical* (Paykull)	4 (7)	2	0	0	0	0	0	0	0	0	1 (1.75)	0	0	0	0
WI[c]	Tenebrionidae	*Corticeus parallelus* (Melsheimer)	5 (41)	6	0.4 (1.5)	0	0.2 (16)	0	0	0	0	0	0.8 (5)	0	0.2 (1)	0.2 (1)	0
WI		*Corticeus parallelus* (Melsheimer)	1 (1)	2	0	0	0	0	0	0	0	0	1 (1)	0	0	0	0
GA		*Hymenorus* sp.	3 (0)	0	--	--	--	--	--	--	--	--	--	--	--	--	--
WI		*Hymenorus* sp.	1 (0)	0	--	--	--	--	--	--	--	--	--	--	--	--	--

GA		*Isomira* sp.	1 (0)	0	--	--	--	--	--	--	--	--	--	--	--	--	--	--
AZ	Trogossitidae	*Temnocheila* sp.	1 (0)	0	--	--	--	--	--	--	--	--	--	--	--	--	--	--
GA	Zopheridae	*Lasconotus* sp.	1 (0)	0	--	--	--	--	--	--	--	--	--	--	--	--	--	--
GA		*Namunaria*	1 (0)	0	--	--	--	--	--	--	--	--	--	--	--	--	--	--
		Total Beetle Species Carrying	--	--	9	3	6	3	3	7	7	9	12	3	9	6	5	

[a] The majority of *Tarsonemus* spp. were *Tarsonemus ips* Lindquist, *Tarsonemus fusari* Coorman were identified in a few samples. Small structural features make conclusive differentiation difficult, so we pool them to genus here.

[b] Most of the *Histiostoma* spp. obtained were *Histiostoma varia* Woodring and Moser, but small structural features make it difficult to confirm that all are *H. varia*.

[c] *Monochamus titallator* (F.) and *Monochamus carolinensis* Oliver are indistinguishable in this region.

[d] Samples were collected in 2011. All samples not marked [c] were collected in 2013.

[e] *Thanasimus dubius* from Wisconsin in 2011 carried 0.14 (1.33) *Schwebia* sp.

[f] *Dendroctonus brevicomis* from Arizona carried 0.04 (1.2) *Mexecheles virginiensis* and 0.1 (3) *Schwebia* sp.

Table 3. Poisson model estimates, *P* values and pairwise *post-hoc* Tukey test *P* values for regional comparisons of individual beetle species phoretic mite abundance on *I. pini* and *I. grandicollis*. Mite species occurring fewer than 10 times on *I. pini* and *I. grandicollis* were removed from model consideration.

		Model Estimates (Mean Mites per Beetle)			***P* values**			
Beetle Species	**Mite Species**	**AZ**	**GA**	**WI**	**Overall**	**GA – AZ**	**WI – AZ**	**WI – GA**
Ips pini	*Iponemus confusus*	6.20	1.59	8.1	<0.001	<0.001	0.004	<0.001
	Histiogaster arborsignis	0.24	0.02	0	<0.001	0.025	> 1	> 1
	Tarsonemus ips	0.20	0.39	0	0.001	0.023	> 1	> 1
	Elattoma sp.	12.91	15.26	0	<0.001	<0.001	0.994	0.994
	Histiostoma spp.	0.10	0.02	0.75	<0.001	0.233	<0.001	0.001
	Ereynetes propescutulis	0.13	0.08	0	0.067	0.608	> 1	> 1
	Trichouropoda australis	0.43	0.08	0.3	<0.001	0.003	0.665	0.089
	Dendrolaelaps quadrisetus	0.83	0	0.85	<0.001	0.999	0.996	0.999
Ips grandicollis	*Iponemus confusus*	8.09	0.63	3.3	<0.001	<0.001	<0.001	<0.001
	Elattoma sp.	8.73	5.36	0	<0.001	<0.001	0.999	0.999
	Histiostoma spp.	0	4.8	10.7	<0.001	0.999	0.999	<0.001
	Trichouropoda australis	0.18	0.77	4.00	<0.001	0.107	<0.001	<0.001
	Dendrolaelaps quadrisetus	0.55	0	0.7	<0.001	> 1	0.88	> 1

Figure Legends

Figure 1. Rarefaction curves for phoretic mite communities on bark beetles: All samples (thick, white), Wisconsin (thin, black), Arizona (thick, black), and Georgia (thin, grey) in 2013. Solid lines represent data from actual samples; dotted lines predict the Hill-rarefaction curves to twice the original sample size. Shaded bands around each curve represent 95% confidence intervals.

Figure 2. Network representation of the average proportion of phoretic mite species on individuals of various beetle species that carried at least one species of phoretic mite. A darker color indicates a higher average proportion of association. Beetles species were pooled between regions and years. Unidentified mites were removed from this representation.

Figure 3. Nonmetric Multidimensional Scaling (NMS) visualization of similarities among phoretic mite communities on subcortical beetle species captured in Wisconsin (squares), Arizona (circles), and Georgia (triangles) in 2013. A) 2D (NMS Stress = 0.188, r^2 = 0.819) and B) 3D (NMS Stress = 0.094, r^2 = 0.927). Data were aggregated by beetle species collected at each region, standardized with the Wisconsin double transformation, resembled to a Bray-Curtis dissimilarity matrix, and ordinated with NMS. Ordinated data are labeled by collection region and beetle species. Phoretic mite communities from different beetle species (ANOSIM R = 0.643, P = 0.01) and collection regions (ANOSIM R = 0.191, P = 0.053) varies significantly. Unidentified beetles, beetles with less than 10 representative individuals and beetles species not co-occurring with at least one phoretic mite species were removed from the ordination. Significant ($P < 0.05$) mite species correlations are overlaid on the 2D NMS.

Figure 4. Rarefied curves for Hill numbers for phoretic mite communities of *D. brevicomis*, *D. frontalis*, and *I. pini* in Arizona; and *H. porculus*, *I. avulsus*, *I. grandicollis*, and *I. pini* in Georgia A) q = 0, B) q = 0.5, C) q = 1, and D) q = 2. Beetles not represented by at least 35 specimens

were removed from rarefied Hill indices. Solid lines represent data from actual samples; dotted lines predict the hill-rarefaction curves to twice the original sample size. Shaded bands around each curve represent 95% confidence intervals.

Figure 5. Proportions of bark beetles *T. dubius* (WI), *P. picivorus* (GA), *I. pini* (WI, AZ, GA), *I. grandicollis* (WI, AZ, GA), *I. avulsus* (GA), *H. porculus* (GA), *D. valens* (WI), *D. frontalis* (AZ) and *D. brevicomis* (AZ)) carrying phoretic mites, and mean number of mites on mite-carrying beetles A) *I. confusus*, B) *H. arborsignis*, C) *T. ips*, D) *Elattoma* sp., E) *Histiostoma* spp., F) *T. australis*, G) *D. quadrisetus*, and H) *Proctolaelaps* sp. Beetle species represented by fewer than 10 samples and mite species found fewer than 40 times are not presented.

Figure 6. Colorometric visualizations of Pearson correlations for paired mite species phoretic on A) *I. grandicollis* -- AZ, B) *I. pini* -- AZ, C) *I. grandicollis* -- GA, D) *I. pini* -- GA, E) *I. grandicollis* -- WI, F) *I. pini* -- WI, G) *D. brevicomis* -- AZ, H) *D. frontalis* -- AZ, I) *H. porculus* -- GA, J) *I. avulsus* -- GA, K) *P. picivorus* -- GA, and L) *T. dubius* -- WI. Correlations closer to 1 or negative 1 are indicated by the darker saturation of blue (positive) or red (negative). Negative associations are also indicated by a negative sign in the upper right corner of each negatively correlated matrix cell. Insignificant correlations are marked with an X.

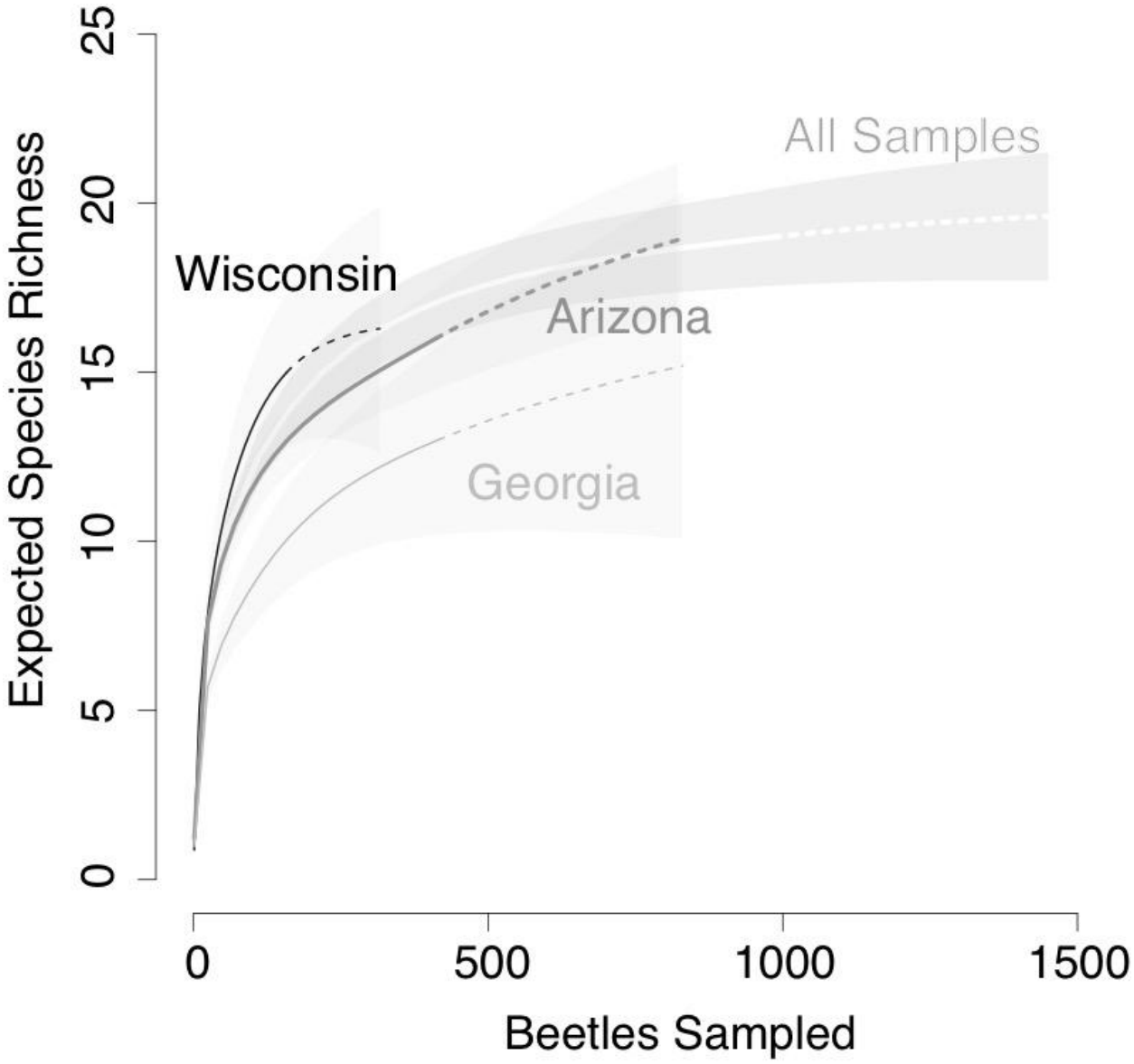
Expected Species Richness
0
5
10
15
20
25
All Samples
Wisconsin
Arizona
Georgia
0
500
1000
1500
Beetles Sampled

Figure 1.

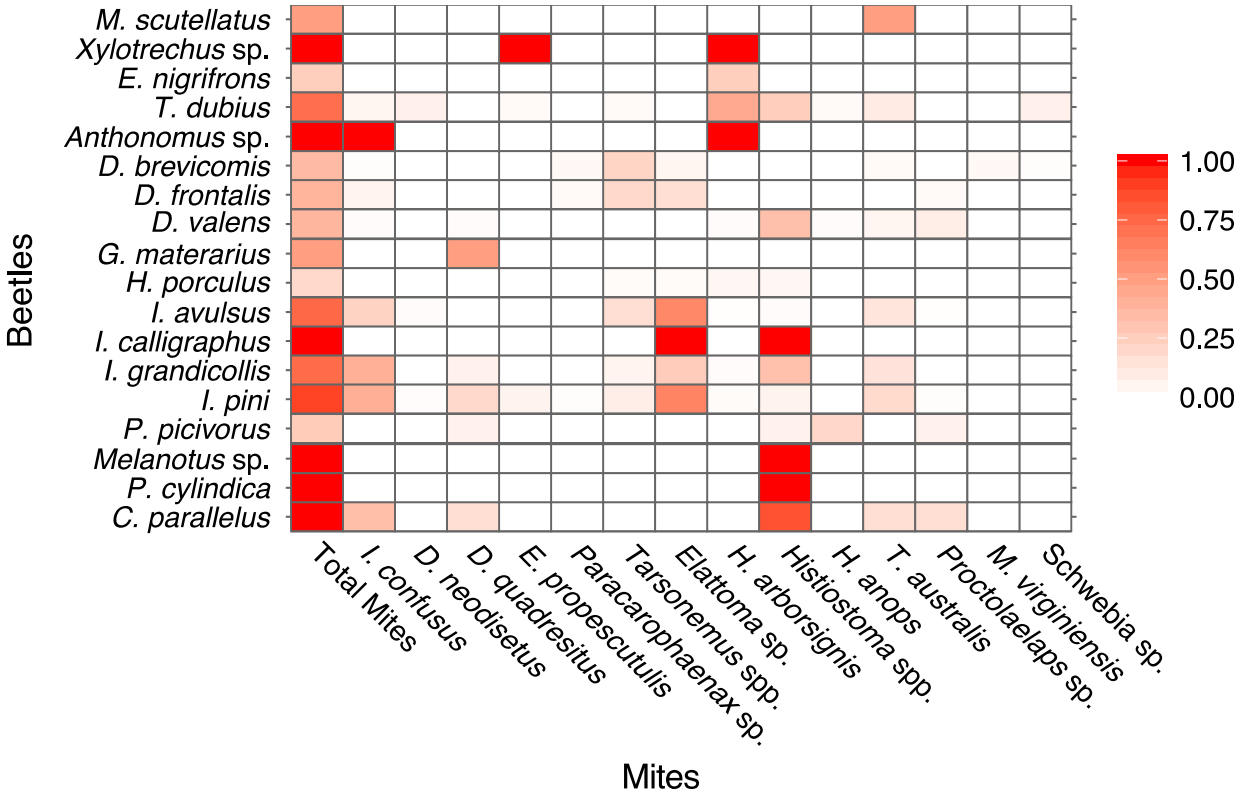
Beetles
M. scutellatus
Xylotrechus sp.
E. nigrifrons
T. dubius
Anthonomus sp.
D. brevicomis
D. frontalis
D. valens
G. materarius
H. porculus
I. avulsus
I. calligraphus
I. grandicollis
I. pini
P. picivorus
Melanotus sp.
P. cylindica
C. parallelus
Total Mites
I. confusus
D. neodisetus
D. quadresitus
E. propescutulis
Paracarophaenax sp.
Tarsonemus spp.
Elattoma sp.
H. arborsignis
Histiostoma spp.
H. anops
T. australis
Proctolaelaps sp.
M. virginiensis
Schwebia sp.
Mites
1.00
0.75
0.50
0.25
0.00

Figure 2.

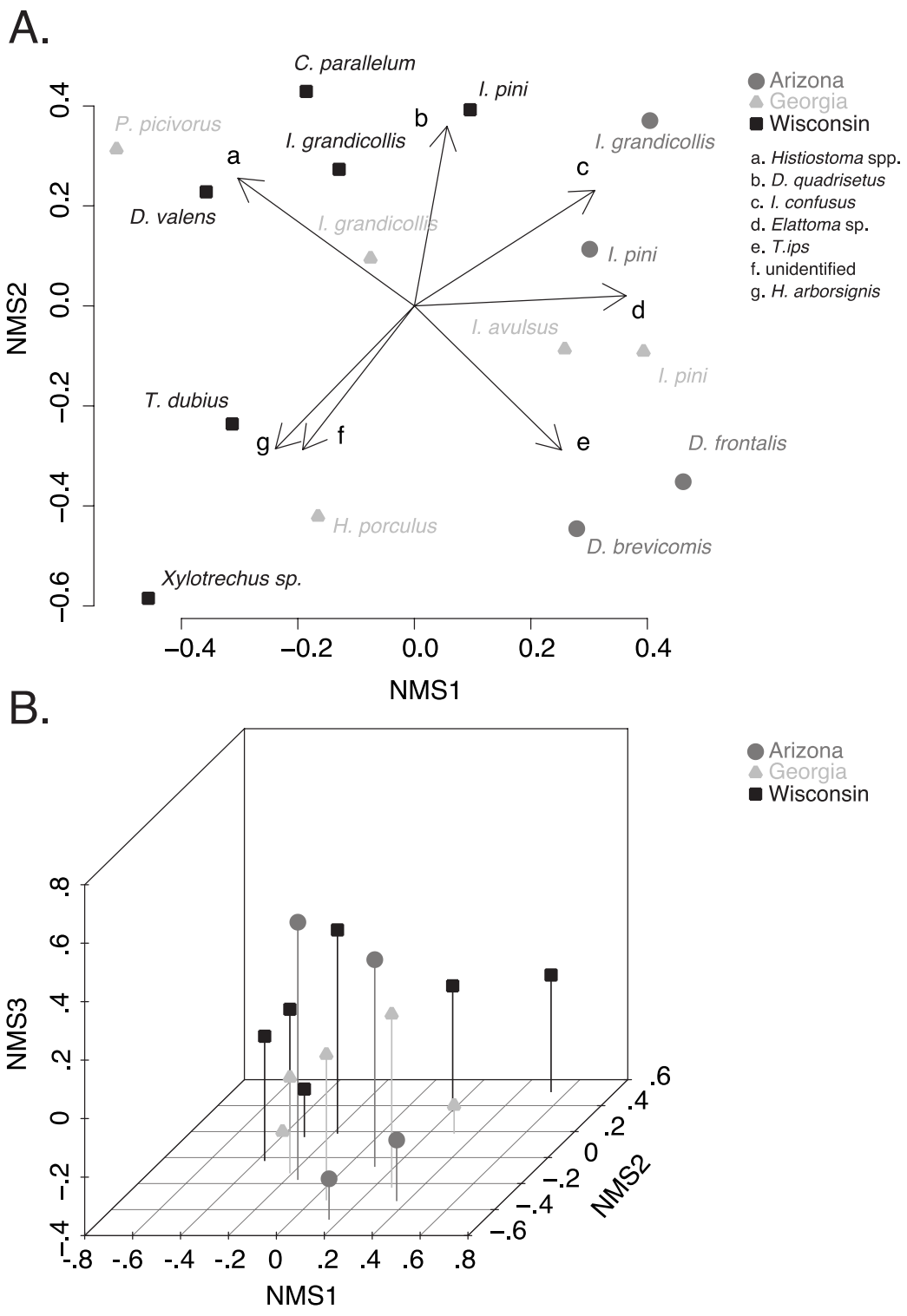
A.
C. parallelum
I. pini
P. picivorus
b
I. grandicollis
I. grandicollis
a
c
D. valens
I. grandicollis
I. pini
d
I. avulsus
I. pini
T. dubius
g
f
e
D. frontalis
H. porculus
D. brevicomis
Xylotrechus sp.
NMS2
NMS1
Arizona
Georgia
Wisconsin
a. Histiostoma spp.
b. D. quadrisetus
c. I. confusus
d. Elattoma sp.
e. T.ips
f. unidentified
g. H. arborsignis
B.
NMS3
NMS1
NMS2
Arizona
Georgia
Wisconsin

Figure 3.

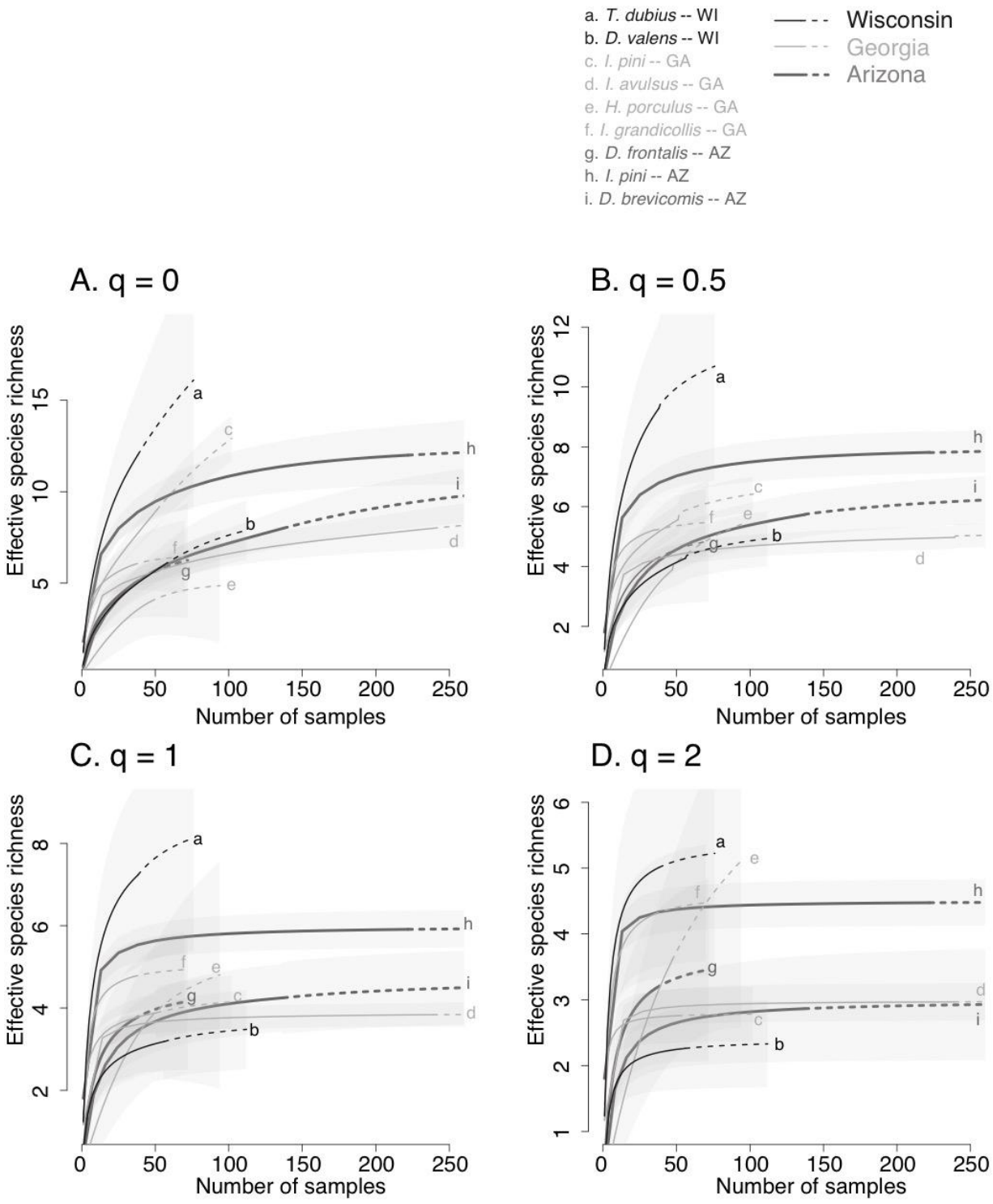
a. T. dubius -- WI
b. D. valens -- WI
c. I. pini -- GA
d. I. avulsus -- GA
e. H. porculus -- GA
f. I. grandicollis -- GA
g. D. frontalis -- AZ
h. I. pini -- AZ
i. D. brevicomis -- AZ
Wisconsin
Georgia
Arizona
A. q = 0
B. q = 0.5
C. q = 1
D. q = 2
Effective species richness
Number of samples

Figure 4.

