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### Do Saproxylic Curculionids Affect the Fitness of Co-occurring Cerambycids?

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**Do Saproxylic Curculionids Affect the Fitness of Co-occurring Cerambycids?**

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A thesis submitted in partial fulfillment of the requirements for the  
Degree of Master of Science in Biology

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## Abstract

Saproxylic insects sometimes coexist in incredibly high numbers under bark and share common resources. Thus, interactions between species are possible and could even explain their coexistence. This study investigates evidence of negative or positive effects of curculionid beetles (Coleoptera: Curculionidae) on cerambycid beetles (Coleoptera: Cerambycidae) that coexisted in dead tree branches in Costa Rica. Co-occurrence analysis and generalized regressions were used to test associations between cerambycid and curculionid species. Three cerambycid species that each co-occurred with a curculionid species were selected to measure fitness. Fitness measures of the cerambycid were compared with abundance of the co-occurring curculionid to assess the possible impact of curculionids on cerambycids. Elytral length and emergence week were measured and compared as proxies for fitness. One-hundred and nineteen species pairs showed significantly positive co-occurrence, and none showed significant negative co-occurrence. The three focal species showed no strong evidence for facilitation or competition with co-occurring curculionids, but there were significant differences in fitness measures and they deserve further study. In a canopy branch of the tree species *Eschweilera biflava*, many species and individuals emerged, and, despite the high density, the cerambycid *N. mutilatus* emerged in high numbers, early and quickly, and with similar elytral lengths as those in other branches. This could be evidence for facilitation by co-occurring ambrosia beetles, or for cryptic variation among conspecific trees.

Keywords: saproxylic, Coleoptera, coexistence, co-occurrence, biotic interactions

## Introduction

The question of how species can coexist in communities has interested ecologists since the early days of the field, and theories explaining the phenomenon are still being posed and scrutinized. Traditionally, niche theory has dominated the discussion. It proposes that species partition resources and those with similar niches will compete with each other until all competitively inferior species are excluded (Hardin 1960). Thus, coexistence of many species can be explained by the development of narrow niches. While niche theory is generally accepted by ecologists, recent theories have challenged its hegemony. Possible explanations for coexistence include trade-offs in competitive ability, (*e.g.*, Duthie et al. 2014, 2015), the intermediate disturbance hypothesis (Wilson 1990), and neutral theory, which was proposed by Hubbell (2000) and posits that species are functionally equivalent in terms of vital rates, and that entirely different factors lead to the assembly of communities (*e.g.*, Andersen 2008).

More recent studies have also included biotic interactions in models of coexistence. Competition is a common focus, due in part to its importance in ecological niche theory (Hardin 1960). Although competition between species is expected to lead to competitive exclusion, when intraspecific competition is stronger than interspecific competition it can promote coexistence (McIntire and Fajardo 2014). Of more recent interest is facilitation, whereby an interaction between two species benefits at least one of the participants and harms neither (Stachowicz 2001). While it is well-established as an important factor in community succession—pioneer species facilitate the establishment of later arriving species—it can also explain surprising patterns of coexistence and increase biodiversity (McIntire and Fajardo 2014) and can result in both broadened niches and positive density-fitness relationships (Bruno et al. 2003, Bulleri et al. 2016). It can also lead to increased abundance and species richness (Gross 2008) particularly in

harsh environments (Xiao et al. 2013). Facilitative interactions even have the potential to ameliorate the impact of climate change on species that are facilitated (Bulleri et al. 2016).

### *Saproxyllic Insects*

Saproxyllic insects (insects that depend directly or indirectly on moribund or dead wood during some part of their life cycle) can coexist at high densities, and so are a useful model system for investigating species coexistence. Saproxyllic insects occupy many parts of a tree including the trunk, branches and stems, stumps or tree hollows, etc. Wood also has several layers that are utilized differently by dead wood-reliant species. From the outside in, the layers are outer bark, inner bark, cambium, sapwood, and heartwood. The outer bark and heartwood are hard to digest, well-defended and have little nutritional value. The inner bark (phloem), on the other hand, has thin-celled walls and is rich in sugars and relatively rich in nutrients (Kirkendall et al. 2015). The cambium is a thin layer of meristematic cells which tends to be consumed quickly. The sapwood transports water and minerals and is soft compared to heartwood.

Resource partitioning—the ghost of competition past—can facilitate coexistence within ephemeral habitats (Takahashi et al. 2005, but see Wertheim et al. 2000). There is strong support for resource partitioning by saproxyllic arthropods, evidenced by clear preferences for certain strata (Ayres et al. 2001, Fassbender et al. 2014, Schlyter and Anderbrant 1993), bark thickness (Grünwald 1986), stem and trunk thickness (Borkowski and Skrzecz 2016, Schlyter and Anderbrant 1993), wood moisture (Iwata et al. 2007), host species and families (Fassbender et al. 2014, Li et al. 2017, Tavakilian et al. 1997), substrate depth (Rotheray 2013) and other physical resources (*e.g.*, Rankin and Borden 1991). Guilds of saproxyllic insects feeding within wood include myelophages (pith feeders), phloophages (phloem feeders), xylophages (wood eaters),

xylomycetophages (fungus farmers), mycetophages (fungus feeders), and predators (Kirkendall et al. 2015, Ulyshen and Šobotník 2018). Thus, even within small branches, saproxylic species can finely partition resources. Species that share niches often opportunistically partition resources by taking up free space (Ayres et al. 2001, Raffa et al. 2015). Within guilds, co-occurring fungivores have been shown to preferentially feed upon specific developmental stages of fungi (Guevara et al. 2000, Kadowaki 2010). Resource partitioning also comes in the form of temporal variation, whereby a species can avoid competition with another by using the resource at a different time (Chesson 2000).

While saproxylic insects do finely partition resources (some utilize sapwood, others feed exclusively on phloem, etc), many species, including those within similar feeding guilds, coexist together in surprisingly high numbers. In data compiled by Brin and Bouget (2018), for example, the number of species that emerged from dead logs ranged from 1 to 64 species. In Costa Rica, one densely colonized branch yielded 3,472 individuals in 59 species (Li et al. 2017; Morillo 2017; Sevilla & Berkov, unpublished data). Species interactions may explain their coexistence.

### *Primary Saproxylic Beetles*

Curculionids (weevils, bark beetles and ambrosia beetles) are common primary saproxylic insects. Bark beetles feed and live primarily within the inner bark of trees and tend to develop quickly (Graham 1925). Some bark beetles and weevils spend their initial larval stages in the phloem, cambium, and sapwood, and then bore deeper into the heartwood as they grow (Kirkendall et al. 2015). Ambrosia beetles, on the other hand, tend to spend their entire larval period in relatively small tunnels in the sapwood because they require little space to cultivate their primary food, ascomycetous fungi (Kirkendall et al. 2015).

Cerambycidae (long-horned beetles) are generally larger beetles that are often early colonizers of dead wood. Cerambycid adults typically oviposit their eggs into dead or moribund wood, and after the eggs hatch they begin to bore into the wood (Tavakilian et al. 1997). Saproxylic cerambycid larvae may feed exclusively on any one of the dead wood layers or a combination of them. Many cerambycids feed initially near the cambium layer, and later bore into the sapwood and sometimes into the heartwood (Ulyshen and Šobotník 2018).

Since curculionids are among the most abundant and earliest colonizers of dead wood (and sometimes are the cause of dead wood), they are likely to shape the ecosystem and impact the insects that follow (Gimmel and Ferro 2018), which could include cerambycids. Observations and experimental studies have revealed both negative and positive interactions between curculionids and cerambycids (Brin and Bouget 2018).

### *Negative Interactions*

Direct competition has been inferred through observed changes in abundance and fitness measures for saproxylic species living in sympatry (Coulson et al. 1976, Rankin and Borden 1991, Victorsson 2012) and has been shown experimentally (Light et al. 1983, Rankin and Borden 1991, Schlyter and Anderbrant 1993). Most evidence for competitive interactions has been shown between bark beetle species (Boone et al. 2008, Light et al. 1983, Rankin and Borden 1991, Schlyter and Anderbrant 1993), probably due to their economic importance, but there is also evidence for competitive interactions between cerambycids and curculionids (Coulson et al. 1976, 1980, Schroeder and Weslien 1994).

Saproxylic insects have strategies to avoid competition. Pheromone emission and detection is a common method of competitor deterrence (Light et al. 1983, Byers 1989, 1993,

Ayres et al. 2001, Mitchell et al. 2015) and avoidance (Gardiner 1957, Byers 1989, Schlyter and Andebrant 1993). Pheromones, however, can also attract heterospecifics (Ayres et al. 2001, Pajares et al. 2004, Allison et al. 2013), which may have positive effects (*e.g.*, Light et al. 1983), but can also increase predation (Boone et al. 2008). Release of host volatiles by early arrivals may also deter heterospecifics (Schroeder 1997). Within dead wood, bark beetles have mechanisms of avoiding direct interactions with each other, reducing inter- and intraspecific competition (Byers 1989), and adults can re-emerge if hosts are too heavily infested by heterospecifics (Byers 1989, Schlyter and Anderbrant 1993).