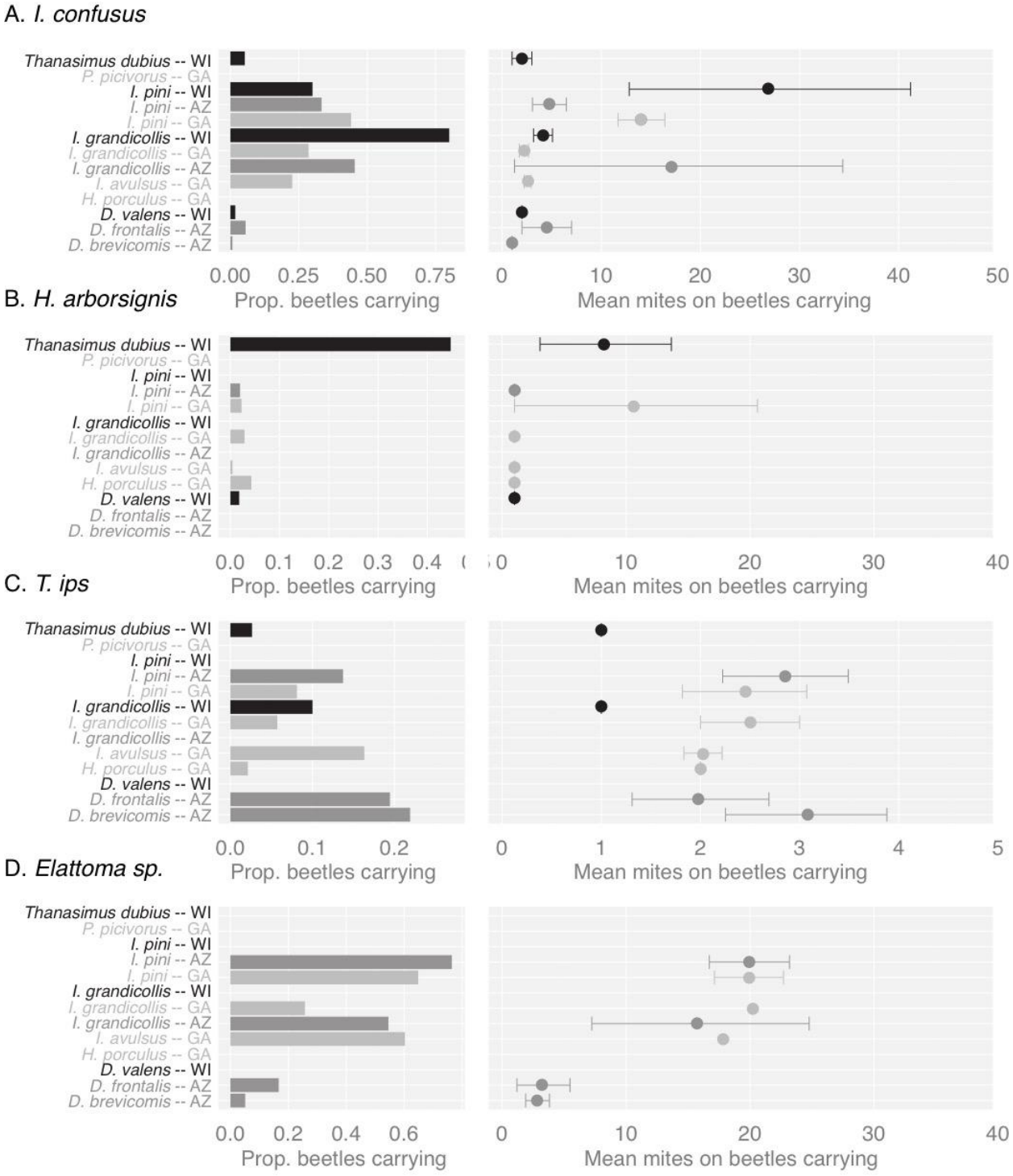
A. I. confusus
Thanasimus dubius -- WI
P. picivorus -- GA
I. pini -- WI
I. pini -- AZ
I. pini -- GA
I. grandicollis -- WI
I. grandicollis -- GA
I. grandicollis -- AZ
I. avulsus -- GA
H. porculus -- GA
D. valens -- WI
D. frontalis -- AZ
D. brevicomis -- AZ
0.00 0.25 0.50 0.75
Prop. beetles carrying
0 10 20 30 40 50
Mean mites on beetles carrying
B. H. arborsignis
0.0 0.1 0.2 0.3 0.4
Prop. beetles carrying
0 10 20 30 40
Mean mites on beetles carrying
C. T. ips
0.0 0.1 0.2
Prop. beetles carrying
0 1 2 3 4 5
Mean mites on beetles carrying
D. Elattoma sp.
0.0 0.2 0.4 0.6
Prop. beetles carrying
0 10 20 30 40
Mean mites on beetles carrying

Figure 5.

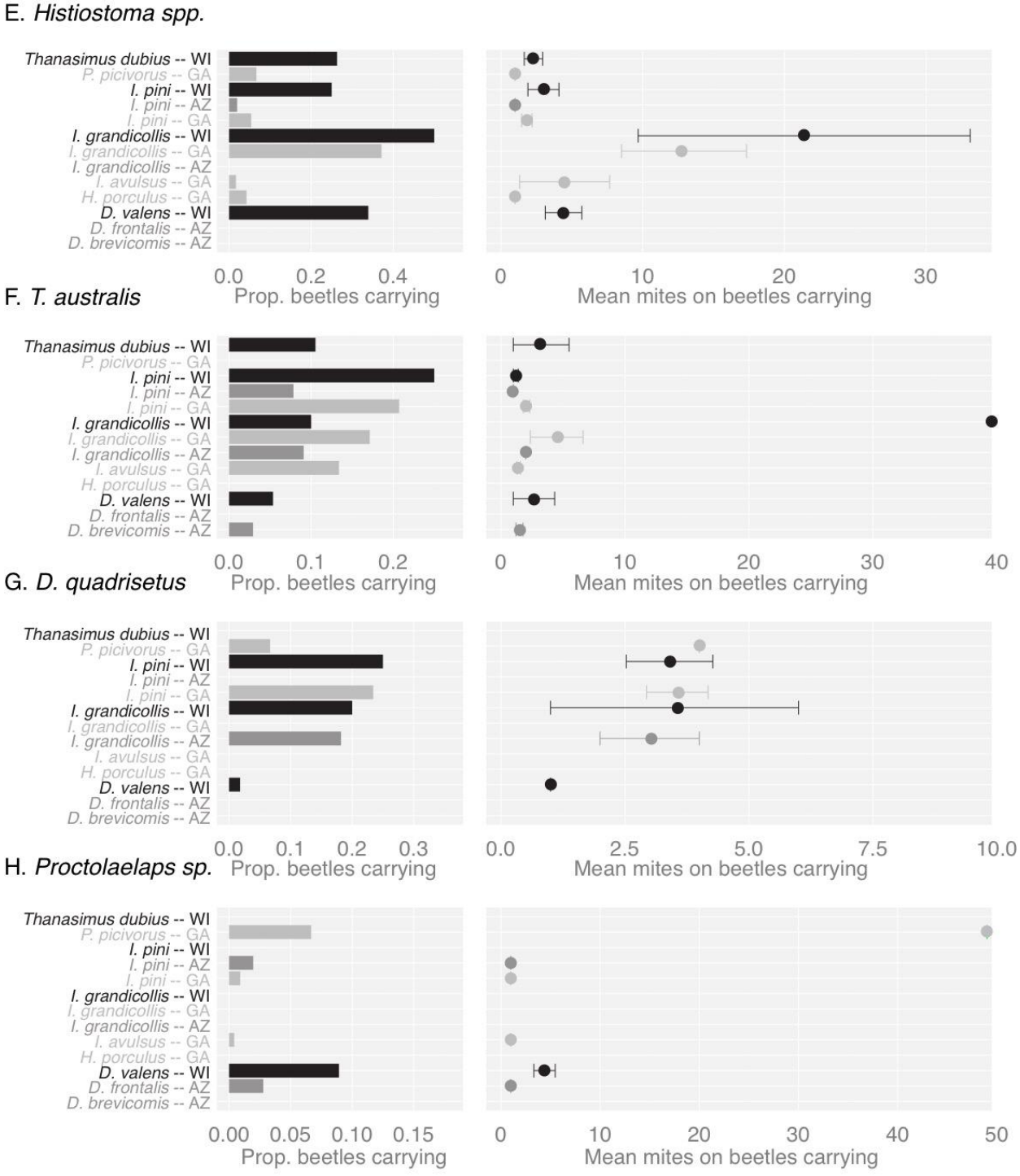

Figure 5. (cont.)

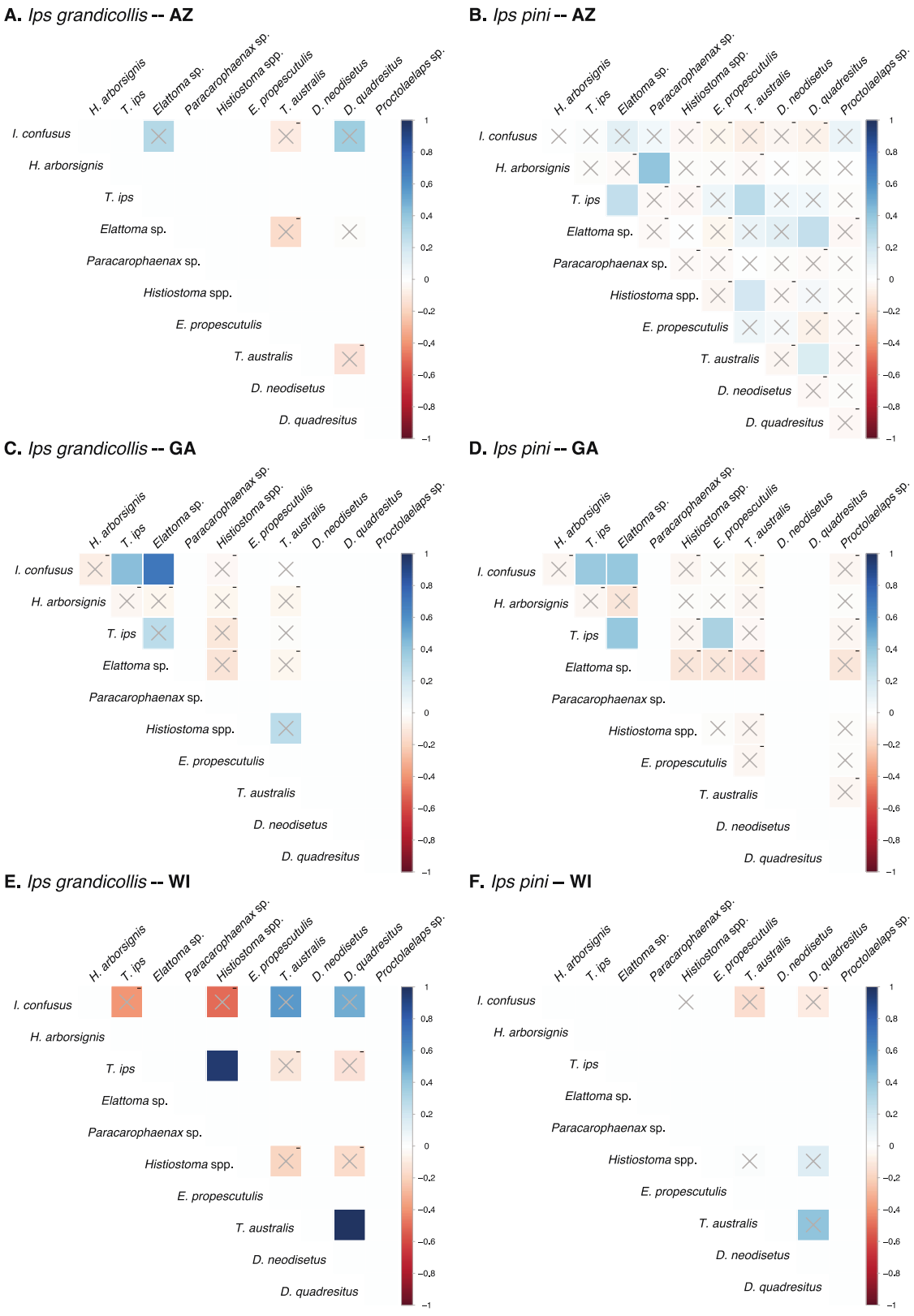
A. Ips grandicollis -- AZ
B. Ips pini -- AZ
C. Ips grandicollis -- GA
D. Ips pini -- GA
E. Ips grandicollis -- WI
F. Ips pini -- WI
H. arborsignis
T. ips
Elattoma sp.
Paracarophaenax sp.
Histiostoma spp.
E. propescutulis
T. australis
D. neodisetus
D. quadresitus
Proctolaelaps sp.
I. confusus
1
0.8
0.6
0.4
0.2
0
−0.2
−0.4
−0.6
−0.8
−1

Figure 6.

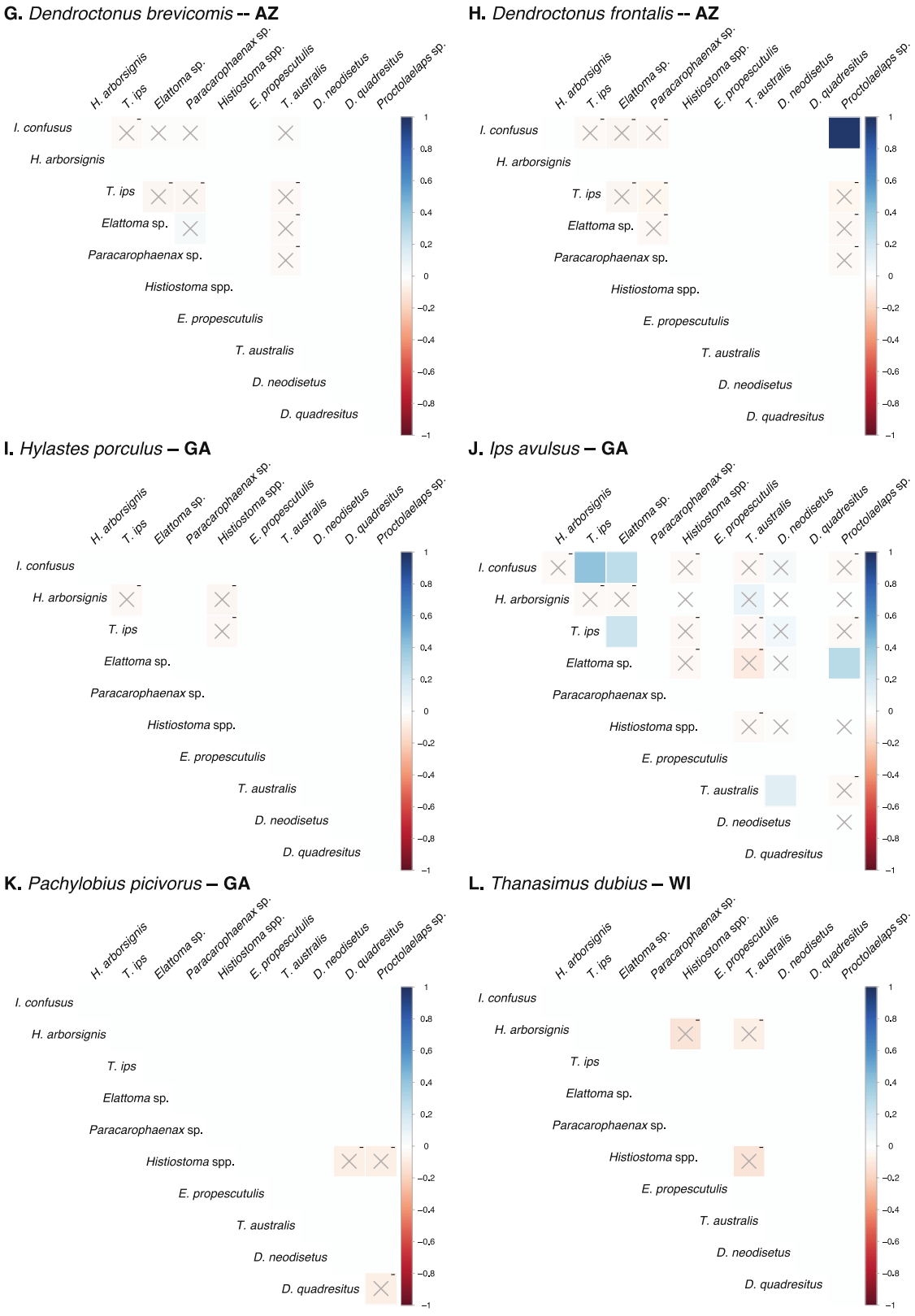

Figure 6. (cont.)

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Chapter 3: Phoretic mite influences on the reproductive success of *Ips grandicollis* (Coleoptera: Curculionidae)

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Keywords: multi-trophic interactions, bark beetles, phoresy, *Iponemus*, *Pinus resinosa*

Abstract

Ips grandicollis (Eichhoff) can be an important pest of plantation trees in the Great Lakes region. Mites are commonly found in phoretic association with this bark beetle, but little is known about their effects on beetle population dynamics. We assessed the effects of phoretic mites on the reproductive success of *I. grandicollis* using complementary correlative and manipulative approaches. First, we allowed beetles to colonize recently felled *Pinus resinosa* (Ait) logs from sites across Wisconsin and reared them in a common laboratory environment. We related the species identities and abundances of mites with beetle production from each log. We found a positive relationship between *I. grandicollis* abundance and the presence of four mite species, *Histiostoma* spp., *Dendrolaelaps quadrisetus* (Berlese), *Iponemus confusus* (Lindquist), *Trichouropoda australis* Hirschmann, and *Tarsonemus* spp. While the abundance of individual mite species was positively correlated with beetle abundance, mite communities, as assessed by measures of species richness and evenness, did not explain beetle reproduction. Next, we introduced beetles that either had a natural complement of mites or whose mites had been mechanically removed into *P. resinosa* logs and assessed whether mites affected beetle reproductive success. We found no difference in colonization rates or total beetle emergence between mite-present and mite-reduced treatments. Collectively, these results suggest a correlative, rather than causal, link between beetle reproductive success and mite incidence and abundances. That is, mites and beetles likely benefit from a commonly suitable environment rather than exerting strong interactions on each other. Thus, although mites may have some effects on *I. grandicollis* reproductive success, they likely play a minimal role in their overall population dynamics in comparison to factors such as tree quality, inter- and intraspecific beetle predation, and weather.

Introduction

Bark beetles play important roles in the functioning of forest ecosystems (Amman 1977, Romme et al. 1986), and cause serious economic and management challenges during outbreaks (Aukema et al. 2006, Safranyik et al. 2007). These subcortical herbivores are becoming increasingly important due to climate change (Waring et al. 2009, Creeden et al. 2014), human transport and land-use change (Kurz et al. 2008, Raffa et al. 2008). The population dynamics of bark beetles can be influenced by multiple interacting abiotic and biotic factors, including temperature, which can directly affect beetle development rates and winter survival (Bentz et al. 1991) physiological stresses in hosts such as drought and defoliation that reduce tree defense (Wallin et al. 2003), natural enemies that can exert density-dependent feedback (Turchin et al. 1999, Erbilgin and Raffa 2002), and symbionts that can either enhance or diminish brood production (Klepzig et al. 2009). Symbionts associated with bark beetles include fungi, bacteria, nematodes, and mites.

Phoretic mites are commonly associated with bark beetles, and have a diverse array of feeding mechanisms and life histories by which they can potentially influence their hosts. Some mite species may directly impact beetle reproductive success. For example, *Iponemus confusus* (Lindquist) parasitizes beetle eggs (Lindquist 1969), and *Resinosa* spp. cause mortality to *Dendroctonus pseudotsugae* Hopkins eggs and early instar larvae (Berryman 1968). Other mite species may indirectly affect beetle reproductive success. For example, *Dendrolaelaps quadrisetus* (Berlese) are nematophagous (Kinn 1984) and may reduce densities of nematodes that parasitize beetles. *Tarsonemus* mites can exert indirect negative effects on *Dendroctonus frontalis* Zimmermann success via mediation of the antagonistic fungus, *Ophiostoma minus* (Hedgecock) (Lombardero et al. 2003). Heavy phoretic mite loads, specifically on small beetle

species such as hydrophilids, may also negatively influence beetle reproductive success during their emergence from trees (Bajerlein and Przewoźny 2012).