### *Positive Interactions*

While negative interactions have long been a focus in ecological studies, positive interactions between species are receiving increasing attention. Within deadwood, facilitation can be expected, because improvements in nutritional quality, for example, are important in communities with poor quality resources (Birkemoe et al. 2018). It might explain coexistence for species with similar niche requirements (Valladares et al. 2015), which is often true of species inhabiting dead wood. Curculionids may facilitate cerambycids in several ways, including: creating habitat, signaling host presence or quality, improving host quality, and predator amelioration (Brin and Bouget 2018).

Aggressive bark and ambrosia beetles can kill trees (Raffa et al. 2015), and therefore be both economically and ecologically destructive, but they may positively impact less aggressive saproxylic species (Økland et al. 2009). By killing trees, they create habitat for wood-boring species which often follow close behind (Flamm et al. 1989). Entrance into a host tree by the first to arrive often results in the release of host volatiles, which may include deterrents (Berkov



et al. 2000), but often attract other saproxylic insects (Schroeder 1997). Additionally, bark beetles emit pheromones that attract conspecifics, but are often picked up by heterospecifics, including cerambycids, which utilize host volatiles for host location (Meurer-Grimes and Tavakilian 1997). This helps those heterospecifics locate suitable hosts, which reduces their foraging effort considerably (Ayres et al. 2001, Allison et al. 2013, Davis and Hofstetter 2009, Molander and Larsson 2018, Pajares et al. 2004, Raffa et al. 2015). Experimental evidence has shown that the pheromones emitted by certain curculionid species in conjunction with host volatiles is more attractive to the cerambycid *Monochamus galloprovincialis* than host volatiles alone (Pajares et al. 2004) and that bark beetle pheromone blends attract cerambycids (Allison et al. 2001).

The earliest arrivers create entrance holes in the bark, through which adult insects either oviposit or enter to lay their eggs (Ulyshen and Šobotník 2018). For insects arriving later, the presence of these entrance holes can be an enormous advantage, as they reduce the energy required to bore into the wood (Grünwald 1986). Entrance holes and tunnels can be used by a wide variety of saproxylic insects (Buse et al. 2008, Calderón-Cortés et al. 2011, Kirkendall et al. 2015, Sydenham et al. 2016, Zuo et al. 2016). For example, the cerambycid species *Acanthocinus aedilis* preferred to oviposit into entrance holes previously made by the bark beetle *Tomicus piniperda* (Schroeder 1997).

Deadwood is low in nutrients (Ulyshen and Šobotník 2018) so any improvement in the quality of the substrate could strongly affect the richness, abundance and fitness of the community. As dead wood-eating insects create tunnels, they leave behind a mixture of chewed wood, fungi, and feces. This frass can be comparatively rich in nitrogen (as some curculionids may fix nitrogen within their guts) (Morales-Jiménez et al. 2013), as well as enzymes that

degrade wood (De Fine Licht and Biedermann 2012). Scarab larvae, for example, produce feces rich in nitrogen and phosphorous and several studies have shown that their activity positively impacts species richness (Micó et al. 2015, Sánchez-Galvan et al. 2014).

Curculionids may positively impact cerambycids indirectly by facilitating the establishment of fungi in dead wood. Ambrosia beetles actively farm fungus within their tunnels, which is their primary food source, and other bark beetles are also commonly associated with fungus (Harrington 2005, Kirkendall et al. 2015, Persson et al. 2011). Entrance holes provide airflow to the interior and even the curculionids that do not actively cultivate fungus often inoculate wood with fungus that they carry from other trees (Birkemoe et al. 2018, Cornwell et al. 2009, Stokland and Siitonen 2012). Fungal mycelia contain much more nitrogen than dead wood (Martin 1987). Fungi also appear to transfer important nutrients from sapwood to phloem (Bleiker and Six 2007) and contain sterols that may aid in insect development. Yi *et al.* (2017) found that a cerambycid beetle grew to a large adult size quickly with an artificial fungal diet. Since cerambycid beetles do not necessarily produce all of the enzymes needed to degrade dead wood (Martin 1992, but see McKenna et al. 2016), the beetles may require fungal enzymes to complete digestion (*e.g.*, Kukor et al. 1988). Several studies suggest that yeasts in the guts of cerambycids aid digestion (Geib et al. 2008, Grünwald et al. 2010, Mohammed et al. 2018, Scully et al. 2012) and their mode of transmission varies (Berkov et al. 2007), with evidence for horizontally transmitted fungal symbionts in several cerambycid species (Haack 2017, Kukor et al. 1988, Martin 1992). Thus, fungi could provide a strong supplement to cerambycid diets, and could aid in digestion. Additionally, fungi may aid beetles by degrading wood, emitting volatiles that insects can use for mate attraction (Leather et al. 2014), detoxifying tree defenses, and even protecting against pathogens by producing antibiotics. (Birkemoe et al. 2018).

## *Hypotheses*

Given the previously mentioned evidence for positive impacts of curculionids on cerambycids and the evidence for competition between them, as well as their dominance in Costa Rica (Li et al. 2017, Morillo 2017), I investigated possible impacts of curculionid species on the fitness of co-occurring cerambycid species. I hypothesized that if competition was occurring, increasing abundance of a curculionid species would correlate with decreased abundance, decreased body size, and increased emergence time of the co-occurring cerambycid. This would imply decreased fitness in the presence of increasing numbers of curculionids. If, on the other hand, curculionids had a positive impact on cerambycids, I predicted that in the presence of increasing numbers of curculionid individuals, coexisting cerambycids would experience increased abundance, increased body size, and decreased emergence time. This would suggest higher fitness in the presence of increasing numbers of curculionids.

## **Methods**

### *Study site*

Beetle specimens were reared at the Piro Biological Station of Osa Conservation (8.24°N, 83.20°W) on the Osa Peninsula, Costa Rica for a project on host specialization. The site contains patches of old-growth forest along with patches of secondary forest regenerated from cattle pasture. The vegetation is described as a lowland moist forest (Li et al. 2017).

### *Rearing Protocol*

Nine tree species in six families were sampled (41 trees). Families included the Brazil nut family (Lecythidaceae), the fig family (Moraceae), the bean family (Fabaceae), the chicle family

(Sapotaceae), the hibiscus family (Malvaceae), and the coffee family (Rubiaceae). Bait branches were cut (one from each tree) and prepared by Christopher Roddick and Lin Li during the transition from the dry to the rainy season. The basal section of a branch (~8cm x 75cm) was severed and suspended in the canopy, while the rest was left on the forest floor. Branches were exposed for 3 months, allowing saproxylic insects the opportunity to colonize, after which the ground branch was cut into three thick segments (~8cm x 75cm) and six thin segments (~3cm x 75cm). The canopy branches, and thick and thin ground branches were placed into separate cages constructed from No-see-um insect netting (625 holes/in<sup>2</sup>; Barre Army Navy Store, Barre, VT) and monitored daily for emerging insects from July 2013 until August 2014, when insects were no longer emerging. Arthropod specimens were stored in 95% ethanol and sent to the City College of New York for sorting and identification. Specimens were initially sorted to subfamily and morphospecies. Lin Li identified cerambycids by comparing specimens to those previously identified by M Monné (MNRJ) or had them identified by L.G. Bezark (Li et al. 2017). Curculionids were initially sorted to subfamily by Jhuni Morillo using a dichotomous key. Bark and ambrosia beetles were identified by Dr. Thomas Atkinson and Dr. Lawrence Kirkendall, and all other curculionids were identified by Dr. Charles O. Brien. Specimens outside of the Cerambycidae and Curculionidae families were sorted to morphospecies by Carlo Sevilla in the Berkov lab. I combined the three datasets into one, which includes all information about the host trees, branch sections, and insect specimens, including the week that they emerged.

### *Study organisms*

I focused on the species that emerged from canopy branches, as they were discrete samples exposed to insects while spatially separated from the remainder of the branch, increasing chances for interspecific interactions to occur. Additionally, the canopy branches chosen yielded fewer species generally, so the confounding impact of multispecies interactions would be weaker in these branches. Those branches were investigated for co-occurring cerambycid/curculionid pairs in high enough abundance to be analyzed. Cerambycid species that emerged generally later or around the same time as the curculionid species were chosen, since those that emerge earlier have less time to interact with coexisting curculionids. I selected three cerambycid species to test the impact of co-occurring curculionid abundance on their fitness.

An undescribed species of cryptorhynchine, hereafter referred to as *Gasterocercini* sp. 17 (Coleoptera: Curculionidae: Cryptorhynchinae) co-occurred with the cerambycid *Gorybia tibialis* (Coleoptera: Cerambycidae: Cerambycinae) in the canopy branches of *Tachigali tessmannii* (Table 1a). Cryptorhynchines are weevils, and a literature search has provided no evidence for a facilitative effect of weevils. Given their long development time coexisting with *G. tibialis*, and their similar emergence times, competition might occur between these two species.

In canopy branches of *Lonchocarpus marcrophyllus* trees, the bark beetle species *Hylocurus elegans* (Coleoptera: Curculionidae: Scolytinae) coexisted with the cerambycid species *Colobothea chontalensis* (Coleoptera: Cerambycidae: Lamiinae) (Table 1b). Species of *Hylocurus* are xylophagous, meaning they bore into and eat sapwood (Kirkendall et al. 2015). This is likely to put them into direct contact with lamiines. *Hylocurus elegans*, like other bark beetles, may provide entrance holes and tunnels making it easier for cerambycids like *C.*

*chontalensis* to enter the wood. Bark beetles may also leave behind nutritious or enzyme rich frass (Morales-Jiménez et al. 2009; De Fine Licht and Biedermann 2012). Bark beetles are often associated with fungus (Martin 1987), and if this is the case for *H. elegans*, they may provide useful gut symbionts and/or an additional source of nutrients. Thus, I hypothesize that *H. elegans* facilitates *C. chontalensis*.

*Eschweilera biflava* was a common host among saproxylic species across several guilds and species (Table 1c). A cerambycid species in the tribe Acanthocinini, *Neoeutrypanus mutilatus* (Coleoptera: Cerambycidae: Lamiinae), was abundant in several branches, along with the non-native ambrosia beetle *Premnobius cavipennis* (Coleoptera: Curculionidae: Scolytinae). Species in the tribe Acanthocinini have been observed using bark beetle emergence holes for oviposition (Schroeder 1997). Ambrosia beetles bore directly into wood, but feed solely on the fungus they farm there (Kirkendall et al. 2015). Since *P. cavipennis* is abundant and associated with fungus, I hypothesize that *P. cavipennis* facilitates *N. mutilatus*.

**Table 1.** Number of individuals (N > 5) that emerged from canopy branches of a) *T. tessmannii*, b) *L. macrophyllus*, and c) *E. biflava*.

a)		Branch				Sum
Family	Species	13	14	18	25	
Cerambycidae	<i>Gorybia tibialis</i>	82	34	57	25	198
	<i>Ozineus moestus</i>	0	6	0	0	6
Curculionidae	Gasterocercini sp. 17	16	58	19	0	93
	<i>Premnobius cavipennis</i>	0	1	14	0	15
Braconidae	Braconidae sp. 8	1	15	3	0	19

b)		Branch				Sum
Family	Species	35	36	37	38	
Cerambycidae	<i>Nyssodectes roseicollis</i>	2	4	5	29	40
	<i>Colobothea chontalensis</i>	0	10	29	9	48
Curculionidae	<i>Macrocopturus ruficolis</i>	0	0	14	0	14
	<i>Trischidias exiguus</i>	0	0	0	21	21
	<i>Hylocurus elegans</i>	0	40	161	127	328
Braconidae	Braconidae sp. 24	0	9	15	18	42

c)

Family	Species	Branch			Sum
		28	29	31	
Cerambycidae	<i>Neoetrypanus mutilatus</i>	133	42	94	269
	<i>Nyssodectes roseicollis</i>	2	8	1	11
	<i>Xenofrea zonata</i>	12	0	8	20
Curculionidae	<i>Monarthrum posticum</i>	16	0	1	17
	<i>Premnobious cavipennis</i>	224	0	230	454
	<i>Xyleborus bispinatus</i>	30	1	5	36
Braconidae	Braconidae sp. 24	102	3	48	153
Tiphiidae	Tiphiidae sp. 7	20	0	0	20

### *Fitness*

To assess fitness of cerambycids, I measured body size. Body size is a common and empirically supported correlate to fitness (Kingsolver and Huey 2008). In cerambycids, larger body size often corresponds with increased fecundity, egg size, and neonate size (Torres-Vila 2017). In other insects it has been shown to correlate with higher survival rates, increased fecundity, larger eggs, higher egg productivity, better reproductive fitness, and other fitness-related traits (Hanks et al. 2005, Kause et al. 1999, Ohgushi 1996, Rodríguez-González et al. 2018, Togashi 2007, Torres-Vila 2017).

Before measuring body size, I determined the sex of each individual. Sexual dimorphism is common in cerambycid beetles, with females usually emerging larger than males (after going through an additional instar), which could potentially skew the results if not addressed. Sex was determined through dissections and then compared with external morphology, so that sex could be determined by observing specimens without dissections. In this study I measured the length of the right elytra of each cerambycid specimen to the nearest 0.1 mm using a Nikon SMZ645 stereo zoom microscope with an eyepiece reticle. This is a common method for measuring

cerambycid beetles (Reagel et al. 2012, Togashi 2007), and correlates strongly with overall length (Hanks et al. 2005).

Mean emergence week was also used as a proxy to assess fitness. Faster development often leads to higher fitness, for example in the form of larger size (Kause et al. 1999), better competitive ability (Krijger et al. 2001) and possibly better predator avoidance (Kause et al. 1999). Emergences were recorded weekly, and each week was designated with a code (sequential starting with 1 from the first week of collection). Julian week after first emergence was used as another estimate of development speed, since precise development time is unknown.

### *Analysis*

To investigate patterns of association, a probability-based co-occurrence analysis was performed on species of Curculionidae and Cerambycidae using the R package *cooccur* (R 3.5.3, Griffith et al. 2016). Probabilistic co-occurrence models compare observed species co-occurrence to the probability of co-occurrence, given by the probability of selecting a sample with one species given that the other species is already there. This method was chosen as it is a relatively simple way to test co-occurrence without creating a null distribution (Veech 2013) and it has low type I and type II error rates (Lavender et al. 2019). Analysis was conducted on a dataset of the cerambycid and curculionid species that emerged from all canopy branches, and also on cerambycid and curculionid species that emerged from complete branches sorted by tree species. Data were converted to presence/absence before analysis. Positive co-occurrence was considered significant at a probability  $<0.05$  that the species observed co-occurrence is greater than what would be expected by chance.



Generalized linear models were used to analyze the impact of curculionid abundance on cerambycid abundance (*Gasterocercini* sp. 17 on *G. tibialis*, *H. elegans* on *C. chontalensis*, and *P. cavipennis* on *N. mutilatus*). Curculionid species abundance, host species, forest type, and partition were included as explanatory variables, by themselves and in combination (factorial to degree two) to assess whether curculionid abundance had a significant effect on cerambycid abundance in relation to other potential factors. Generalized Regression in JMP ver. 12.1.0 was used for predictor choice. It is useful for datasets like mine because a) it does not require normal distributions for responses, and b) it performs variable selection by penalizing estimates in order to reduce variance; this results in estimate shrinkage (sometimes down to zero) and thus variable selection. The zero-inflated Poisson distribution was chosen for each analysis given the high number of 0's in our dataset. The Elastic Net penalization method was used as it works well even in the face of multicollinearity. The corrected Akaike Information Criterion (AICc) was used for model selection.

For each cerambycid species, differences in fitness measures between branches were analyzed by Analysis of Variance (ANOVA) followed by post-hoc Tukey HSD pairwise comparisons (JMP Pro 12.1.0) to compare: elytral length; emergence week; and Julian week after first emergence for females and males separately. Tukey HSD comparisons were considered significantly different at  $\alpha = 0.05$ .

## **Results**

The 41 branches sampled yielded 19,894 individual insects from 256 morphospecies. These came from five different orders: Coleoptera (beetles), Diptera (flies), Hemiptera (true bugs), Hymenoptera (wasps), and Thysanoptera. Coleoptera was by far the most species rich and

abundant, amounting to 71.5% of the species found, and 95% of the individuals. Within Coleoptera, the families Cerambycidae and Curculionidae were the most abundant and species rich, with 3,549 individuals in 49 species (Li et al. 2017) and 8,761 individuals in 91 species respectively (Morillo 2017).

### *Co-occurrence*

Canopy branches yielded 78 species of curculionids and cerambycids; 3003 species combinations were analyzed for significant co-occurrence. No species pairs had significantly negative co-occurrences with each other, while 119 species pairs had significantly positive associations; the remaining pairs were neutral (Appendix 1).

### *Interactions within Tachigali tessmannii*

Within canopy branches, co-occurrence analysis indicates that the cerambycid species *G. tibialis* and the curculionid species *Gasterocercini* sp. 17 co-occurred more often than would be expected by chance (Appendix 1, 2). Co-occurrence analysis confined to host species did not show a significant association between the two species. Generalized regression returned two parameters (plant species (*T. tessmannii*) and plant species (*T. tessmannii*) \* partition (canopy)) with a significant positive impact on the abundance of *G. tibialis*. *Gasterocercini* sp. 17 had no significant impact on the abundance of *G. tibialis* (Appendix 3).