While individual mite species have the capability to impact beetle reproductive success, their importance in influencing beetle populations is largely unknown. Attempts to utilize mites in biological control of bark beetles have been largely unsuccessful (Moser 1975). In addition, mites do not typically occur on bark beetles as individual species, but rather as communities within which mites interact. More information is needed to understand the role of phoretic mite communities on beetle reproductive success. Surveys of mites on individual bark beetle species have recently become more frequent, and include *Scolytus multistriatus* (Marsham) (Moser et al. 2005), *D. pseudotsugae* (Pernek et al. 2007), *Dendroctonus rufipennis* Kirby (Cardoza et al. 2008), *D. frontalis* (Moser and Roton 1971, Moser et al. 1974), and *Ips pini* (Say) (Pfammatter et al. 2013). Pfammatter (2015) recently surveyed the phoretic mites on the subcortical community of beetles associated with pine forests in Wisconsin, Arizona and Georgia, including the bark beetles *Dendrocotnus valens* LeConte, *Ips grandicollis*, (Eichhoff) and *I. pini*, and their predator *Thanasimus dubius* (F.) (Cleridae).

The purpose of this research is to assess the effects of phoretic mites on the reproductive success of *I. grandicollis*. *Ips grandicollis* is common throughout the United States where, similar to *I. pini*, it colonizes trees that are weakened or dying due to stressors such as disease and drought (Person 1931). *Ips grandicollis* can become problematic in its native range following stresses such as defoliation (Wallin and Raffa 2001) or when introduced to other, non-native, regions such as Australia (Morgan 2013). Males select a suitable host tree, excavate a nuptial chamber, and produce pheromones (Vité and Renwick 1971) that attract up to six mates (Thomas 1961). Females oviposit along galleries they construct away from the nuptial chamber,

the larvae dig tunnels away from the ovipositional gallery and consume phloem and fungi, pupae complete development in terminal chambers, and the adults emerge through the bark and fly to near-by trees (Thomas 1961). Development time varies with temperature, with the full reproductive cycle taking approximately 33 days in the laboratory (Aukema and Raffa 2002).

Mites can be found in close proximity to or interacting with all developmental stages of *I. grandicollis* except pupae. Mites, such as the beetle egg parasitizing *I. confusus* which is commonly found in association with *Ips* in the Great Lakes region (Pfammatter 2015), may have the potential to influence beetle reproductive success during these associations. Also, in a study of the phoretic mites on *I. pini*, the three most common mite associates, the scavenger/fungivore *Histiostoma* spp., the egg predator *I. confusus* and the nematode predator *D. quadrisetus*, all have life histories with the potential to influence beetle reproductive success either directly or individually (Pfammatter et al. 2013).

We assessed the effects of phoretic mite communities on the reproductive success of *I. grandicollis*. We employed complementary correlative and manipulative approaches to assess the impacts of phoretic mite communities and individual species on beetles in natural field populations and laboratory assays, respectively.

Materials and Methods

In 2009, we felled 24 *Pinus resinosa* Ait trees from eight sites in Wisconsin, allowed them to be naturally infested with *I. grandicollis*, reared them in a common facility, and related trends between beetle and mite populations. In 2013, we manipulated phoretic mite populations on *I. grandicollis* developing in *P. resinosa* logs. Pairs of *I. grandicollis* with either a full complement of phoretic mites or with phoretic mites removed, were mated and allowed to reproduce. We compared the reproductive success of emerging brood adults between mite-

present and mite-removed (because 100% removal proved impossible, we hereafter refer to this treatment as mite-reduced) treatments. We also related mite incidence and abundance of both colonizing adults and emerging brood beetles within the mite-present treatment. The diagram in Figure 1 illustrates the flow of our experiments.

Does reproductive success of field-collected beetles vary with phoretic mite abundance?

At each of eight *P. resinosa* stands (Table 1), three healthy, mature trees were felled, cut into 1 *m* bolts, stacked, and baited with 40 *mg* $50^{+}/50^{-}$ racemic ipsdienol and 4 *mg* lanierone bubble cap lures (Contech, BC, Canada). Trees were felled between 17 May and 3 Jun 2009, and left in the field for approximately 14 days until colonization by *Ips* was indicated by boring holes. Bolts were taken to the laboratory, sectioned into 30 *cm* logs and placed in approximately 25 *cm* diameter × 45 *cm* tall, 19 *L* metal rearing chambers in which beetles were allowed to develop and emerge naturally. A description of the chambers and bark beetle rearing techniques are detailed in Aukema and Raffa (2004). Beetles emerged in collection cups attached to the rearing chambers and were individually stored in ethanol. This storage processes instantly kills the beetles and associated mites, allowing for analysis of mites on a per beetle basis and preventing mite departure from dying beetles (Pfammatter et al. 2013). Although the pheromones we deployed were intended to target *I. pini*, logs were primarily colonized by *I. grandicollis*, so we focused on the latter species.

After beetle emergence ceased, we destructively sampled logs and tabulated nuptial chambers, pupal chambers, fungal coverage and *Monochamous* (Coleoptera: Cerambycidae) activity. Fungal coverage was measured by visual estimates (Bleiker and Six 2007) of the percent of phloem stained with a blue-black hue, and is hereafter referred to as the percent blue-

stain fungal coverage. This estimate is intended to assess growth of the fungus *Ophiostoma ips* (Rumbold). We also tabulated *Monochamous* beetle presence or absence in each log.

Mites from each beetle were removed using a size one insect pin affixed to a Pasteur pipette and then mounted on a single 75 × 25 *mm* glass microscope slide (Corning Glass Works, Corning, NY). Mites were mounted with clearing fluid (\#6373A, Bioquip, Rancho Dominguez, CA), secured with a glass coverslip, and placed in a drying oven at 80 ^{o}C for a minimum of 24 *h* to allow for lipid digestion. Digested mite specimens were counted and identified using reference samples identified by John Moser.

All analyses and visualizations were performed using R statistical software v3.0.2 (R Core Team 2014). Phoretic mite communities on *I. grandicollis* were visualized using two- and three-dimensional Nonmetric Multidimensional scaling (2D, 3D NMS) (function: nmds, package: ecodist, 300 runs, random start configuration) (Shepard 1962, Kruskal 1964) and labeled by capture region and beetle species. Analysis of Similarities (ANOSIM) (function: anosim, package: vegan, 999 permutations) was used to test for significance of separation of mite communities on beetles from different collection sites. Significant ($P < 0.05$) mite species correlation vectors (Jongman et al. 1995) (function: vf, package: ecodist) were overlaid on the 2D NMS visualization to provide associations between visual patterns of mite communities and individual mite species abundances. All ordination (NMS, ANOSIM and vector correlations) analyses were performed on additively aggregated (function: aggregate, constraints: site and unique tree × log), square root transformed, Wisconsin double-standardized (function: wisconsin) (Bray and Curtis 1957) phoretic mite community abundance data resembled to a Bray-Curtis dissimilarity matrix (function: distance, package: ecodist). Aggregated samples

without at least three phoretic mite species and mite species not represented by at least three samples were removed from the data matrix prior to dissimilarity resemblance.

We analyzed individual phoretic mite species in relation to beetle reproductive success (total beetles emerged per log) with a two-step approach. First, for each mite species independently and in sum, we categorically assessed beetle reproductive output between logs with mite-carrying vs. mite-absent beetles. We utilized t-tests (function: t.test) to compare means between mite-present and absent groupings. Next, in a continuous assessment of logs with beetles carrying at least one mite, the mean number of mites were regressed against the total beetles emerged per log. We used linear mixed models with random effects for site and log to relate mean phoretic mite abundance with beetle emergence. We also explored utilization of nuptial and pupal chamber counts, percent fungal coverage, and presence or absence of *Monochamous,* in all analyses but did not describe them in our results as none contributed to explanation of the experimental variance.

How do mites affect beetle reproductive success in manipulative laboratory experiments?

Ips grandicollis were captured from baited and naturally infested *P. resinosa* logs. Logs were infested at the Mazomanie, WI site in Table 1, brought to the laboratory, and placed in rearing cans from which brood beetles later emerged. Live, emerging beetles were stored in size zero gel capsules (Nutraceutical Corporation, Park City, UT) and refrigerated at 4 ^{o}C for no more than 48 hours prior to processing. *Ips grandicollis* were sexed, and mites were tabulated and identified before beetles were returned to their gel capsules. Male and female beetles were randomly assigned to either mite-reduced or un-manipulated, mite-present treatments. While we were able to remove mites on the surface of beetles, we were unable to remove those attached beneath elytra without killing beetles. Thus, some mite-reduced beetles still carried a small

number of phoretic mites. All instances of the words 'mite-reduced' indicate the potential for low numbers of phoretic mites while we use the phrases 'mite-absent' or 'mite-free' to indicate truly mite-free situations. We summarized the mite community of mite-present and mite-reduced treatment on both adult and brood beetles in Table 2. Values presented in Table 2 for colonizing beetles in the mite-reduced treatment represent the average number of mites removed during the experiment and are provided as an assessment of initial mite load prior to mite mechanical removal.

Two male-female pairs from within a treatment were introduced 180^{o} apart from one another on each fresh 30 × 10-12 *cm P. resinosa* log. Logs were then placed in rearing chambers at approximately 24 ^{o}C and ambient humidity, and beetles were allowed to develop naturally. After three weeks, we began monitoring chambers every other day to check for beetle emergence. Emerging brood beetles were collected into individual size 0 gel capsules and immediately frozen. Brood beetles were assessed for mite abundance and composition using the same methods as described in the previous section. After approximately six weeks of beetle emergence, logs were peeled of their bark and all remaining beetles were removed from the phloem, frozen and likewise examined for phoretic mites. We photographed phloem for later checks of bark beetle reproductive metrics and confirmation of tunneling and nuptial gallery success.

We assessed the frequency of *I. grandicollis* tunneling success between mite-present and mite-reduced treatments with a χ^2 goodness-of-fit test (function: chisq.test). We compared beetle initial tunneling success among the *post-hoc* categories of both, one or neither pair of colonizing beetles succeeding at laying at least one egg using ANOVA. We compared the average number of brood beetles that emerged between mite-present and mite-reduced groupings using t-tests.

We assessed the relationship between mite incidence and abundance on colonizing and brood beetles and beetle reproductive success, using a similar approach to that previously described for the correlative experiment, i.e. a two step, categorical and continuous assessment.

Results

Does reproductive success of field-collected beetles vary with phoretic mite abundance?

A total of 2,530 beetles, mainly *I. grandicollis*, emerged from logs in all collection sites. Logs from each site produced an average of 316.3 beetles per site, with emergence ranging from 3 to 755 (Table 3). One site, Dairymens, was removed from further analyses due to low beetle capture rates. Logs from all remaining sites had beetles emerge that carried at least one phoretic mite, with these beetles carrying an average of 19.9 mites. In total, we found nine mite species or species groups, including *D. quadrisetus*, *Dendrolaelaps neodisetus* (Hurlbutt), *Ereynetes prospecutulis* Hunter, *Histiostoma* spp., *Histiogaster arborsignis* Woodring, *I. confusus*, *Tarsonemus* spp., *Trichouropoda australis* Hirschmann, and *Elattoma* sp. The majority of *Tarsonemus* spp. were *Tarsonmeus ips* Lindquist and the remaining were *Tarsonemus fusari* Coorman. Most of the *Histiostoma* spp. obtained were *Histiostoma varia* Woodring and Moser. Small structural features on *Tarsonemus* and *Histiostoma* mites make conclusive differentiation to species difficult, so we pooled these to genus. Beetles from each site were found to have at least five of the nine total mite species. Beetles from Plum Creek (PC) carried the greatest number of phoretic mites, while beetles from Wild Rose (WR) and Boulder Junction (BJ) carried the fewest, averaging less than three mites per beetle (Table 3). *Histiostoma* mites were found more commonly than any other mite species, averaging 6.0 mites per beetle. We found less than five each of *Proctolaelaps* sp., *Paracarophaenax* sp., *Schwebia* sp., and *Mexecheles virginiensis*

as well as two unidentified species of mites and therefore did not included these mites in any statistical analyses.

The relative abundance of individual mite species varied among collection sites (Table 3, Fig. 1) (ANOSIM $R = 0.323$, $P = 0.012$). *Dendrolaelaps quadrisetus* occurred most commonly on beetles from PC, frequently on beetles from MZ, and in low abundances or not at all on beetles from the remaining sites (Table 3). *Dendrolaelaps neodisetus* occurred in low abundances on beetles from MZ, Spring Green (SG) and PC, but did not occur on beetles from other sites. *Ereynetes prospecutulis*, *Histiostoma* spp., *I. confusus* and *Tarsonemus* spp. occurred on beetles from seven of eight sites (Table 3). Of these four species, *E. prospecutulis* occurred in relatively consistent abundance among sites. *Histiogaster arborsignis* occurred in consistently low abundances on beetles from five sample locations and were absent from the other three sites (Table 3). *Elattoma* sp. only occurred on beetles from three sites of which they were most abundant on beetles from PC (Table 3).

The phoretic mite community, viewed as a whole, was most consistent in species membership and evenness within and between beetles from MZ and PC as compared to other sites (Fig. 1). Correlations between individual mite species abundances and phoretic mite community dissimilarity between sites exist for *I. confusus*, *D. quadrisetus*, *E. prospecutulis*, *T. australis*, and *H. arborsignis*. However, interpreting biological meaning from site-level patterns indicated from the correlations overlaid on 2D NMS is difficult. Three-dimensional NMS visualization depicts phoretic mite community clustering by site more effectively than a two-dimensional depiction (Fig. 1), and species correlations overlaid on 3D NMS might be more useful than on 2D visualizations. However, due to software limitations we were unable to superimpose vector correlations on the 3D NMS. On whole, we did not observe any relationship

between beetle productivity and the phoretic mite community, as estimated by Bray-Curtis dissimilarity (Fig. 1).

Logs from which emerging *I. grandicollis* brood adults carried phoretic mites were, on average, 6× more productive than logs from which emerging beetles were mite-free (Fig. 2A). The presence of the mite species *Histiostoma* spp., *D. quadrisetus*, *I. confusus*, *T. australis*, and *Tarsonemus* spp. mites on brood *I. grandicollis* were positively associated with beetle emergence (Fig. 2B, Table 4). Logs from which emerging brood beetles carried *Elattoma* sp. tended to have a 2× increase in total beetle emergence as compared to those without mites, although this relationship was not statistically significant (Fig. 2) (Table 4). The presence of *H. arborsignis* had no effect on the number of beetles emerged per log (Fig. 2). In logs from which emerging brood *I. grandicollis* carried *Histiostoma* spp., an increase of one *Histiostoma* spp. per log was associated with the production of approximately seven more beetles (Fig. 2B) (Table 4). On beetles that carried *Elattoma* sp. and *H. arborsignis*, an increase in the average number of phoretic mites on brood beetles emerging from logs was negatively correlated with overall beetles emergence (Fig. 2E, F) (Table 4). The abundance of all other mite species was not related to beetle emergence (Table 4). Blue-stain fungi phloem coverage and *Monochamous* presence were not associated with beetle reproductive success.

How do mites affect beetle reproductive success in manipulative laboratory experiments?

Beetles emerged at a relatively constant rate from 11 Aug to 4 Sep 2014, with the highest beetle emergence occurring prior to 25 Aug. We found no difference in the rate of beetle emergence between mite-present and mite-reduced treatments over the course of the experiment (Fig. 3). Brood *I. grandicollis* emerging from logs in the mite-present treatment carried 1.3 mites per beetle as compared to 9.0 mites per beetle on the colonizing beetles. Brood *I. grandicollis*

emerging from logs in the mite-reduced treatment carried an average of 0.6 mites per beetle as compared to 6.2 mites per beetle on colonizing beetles in the same treatment category. Abundance of almost all mite species was significantly lower on brood beetles as compared to colonizing individuals regardless of treatment category (Table 2).

We observed no difference in the initial tunneling success between mite-present, 36 of 50 pairs, and mite-reduced, 31 of 44 pairs, treatments ($\chi^2 = 0$, df = 1, *P* value = 1). We found no relationship between the average abundance of phoretic mites per log, for any mite species, when comparing the *post-hoc* beetle tunneling success categories of one, both or no pairs of beetles successfully colonizing logs (Table 5).

We found an average of just under 20 *I. grandicollis* per log emerging from both mite-present and mite-reduced treatments (*T* = 0.590, df = 46.56, *P* value = 0.558) (Fig. 4). We found no relationship between the presence or absence of any mite species on colonizing and brood beetle emergence (Fig. 5, Table 6). Likewise, we found no relationship between the present or absence of any mite species on brood beetles and brood beetle emergence (Fig. 5, Table 6). We found a marginally significant trend towards the presence of *Histiostoma* spp. on emerged beetles being associated with increased beetle brood emergence per log (Fig. 6, Table 6). For beetles that carried mites, we found no patterns relating mite abundance to total brood beetle emergence (Table 6). While data for *I. confusus* and *Tarsonemus* spp. were presented as pooled in this analysis, models considering these species separately gave similar results.

Discussion

Overall, logs from which emerging adult beetles carried mites yielded higher densities of brood beetles than logs from which emerging beetles did not carry mites. This positive association held for several prominent mite species, and for all mites collectively. No mite

species were negatively associated with beetle emergence, including those such as the beetle egg predator, *I. confusus*, which would seem most likely to exert an adverse effect. While mite presence was positively associated with beetle emergence, we did not see consistent evidence of relationships between continuous measures of mite abundance and beetle performance. That is, increasing mite densities were either not or only weakly associated, with improved beetle performance. Although this could potentially arise from excessive mite densities having an adverse effect beyond a moderate level that benefits beetles, we rarely saw evidence of these data fitting well to the parabolic models that would be indicative of these types of relationships between mite and beetle abundances. Further, we did not see strong positive effects of mites in the manipulative approach, although some mite species showed trends similar to those in the correlative experiment. For example, we found a positive trend between the presence of *Histiostoma* spp. and beetle emergence in both the manipulative and correlative experiments. Collectively, these results suggest the persistent associations between presence of phoretic mites and host beetle performance are more likely reflective of the mutual suitability of host plant substrates for both beetles and mites, rather than causal relationships.

The absence of significant treatment effects in the manipulative approach, when mites were artificially removed from beetles, is consistent with a lack of underlying causalities in positive beetle-mite associations. There are several ecological factors that may explain our findings. Phoretic mites on *I. grandicollis* are largely generalist in their associations with beetle hosts (Pfammatter 2015). Diffuse relationships between any single mite-beetle species combination likely arose from this large set of mite-beetle interactions (Pfammatter 2015). Because of these loose associations, and roles played by other subcortical beetles in the system, positive or negative influences of phoretic mites may be highly variable and ultimately diluted.

Second, indirect drivers linking phoretic mites and beetle reproductive success in other systems may be less prominent in this system. For example, southern pine beetle populations are indirectly driven by *Tarsonemus* mites that modulate the antagonistic fungus *O. minus* (Lombardero et al. 2003). In contrast, fungal associates of *I. grandicollis*, which consist predominantly of *O. ips*, have generally positive or neutral effects on beetle development (Kopper et al. 2004). Without a common, strong antagonistic fungus, we would expect relationships between mite and beetle reproductive success to be positively but not necessarily causally related. Experimental factors during the manipulative approach, such as low overall mite abundances, high variation of mite species and abundance between replicates, and inability to remove all mites could also contribute to the lack of treatment effects. Because we were unable to consistently remove all mites from our intended mite-eliminated treatments, we focused on the incidence and abundance of mites, and their influence on brood production in the mite-present treatments, rather than relying soley on between-treatment differences. The agreement between our results between the trends in the mite-present group and the difference between the mite-present and mite-reduced treatments adds confidence to our conclusions. However, future work is needed to more fully understand roles of mites in reproductive performance by *Ips* species.

Two mite species are worth noting in regard to their potential effect on beetle reproductive success. Egg predation by *I. confusus*, which presumably has an immediate and direct adverse effect on *I. grandicollis* may also release remaining larvae from intraspecific competition, which is intense among bark beetles occupying a finite resource (Anderbrant et al. 1985). For example, predation of *I. pini* adults by *T. dubius* reduces interspecific competition under similar experimental conditions as our manipulative study (Aukema and Raffa 2002).

Also, we have reared *I. confusus* in the laboratory without providing them consistent access to beetle eggs, indicating this species may be more generalist than previously thought. The second species, nematophagous *Dendrolaelaps* mites, has the potential to indirectly mediate beetle abundance by reduction of antagonist nematode populations (Massey 1966, Tomalak et al. 1984). We found a positive relationship between the presence of *D. quadrisetus* and beetle emergence in our correlative approach, however, we were unable to effectively test the effects of *D. quadrisetus* on bark beetle reproductive success due to low mite incidences and the inability to remove these subelytral mites in our manipulative experiment without damaging their hosts.

The positive or neutral role that most mite species play on *I. grandicollis* reproductive success appears to resemble mite interactions with *Nicophorus* burying beetles. Normal mite population levels have no negative effects on *Nicophorus* reproductive success (Wilson and Knollenberg 1987). Wilson and Knollenberg (1987) also found that *Nicophorus* beetles could be negatively impacted by phoretic mites under abnormally high population densities. While our continuous models of mite abundance in relation to beetle brood production were largely insignificant, trends suggested that overabundances of some mites may have negative effects on *I. grandicollis*. Temporal scale is also pertinent and mites occasionally show positive effects on *Nicophorus*, but these benefits require a long time to manifest (Wilson and Knollenberg 1987). Perhaps in our system, mites play a long-term role that may not be strong enough to quantify in experiments that span a single generation.

Our results suggest that mite effects on *I. grandicollis* reproductive success are less likely to affect beetle population dynamics than are several other processes. For example, bark beetle population dynamics are strongly influenced by biotic factors such as inter- and intraspecific competition within tree galleries (Coulson 1979, Schlyter and Anderbrant 1993, Rankin and

Borden 2011). Other drivers, such as the availability, susceptibility and substrate quality of host trees (Klepzig et al. 1991, Cudmore et al. 2010), predators (Erbilgin and Raffa 2002), and weather (Safranyik et al. 2012) can play highly influential roles. Ultimately, *I. grandicollis* and mite reproductive success, while linked, appear largely independent, owing mostly to phoretic mites achieving higher reproductive success in environments that are most suitable for beetle reproductive success. The positive association between mite and beetle abundances from our correlative approach in conjunction with our lack of significant effects in the manipulative approach, supports this conclusion. Future experiments, studying the effects of individual mite species such as *I. confusus* and *D. quadrisetus*, and manipulating mite densities over multiple beetle generations are needed to further elucidate the effects of phoretic mites on bark beetle reproductive success.

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Table 1. Collection sites in Wisconsin at which *P. resinosa* logs were felled and naturally infested by *Ips* beetles.