Canopy branches of *T. tessmannii* had a significant effect on mean elytral lengths of *G. tibialis* for both males ( $F(3,80) = 10.2288$ ,  $p < 0.0001$ ) and females ( $F(3,91) = 6.9382$ ,  $p = 0.0003$ ). Mean elytral length of females was significantly higher in branch 14 than branches 13 ( $p = 0.0002$ ) and 25 ( $p = 0.0093$ ). Males had significantly longer elytra in branches 14 and 18

than in branches 13 and 25 (branches 14-13  $p = 0.0002$ , branches 14-25  $p = 0.0004$ , branches 18-13  $p = 0.0058$ , branches 18-25  $p = 0.0079$ ) (Fig. 1a).

Branches also had a significant effect on mean emergence week for both males ( $F(3,80) = 7.5311$ ,  $p = 0.0002$ ) and females ( $F(3,91) = 7.5083$ ,  $p = 0.0002$ ). Mean emergence week was significantly later in branch 14 for males (14-18  $p = 0.0002$ , 14-13  $p = 0.0004$ , 14-25  $p = 0.0055$ ) and females (14-18  $p = 0.0001$ , 14-13  $p = 0.0019$ , 14-25  $p = 0.0091$ ), which also yielded the most individuals of *Gasterocercini* sp. 17 (Fig. 1b). Mean Julian weeks since first emergence was not significantly different for females ( $F(3,91) = 1.0725$ ,  $p = 0.3648$ ), but was significantly different for males ( $F(3,80) = 4.2196$ ,  $p = 0.008$ ). Males emerged significantly faster from branches 18 and 25 than branch 13 (18-13  $p = 0.0298$ , 25-13  $p = 0.0347$ ) (Appendix 4). A summary of these results can be found in Appendix 7.

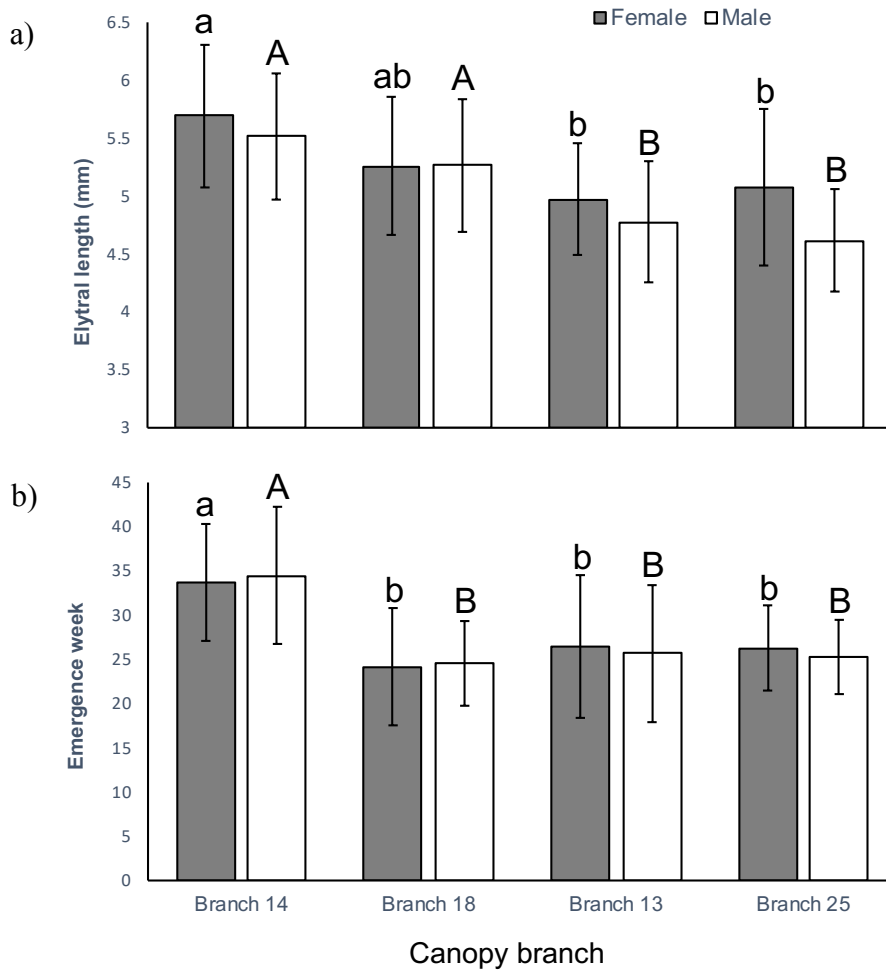


Figure 1. a) Mean elytral length (mm) and b) mean emergence week of individuals of *G. tibialis* emerging from canopy branches of *T. tessmannii*. Different letters represent significant differences ( $p < 0.05$ ). Lowercase letters pertain to females; uppercase letters pertain to males. Error bars represent standard deviation. Branches are ordered from the branch that yielded the highest abundance of Gasterocercini sp. 17 (58; branch 14) to the branch that yielded the fewest (none; branch 25).

#### *Interactions within Lonchocarpus macrophyllus*

Within canopy branches, *H. elegans* and *C. chontalensis* co-occurred more often than would be expected by chance (Appendix 1, 2). Within branches of *Lonchocarpus macrophyllus*, the two species also co-occurred more often than would be expected by chance (Fig. 2)

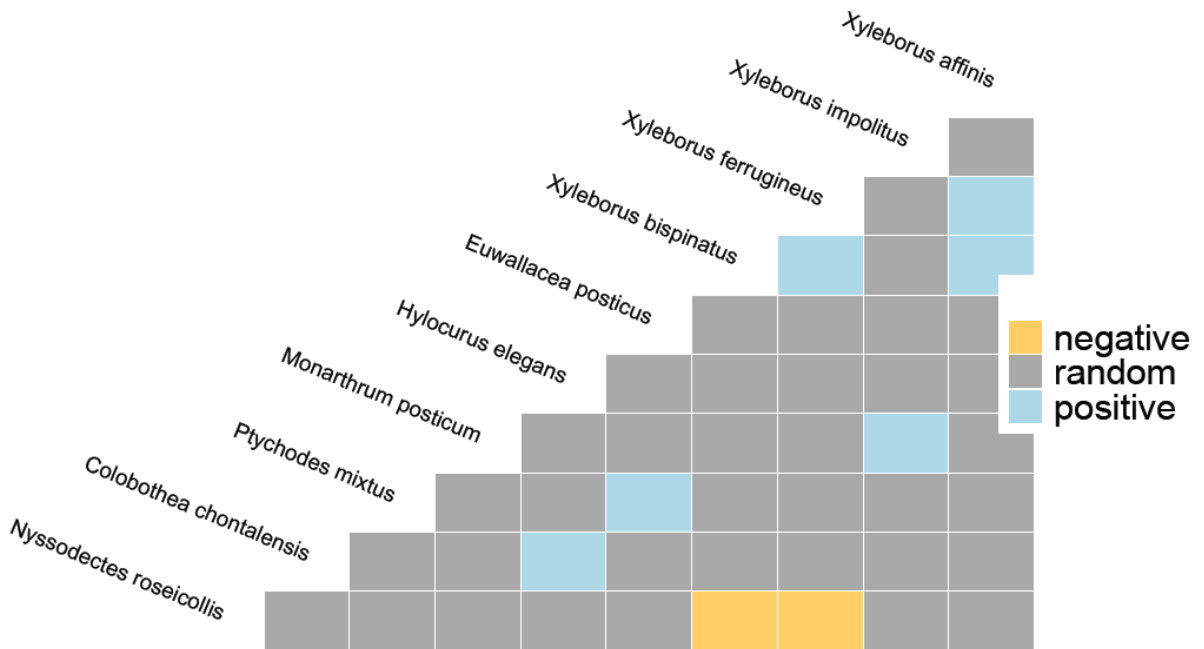


Figure 2. Co-occurrence analysis results within *L. macrophyllus* branches only, including canopy and large and small ground sections. Species pairs that co-occurred significantly more often than would be expected by chance in blue, species pairs that co-occurred significantly less often than would be expected by chance in yellow, and species pairs that did not co-occur more or less often than would be expected by chance in grey.

Generalized regression indicated that although the effect was not as pronounced as plant species, *H. elegans*, by itself and in conjunction with large ground branches, had a significant positive effect on the abundance of *C. chontalensis* (Table 2).

**Table 2.** *Colobothea chontalensis* generalized regression results

Parameter	Estimate	SE	Wald X <sup>2</sup>	p > X <sup>2</sup>
Intercept	-4.254	0.443	92.163	<0.0001
Plant species ( <i>L. macrophyllus</i> )	4.126	0.672	37.698	<0.0001
Partition (L ground) * <i>H. elegans</i>	0.514	0.113	20.818	<0.0001
Plant species ( <i>E. biflava</i> ) * Partition (L ground)	3.658	1.317	7.713	0.0055
<i>H. elegans</i>	0.008	0.003	7.129	0.0076
Plant species ( <i>E. biflava</i> ) * Forest type (primary)	2.468	0.966	6.527	0.0106
Plant species ( <i>L. macrophyllus</i> ) * Partition (canopy)	1.942	0.784	6.141	0.0132
Zero inflation	0.461	0.229	4.047	0.0442

Effects of plant species, partition, forest type, and *H. elegans* and combined factors on abundance of *C. chontalensis*. L ground = large ground sections. SE = standard error. R<sup>2</sup>=0.5022

No fitness measures were significantly different by branch as determined by one-way ANOVAs (Females: mean emergence week  $F(2,26) = 0.3691$ ,  $p = 0.6949$ , mean elytral length:  $F(2,26) = 2.1080$ ,  $p = 0.1418$ ; Males: mean emergence week,  $F(2,9) = 0.8485$ ,  $p = 0.4596$ , mean elytral length:  $F(2,9) = 2.5895$ ,  $p = 0.1293$ ). For both males and females, individuals of *C. chontalensis* that emerged from the branch that yielded the highest number of *H. elegans* (37) tended to be slightly smaller and emerge slightly earlier (Table 3). A summary of these results can be found in Appendix 7.