Site ID	County	GPS Coordinates	Region	Soil Type	% Forest Area[a]	Stand Age (Years)
Mazomanie (MZ)	Dane	43.214306, -89.793194	Southern	silty	13	37-39
Spring Green (SG)	Sauk	43.180194, -90.155444	Southern	silty	32	35-45
Wild Rose (WR)	Waushara	44.259528, -89.314000	Central	sandy	43	38-50
Plum Creek (PC)	Adams	44.236222, -89.820833	Central	sandy	65	25
Black River Falls (BRF)	Jackson	44.247417, -90.586000	Central	sandy	58	34-35
Spooner (SP)	Washburn	45.781472, -91.944944	Northern	sandy-loamy	68	35
Boulder Junction (BJ)	Vilas	45.996583, -89.678778	Northern	sandy-loamy	88	55
Dairymens (DM)	Vilas	46.151028, -89.655444	Northern	sandy-loamy	88	30

[a] On county basis.

Table 2. Summary of phoretic mites on colonizing and brood beetles used in the manipulative approach experiment. Data for colonizing mite-reduced beetles represent average populations prior to removal. All other groups represent average mite populations at the time of experimentation.

Generation	Treatment	Total Mites	*I. confusus* / *Tarsonemus* spp.	*Histiostoma* sp.	*Dendrolaelaps* spp.	*T. australis*	Other Species	*I. confusus*	*Tarsonemus* spp.
Colonizing	Mite-Present	9.0 ± 1.5	6.1 ± 1.3	0.2 ± 0.1	0.3 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	NA	NA
	Mite-reduced	6.2 ± 0.9	3.3 ± 0.6	0.1 ± 0.04	0.3 ± 0.1	0.1 ± 0.1	0	NA	NA
Brood	Mite-Present	1.3 ± 0.2	0.4 ± 0.1	0	0.1 ± 0.02	< 0.1	0.1 ± 0.1	0.3 ± 0.1	0.4 ± 0.1
	Mite-reduced	0.6 ± 0.2	0.6 ± 0.2	< 0.1	0.1 ± 0.03	0	0	0	0

Table 3. Summary of bark beetles and mites, that emerged in a common laboratory environment from *P. resinosa* that were naturally infested by *I. grandicollis* at eight sites in Wisconsin. At each site, a random subsample of *I. grandicollis* was chosen for a survey of the phoretic mite community. Average mite load ± standard error of nine mite species on *I. grandicollis* emerging from logs at each site are presented.

Site[a]	Total *Ips* Emerged	Total *Ips grandicollis* Sampled	Total Mites Sampled	*Dendrolaelaps quadrisetus* (Berlese)	*Dendrolaelaps neodisetus* (Hurlbutt)	*Ereynetes propescutulis* Hunter	*Histiostoma* spp.[b]	*Histiogaster arborsignis* Woodring	*Iponemus confusus* Lindquist	*Tarsonemus* spp.[c]	*Trichouropoda australis* Hirschmann	*Elattoma* sp.
MZ	436	52	18.33 ± 2.59	2.73 ± 0.53	0.67 ± 0.26	0.42 ± 0.14	9.29 ± 1.7	0.15 ± 0.06	1.21 ± 0.31	1.02 ± 0.27	2.5 ± 0.78	0.04 ± 0.03
SG	268	33	14.09 ± 4.74	0.12 ± 0.07	0.18 ± 0.11	0.03 ± 0.03	2.76 ± 0.88	0.09 ± 0.07	0.97 ± 0.48	4.79 ± 2.07	0.88 ± 0.49	0 ± 0
WR	256	26	2.81 ± 0.76	0.04 ± 0.04	0 ± 0	0.19 ± 0.12	1 ± 0.51	0 ± 0	0.54 ± 0.26	0.46 ± 0.24	0.08 ± 0.05	0 ± 0
PC	664	65	37.55 ± 3.28	6.85 ± 0.86	0.06 ± 0.05	0.31 ± 0.1	5.66 ± 0.92	0.34 ± 0.14	3.42 ± 0.7	0.66 ± 0.16	1.72 ± 0.4	6.75 ± 1.15
BRF	63	10	5.5 ± 2.8	0 ± 0	0 ± 0	0.2 ± 0.2	3.9 ± 2.4	0.3 ± 0.15	0.1 ± 0.1	0.1 ± 0.1	0.5 ± 0.4	0 ± 0
SP	755	41	18.44 ± 3.48	1.02 ± 0.2	0 ± 0	0.44 ± 0.23	10.37 ± 2.42	0 ± 0	1.85 ± 0.48	0.71 ± 0.22	0.2 ± 0.13	0.39 ± 0.13
BJ	85	11	2 ± 0.65	0 ± 0	0 ± 0	0.09 ± 0.09	0.82 ± 0.38	0.09 ± 0.09	0.18 ± 0.12	0.64 ± 0.39	0 ± 0	0 ± 0

[a]DM had only three *Ips* emerge from logs, and these carried no mites, so this site is excluded.

[b]Most *Histiostoma* spp. obtained were *Histiostoma varia* Woodring and Moser, but small structural features made confirmation difficult, so we used the genus designation to be cautious.

[c]Most *Tarsonemus* spp. were *Tarsonmeus ips* Lindquist. *Tarsonemus fusari* Coorman were identified in a few samples. Small structural features make conclusive differentiation difficult, so we used the genus designation to be cautious.

We also found less than five of *Proctolaelaps* sp., *Paracarophaenax* sp., *Schwebia* sp., *Mexecheles virginiensis*, and two species of unidentified mites. These were not included in any analyses because of their low numbers.

Table 4. Statistical summaries for categorical (presence/absence) and continuous (beetles with mites present only) models relating mite and beetle abundances for *I. grandicollis* emerging from naturally infested *P. resinosa* logs at eight sites in southern Wisconsin in 2009. Mite presence (categorical) is positively associated with emergence of brood *I. grandicollis* for all mite species measured except *Elattoma* sp. and *H. arborsignis*. Mite abundance was significantly related to beetle emergence (continuous) for *Histiostoma* spp., *Elattoma* sp., and *H. arborsignis*

	Presence/Absence Models			**Presence Only Models**		
Mite Species	***T***	**df**	***P* value**	χ^2	**df**	***P* value**
Total Mites	**4.08**	**32.03**	**< 0.001**	0.856	1	0.355
Histiostoma spp.	**2.97**	**33.01**	**0.005**	**7.261**	1	**0.007**
D. quadrisetus	**2.69**	**18.32**	**0.017**	0.392	1	0.531
I. confuses	**2.48**	**30.14**	**0.018**	2.498	1	0.114
Elattoma sp.	1.65	5.23	0.158	**7.619**	1	**0.006**
H. arborsignis	0.40	27.84	0.692	**17.767**	1	**< 0.001**
T. australis	**2.54**	**16.71**	**0.021**	1.418	1	0.234
Tarsonemus spp.	**3.20**	**27.45**	**0.003**	0.691	1	0.406

Table 5. Summary of the average mite abundances per *P. resinosa* log for pooled mite species, *Iponemus*/*Tarsonemus*, *Histiostoma* spp., and *H. arborsignis* emerging on brood beetles in mite-present treatment of the manipulative experiment. We compared the average mite abundance per log between post-hoc tunneling success categories, where both, one, or neither pair of *I. grandicollis* successfully tunneled. Mating success comparisons for all species are insignificant.

Mites	Tunneling Success[a]			*F*	*P* value
	Both Pairs (n = 12)	**One Pair (n = 5)**	**Neither Pair (n = 4)**		
Pooled Mite Species	33.25	47.20	27.25	0.616	0.55
Iponemus/Tarsonemus	10.83	5.20	9.50	0.378	0.69
Histiostoma spp.	20.58	37.20	16.50	0.765	0.48
H. arborsignis	0.83	2.20	1.00	0.864	0.44

[a]Tunneling success is based on a beetle pair successfully ovipositing at least one egg.

Table 6. Statistical summaries for categorical (presence/absence) and continuous (beetles with mites present only) models relating mite and *I. grandicollis* abundances. Presence or absence of phoretic mites on colonizing and brood *I. grandicollis* emerging from logs did not predict total beetle emergence for any mite species, although trends in Histiostoma mite presence on brood beetles emerging from *P. resinosa* logs was significant at $\propto = 0.10$. We found no relationship between mite abundance on colonizing or brood beetles that carried mites and *I. grandicollis* brood emergence. NA's represent are presented where mites were categorically absent or too infrequent to perform a statistical analysis within a presence or absence grouping. Only data from the mite-present treatment used.

		Presence/Absence Models			**Presence Only Models**		
Generation	**Mite Species**	***T***	**df**	***P* value**	***F***	**Df**	***P* value**
Colonizing	Total Mites	NA	NA	NA	1.18	1, 19	0.291
	Iponemus/Tarsonemus	1.687	7.796	0.130	0.137	1, 14	0.717
	Histiostoma spp.	NA	NA	NA	1.528	1, 19	0.231
	H. arborsignis	0.475	15.16	0.642	0.224	1, 6	0.652
Brood	Total Mites	NA	NA	NA	0.479	1, 13	0.501
	Iponemus/Tarsonemus	0.021	10.566	0.984	0.013	1, 7	0.921
	Histiostoma spp.	2.001	5.525	0.096	0.654	1, 7	0.445
	H. arborsignis	0.046	2.715	0.99	9.942	1, 1	0.196
	Tarsonemus spp.	1.320	7.186	0.228	0.165	1, 3	0.712
	I. cinfusus	1.21	12.997	0.248	0.961	1, 5	0.372

Figure Legends

Figure 1. Overall flow of correlative (left) and manipulative (right) experiments evaluating potential effects of phoretic mites on reproductive success of *I. grandicollis*. Note that initial tree colonization was done on separate occasions for the two experiments.

Figure 2. Assessment of community variation of phoretic mites on *I. grandicollis* naturally infesting *P. resinosa* logs from eight sites in southern Wisconsin. Nonmetric Multidimensional scaling (NMS) visualizations represent the dissimilarity of phoretic mite communities on *I. grandicollis* displayed in A) 2D (NMS Stress = 0.240, r^2 = 0.722). and B) 3D (NMS Stress = 0.146, r^2 = 0.841). Relative abundances of mite community members differed among sites (ANOSIM R = 0.323, P = 0.012). Aggregated data (by collection site and unique tree log) used for ordination analyses were standardized using the Wisconsin-double Standardization, resembled to a Bray-Curtis dissimilarity matrix, and labeled by collection site (color) and total beetle emergence per log. Changes in phoretic mite relative abundance are indicated by arrows were generated from significant ($P < 0.05$) mite species correlations overlaid on the 2D NMS.

Figure 3. Correlative assessment of discrete (left) and continuous (right) measures of mite abundance on emergence of *I. grandicollis* brood adults. Mite presence (left) is positively associated with beetle emergence for all mite species except *Elattoma* sp. and *H. arborsignis*. The relationship between beetle emergence and mite species abundance (right) is not linear. A) Total Mites, B) *Histiostoma* sp., C) *Dendrolaelaps quadrisetus*, D.) *Iponemus confusus*, E) *Elattoma* sp., F) *Histiogaster arborsignis*, G) *Trichouropoda australis*, and H) *Tarsonemus* spp. logs were naturally infested at sites in southern Wisconsin in 2009.

Figure 4. *Ips grandicollis* emergence over the course of the manipulative experiment where beetles with a natural complement of phoretic mites or mites mechanically removed were

introduced into logs in order to assess mite effects on beetle reproductive success. Beetle emergence over time does not show strong relationships to mite abundance, either within or between mite-present or mite-reduced treatments. Beetles emerged from 11 Aug to 04 Sep for both the mite-present (black) and mite-reduced (grey) treatments. Filled in circles and connecting lines represent mean beetle emergence and mites per beetle for each treatment.

Figure 5. Average *I. grandicollis* emergence compared between mite-present and mite-absent treatments from the manipulative experiment in which beetles with a natural complement of phoretic mites or mites mechanically removed were introduced into logs to assess mite effects on beetle reproductive success. The average number of *I. grandicollis* emerged per log did not differ between mite-present (n = 25) or mite-absent (n = 22) experimental treatments ($T = 0.590$, df = 46.56, P value = 0.558). Box plots represent the mean, 25^{th} and 75^{th} quantile for emerged *I. grandicollis*.

Figure 6. Assessment of the effects of mite presence or absence (left) and the abundance of mites on mite-carrying beetles (right) on beetle reproductive success in manipulative experiment where beetles with a natural complement of phoretic mites or mites mechanically removed were introduced into logs. This figure presents data on the mite communities of colonizing adult (F1) (Fig. 1) *I. grandicollis*. Presence or absence of phoretic mites (categorical) on colonizing *I. grandicollis* from logs was not influential in predicting total *I. grandicollis* emergence per log. The relationship between beetle emergence and mite species abundance (continuous) is best described as not linear. Plots represent A) total mites, B) combined *I. confusus* and *Tarsonemus* spp., C) *Histiostoma* spp., D) and *H. arborsignis* from the phoretic mite community manipulation experiment. Sample sizes are variable and depend on mite populations. NA's represent are

presented where mites were categorically absent or too infrequent to perform a statistical analysis within a presence or absence grouping.

Figure 7. Assessment of the effects of mite presence or absence (left) and the abundance of mites on mite-carrying beetles (right) on beetle reproductive success in manipulative experiment where beetles with a natural complement of phoretic mites or mites mechanically removed were introduced to logs. This figure presents data on the mite communities found on emerging brood adult *I. grandicollis*. Presence or absence of phoretic mites (categorical) on brood *I. grandicollis* emerging from logs was not influential in predicting total *I. grandicollis* emergence for all mite besides *Histiostoma* spp. *Histiostoma* presence on brood beetles was moderately positively related to beetle emergence. The relationship between beetle emergence and mite species abundance (continuous) is best described as not linear. Plots represent A) total mites, B) combined *I. confusus* and *Tarsonemus* spp., C) *Histiostoma* spp., D) and *H. arborsignis* from the phoretic mite community manipulation experiment. Sample sizes are variable and depend on mite populations. NA's represent are presented where mites were categorically absent or too infrequent to perform a statistical analysis within a presence or absence grouping.

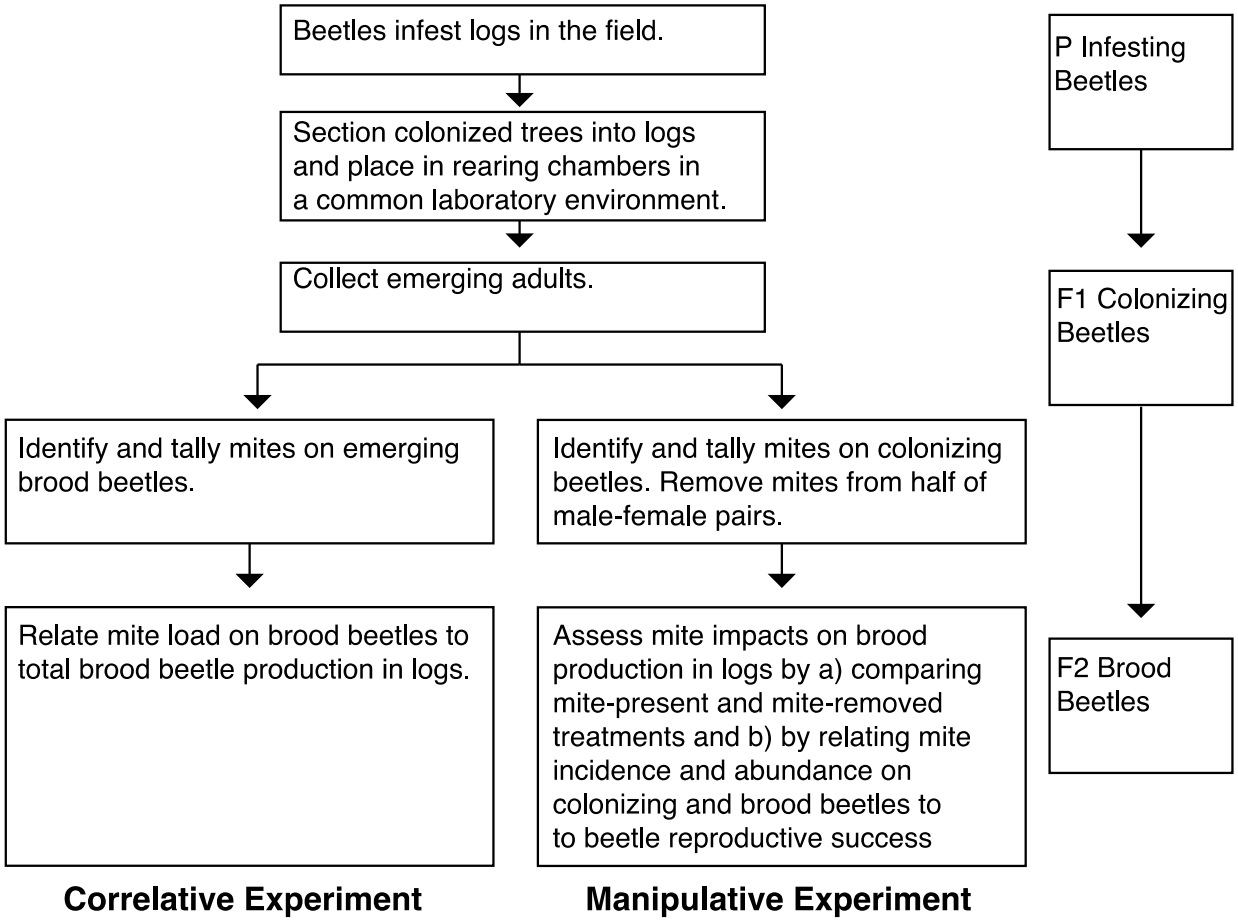
Beetles infest logs in the field.
Section colonized trees into logs and place in rearing chambers in a common laboratory environment.
Collect emerging adults.
Identify and tally mites on emerging brood beetles.
Identify and tally mites on colonizing beetles. Remove mites from half of male-female pairs.
Relate mite load on brood beetles to total brood beetle production in logs.
Assess mite impacts on brood production in logs by a) comparing mite-present and mite-removed treatments and b) by relating mite incidence and abundance on colonizing and brood beetles to to beetle reproductive success
Correlative Experiment
Manipulative Experiment
P Infesting Beetles
F1 Colonizing Beetles
F2 Brood Beetles

Figure 1.

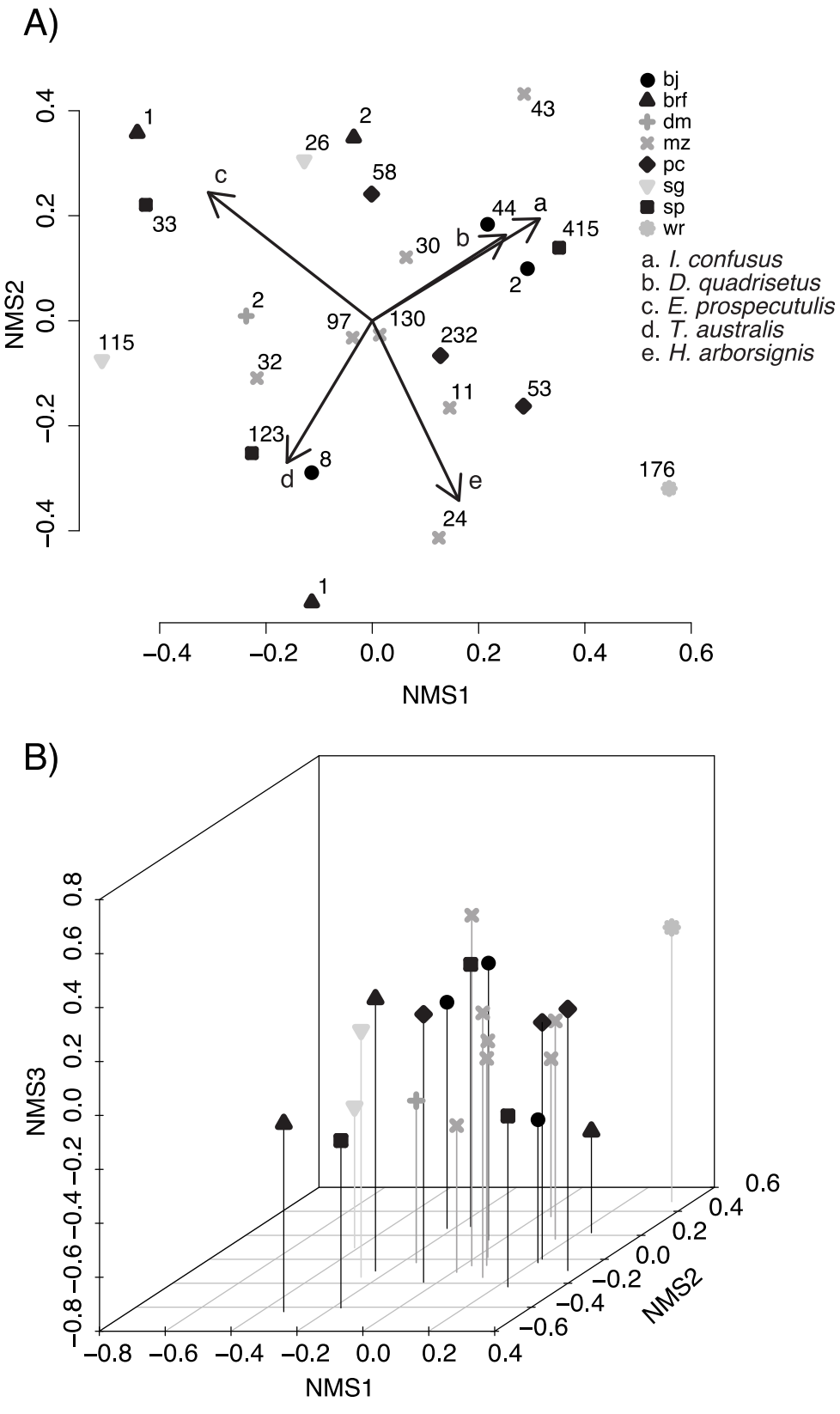
A)
bj
brf
dm
mz
pc
sg
sp
wr
a. I. confusus
b. D. quadrisetus
c. E. prospecutulis
d. T. australis
e. H. arborsignis
NMS1
NMS2
B)
NMS1
NMS2
NMS3

Figure 2.