**Table 3.** Fitness measures for *C. chontalensis* individuals that emerged from branches of *L. macrophyllus*.

Sex	Branch	Abundance of <i>H. elegans</i>	Elytral length (mean)	Emergence week (mean)	N
Female	37	161	8.12±0.16	29.53±1.14	19
	38	127	8.68±0.32	31.2±2.23	5
	36	40	8.76±0.32	31.2±2.23	5
Male	37	161	6.81±0.27	27.86±2.42	7
	38	127	7.9±0.41	30±3.70	3
	36	40	6.9±0.50	34.5±4.53	2

### *Interactions within Eschweilera biflava*

Co-occurrence analysis across canopy branches indicates that the cerambycid species *N. mutilatus* and the curculionid species *P. cavipennis* co-occurred more often than would be expected by chance (Appendix 1, 2). The two species did not co-occur more often than would be expected by chance by host species. *Premnobius cavipennis* alone did not have a significant impact on the abundance of *N. mutilatus*. *Premnobius cavipennis* in conjunction with large ground branches had a small negative correlation with *N. mutilatus* abundance (Appendix 5).

Mean elytral length was affected by branch for females ( $F(2,102) = 3.4052$ ,  $p = 0.0370$ ) and males ( $F(2, 141) = 6.5952$ ,  $p = 0.0018$ ). Males from branches 31 and 28 emerged significantly larger than males from branch 29 (31-29  $p = 0.0013$ , 28-29  $p = 0.0092$ ) (Fig. 3a). Mean emergence week of *N. mutilatus* was significantly different among branches for both males ( $F(2, 141) = 30.0321$ ,  $p < 0.0001$ ) and females ( $F(2, 102) = 46.9916$ ,  $p < 0.0001$ ). Mean emergence week was significantly earlier for both males and females emerging from branch 28 (males: 28-31  $p < 0.0001$ , 28-29  $p < 0.0001$ ; females: 28-31  $p < 0.0001$ , 28-29  $p < 0.0001$ ) (Fig 3b). Mean Julian week after first emergence was significantly different between branches for both males ( $F(2, 141) = 10.7457$ ,  $p < 0.0001$ ) and females ( $F(2,102) = 7.3203$ ,  $p = 0.0011$ ). Mean Julian week since first emergence for males and females was significantly earlier from branch 28 than from the other two branches (males: 28-31,  $p = 0.0003$ , 29,  $p = 0.0032$ ; females: 31,  $p = 0.0031$ , 29,  $p = 0.0018$ ) (Appendix 6). A summary of these results can be found in Appendix 7.

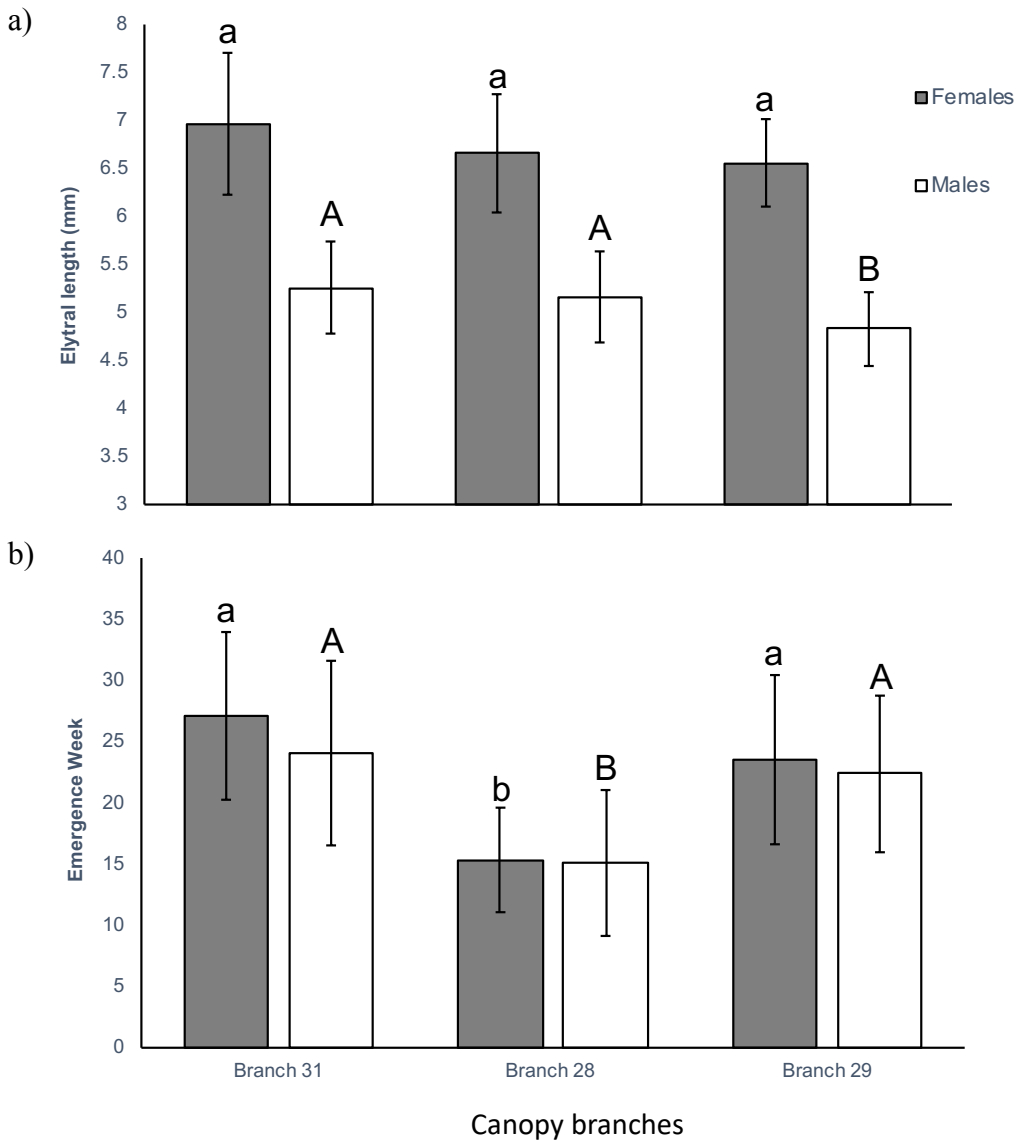


Figure 3. a) Mean elytral length (mm) of individuals of *N. mutilatus* that emerged from canopy branches of *E. biflava*. Different letters represent significant differences ( $p < 0.05$ ). Lowercase letters pertain to females; uppercase letters pertain to males. Error bars represent standard deviation. Branches are ordered from the branch that yielded the highest abundance of *P. cavipennis* (230; branch 31) to the branch that yielded the fewest (none; branch 29)

## Discussion

Understanding biotic interactions is essential for a complete understanding of communities. While negative interactions have been the focus of many previous studies, positive interactions are widespread and can explain many pervasive ecological patterns in communities,



including species coexistence (Gross 2008). Studying biotic interactions also contributes to improved conservation strategies, especially in the face of climate change (Valladares et al. 2015). Saproxylic communities are often incredibly species rich given the confined spaces and nutritionally deficient resources upon which these communities rely (Brin and Bouget 2018). Competition seems likely in such communities where many individuals share space. Even so, facilitation is relatively common in saproxylic communities (Brin and Bouget 2018). In this study I investigated whether competition or facilitation exists between saproxylic beetles coexisting in dead wood.

This study has added to the growing body of literature suggesting that positive interactions are common. In this study I have found many instances of positive co-occurrence between cerambycid and curculionid species (Appendix 1, Fig 1), and some evidence for positive interactions. With increasing abundance of *Gasterocercini* sp 17., *G tibialis* individuals had higher body size, suggesting a positive relationship (Fig. 2). *Hylocurus elegans* abundance correlated positively with *C. chontalensis* abundance (Table 2) and *C. chontalensis* individuals had earlier emergence times when co-existing with a higher abundance of *H. elegans*, though this corresponded with lower body sizes (these results were not significant) (Table 3).