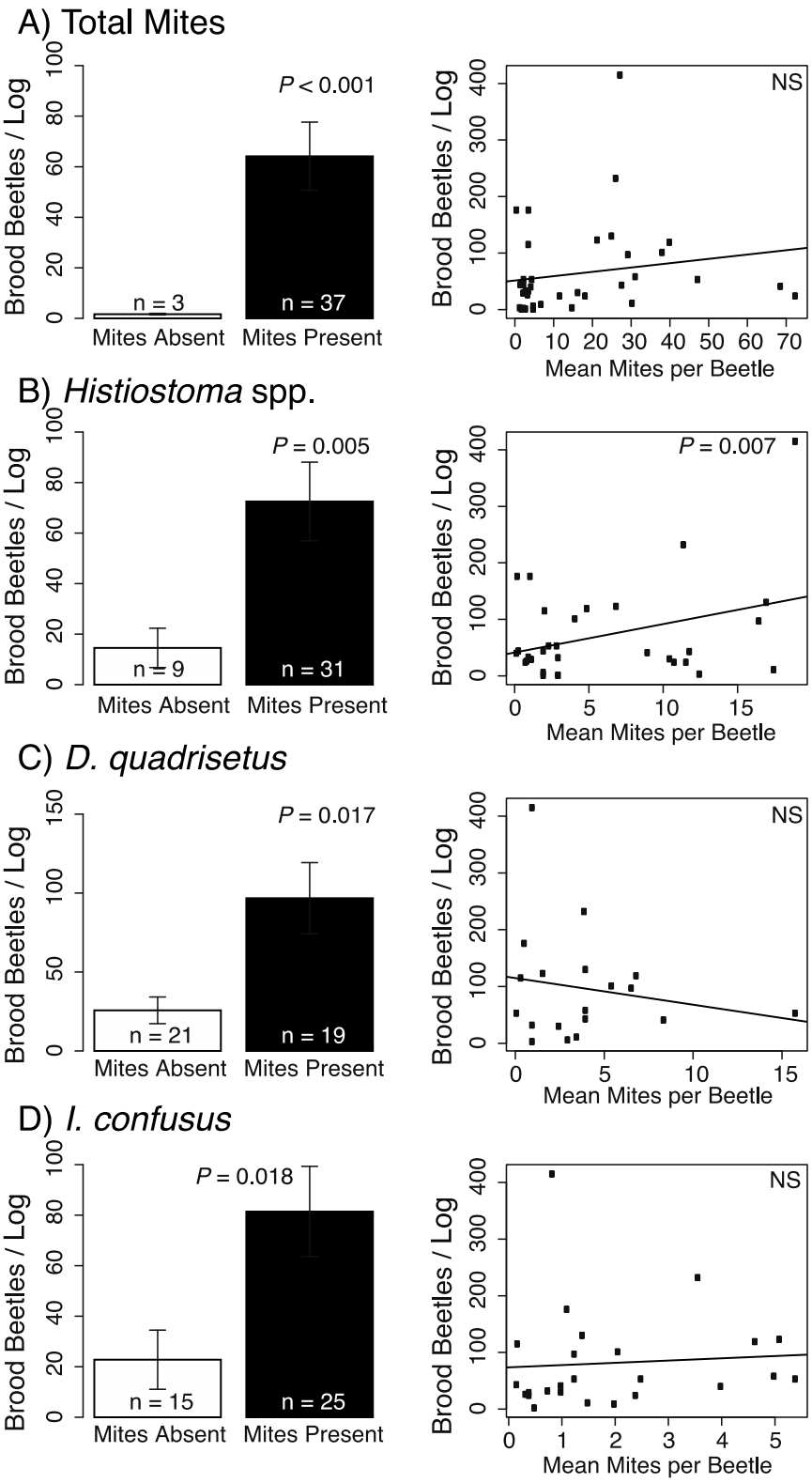
A) Total Mites
Brood Beetles / Log
P < 0.001
n = 3
n = 37
Mites Absent
Mites Present
NS
Mean Mites per Beetle
B) Histiostoma spp.
P = 0.005
n = 9
n = 31
P = 0.007
C) D. quadrisetus
P = 0.017
n = 21
n = 19
D) I. confusus
P = 0.018
n = 15
n = 25

Figure 3.

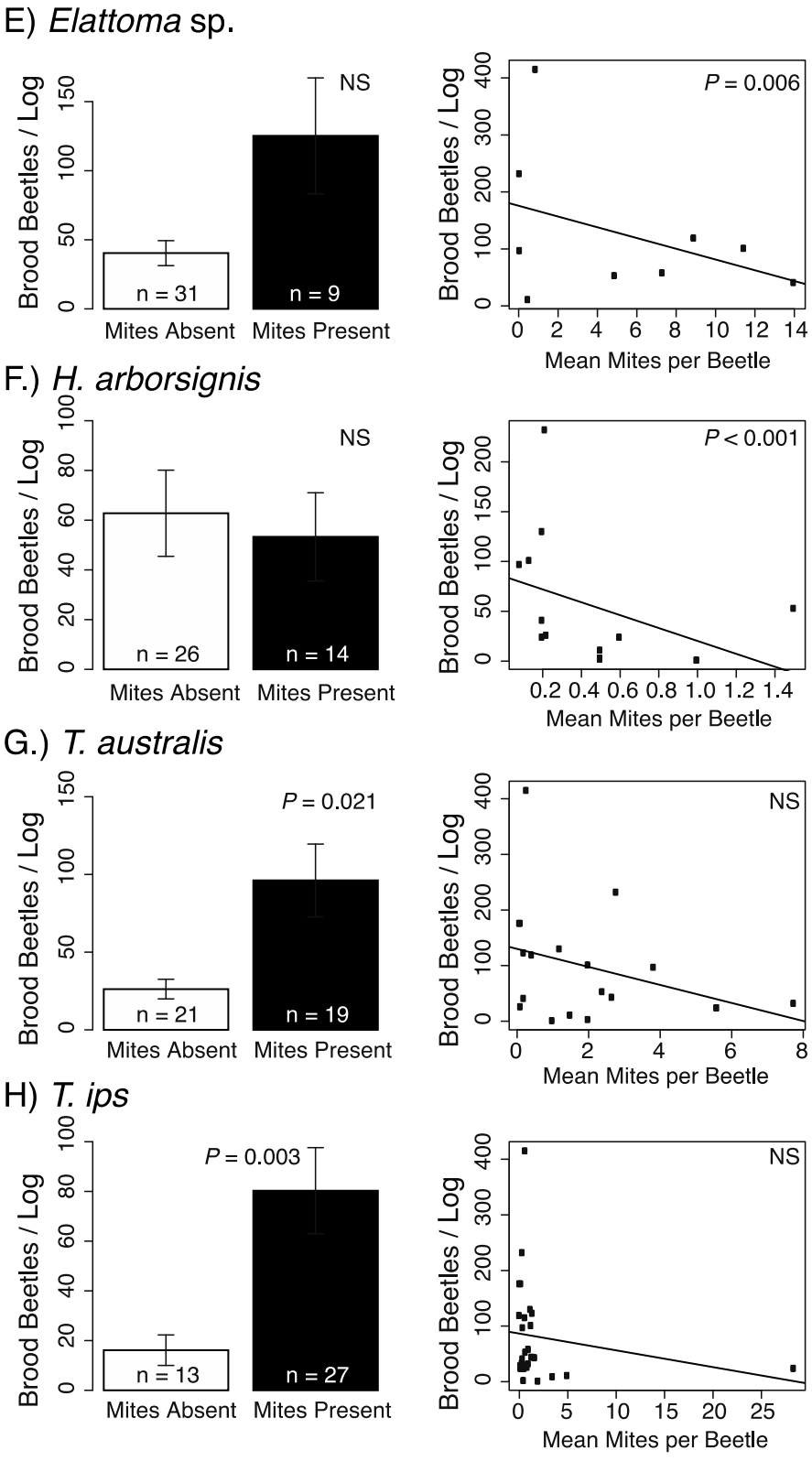

Figure 3. (cont.)

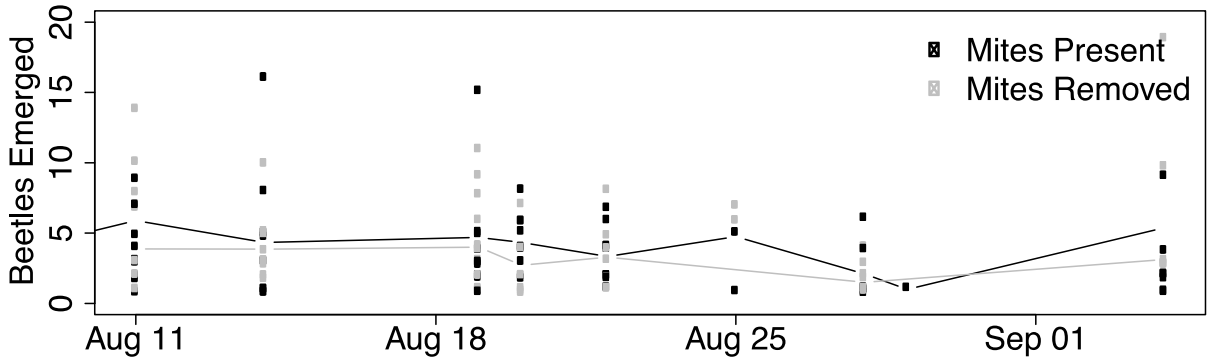
Beetles Emerged
0
5
10
15
20
Aug 11
Aug 18
Aug 25
Sep 01
Mites Present
Mites Removed

Figure 4.

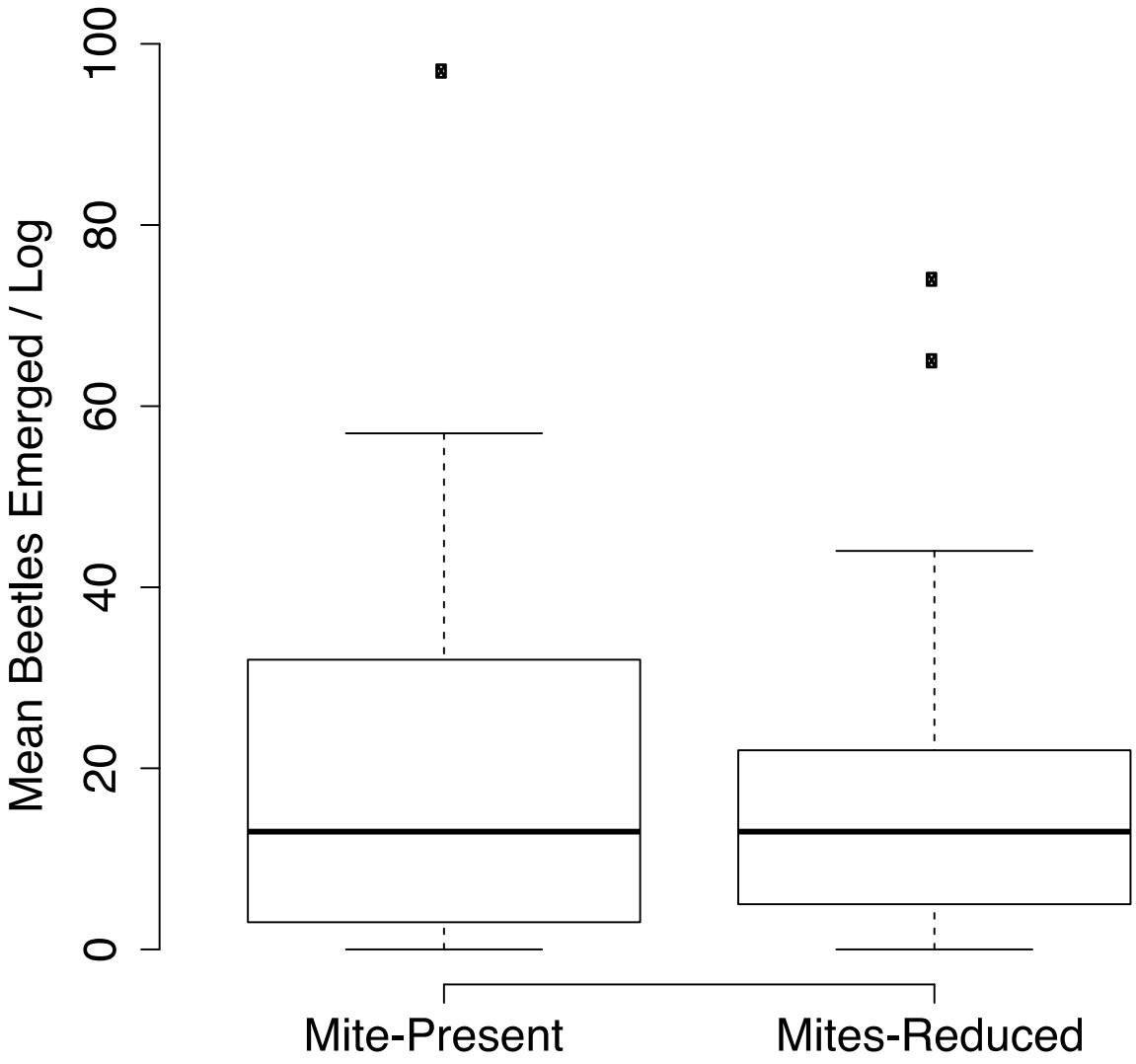
Mean Beetles Emerged / Log
0
20
40
60
80
100
Mite-Present
Mites-Reduced

Figure 5.

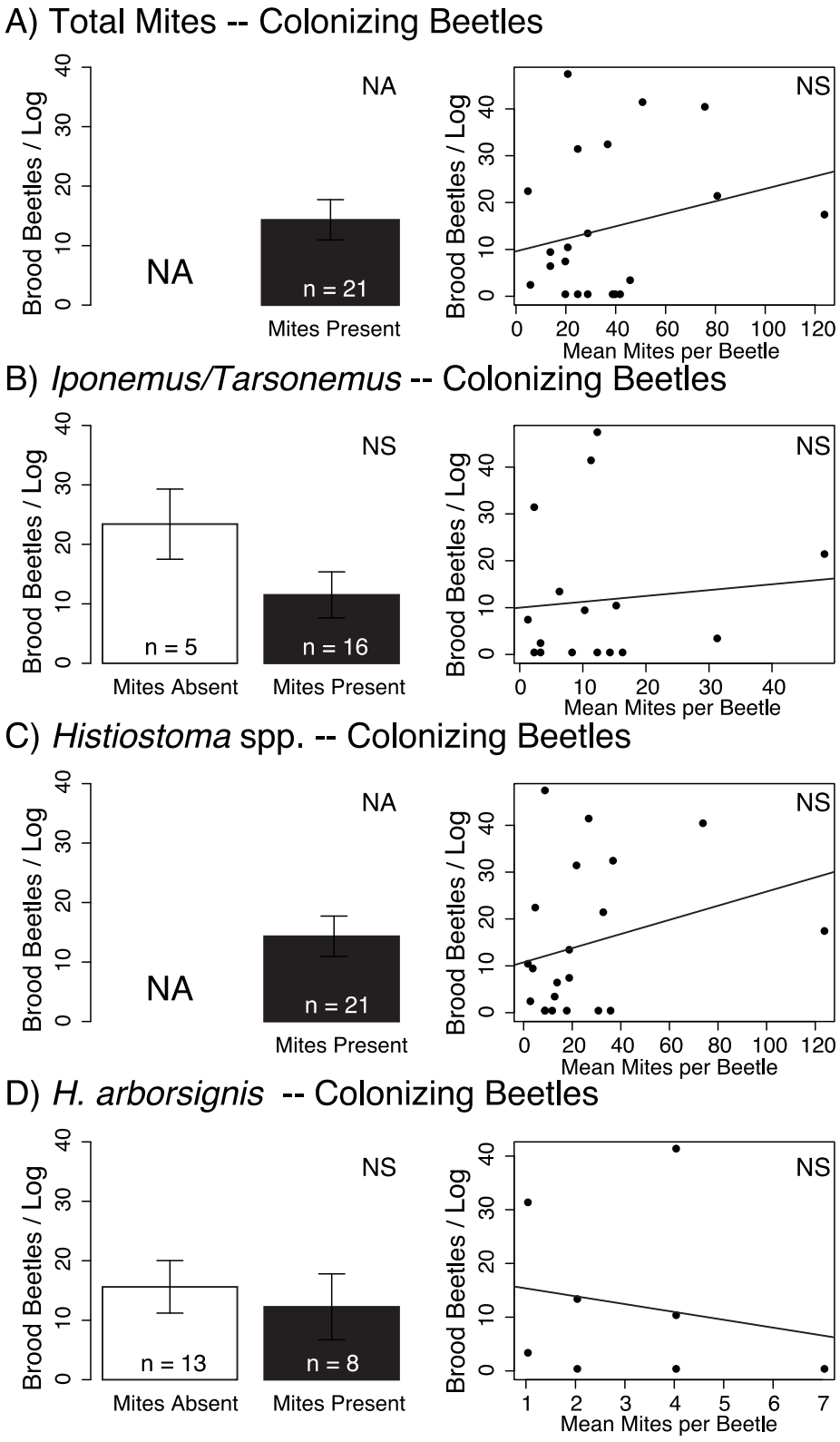
A) Total Mites -- Colonizing Beetles
Brood Beetles / Log
NA
NA
n = 21
Mites Present
NS
Mean Mites per Beetle
B) Iponemus/Tarsonemus -- Colonizing Beetles
NS
n = 5
n = 16
Mites Absent
Mites Present
C) Histiostoma spp. -- Colonizing Beetles
NA
NA
n = 21
Mites Present
D) H. arborsignis -- Colonizing Beetles
NS
n = 13
n = 8
Mites Absent
Mites Present

Figure 6.

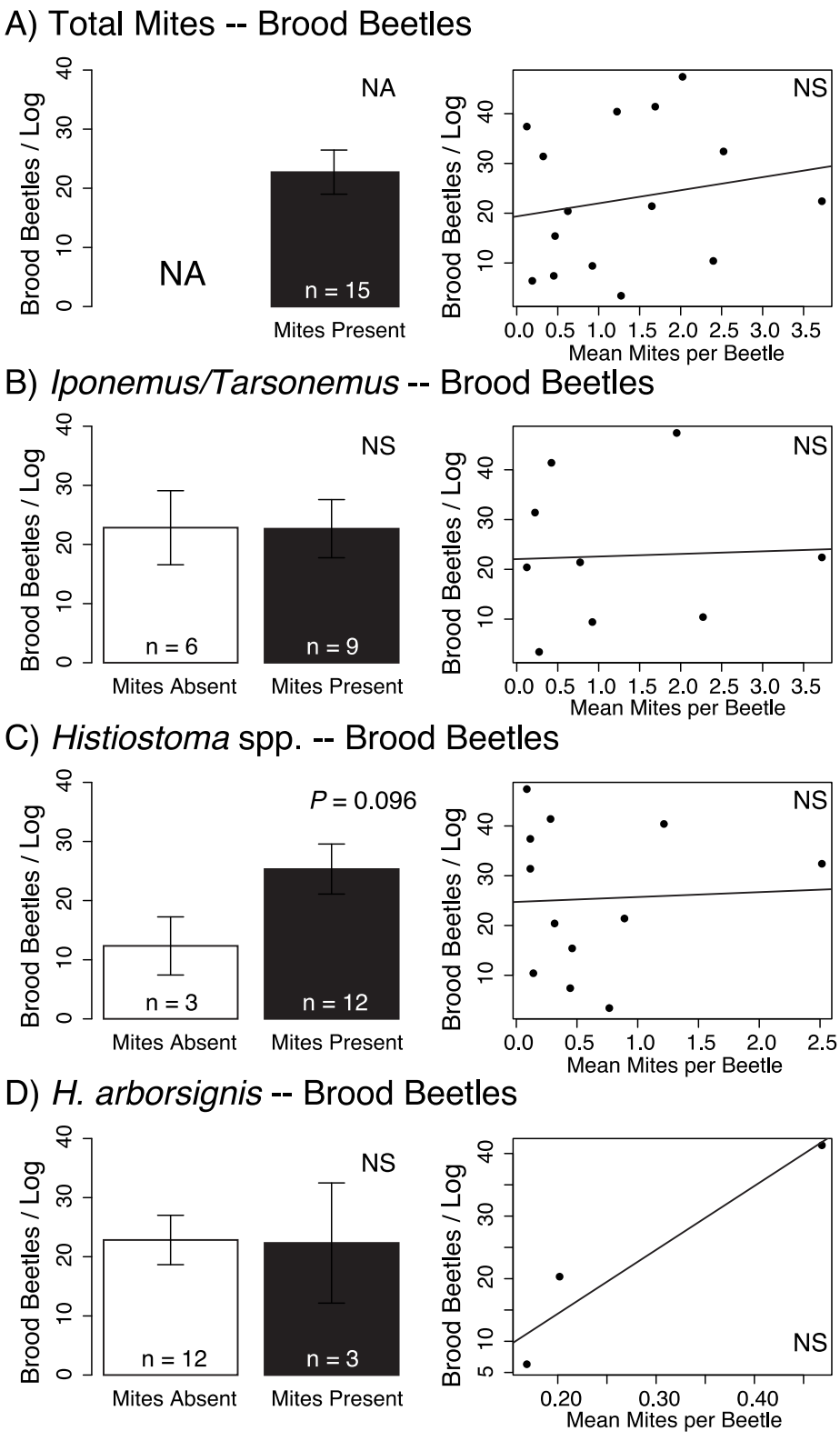
A) Total Mites -- Brood Beetles
Brood Beetles / Log
NA
NA
n = 15
Mites Present
NS
Mean Mites per Beetle
B) Iponemus/Tarsonemus -- Brood Beetles
NS
n = 6
n = 9
Mites Absent
Mites Present
NS
C) Histiostoma spp. -- Brood Beetles
P = 0.096
n = 3
n = 12
Mites Absent
Mites Present
NS
D) H. arborsignis -- Brood Beetles
NS
n = 12
n = 3
Mites Absent
Mites Present
NS

Figure 7.