There was less evidence for competition in this study. Co-occurrence analysis revealed few instances of significantly negative co-occurrences, and none in canopy branches across the dataset (Appendix 1). However, *P. cavipennis* in conjunction with large ground branches had a significantly negative impact on the abundance of *N. mutilatus*, which could suggest competition (Appendix 5). Generally, high body size (indicating high fitness) has correlated with later emergence time (indicating lower fitness), which makes interpretation difficult. However, body size has a stronger correlation with fitness, since early emergence time could potentially be

detrimental. For example, saproxylic insects often prefer certain climatic conditions for emergence (lamiines seem to prefer wetter conditions, while cerambycines seem to prefer drier conditions), and early emergence could put adults into less favorable conditions (Berkov 2018). Also, adult beetles search for mates and a new host after emergence, so emerging earlier could put them out of sync with potential mates or with other phenological processes that would otherwise be favorable.

The high fitness of individuals in branch 28 of *E. biflava* is of interest. Branches 28 and 31 had similarly high numbers of *P. cavipennis* and both also yielded more individuals of *N. mutilatus* than branch 29 (Table 1c). Branch 28, however, yielded the highest abundance of *N. mutilatus* which also emerged significantly larger, earlier and more quickly than those in the other branches (Fig. 3, Appendix 6). This intriguing finding did not correspond clearly with the abundance of *P. cavipennis* since branch 31 and branch 28 yielded similar numbers (Table 1c). It is also surprising given the high abundance of both *N. mutilatus* and other species in branch 28, because in that situation, one might expect intra- or interspecific competition, but instead, *N. mutilatus* was more fit in branch 28.

The high fitness of *N. mutilatus* in branch 28 could be explained by intraguild predation. Larvae of some cerambycids engage in facultative intraguild predation by consuming smaller curculionid larvae they come across (Dodds et al. 2001, Schoeller et al. 2012). Intraguild predation can have positive impacts on cerambycids through increasing nutritional value, which could be indistinguishable from facilitative impacts. Intraguild predation is possible in branch 28 of *E. biflava*, given the high abundance of curculionids. Indeed, consumption of animal tissue has been shown to increase the fitness of insects and reduce their larval development time

(Wissinger et al. 2004). In future studies, analysis of gut contents would help determine whether intraguild predation was occurring.

The high abundance and richness in branch 28, could suggest that the branch was particularly attractive—on the day the branches were cut, individuals 28 and 31 were in bud, and floral aromas may have sent out stronger signals (Amy Berkov, pers. comm.)—or nutritious and thus yielded more individuals and species. Whether the tree was more nutritious could be tested by measuring the size and assessing emergence week by sex for the co-occurring species in each canopy branch of *E. biflava*. If co-occurring species also have higher fitness in branch 28, this could indicate that the branch was more nutritious overall. If, instead, co-occurring species have lower fitness attributes than *N. mutilatus*, this could indicate inter- or intraspecific competition, which would suggest that *N. mutilatus* received some benefit from co-existing with many individuals or received some benefit from the branch that was not shared by other species.

Besides interactions with the species I focused on, the fitness differences between the cerambycids in different branches could be explained by interactions with other co-existing species. I chose branches that yielded few individuals in addition to the focal curculionid/cerambycid pairings, but some confounding factors could not be avoided. All branches yielded other species, and *E. biflava* was particularly densely populated. In such conditions, accurate interpretations are difficult. And while arthropods are a potential interactor, other interactions may be even more informative. Bacteria and fungi are important parts of saproxylic communities and their abundance can vary widely across hosts. For cerambycids, both can provide additional nutrition and can provide additional enzymes that help degrade wood (Martin 1987). We did not collect bacteria or fungi from the sampled branches, so we cannot assess their diversity or abundance.

Curculionids and cerambycids may interact in other ways. In our system, I have assumed that curculionids affected cerambycids, but it is quite possible that cerambycids have stronger impacts on curculionids. We do not know which insects arrived first, so cerambycids may well have arrived first, and priority often impacts the nature of an interaction (Victorsson 2012). There is evidence of cerambycids having a facilitative impact on other saproxylic insects (Calderón-Cortés et al. 2011). Cerambycids have also been observed negatively impacting curculionids through competition (Coulson et al. 1976, 1980, Miller 1985) or through intraguild predation (Dodds et al. 2001, Schoeller et al. 2012).

Intraspecific competition is also possible. It is unclear whether the different species sampled in this study compete for resources. Many species utilize very small portions of the inner bark during their larval stages, and curculionids often develop quickly. Wood-borer larvae are often small and not especially mobile. Ambrosia beetles make small galleries because they feed mostly on the fungus they cultivate (Kirkendall et al. 2015). Also, many saproxylic larvae have methods for avoiding the galleries of other larvae entirely (Byers 1989), and many bark beetle larvae engage in frass packing (packing their frass into a space in their gallery to create a plug (Kirkendall et al. 2015), which can further prevent interactions with other saproxylic larvae. Individuals within the same species, however, do require similar resources, and thus are more likely to compete. While individual larvae most likely have methods for avoiding conspecifics, as densities increase, scramble competition becomes more likely. At high densities, individuals can emerge with lower body size and reduced progeny (Anderbrant et al. 1985) or have lower survivorship over the long run (Hanks et al. 2005). In *L. macrophyllus*, for example, individuals from the most densely colonized wood emerged earlier and smaller than those from less densely

colonized wood (Table 3), which could suggest intraspecific competition, though small sample sizes render these findings inconclusive.

Another possible explanation for the observed differences in cerambycid emergence time and body size is cryptic variability of the host branches. Dead wood can vary in many ways that were unmeasured in this study. Between species, wood varies considerably in density, moisture content, physical and chemical defenses, nitrogen content, and many other traits (Seibold et al. 2016). Microclimates can vary considerably on a single log (Birkemoe et al. 2018). Moist wood may be preferred by certain species including lamiines and some scolytines (Torres 2018).

It is possible that resources are actually plentiful and thus the branches under consideration were not saturated with species or individuals, which would make competition unlikely. Undersaturation is common for resources that are ephemeral and patchy; such is the case for one community of fig wasps (Hawkins and Compton 1992). Lack of saturation can be expected in more specialized guilds because they are likely to have few competitors for their specific resource (Novotny et al. 2012). The relationship between species richness and abundance is positive and does not seem to reach an asymptote, and there is wide variation in abundance and richness among branches even within the same species, which could suggest that dead tree branches in Costa Rica were not saturated.

Co-occurrence analysis has revealed 119 pairs of species that co-occurred significantly more often than would be expected by chance, within the canopy branches alone (Appendix 1), and there were many instances of positive and negative associations among species within host species (ex: Fig 1). These pairs could be investigated further, while considering the phylogenetic relatedness, biology, and behavior of the organisms in question, along with habitat preferences and potential multispecies interactions. This could reveal more about the ways in which co-

existing saproxylic insects interact. Guilds could be studied specifically in order to investigate the ways different functional groups may impact others.

This study helps emphasize the importance of collecting ample data when studying saproxylic communities. In the future, host traits could be analyzed since there may be substantial differences between seemingly similar branches. Fungi and bacteria could also be sampled for a more complete understanding of the community. Gut contents could be analyzed in order to ascertain the source of nutrition for these insects. And of course, experimental studies would illuminate the factors that impact community structure and fitness differences.

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## References Cited

**Allison, J. D., Borden, J. H., McIntosh, R. L., de Groot, P., and Gries, R. 2001.** Kairomonal response by four *Monochamus* species (Coleoptera: Cerambycidae) to bark beetle pheromones. *J. Chem. Ecol.* 27: 633-646.

**Allison, J. D., McKenney, J. L., Miller, D. R., Gimmel, M. L. 2013.** Kairomonal responses of natural enemies and associates of the southern *Ips* (Coleoptera: Curculionidae: Scolytinae) to Ipsdienol, Ipsenol and *Cis*-Verbenol. *J. Insect Behav.* 26: 321-335.

**Anderbrant, O., Schlyter, F., and Birgersson, G. 1985.** Intraspecific competition affecting parents and offspring in the bark beetle *Ips typographus*. *Oikos.* 45: 89-98.

**Andersen, A. N. 2008.** Not enough niches: non-equilibrial processes promoting species coexistence in diverse ant communities. *Austral Ecol.* 33: 211-220.

**Ayres, B. D., Ayres, M. P. Abrahamson, M. D., and Teale, S. A. 2001.** Resource partitioning and overlap in three sympatric species of *Ips* bark beetles (Coleoptera: Scolytidae). *Oecologia.* 128: 443-453.

**Berkov, A., Meurer-Grimes, B., and Purzycki, K. L. 2000.** Do Lecythidaceae specialists (Coleoptera, Cerambycidae) shun fetid tree species? *Biotropica.* 32: 440-451.

**Berkov, A., Feinstein, J., Small, J., and Nkamany, M. 2007.** Yeasts isolated from neotropical wood-boring beetles in SE Peru. *Biotropica.* 39: 530-538.