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Chapter 4: Behaviors of phoretic mites (Acari) during host colonization and development by *Ips pini* and *Ips grandicollis* (Coleoptera: Curculionidae)

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Abstract

Studies on interactions between bark beetles and phoretic mites have focused largely on dispersal, during which specialized life stages gain transport between food resources. However, many interactions between beetles and mites occur within trees, where beetles and mites develop concurrently. These poorly understood within-tree interactions are potentially important to beetle and mite reproductive success, and beetle-mite phoretic pairings. We performed a series of experiments to characterize the behaviors of mites commonly associated with *Ips pini* (Say) and *Ips grandicollis* (Eichhoff) during non-dispersal life-stages. We found:

1. Mites are highly specific in choosing the location on which they attach to host beetles. Various species preferentially attach within the beetles' elytral declivities, legs and ventral thorax region, or beneath the elytra.
2. Detachment from beetles is elicited by cues associated with both beetle and tree hosts. For example, beetle host health can trigger rapid mite departure. *Iponemus confusus* detach more rapidly from beetles colonizing tissue amended with the host tree secondary compound α-pinene than in unamended tissue.
3. Within-plant movement by detached mites is elicited by host plant volatiles. In olfactometer assays, *D. quadrisetus* preferred volatiles from fresh phloem and beetle-infested phloem over blank controls, and volatiles from infested phloem over fresh phloem.
4. Within host plant tissue, fungal symbionts of beetles provide a resource for mites. *Histiogaster arborsignis* Woodring fed and reproduced more successfully on *P. resinosa* phloem inoculated with the symbiotic fungus *Ophiostoma ips* (Rumbold), than on phloem with the opportunistic fungus *Aspergillus fumigatus* Fresen or broth control.

Introduction

Phoretic mites of bark beetles utilize their hosts for transport between ephemeral tree resources (Houck and OConnor 1991). Mite life cycles can be grouped into two phases, within-tree colonization and and between-tree dispersal. During tree colonization, phoretic mites detach from their host beetles and inhabit subcortical galleries that the beetles construct. Once inside the galleries, mites develop into adults and oviposit. Brood mites feed, develop and reproduce within galleries, often having multiple generations during a single beetle generation. Developing mites feed on an array of substrates, including bacteria, fungi, nematodes, other mites, and eggs or early instar larvae of beetles (OConnor 1982). During their reproductive cycle, most mites bypass transforming into a phoretic life-stage. However, some individuals facultatively transform, attach to emerging beetles, and gain dispersal to new tree resources. During their dispersal phase, most mites are dorsal-ventrally flattened, desiccation resistant, and often have reduced non-feeding mouthparts and specialized attachment structures (Hughes 1959). Mite reproduction and dispersal processes vary among species (Binns 1982). Some species such as *Dendrolaelaps quadrisetus* (Berlese) disperse during their adult stage and thus do not require transformation into dispersal-specific stage (Binns 1982). Various factors can trigger mite transformation into a dispersal stage, such as fluctuations in moisture (Wallace 1960) and food resources (Corente and Knülle 2003), or overcrowding (Houck and OConnor 1991). A combined schematic and photographic representation of a generalized life-history of mites is shown in Figure 1.

Numerous surveys have characterized the dispersal stage associations of mites associated with bark beetles, including on *Scolytus multistriatus* (Marsham) (Moser et al. 2005), *Dendroctonus pseudotsugae* Hopkins (Pernek et al. 2007), *Dendroctonus rufipennis* Kirby

(Cardoza et al. 2008), *Dendrocotnus frontalis* Zimmerman (Moser and Roton 1971, Moser et al. 1974), *Ips grandicollis* (Eichoff), and *Ips pini* (Say) (Pfammatter et al. 2013). These surveys provide a baseline for our current understanding of bark beetle-phoretic mite systems. These snapshots represent the relatively short dispersal phase of beetles and mites. However, both spend of these groups spend the majority of their lives developing concurrently within tree tissues. Interactions during this period may therefore be critically important in shaping aspects of beetle and mite reproductive success and beetle-mite phoretic pairings. Due to the challenges of sampling, maintaining, and manipulating mites, research on mite behavior within their subcortical environments has been relatively limited compared to the phoretic stage. Additionally, mite behaviors are highly context-dependent, requiring substantial understanding of life histories, which is often lacking.

Behavioral studies on the attachment and detachment of mites are relatively sparse, yet necessary for our understanding of how symbiotic communities and relationships arise. Attachment may be elicited by factors associated with phoretic hosts (Krantz and Mellott 1972), with cues showing a broad range of specificity. For example, *Polyphagotarsonemus latus* (Banks) has high phoretic fidelity to the whitefly *Bemisia tabaci* (Gennadius) and uses its cuticular waxes as cues for attachment (Soroker et al. 2003). Other mites, such as the generalist *Histiostoma laboratorium* Hughes, are induced to attach by non-specific contact with forelegs (Schulze 1924). Attachment mechanisms range from morphological modifications, such as the anal suckers of *Histiostoma* spp., anal stalks of *Trichouropoda* spp., and transformed claw-like forelegs of *Elattoma* spp., to behavioral orientations, such as the alignment of *D. quadrisetus* underneath beetle elytra, and positioning of *Tarsonemus* spp. in the elytral declivity of bark beetles.

After attachment to their host beetels and subsequent travel to a new tree resources, mites must detach in order to colonize new tree resources. The factors that influence the detachment of mites during plant colonization by host insects are largely unknown. Most mites require plant- or insect-specific cues to initiate detachment and simple mechanical removal of mites is often insufficient to elicit a shift from phoretic to within-plant behaviors (Binns 1982). For example, *Digamasellus fallax* Leitner mechanically removed from their host in the absence of the cues normally associated with natural colonization reattach to their host flies (Binns 1973) rather than seek food resources. Factors that can elicit mite detachment and colonization of host trees may be related to their feeding prefrences.

Phoretic mites of bark beetles comprise a range of feeding guilds. For example, *Iponemus confusus* Lindquist feed on eggs and early instar larvae of beetles (Berryman 1968, Lindquist 1969), *D. quadrisetus* feed on beetle-associated nematodes (Kinn 1984), and *Histiogaster arborsignis* Woodring feed on fungal spores (Fig. 1C). Thus, mites can potentially affect bark beetles both directly, such as through predation (Lindquist 1969), or indirectly, as mediated through associations with other subcortical organisms. For example, bark beetles and their mite associates can vector fungi (Bridges and Moser 1986), which can play a variety of roles ranging from nutritional resource provision to reduced beetle fecundity (Ayres et al. 2000). The fungus *Ophiostoma minus* (Hedgcock) can out compete beetle-mutualistic fungi for space and resources (Six and Paine 1998) which, in turn, negatively affects the fecundity of *D. frontalis* (Hofstetter et al. 2006). *Tarsonemus* mites have been shown to increase the abundance of *O. minus* within trees, resulting in a net negative effect on *D. frontalis* reproductive success (Lombardero et al. 2003).

We studied behaviors of mites associated with *I. pini* and *I. grandicollis* that may be influential in the formation of relationships between beetles and mites. *Ips grandicollis* and *I. pini* are the predominant subcortical insects associated with mature red pines, *Pinus resinosa* (Ait), in the Great Lakes region. These beetles colonize weakened and dying mature trees in the genus *Pinus*, and when successful kill the host trees. The reproductive cycle of beetles lasts around 33 days in the laboratory (Aukema and Raffa 2002), and there are typically two to three generations per year in the field. Colonizing beetles produce pheromones that attract members of both sexes, and also predators such as beetles in the genus *Thanasimus* (Cleridae) (Mizell et al. 1984), and competitors such as beetles in the genus *Monochamus* (Cerambycidae) (Miller and Asaro 2005). *Ips* vector fungi, predominantly *Ophiostoma ips* (Rumbold) which colonize beetle galleries and confer some benefits to beetles. Opportunistic fungi such as *Aspergillus fumigatus* Fresen colonize killed trees are harmful to beetles (Cardoza et al. 2006). Bark beetles, in conjunction with other subcortical beetles, convert live trees into suitable habitat for colonization by beetle-vectored organisms including fungi, nematodes, and phoretic mites.

Here, we present a series of experiments investigating interactions between phoretic mites and bark beetles in host-tree environments during non-dispersal life-stages. Our experiments group into four stages of mite behavior that span mite and beetle life histories. These include attachment to host beetles (Obj. 1), detachment from beetles as they colonize new tree hosts (Obj. 2), dispersal within the host tree (Obj. 3), and development within the host tree (Obj. 4). These objectives are superimposed onto the life-history schematic of mites in Figure 1.

Materials and Methods

Beetle and Mite Collection

Beetles used for experiments were either live-trapped using baited multiple-funnel traps (Lindgren 1983), or collected from field infested *P. resinosa* logs that were brought to the laboratory prior to beetle emergence (herein refered to as laboratory, laboratory-emerged, or, laboratory colony beetles). Methods and rationale for live-trapping are detailed in Pfammatter et al. (2013). Briefly, beetles were attracted to traps baited with 40 *mg* 50+/50- racemic ipsdienol and 4 *mg* lanierone bubble cap lures (Contech, BC, Canada) and individually collected upon entering. Live-trapping sessions ended when the requisite number of beetles was captured for each objective, and never lasted longer than 8 hours. Beetles collected during emergence from *P. resinosa* logs were reared using techniques described in Aukema and Raffa (2004a). Briefly, healthy trees were felled, baited with 40 *mg* 50+/50- racemic ipsdienol and 4 *mg* lanierone bubble cap lures (Contech, BC, Canada), allowed to become naturally infested with bark beetles for approximately two weeks, and brought to the laboratory where they were placed in ~ 25 *cm* diameter × 45 *cm* tall, 19 *L* metal rearing chambers until beetle emergence. To ensure good health, beetles were collected at least every other day from rearing chambers. Unless otherwise noted, all trapped and emerging beetles originated from a mature *P. resinosa* stand in Mazomanie, WI (43^{o} 12’ 51.5”, -89^{o} 47’ 35.5”).

Objective 1: Do mites show preferences for attachment on specific host beetle body parts?

We sampled the attachment locations of mites on 20 *I. grandicollis*. Emerging adult beetles were captured live from our laboratory colony, immediately placed in individual Eppendorf vials, and immersed in 70% EtOH. Beetles and their phoretic mites were removed from the vials, placed in deep well slides with specimen clearing fluid (BioQuip, Rancho Dominguez, CA), and baked at 80°C to allow for lipid digestion. Mites were then removed from the beetles and placed on separate slides, based on the location on which they were attached to

their host beetles. Attachment locations were specified as one of four categories; within the elytral declivity, attached to legs or ventral thorax region, underneath elytra, or on any remaining part of the exoskeleton. Mites that had fallen from beetles during processing were discounted. Detailed methods for the collection, curation and identification of phoretic mites are described in Pfammatter et al. (2013).

We compared average abundance of mites among each of the four attachment locations with Analysis of Variance (ANOVA) (function: Anova, library: 'car') for each mite species, individually. Statistical analyses for all experiments were performed in R statistical computing software (R Core Team 2014).

Objective 2: Do cues associated with host beetles and trees mediate detachment?

We assessed mite responses to morbidity and mortality of *I. pini*. We administered one of the following treatments to laboratory-reared male *I. pini*; 1) control: beetles handled but uninjured, 2) decapitation: beetles' head capsule removed, 3) injury: beetles had a metal probe inserted into their head capsule, 4) toxicity: beetles had four 0.25 μL aliquots of ethyl acetate applied to their mouthparts, 5) injury + toxicity, 6) decapitation + toxicity. Treatments including ethyl acetate were intended to cause beetle mortality without release of hemolymph or other internal chemicals. However, the ethyl acetate treatments caused unintended mortality to mites and were thus removed from further consideration.

Beetles emerging from the laboratory colony were collected individually and immediately refrigerated at 4°C for up to two days prior to experimentation. Male beetles were randomly assigned to one of the six treatments, and their phoretic mites were tabulated and classified. Beetles were placed in individual experimental arenas and dorsally affixed to the arena surface with silicon caulk, in order to prevent beetle movement. Attempts to affix beetles

on their ventral size were unsuccessful due to the beeles; leg movements. Each arena was constructed with a round, 20 *mm* diameter filter paper stage affixed to a 30 *mm* diameter sticky card (Seabright Laboratories, Emeryville, CA) and placed in a 100 *mm* Petri-dish. After healthy beetles were affixed in the experimental arenas, we performed treatments and conducted three hours of continuous observation. Mite counts and locations were tracked every ten minutes throughout each trial for *D. quadrisetus* and *H. arborsignis*, and as a pooled grouping of *Tarsonemus* spp., *I. confusus*, *Elattoma* sp. and other small mite species. Upon completion of each trial, each beetle was examined beneath the elytra and membranous wings for any remaining mites that could not be tabulated prior to experimentation.

We compared the proportions of beetles from which mites detached, the proportion of mites detaching from beetles from which at least one mite had detached, and average time to initial mite detachment for beetles among treatments. We used χ^2 goodness-of-fit tests (function: chisq.test) to compare measures of proportional mite detachment among treatments. We compared the percentage of mite deteachment and mean time to initial mite detachment among groups with Kruskal-Wallis tests (function: kruskal.test). Due to low sample sizes of some mite species and apparent dichotomy of response types, we pooled mite species groups for analysis into two categories; *D. quadrisetus* or all other mite species. Beetles without *D. quadrisetus* were removed from analysis.

We investigated the effects of the predominant monoterpene in *P. resinosa*, α-pinene, on the detachment of phoretic mites during tunneling by *I. grandicollis*. We allowed beetles to tunnel for 3, 6, 9, 12, 15, 18, 21, or 24 hours in one of these substrates: natural phloem, autoclaved phloem, or autoclaved phloem amended with 25 μL of + α-pinene (Sigma Aldrich, St.

Louis, MO). We measured the rate of mite detachment for *D. quadrisetus*, *H. arborsignis*, *Elattoma* sp., and a pooled grouping of *Tarsonemus* spp. and *I. confusus*.

Upon collection, we tabulated species composition and membership of the mites on each beetle. Beetles were randomly assigned to one of 24 treatment (3 time × 8 category) combinations, with five per treatment. Male beetles were introduced in to 5×5 *cm* 'phloem sandwiches', allowed to tunnel for the predetermined time, and immediately frozen at -30°C for later assessment of mite detachment. Detailed methods for construction of phloem sandwiches are in Kim and Miller (1981). Briefly, phloem strips were placed between two 4 *mm* thick Lexan^{TM} plastic sheets and sealed with Parafilm M(R) (Neenah, WI). A 5 *mm* hole was drilled into one of the phloem strips to allow for beetle introduction during trials. *Pinus resinosa* phloem was collected from field sites near Flagstaff, AZ. Techniques for phloem collection are in Aukema and Raffa (2004b). Briefly, healthy *P. resinosa* trees were felled, their bark was peeled of phloem in large continuous sheets, and the phloem was vacuum-sealed and refrigerated at approximately 4°C for 48 *h* until use in experimentation. *Ips grandicollis* were collected from logs infested near Flagstaff, AZ, and allowed to emerge in a laboratory at Northern Arizona University.

At the conclusion of this experiment, we allowed phloem sandwiches to thaw, measured the distance beetles tunneled from their initial entry point, excavated beetles, and re-tabulated the mite community remaining on each beetle. We individually compared measures of beetle tunneling length and mean proportion of mite detachment for each mite species or species grouping and experimental duration treatment categories with ANCOVA. Models describing mean proportion of mite detachment fit best when a numerical factor for beetle tunnel distance was included in the structure.

Objective 3: Do cues associated with host trees mediate mite dispersal within trees?

We exposed the phoretic mite, *D. quadrisetus*, to a series of two-way choice assays in an olfactometer to assess the attractiveness of volatiles of beetle-colonized and uncolonized phloem. Phloem trials consisted of pairwise choices among the following odor sources: fresh *P. resinosa* phloem, beetle-colonized phloem, or blank control. Pairwise comparisons included control vs. control, control vs. fresh phloem, control vs. colonized phloem, and fresh phloem vs. colonized phloem. Fresh phloem was cut from healthy mature *P. resinosa* near Mazomanie, WI, and stored at approximately 4°C for less than one month. Infested phloem was collected from *P. resinosa* logs housing laboratory beetle colonies. All phloem was presented as 4×10 *mm* strips placed in odor chambers. Controls were presented as empty odor chambers.

Behavioral two-way choice assays were conducted in a glass y-tube olfactometer. The olfactometer system contained the following components assembled in-line from a closed system starting at an air supply and ending at a vacuum line; a carbon filter (VAS Systems, Rensseaer, NY), a three-part air filter (VAS Systems), a humidification chamber, a multichannel air flow regulator (VAS Systems), glass odor source chambers handblown from 1 *cm* borosilicate threaded stock (UW Glass shop, Madison WI), and a handblown glass y-tube arena with 5 *cm* arms. All air filtration components were connected with brass tubing and the remaining olfactometer components were connected with Teflon^{TM} tubing (VAS Systems).

All trials were conducted at 25°C under red lighting to mimic the sensation of dark conditions within beetle galleries (Belan and Bull 1995, Briscoe and Chittka 2003). Adult *D. quadrisetus* were mechanically removed from *I. pini* that emerged from the laboratory colony, and refrigerated for up to three hours. All mites were allowed a minimum of 15 minutes to acclimate to assay conditions prior to experimentation. Mites were introduced into the base of

the y-tube olfactometer using a paintbrush modified so that only a few bristles remained. Mites were presented with a choice of odor sources at 250 μL per hour from each arm of the olfactometer. After introducing assay mites, we sealed off the olfactometer with a vacuum line drawing 500 μL per hour.

During trials, mites were faced with two sets of dichotomous choices. First, mites could move either towards or away from the odor sources. Next, mites that moved towards the odor sources could demonstrate a preference for a particular odor source by moving into the chamber emitting that odor. Trials ended when a mite either moved away from the odor sources and exited the y-tube arena, or entered a chamber containing an odor source. Any mite that did not make either set of choices after 10 minutes was removed from the arena and the trial was terminated. Each mite was used for a single trial. Selection of the pairwise comparison and the orientation of odor sources within the olfactometer were randomized. All components of the olfactometer were cleaned with Alconox cleaner (White Plains, NY), rinsed with de-ionized distilled water followed by 95% EtOH, and allowed to thoroughly air dry between trials.

Mite responses to each set of paired odor sources were scored in two steps. Initial entry or exit frequencies were compared with χ^2 goodness-of-fit tests. For individual mites that entered the arena, choice frequencies were compared between the odor sources with χ^2 goodness-of-fit. Mites that entered the arena but made no choice between odor sources were removed from the analyses.

Objective 4: How do fungi that colonize trees killed by bark beetles influence mite success?

We assessed the effects of symbiotic and opportunistic fungi on the feeding and oviposition success of *H. arborsignis* by allowing mites to develop on *P. resinosa* phloem inoculated with one of five treatments in no-choice assays: *Ophiostoma ips* (Rumbold), three

morphotypes of *A. fumigatus*, or a broth control (non-specific fungal growth). Square 5×5 *cm* pieces of *P. resinosa* phloem were cut from fresh logs from trees felled near Mazomanie, WI. We cut an approximately 2×2 *mm* central notch half way through the total thickness in each piece of phloem. Phloem pieces were then spread-inoculated with 500 μL of liquid cultures of either *O. ips*, wild type *A. fumigatus* (WT), *A. fumigatus* with overexposed mycotoxin (OE) or *A. fumigatus* with a mutated LaeA gene and minimal mycotoxin production (ΔLaeA), or with 2% malt extract broth (control). All inoculations occurred in a laminar flow hood, and phloem was then immediately placed in Parafilm M(R)-sealed 100 *mm* pyrex Petri dishes for seven days at 25°C in a 0:24 light:dark growth chamber. Liquid fungal cultures of *O. ips* and *A. fumigatus* (wild type, OE, and ΔLaeA) were maintained in 2% malt extract broth. All fungal strains were acquired from N.P. Keller at the University of Wisconsin – Madison. A full description of the *A. fumigatus* WT, OE and ΔLaeA strains are provided in Bok and Keller (2004).

Ten *H. arborsignis* were introduced into the central notch of each piece of colonized phloem and placed in 100 *mm* glass Petri dishes sealed with Parafilm M(R). The *Histiogaster arborsignis* for this assay were reared on naturally colonized phloem collected from laboratory-reared *Ips* colonies or on *A. fumigatus* OE colonized phloem, and thus were experienced on either *Ips*- or *A. fumigatus* OE-colonized phloem. Petri dishes were then sealed with parafilm, and placed back in the growth chamber. After 21 days, all samples were frozen at -20°C to stop mite reproduction and movement. We counted all mites (all life-stages besides eggs) and egg abundance at nine evenly spaced 3 *mm* circular regions placed in a grid pattern over the phloem. We also estimated the percentage of phloem visibly covered by fungi on each piece of phloem. We compared the average number of mites and mite eggs between treatments with analysis of

co-variance (ANCOVA), where percent fungal growth and previous feeding experience of mites were incorporated as a standardizing covariate.

Results

Objective 1: Do mites show preferences for attachment on specific host beetle body parts?

Most mite species demonstrated a distinct preference for a particular location on which they attach to *I. grandicollis* (Table 1). For all mite species pooled, the average numbers of phoretic mites ± standard error per beetle were 12.9 ± 1.7 on the elytral declivity, 7.2 ± 0.8 underneath elytra, 4.4 ± 0.5 on legs or ventral abdomen near where the legs attach, and 2.7 ± 0.5 elsewhere on the outer body (Fig. 2A).

We found an average of 6.6 ± 0.9 *Dendrolaelaps* spp. per beetle underneath elytra, which was 18× more abundant than any other location (Fig. 2B). An average of 9.7 ± 1.5 *I. confusus* were found in the elytral declevity of host beetles, which was 13.5× more than in any other attachment location (Fig. 2C). *Tarsonemus* spp., *Histiostoma* spp. and *T. australis* were found in relatively low abundances on beetles as compared to other mite species. *Tarsonemus* spp. (1.9 ± 0.5 mites per beetle) attached to mite legs or ventral abdomen near where the legs attach 9.5× more often than on the elytral declivity or elsewhere on the outer body (Fig. 2D). *Tarsonemus* spp. were not found underneath the elytra on any beetles (Fig. 2D). *Histiostoma* spp. occurred more commonly on the legs or ventral abdomen near where the legs attach (1.5 ± 0.4 mites per beetle) than on either the elytral declivity or underneath the elytra (Fig. 2E). *Histiostoma* spp. distribution was relatively nonspecific compared to other phoretic mite species (Fig. 2E). *Trichouropoda australis* was found at 2.1 ± 0.5 mites per beetle in the elytral declivity and was rarely found elsewhere on beetles (Fig. 2F).

Objective 2: Do cues associated with host beetles and trees mediate detachment?

Dendrolaelaps quadrisetus detached from decapitated and injured beetles more often than uninjured control beetles (χ^2 = 11.44, df = 2, *P* = 0.003). At least one *D. quadrisetus* detached from decapitated *I. pini* in 16 of 16 trials, and from injured beetles in 13 of 14 trials, compared to none from uninjured control beetles in 14 trials (Fig. 3A). Detachment of *D. quadrisetus* was highest from decapitated beetles at approximately 97%, while only 73% detached from injured beetles (Kruskal-Wallis χ^2 = 7.16, df = 1, *P* = 0.007) (Fig. 3B). Those *D. quadrisetus* that detached left decapitated beetles almost 20 minutes sooner than injured beetles, although this was only marginally statistically significant (Kruskal-Wallis χ^2 = 2.75, df = 1, *P* = 0.097) (Fig. 3C). Other mite species showed no difference in detachment rate (χ^2 = 1.37, df = 2, *P* = 0.505) (Fig. 3D), proportion of mites detaching (Kruskal-Wallis χ^2 = 1.74, df = 2, *P* = 0.420) (Fig. 3E), or time before detachment (Kruskal-Wallis χ^2 = 1.67, df = 2, *P* = 0.435) (Fig. 3F) among decapitated, injured, and uninjured beetles.

Iponemus confusus detachment during host beetle colonization experiments was influenced by phloem treatments (*F* = 9.80, df = 2, *P* = < 0.001), tunneling duration (*F* = 14.06, df = 1, *P* = < 0.001), and beetle tunnel length (*F* = 0.82, df = 1, *P* = < 0.001) (Fig 4A). Across all treatment types, mites detached from beetles at a rate of 1.59% per hour and 3.90% per millimeter tunneled by host beetles. While mite detachment was similar across the duration of the experiment, a 2.5× higher proportion of mites detached from beetles in autoclaved phloem amended with α-pinene than in other treatment types. For example, after the first three hours of tunneling, model estimates show approximately 16-17% of *I. confusus* detached from host beetles in control and autoclaved phloem, while nearly 43% had detached from host beetles in autoclaved phloem amended with α-pinene. We found no evidence for interactive effects between phloem treatments, tunneling duration and beetle tunnel length. As *I. confusus* was by

far the most abundant phoretic mite species in this experiment, patterns of total mite detachment largely reflect this species, so pooled analysis is not shown. The only other species with high enough abundance to analyze was *Elattoma* sp., which demonstrated erratic behavior over both time and phloem treatments (Fig. 4B). The tunneling distance of beetles (Fig. 4C) was influenced by time (F = 92.45, df = 1, P = 0.006), with beetles tunneling approximately 0.14 *mm* per hour. Beetle tunneling rate was not influenced by phloem treatment type (F = 15.21, df = 2, P = 0.525). We did not observe any differences in beetle or mite behavior or condition among treatments.

Objective 3: Do cues associated with host trees mediate mite dispersal within trees?

Dendrolaelaps quadrisetus was attracted to volatiles from host tree phloem in the y-tube olfactometer (Fig. 5). Its attraction was stronger to previously infested over un-infested phloem. Mites that oriented into the experimental arena chose fresh phloem over control in 100% of the trials (χ^2 = 8.00, df = 1, P = 0.005), infested phloem over control in 89% of the trials (χ^2 = 5.44 , df = 1, P = 0.020), and infested over fresh phloem in 86% of the trials (χ^2 = 3.57, df = 1, P = 0.059) (Fig. 5). *Dendrolaelaps quadisetus* that entered the experimental arena during the control vs. control trials, oriented randomly (χ^2 = 0.14, df = 1, P = 0.706) (Fig. 5).

Objective 4: How do fungi that colonize trees killed by bark beetles influence mite success?

After 21 days, the abundance (F = 4.84, df = 4, P = 0.003) (Fig. 6A) and egg density (F = 9.84, df = 4, P < 0.001) (Fig. 6B) of *H. arborsignis* varied significantly among treatments with varying sources of fungi. *Histiogaster arborsignis* adult density was highest on *O. ips* (23.7 ± 8.67 mites per mm^2) colonized phloem and lowest on broth control (2.70 ± 1.73 mites per mm^2) phloem. *Histiogaster arborsignis* egg density was almost 50× higher on *O. ips* than any other

treatment type. We found no difference in *H. arborsignis* abundance or egg density among the three varieties of *A. fumigatus*. There was no visual difference in percent fungal coverage among treatments (Fig. 6C).

Mites that experienced *A. fumigatus* OE-inoculated phloem prior to experimentation were not substantially different from those that previously experienced naturally infested phloem (F = 2.67, df = 1, P = 0.109). We found no interaction between fungal treatment and mite experience in relation to *H. arborsignis* density among treatments (F = 0.266, df = 4, P = 0.899). We found no interaction between mite experience (F = 0.315, df = 1, P = 0.577) or fungal treatment type (F = 0.402, df = 4, P = 0.806) in relation to egg densities.

Discussion

These results show that phoretic mites use cues associated with both host beetles and trees during detachment, attachment, and within-tree movement. The behavioral responses and exploitation of cues by mites are context-dependent, and vary among mite species. During their development within host trees, consumption of fungi vectored by the beetle hosts can improve reproductive performance of some mite species. In contrast, consumption of the opportunistic fungi *A. fumigatus*, that invade beetle galleries, confers phoretic mites with little to no benefit.

Most mite species are highly selective in the location on which they attach to their host beetles. This selective behavior appears to correspond to specializations in mite morphology and functional roles. For example, *Dendrolaelaps* spp. which actively feed on nematodes during phoretic transport (Moser 1975, Kinn 1984), were found mainly underneath the elytra, where nematodes are abundant (Cardoza et al. 2008). *Iponemus confusus* are relatively small and have few morphological adaptations for host attachment compared to other mites. *Iponemus confusus* are most often found in the spatial crease provided by the elytral declivity of their host *Ips* spp.

Attachment in the elytral declivity may reduce incidental mechanical removal during beetle flight and host searching. *Trichouropoda* spp., though dorsal-ventrally flattened in a manner similar to other mites, are generally much larger and would be mechanically removed due to perturbation during phoretic transport if not for selective attachment in the elytral declivity as well as strong attachment via a glue-like substance secreted from their haustorial stalk (Bajerlein and Witaliński 2012).

Specialization in the locations on which mites attach to beetles provides each mite with a spatial niche during phoretic transport, when attachment space could be a limiting resource. For example, mites on *Nicrophorus* beetles show specialization in the selection of their phoretic hosts (Brown and Wilson 1992). Mites associated with pine-colonizing bark beetles are mainly generalists in their host beetle selection (Pfammatter 2015), and as such are prone to interspecific competition. Some mites phoretic on other types of arthropods, such as those parasitic on harvestmen, are known to reduce interspecific competition by specializing in the location on which they attach to their hosts (McAloon and Durden 2000). Mites in our system may spatially partition attachment location to mediate interspecific competition during phoresy.

Cues resulting from beetle decapitation and injury elicit detachment behavior, suggesting that mites can assess the condition of their beetle host and detach when it can no longer transport them to an environment suitable for feeding and reproduction. Mites in other systems, such as *Poecilochirus* phoretic on *Nicrophorus* burying-beetles, are able to select and attach to hosts associated with higher fecundity (Grossman and Smith 2008). However, less is known about the ability of mites to assess the relative quality of beetle hosts on which mites have already attached, and what cues orient mites after they detach. Once mites detach due to degrading host quality, they may reattach to another host beetle or enter the tree for feeding and reproduction.

For example, when *Ips* colonize trees, the predator *Thanasimus dubius* (F.) is strongly attracted to its pheromones, and frequently decapitate them. Mites that detach quickly from their decapitated host would have the opportunity to attach to these predators to gain transport to food resources, or access the phloem through beetle entrance holes. Further research is needed to identify the specific cues that trigger detachment during beetle host degradation and to determine mite behavior post detachment.

Both mite detachment from their beetle hosts and dispersal within their host trees are mediated by tree-related cues. Mites use cues from their environment, including moisture content, food availability, and overcrowding to trigger transformations in morphology and phoretic transport (Binns 1982). For example, *Polyphagotarsonemus* mites that are phoretic on whiteflies detach more readily when whiteflies are near plant hosts that are correlated with increased mite reproductive success (Alagarmalai et al. 2009). Similarly, we found that the major secondary volatile of red pine, α-pinene, can provide a detachment cue for *I. confusus*. However, based on our results, responses to α-pinene appear to be largely species dependent.

Once detached from their host beetles, *D. quadrisetus* are attracted to volatiles from host tree tissue, particularly from beetle-colonized phloem. While we did not identify specific chemicals associated with these behaviors, both cues originating from trees and beetles appear to be important. Future experiments should investigate responses to specific host compounds and context-dependent behaviors such as how mites respond to tree associated cues under different stages of beetle association.

Fungi that are vectored by beetles and subsequently colonize beetle-killed trees can benefit mite reproductive success. In addition, fungal distribution within host trees may be influential to mites during colonization. Fungi vectored by beetles have been shown to confer

nutritional benefits to many species of bark beetles (Six and Paine 1998, Bleiker and Six 2007) and to some of their associated mites (Hofstetter et al. 2006). However, mites did not benefit from the antagonistic fungus *A. fumigatus*, which opportunistically invades *Ips* galleries. Beneficial and antagonistic fungi often have patchy distributions throughout mite habitat. While we did not perform any choice assays to determine mite preference for egg laying and feeding between fungal patches, we did observed mite avoidance of non-specific fungal growth within *O. ips* no-choice experimental samples. Our findings suggest that mites benefit from an ability to discriminate between patches containing *O. ips* versus other fungi.

These results provide insights into behaviors of mites associated with *I. grandicollis* and *I. pini* during the colonization and development phases of both beetles and mites. However, we currently lack mechanistic understanding of these behaviors. Future investigations should be directed at improving linkages among these various behaviors, ecological pathways, and fitness consequences. Further experimentation with additional mite species would prove insightful into the community context of these relationships.

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Table 1. ANOVA statistics and pairwise Tukey *P* values for locations on *I. grandicollis* where various species of phoretic mites attached. N = 20 adult beetles obtained from *P. resinosa* stands in Wisconsin in 2009. Beetles' bodies were categorized into four attachment locations: legs and nearby ventral thorax where the legs attach (Legs), elytral declivity, outer body (Body), and under elytra.

	ANOVA			**Pairwise Comparison *P* values**					
Mite Species	**F**	**df**	***P* value**	**Legs – Elytral Declivity**	**Body vs. Elytral Declivity**	**Under Elytra vs. Elytral Declivity**	**Body vs. Legs**	**Under Elytra vs. Legs**	**Under Elytra vs. Body**
All Mites	20.035	3	<0.001	<0.001	<0.001	<0.001	0.627	0.191	0.010
Dendrolaelaps spp.	37.898	3	<0.001	0.943	>1	<0.001	0.961	<0.001	<0.001
I. confusus	31.017	3	<0.001	<0.001	<0.001	<0.001	0.996	0.941	0.860
Tarsonemus spp.	10.534	3	<0.001	<0.001	0.994	0.981	<0.001	<0.001	0.919
Histiostoma spp.	4.567	3	0.005	0.032	0.662	0.920	0.353	0.005	0.289
T. australis	15.700	3	<0.001	<0.001	<0.001	<0.001	>1	0.993	0.993

Figure Legends

Figure 1. Interactions among mites, beetles and their host trees. A) Phoretic mites, mostly *H. arborsignis*) attached to an adult *I. pini.* B) *Histiogaster arborsignis* transforming from protonymph (left) to duetonymph (right). C) *Histiogaster arborsignis* feeding on blue-stain fungi inoculated *P. resinosa* phloem. D) A flowchart describing the stages of mite and beetle development and their relationship with our experimental objectives.

Figure 2. Average numbers ± standard error of phoretic mites attached to various locations on *I. grandicollis* (n = 20) captured in Wisconsin *P. resinosa* stands in 2009: within the elytral declivity, on the legs and ventral thorax (Legs), on any remaining part of the outer body (Body), and underneath the elytra. Average mite loads per location are presented for A) Total Mites, B) *Dendrolaelaps* spp., C) *I. confusus*, D) *Tarsonemus* spp., E) *Histiostoma* spp. and F) *T. australis.* Letters above bars represent means separation as calculated with pairwise Tukey comparisons.

Figure 3. Effect of host beetle condition on detachment of phoretic mites. Data are presented for the proportion of beetles from which phoretic mites detached (top row), the proportion of mites detaching from beetles (middle row), and the time to first mite detachment among treatments (bottom row) for *D. quadrisetus* (left column) and mite species other than *D. quadrisetus* (right column).

Figure 4. Effect of host tree chemistry on detachment of phoretic mites from *I. pini.* Phloem was un-manipulated (control), autoclaved, or autoclaved and amended with racemic □-pinene. A higher proportion of *I. confusus* detached from beetles that were introduced into autoclaved phloem amended with □-pinene than from beetles introduced to un-manipulated or autoclaved phloem (F = 9.80, df = 2, P = < 0.001). A) Detachment increased with the duration of trials (F = 14.06, df = 1, P = < 0.001). B) Patterns of detachment by *Elattoma* sp. were unrelated to phloem

category or duration of beetle tunneling. C) Beetle tunnel lengths did not differ among phloem treatments (F = 15.21, df = 2, P = 0.525), but increased with time (F = 92.45, df = 1, P = 0.006). n = 5 *I. pini* per time × treatment combination. Lines on each curve display linear model estimates for the relationship between proportions of mites detaching or beetle tunneling distance and phloem treatments over time.

Figure 5. Response of *D. quadrisetus* to phloem volatiles in a two-way choice assay using a y-tube olfactometer. Data show the number of mites that selected each odor within each pairwise comparison. The proportion of mites that chose to enter the y-tube arena for each group of experiments was 3 of 10 for control vs. control (χ^2 = 1.60, df = 1, P = 0.206), 2 of 10 for control vs. fresh phloem (χ^2 = 3.60, df = 1, P = 0.058), 3 of 12 for control vs. infested phloem (χ^2 = 3.00, df = 1, P = 0.083), and 4 of 11 for fresh vs. infested phloem (χ^2 = 0.82, df = 1, P = 0.366).

Figure 6. Effects of fungi on reproductive success of *H. arborsignis* under no-choice conditions, Phloem squares were inoculated with one of five treatments: broth (control, nonspecific fungal growth) (n = 10), *O. ips* (n = 10), *A. fumigatus* wild type (A-WT) (n = 11), *A. fumigatus* ΔLeaA (n = 11), and *A. fumigatus* over exposed toxin (A-OE) (n = 11) fungal types. Mites reproduced more successfully on *O. ips* than any other fungal treatment, based on by measures of A) mite density (F = 2.649, df = 4, P = 0.045) and B) egg density (F = 5.488, df = 4, P = 0.001). C) Percent fungal growth (percent visual coverage) did not differ among fungal treatments.

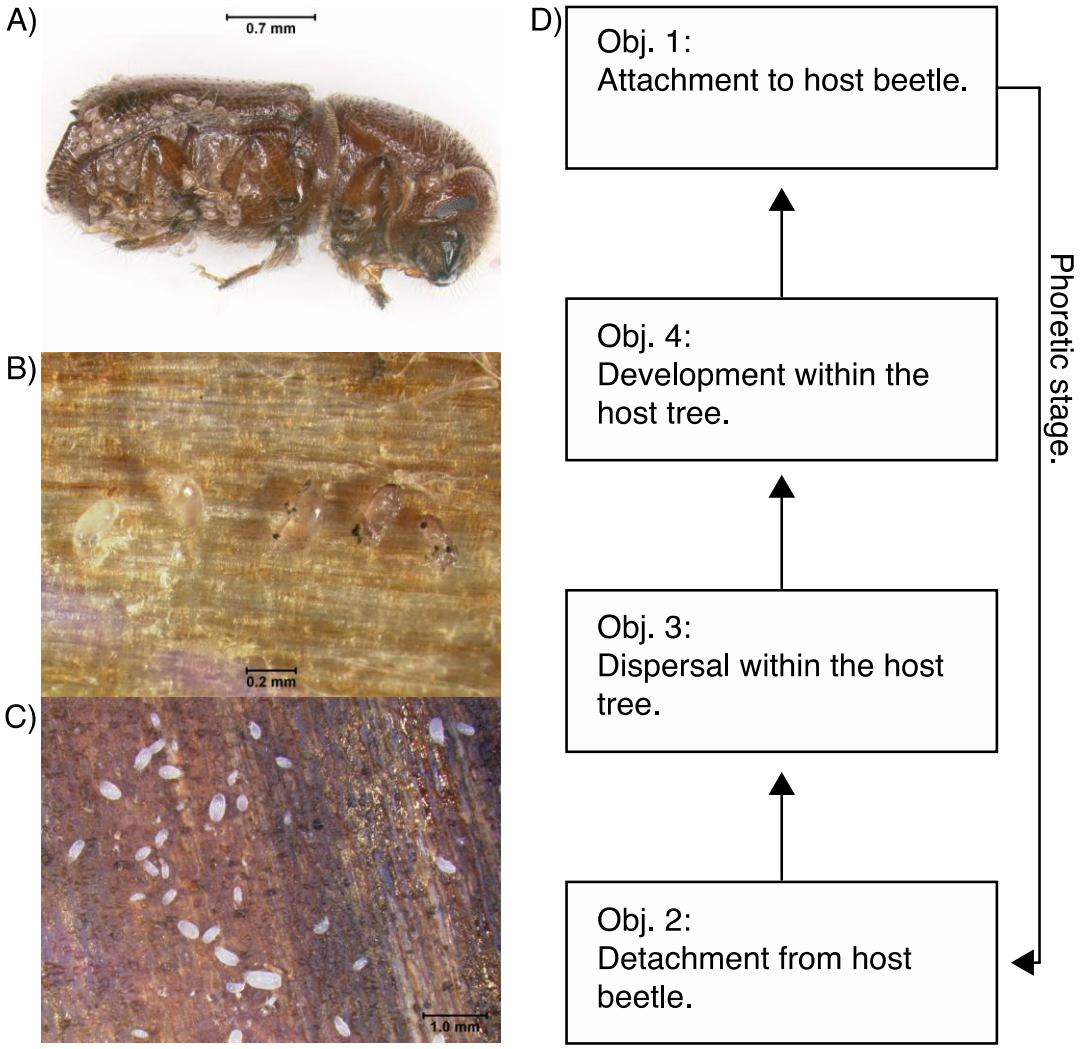
A)
0.7 mm
B)
0.2 mm
C)
1.0 mm
D)
Obj. 1:
Attachment to host beetle.
Obj. 4:
Development within the host tree.
Obj. 3:
Dispersal within the host tree.
Obj. 2:
Detachment from host beetle.
Phoretic stage.

Figure 1.

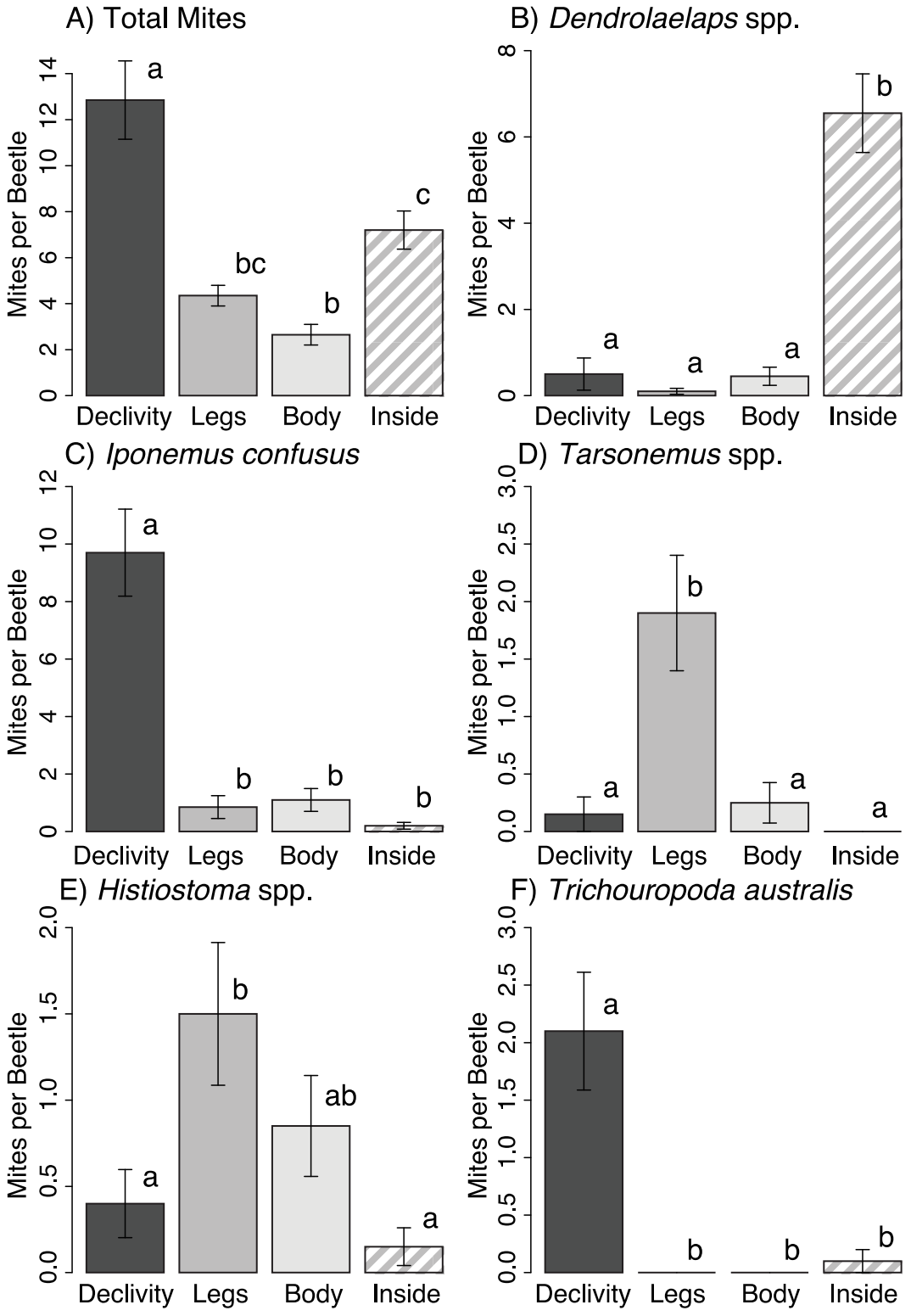
A) Total Mites
Mites per Beetle
0 2 4 6 8 10 12 14
a
bc
b
c
Declivity Legs Body Inside
B) Dendrolaelaps spp.
Mites per Beetle
0 2 4 6 8
a
a
a
b
Declivity Legs Body Inside
C) Iponemus confusus
Mites per Beetle
0 2 4 6 8 10 12
a
b
b
b
Declivity Legs Body Inside
D) Tarsonemus spp.
Mites per Beetle
0.0 0.5 1.0 1.5 2.0 2.5 3.0
a
b
a
a
Declivity Legs Body Inside
E) Histiostoma spp.
Mites per Beetle
0.0 0.5 1.0 1.5 2.0
a
b
ab
a
Declivity Legs Body Inside
F) Trichouropoda australis
Mites per Beetle
0.0 0.5 1.0 1.5 2.0 2.5 3.0
a
b
b
b
Declivity Legs Body Inside

Figure 2.