**Berkov, A. 2018.** Seasonality and stratification: neotropical saproxylic beetles respond to a heat and moisture continuum with conservation and plasticity, pp. 547-578. In M. D. Ulyshen (ed.), *Saproxylic insects: diversity, ecology, and conservation.* Springer, Cham, Switzerland.

**Birkemoe, T., Jacobsen, R. M., Sverdrup-Thygeson, A., and Biedermann, P. H. W. 2018.** Insect-fungus interactions in dead wood systems, pp. 377-428. In M. D. Ulyshen (ed.), *Saproxylic insects: diversity, ecology, and conservation.* Springer, Cham, Switzerland

**Bleiker, K. P., and Six, D. L. 2007.** Dietary benefits of fungal associates to an eruptive herbivore: potential implications of multiple associates on host population dynamics. *Environ. Entomol.* 36: 1384-1396.

**Boone, C. K., Six, D. L., and Raffa, K. F. 2008.** The enemy of my enemy is still my enemy: competitors add to predator load of a tree-killing bark beetle. *Agr. Forest Entomol.* 10: 411-421.

- Borkowski, A., and Skrzecz, I. 2016.** Ecological segregation of bark beetle (Coleoptera, Curculionidae, Scolytinae) infested Scots pine. *Ecol. Res.* 31: 135-144.
- Brin, A., and Bouget, C. 2018.** Biotic interactions between saproxylic insect species, pp. 471-514. In M. D. Ulyshen (ed.), *Saproxylic insects: diversity, ecology, and conservation*. Springer, Cham, Switzerland.
- Bruno, J. F., Stachowicz, J. J., and Bertness, M. D. 2003.** Inclusion of facilitation into ecological theory. *Trends Ecol. Evol.* 18: 119-125.
- Bulleri, F., Bruno, J. F., Silliman, B. R., and Stachowicz, J. J. 2016.** Facilitation and the niche: implications for coexistence, range shifts and ecosystem functioning. *Funct. Ecol.* 30: 70-78.
- Buse, J., Ranius, T., and Assmann, T. 2008.** An endangered longhorn beetle associated with old oaks and its possible role as an ecosystem engineer. *Conserv. Biol.* 22: 329-337.
- Byers, J. A. 1989.** Behavioral mechanisms involved in reducing competition in bark beetles. *Holarctic Ecol.* 12: 466-476.
- Byers, J. A. 1993.** Avoidance of competition by spruce bark beetles, *Ips typographus* and *Pityogenes chalcographus*. *Experientia.* 49: 272-275.
- Calderón-Cortés, N., Quesada, M., and Escalera-Vásquez, L. H. 2011.** Insects as stem engineers: interactions mediated by the twig-girdler *Oncideres albomarginata chamela* enhance arthropod diversity. *PLoS ONE*, 6: e19083.
- Chesson, P. 2000.** Mechanisms of maintenance of species diversity. *Annu. Rev. Ecol. Syst.* 31: 343-366.
- Cornwell, W. K., Cornelissen, J. H. C., Allison, S. D., Bauhuss, J., Eggleton, P., Preston, C. M., Scarff, F., Weedon, J. T., Wirth, C., and Zanne, A. E. 2009.** Plant traits and wood fates across the globe: rotted, burned, or consumed? *Global Change Biol.* 15: 2431-2449.
- Coulson, R. N., Mayyasi, A. M., Foltz, J. L., and Hain, F. P. 1976.** Interspecific competition between *Monochamus titillator* and *Dendroctonus frontalis*. *Environ. Entomol.* 5: 235-247.
- Coulson, R. N., Pope, D. N., Gagne, J. A., Fargo, W. S., Pulley, P. E., Edson, L. J., and Wagner, T. L. 1980.** Impact of foraging by *Monochamus titillator* [Col.: Cerambycidae] on within-tree populations of *Dendroctonus frontalis* [Col: Scolytidae]. *Entomophaga.* 25: 155-170.
- Davis, T. S., and Hofstetter, R. W. 2009.** Effects of gallery density and species ratio on the fitness and fecundity of two sympatric bark beetles (Coleoptera: Curculionidae). *Environ. Entomol.* 38: 639-650.



**De Fine Licht, H. H., and Biedermann, P. H. W. 2012.** Patterns of functional enzyme activity in fungus farming ambrosia beetles. *Front. Zool.* 9: <https://doi.org/10.1186/1742-9994-9-13>

**Dodds, K. J., Graber, C., and Stephen, F. M. 2001.** Facultative intraguild predation by larval Cerambycidae (Coleoptera) on bark beetle larvae (Coleoptera: Scolytidae). *Environ. Entomol.* 30: 17-22.

**Duthie, A. B., Abbott, K. C., and Nason, J. D. 2014.** Trade-offs and coexistence: a lottery model applied to fig wasp communities. *Am. Nat.* 183: 826-841.

**Duthie, A. B., Abbott, K. C., and Nason, J. D. 2015.** Trade-offs and coexistence in fluctuating environments: evidence for a key dispersal-fecundity trade-off in five nonpollinating fig wasps. *Am. Nat.* 186: 151-158.

**Fassbender, J., Baxt, A., and Berkov, A. 2014.** Niches of saproxylic weevils (Coleoptera: Curculionidae) in French Guiana. *Coleopt. Bull.* 68: 689-699.

**Flamm, R. O., Coulson, R. N., Beckley, P., Pulley, P. E., and Wagner, T. L. 1989.** Maintenance of a phloem-inhabiting guild. *Environ. Entomol.* 18: 381-387.

**Gardiner, L. M. 1957.** Deterioration of fire-killed pine in Ontario and the causal wood-boring beetles. *Can. Entomol.* 89: 241-263

**Geib, S. M., Filley, T. R., Hatcher, P. G., Hoover, K., Carlson, J. E., Jimenez-Gasco, M. M., Nakagawa-Izumi, A., Sleighter, R. L., and Tien, M. 2008.** Lignin degradation in wood-feeding insects. *PNAS.* 105: 12932-12937.

**Gimmel, M. L., and Ferro, M. L. 2018.** General overview of saproxylic Coleoptera, pp. 51-128. In M. D. Ulyshen (ed.), *Saproxylic insects: diversity, ecology, and conservation*. Springer, Cham, Switzerland.

**Graham, S. A. 1925.** The felled tree trunk as an ecological unit. *Ecology.* 6: 397-411.

**Griffith, D. M., Veech, J. A., and Marsh, C. J. 2016.** Cooccur: probabilistic species co-occurrence analysis in R. *J. Stat. Softw.* 69.

**Gross, K. 2008.** Positive interactions among competitors can produce species-rich communities. *Ecol. Lett.* 11: 929-936.

**Grünwald, M. 1986.** Ecological segregation of bark beetles (Coleoptera, Scolytidae) of spruce. *J. Appl. Ent.* 101: 176-187.

**Grünwald, S., Pilhofer, M., and Höll, W. 2010.** Microbial associations in gut systems of wood- and bark-inhabiting longhorned beetles [Coleoptera: Cerambycidae]. *Syst. Appl. Microbiol.* 33: 25-34.

- Guevara, R., Hutcheson, K. A., Mee, A. C., Rayner, A. D. M., and Reynolds, S. E. 2000.** Resource partitioning of the host fungus *Coriolus versicolor* by two ciid beetles: the role of odour compounds and host ageing. *Oikos*. 91: 184-194.
- Haack, R. A. 2017.** Feeding biology of cerambycids, pp. 105-132. In Q. Wang (ed.), *Cerambycidae of the world: biology and pest management*. CRC Press Boca Raton, FL.
- Hanks, L. M., Paine, T. D., and Millar, J. G., 2005.** Influence of the larval environment on performance and adult body size of the wood-boring beetle *Phoracantha semipunctata*. *Entomol. Exp. Appl.* 114: 25-34.
- Hardin, G. 1960.** The competitive exclusion principle. *Science*. 131: 1292-1297.
- Harrington, T.C. 2005.** Ecology and evolution of mycophagous bark beetles and their fungal partners, pp. 257-291. In Vega, F. E., and Blackwell, M. (eds.), *Ecological and evolutionary advances in insect-fungal associations*. Oxford University Press, New York, NY
- Hawkins, B. A., and Compton, S. G. 1992.** African fig wasp communities: undersaturation and latitudinal gradients in species richness. *J. Anim. Ecol.* 61: 361-372.
- Hubbell, S. P. 2000.** *The unified neutral theory of biodiversity and biogeography*. Princeton University Press, Princeton, NJ.
- Iwata, R., Maro, T., Yonezawa, Y., Yahagi, T., and Fujikawa, Y. 2007.** Period of adult activity and response to wood moisture content as major segregating factors in the coexistence of two conifer longhorn beetles, *Callidiellum rufipenne*, and *Semanotus bifasciatus* (Coleoptera: Cerambycidae). *Eur. J. Entomol.* 104: 341-345.
- Kadowaki, K. 2010.** Species coexistence patterns in a mycophagous insect community inhabiting the wood-decaying bracket fungus *Cryptoporus volvatus* (Polyporaceae: Basidiomycota). *Eur. J. Entomol.* 107: 89-99.
- Kause, A., Saloniemi, I., Haukioja, E., and Hanhimäki, S. 1999.** How to become large quickly: quantitative genetics of growth and foraging in a flush feeding lepidopteran larva. *J. Evol. Biol.* 12: 471-482.
- Kingsolver, J. G., and Huey, R. B. 2008.** Size, temperature, and fitness: three rules. *Evol. Ecol. Res.* 10: 251-268.
- Kirkendall, L. R., Biedermann, P. H. W., and Jordal, B. H. 2015.** Evolution and diversity of bark and ambrosia beetles, pp. 85-156. In F. Vega, and R. Hofstetter (eds.), *Bark beetles: biology and ecology of native and invasive species*. Academic Press, Cambridge, MA.
- Krijger, C. L., Peters, Y. C., and Sevenster, J. G. 2001.** Competitive ability of neotropical *Drosophila* predicted from larval development times. *Oikos*. 92: 325-332.