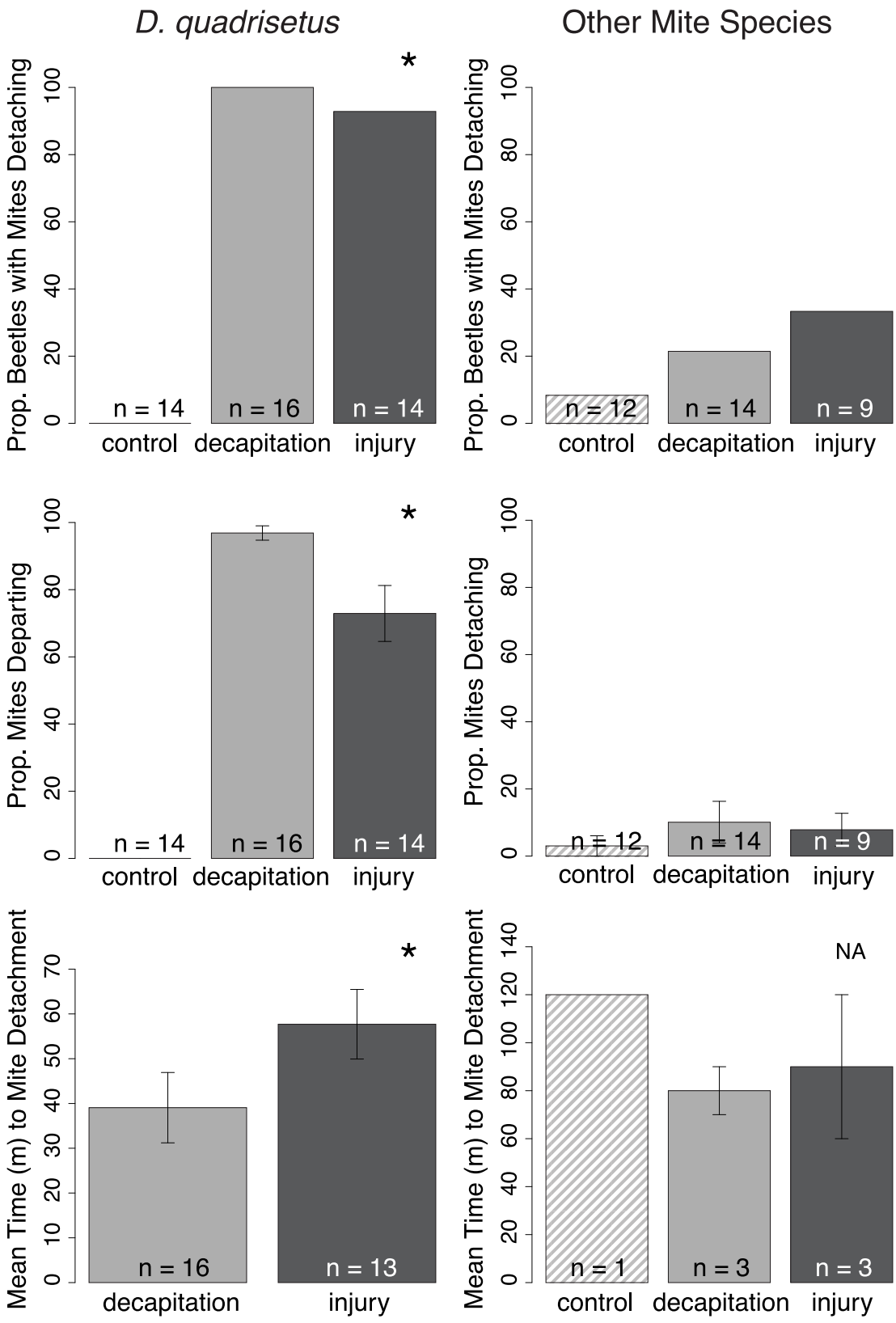
D. quadrisetus
Other Mite Species
Prop. Beetles with Mites Detaching
Prop. Mites Departing
Prop. Mites Detaching
Mean Time (m) to Mite Detachment
n = 14
n = 16
n = 14
n = 12
n = 14
n = 9
n = 16
n = 13
n = 1
n = 3
n = 3
control
decapitation
injury
*
NA

Figure 3.

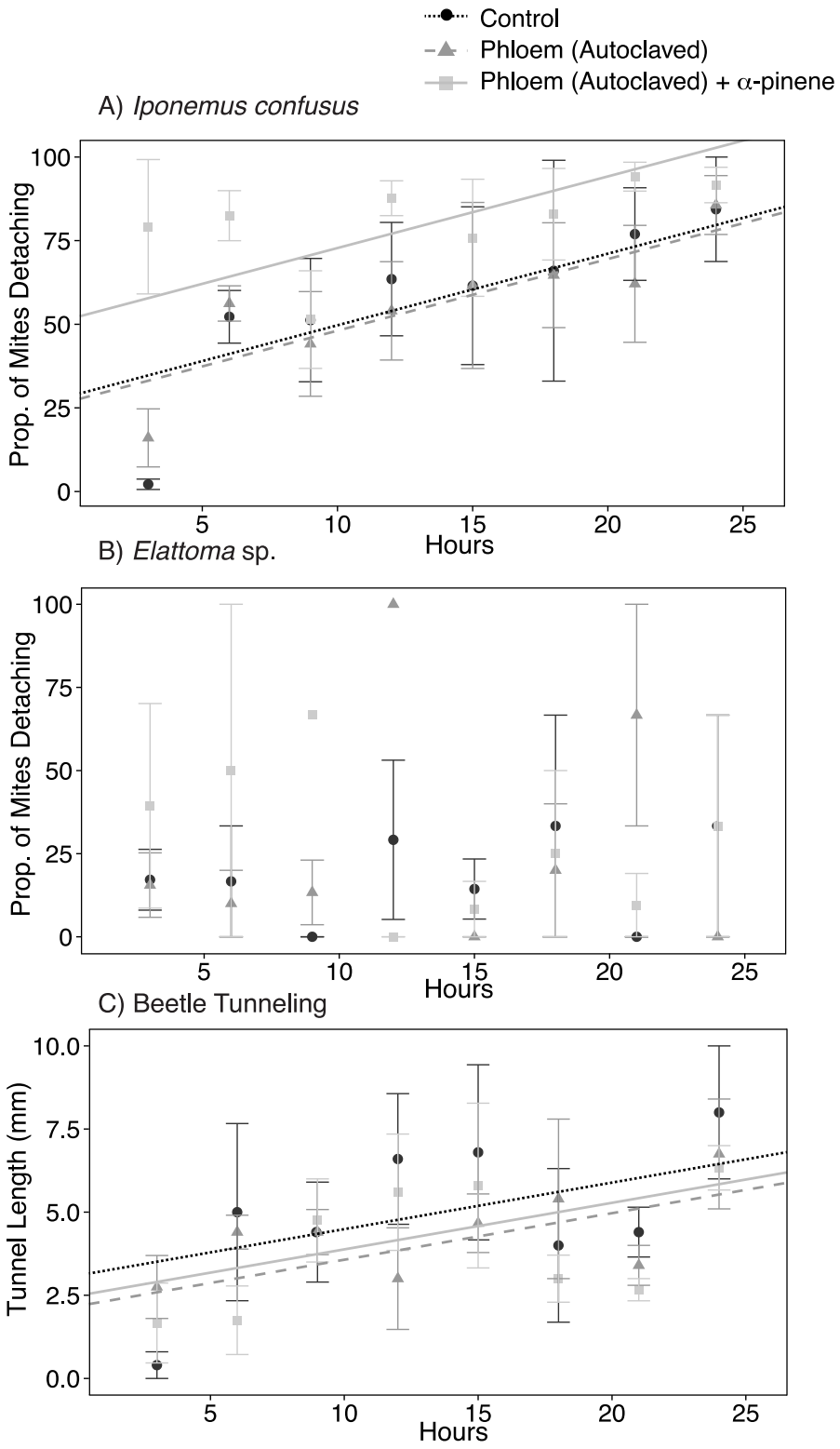
Control
Phloem (Autoclaved)
Phloem (Autoclaved) + α-pinene
A) Iponemus confusus
Prop. of Mites Detaching
Hours
B) Elattoma sp.
Prop. of Mites Detaching
Hours
C) Beetle Tunneling
Tunnel Length (mm)
Hours

Figure 4.

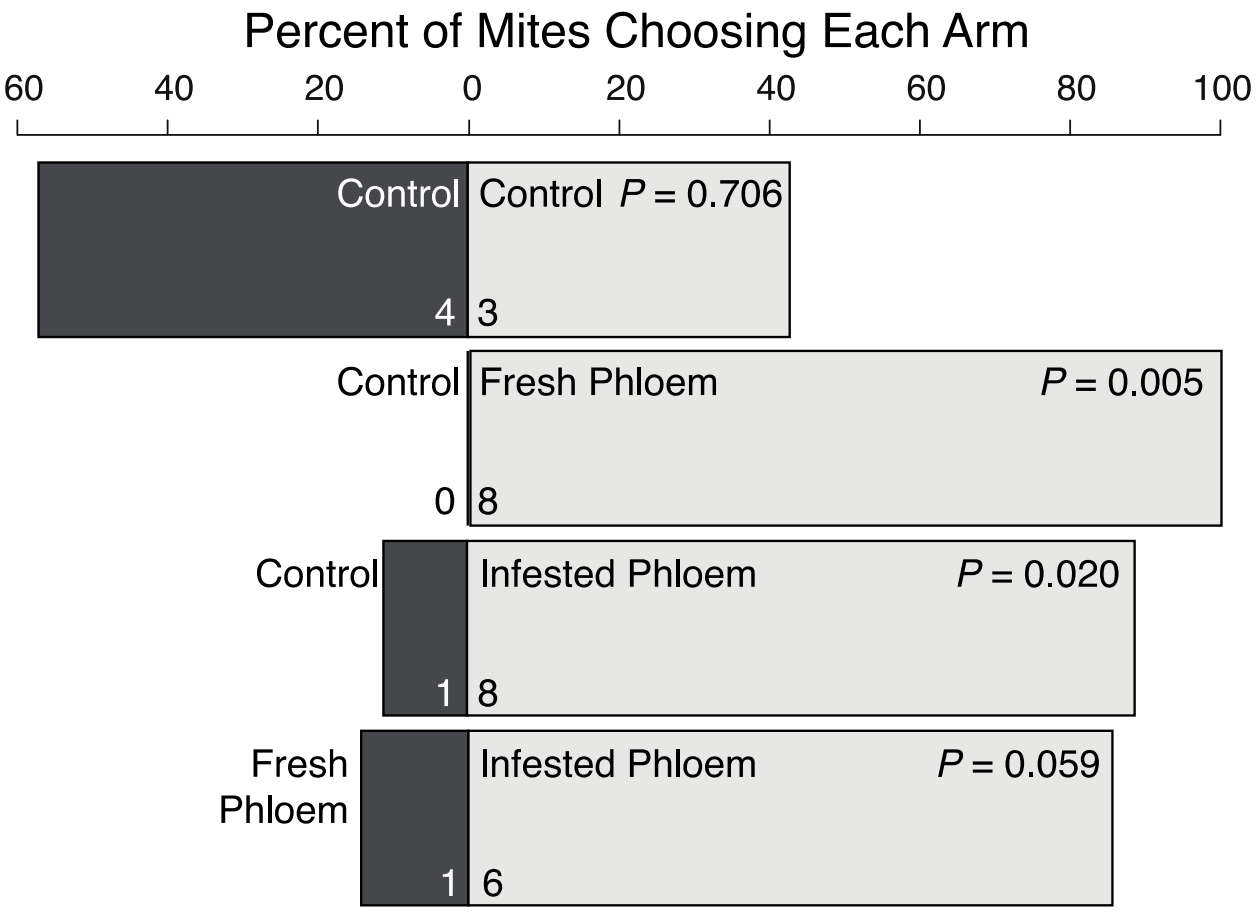
Percent of Mites Choosing Each Arm
60
40
20
0
20
40
60
80
100
Control
Control P = 0.706
4
3
Control
Fresh Phloem
P = 0.005
0
8
Control
Infested Phloem
P = 0.020
1
8
Fresh Phloem
Infested Phloem
P = 0.059
1
6

Figure 5.

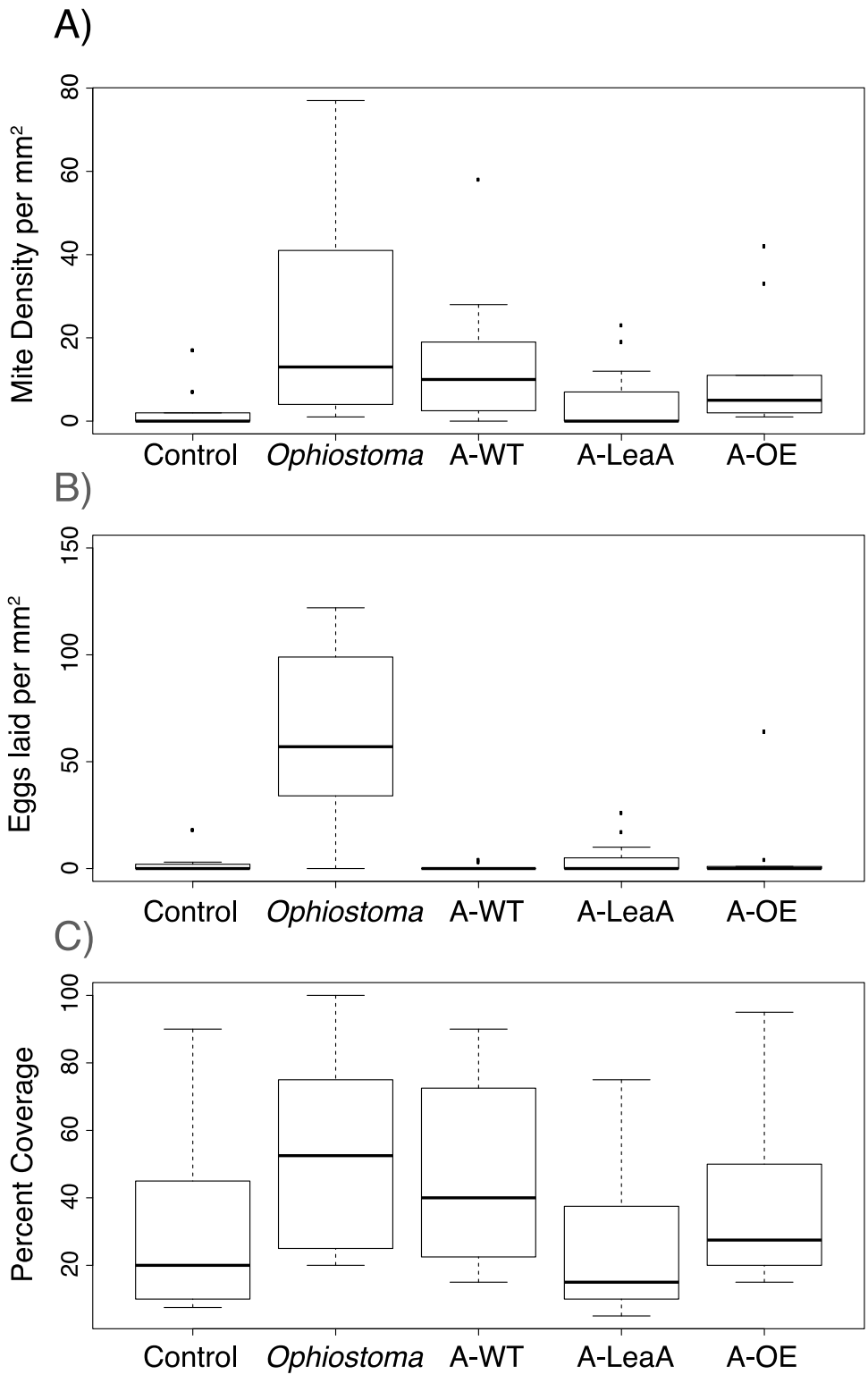
A)
Mite Density per mm²
0
20
40
60
80
Control
Ophiostoma
A-WT
A-LeaA
A-OE
B)
Eggs laid per mm²
0
50
100
150
Control
Ophiostoma
A-WT
A-LeaA
A-OE
C)
Percent Coverage
20
40
60
80
100
Control
Ophiostoma
A-WT
A-LeaA
A-OE

Figure 6.

Thesis Conclusions

1. **Most subcortical beetles visiting pine habitat are associated with phoretic mites.** A diverse community of phoretic mites was recovered from numerous genera of pine-associated beetles, including but not limited to *Dendroctonus*, *Ips*, and *Thanasimus*. Approximately 75% of *Ips pini* in Wisconsin carried mites, representing 20 species.
2. **Patterns of pairing between beetles and mites fluctuate seasonally and with geographic region.** Seasonal effects and geography contribute to variation in the incidence and abundances of phoretic mites on pine engravers in Wisconsin. Beetles emerging later in the season had higher abundances of phoretic mites. These increases are likely due to improved environmental conditions (i.e. temperature and moisture) within mite habitat throughout the course of the season. Effects of geographic variation on beetle-mite pair formation are likely driven by differences in weather, beetle species colonization, and tree species availability.
3. **The community of bark beetles associated with pine habitat has a highly overlapping set of phoretic mites.** We characterized the phoretic mite communities of 36 bark beetle species in three regions: southern Wisconsin, northern Arizona, and northern Georgia. The species richness of the community of mites that these beetles harbor is similar to that found on *I. pini* alone. Collectively, our studies demonstrate substantial overlap in phoretic mite community membership among bark beetle species. Most phoretic mites were found associated with at least three, and up to twenty, beetle species.

4. **Highly specialized phoretic relationships between individual beetle and mite species are uncommon.** Phoretic associations between beetles and mites were found to be relatively diffuse within and across both beetle species and regions. Mites appear to be largely generalist in their selection of a host beetle for transport. Generalist behavior by mites in host selection of beetles is likely related to the common usage of pine habitat by multiple beetle species, and the overlapping availability of insect vectors between tree resources in both space and time. Thus, particular phoretic pairings between beetles and mites are difficult to predict among various regions and beetle species.
5. **Reproductive success of *Ips* does not seem to be strongly influenced by phoretic mites.** In field populations, I found positive correlations between the incidence and abundance of several species of mites, including *Histiostoma* spp., *D. quadrisetus*, *I. confusus*, *T. australis*, and *Tarsonemus* spp., and the emergence of *Ips* from trees. Alternately, in laboratory experiments in which I manipulated mite prevalence, there were no strong positive or negative interactions between phoretic mites and bark beetles. This suggests little causal link between mites and beetle reproductive success. Rather, the reproductive success of most phoretic mites appears largely driven by conditions that likewise favor reproduction of their host bark beetles, suggesting primarily commensal relationships between the two groups. Biotic factors such as inter- and intra-specific competition within tree galleries, and other drivers, such as the availability, susceptibility and substrate quality of host trees, predators, and weather, likely play more influential roles in beetle reproductive success than phoretic mites.

6. **Most mite species are highly selective in the location onto which they attach to host beetles.** While mites are largely generalist in their selection of host beetle species during phoretic events, they are highly specialized in the mechanisms by which they attach to beetles. Specialization in the locations onto which mites attach to beetles provides each mite with a spatial niche during phoretic transport. This spatial partitioning may reduce intraspecific competition on a resource in which space could be limiting.
7. **Mites use cues associated with both host beetles and trees during detachment and within-tree movement.** Cues arising from beetle decapitation and injury elicit detachment behavior, suggesting that mites can assess the condition of their beetle host, and detach when it can no longer transport them to an environment suitable for feeding and reproduction. Mites utilize cues from their host trees to trigger detachment from beetles, and to orient movement within trees. For example, the predominant secondary volatile in red pine, α-pinene, can elicit detachment by *I. confusus*. Once detached from their host beetles, *D. quadrisetus* are attracted to volatiles from host tree tissue, particularly from beetle-colonized phloem. Given their largely generalist host selection strategies, mites are more likely to utilize cues associated with general bark beetle behavior than species-specific cues. The behavioral responses and exploitation of cues by mites are context-dependent, and vary among mite species.
8. **Mites can benefit from beetle-vectored, but not opportunistic, fungal symbionts.** During their development within host trees, consuming fungi vectored by the beetle hosts can improve reproductive performance of some mite species.

In contrast, consumption of opportunistic fungi that invade beetle galleries confers phoretic mites with little to no benefit.

9. **Mites appear largely driven by bottom-up ecological processes and so may be more likely to be habitat- than vector-specific.** Collectively, these experiments appear to describe a system in which mites largely benefit from beetle transport, but have a relatively low impact on beetle activities and reproductive success. Mites in this system can generally be classified as commensal in their relationship with bark beetles.

Appendix A

Phoretic mites associated with bark beetles feed and reproduce within beetle-engineered habitats. Mites are poorly suited for transport between the patchy, island-like tree resources where they feed and as a result have developed phoretic mechanisms of dispersal. While transport between food resources of these mites is thought to be entirely via their phoretic associations with beetles, it is possiblet that some mite species disperse independently. It is also possible, that the duff environment is suitable for successful feeding and reproduction of mites usually associated with trees colonized by bark beetles. We sampled the duff of red pine sites in Wisconsin where whe had previously found mites phoreteically associated with beetles captured in flight traps and in killed trees.

We collected duff samples from sites near Boulder Junction and Almond Wisconsin in September 2010. One liter duff samples, which consisted mainly of red pine needles and the top 5 *mm* of soil, were collected from each of 20 randomly selected locations in the pine sites. Samples were brought to the laboratory and placed in Berlese funnels, from which mites migrated into ethanol collection jars. The collection jars were assessed for the presence of mites phoretically associated with bark beetles.

In the 40 samples take across the two sites, we found two instances of individual *H. varia* in different samples from a single site, and no other mites that have been found to be regualarly phoretically associated with pine bark beetles.