- Kukor, J. J., Cowan, D. P., and Martin, M. M. 1988.** The role of ingested fungal enzymes in cellulose digestion in the larvae of cerambycid beetles. *Physiol. Zool.* 61: 364-371.
- Lavender, T. M., Schamp, B. S., Arnott, S. E., and Rusak, J. A. 2019.** A comparative evaluation of five common pairwise tests of species association. *Ecology.* 100: e02640.
- Leather, S. R., Baumgart, E. A., Evans, H. F., and Quicke, D. L. J. 2014.** Seeing the trees for the wood – beech (*Fagus sylvatica*) decay fungal volatiles influence the structure of saproxylic beetle communities. *Insect Conserv. Diver.* 7: 314-326.
- Li, L., Aguilar, R. and Berkov, A. 2017.** What shapes cerambycid beetle communities in a tropical forest mosaic? Assessing the effects of host tree identity, forest structure, and vertical stratification. *Biotropica.* 49: 675-684.
- Light, D. M., Birch, M. C., and Paine, T. D. 1983.** Laboratory study of intraspecific and interspecific competition within and between two sympatric bark beetle species, *Ips pini* and *I. paraconfusus*. *Z. ang. Ent.* 96: 233-241.
- Martin, M. M. 1987.** Invertebrate-microbial interactions: ingested fungal enzymes in arthropod biology. Cornell University Press, Ithaca, NY.
- Martin, M. M. 1992.** The evolution of insect-fungus associations from contact to stable symbiosis. *Amer. Zool.* 32: 593-605.
- McIntire, E. J. B., and Fajardo, A. 2014.** Facilitation as a ubiquitous driver of biodiversity. *New Phytol.* 201: 403-416.
- McKenna, D.D., Scully, E.D., Pauchet, Y., Hoover, K., Kirsch, R., Geib, S.M., Mitchell, R.F., Waterhouse, R.M., Ahn, S.J., Arsal, D., Benoit, J.B., Blackmon, H., Bledsoe, T., Bowsher, J.H., Busch, A., Calla, B., Chao, H., Childers, A.K., Childers, C., Clarke, D.J., Cohen, L., Demuth, J.P., Dinh, H., Doddapaneni, H., Dolan, A., Duan, J.J., Dugan, S., Friedrich, M., Glastad, K.M., Goodisman, M.A., Haddad, S., Han, Y., Hughes, D.S., Ioannidis, P., Johnston, J.S., Jones, J.W., Kuhn, L.A., Lance, D.R., Lee, C.Y., Lee, S.L., Lin, H., Lynch, J.A., Moczek, A.P., Murali, S.C., Muzny, D.M., Nelson, D.R., Palli, S.R., Panfilio, K.A., Pers, D., Poelchau, M.F., Quan, H., Qu, J., Ray, A.M., Rinehart, J.P., Robertson, H.M., Roehrdanz, R., Rosendale, A.J., Shin, S., Silva, C., Torson, A.S., Jentsch, I.M., Werren, J.H., Worley, K.C., Yocum, G., Zdobnov, E.M., Gibbs, R.A., and Richards, S.. 2016.** Genome of the Asian longhorned beetle (*Anoplophora glabripennis*), a globally significant invasive species, reveals key functional and evolutionary innovations at the beetle-plant interface. *Genome Biol.* 17: 227
- Meurer-Grimes, B. and Tavakilian, G. 1997.** Chemistry of cerambycid host plants. Part 1: survey of Leguminosae—a study in adaptive radiation. *Bot. Rev.* 63: 356-394.

- Micó, E., García-López, A., Sánchez, A., Juárez, M., and Galante, E. 2015.** What can physical, biotic and chemical features of a tree hollow tell us about their associated diversity? *J. Insect Conserv.* 19: 141-153.
- Miller, M. C. 1985.** The effect of *Monohamus titillator* (F.) (Col., Cerambycidae) foraging on the emergence of *Ips calligraphus* (Germ.) (Col., Scolytidae) insect associates. *Z. ang. Ent.* 100: 189-197.
- Mitchell, R. F., Reagel, P. F., Wong, J. C. H., Meier, L. R., Silva, W. D., Mongold-Diers, J., Millar, J. G., and Hanks, L. M. 2015.** Cerambycid beetle species with similar pheromones are segregated by phenology and minor pheromone components. *J. Chem. Ecol.* 41: 431-440.
- Mohammed, W. S., Ziganshina, E. E., Shagimardanova, E. I., Gogoleva, N. E. and Ziganshin, A. M. 2018.** Comparisons of intestinal bacterial and fungal communities across various xylophagous beetle larvae (Coleoptera: Cerambycidae). *Sci. Rep.* 8: 1-12.
- Molander, M. A., and Larsson, M. C. 2018.** Identification of the aggregation-sex pheromone of the cerambycid beetle *Phymatodes pusillus* ssp. *pusillus* and evidence of a synergistic effect from a heterospecific pheromone component. *J. Chem. Ecol.* <https://doi.org/10.1007/s10886-018-1008-3>.
- Morales-Jiménez, J., Zúñiga, G., Villa-Tanaca, L., and Hernández-Rodríguez, C. 2009.** Bacterial community and nitrogen fixation in the red turpentine beetle, *Dendroctonus valens* LeConte (Coleoptera: Curculionidae: Scolytinae). *Microb. Ecol.* 58: 879-891.
- Morillo, J. 2017.** Are weevils picky eaters? Community structure and host specificity of Neotropical saproxylic beetles (Coleoptera: Curculionidae). CUNY Academic Works. [http://academicworks.cuny.edu/cc\\_etds\\_theses/664](http://academicworks.cuny.edu/cc_etds_theses/664)
- Novotny, V., Miller, S. E., Hrcek, J., Baje, L., Bassett, Y., Lewis, O. T., Stewart, A. J. A., and Weiblen, G. D. 2012.** Insects on plants: explaining the paradox of low diversity within specialist herbivore guilds. *Am. Nat.* 179: 351-362.
- Ohgushi, T. 1996.** Consequences of adult size for survival and reproductive performance in an herbivorous ladybird beetle. *Ecol. Entomol.* 21: 47-55.
- Økland, B., Skarpaas, O., and Kausrud, K. 2009.** Threshold facilitations of interacting species. *Popul. Ecol.* 51: 513-523.
- Pajares, J. A., Ibeas, F., Diez, J. J., and Gallego, D. 2004.** Attractive responses by *Monochamus galloprovincialis* (Col., Cerambycidae) to host and bark beetle semiochemicals. *JEN*, 128: 633-638.
- Persson, Y., Ihrmark, K., and Stenlid, J. 2011.** Do bark beetles facilitate the establishment of rot fungi in Norway spruce? *Fungal Ecol.* 4: 262-269.

- Raffa, K. F., Grégoire, J., and Lindgren, B. S. 2015.** Natural history and ecology of bark beetles, pp. 1-40. In F. Vega, and R. Hofstetter (eds.), *Bark beetles: biology and ecology of native and invasive species*. Academic Press, Cambridge, MA.
- Rankin, L. J., and Borden, J. H. 1991.** Competitive interactions between the mountain pine beetle and the pine engraver in lodgepole pine. *Can. J. For. Res.* 21: 1029-1036.
- Reagel, P. F., Smith, M. T., Hanks, L. M. 2012.** Effects of larval host diameter on body size, adult density, and parasitism of cerambycid beetles. *Can. Entomol.* 144: 435-438.
- Rodríguez-González, A., Peláez, H. J., González-López, Ó., Mayo, S., and Casquero, P. A. 2018.** Effects of adult body size and larvae diet on the fecundity and percent fertility of eggs laid by *Xylotrechus arvicola* (Coleoptera: Cerambycidae) females, insect pest in Spanish vineyards. *Entomol. Res.* 48: 83-91.
- Rotheray, E. L. 2013.** Differences in ecomorphology and microhabitat use of four saproxylic larvae (Diptera, Syrphidae) in Scots pine stump rot holes. *Ecol. Entomol.* 38: 219-229.
- Sánchez-Galván, I. R., Quinto, J., Mico, E., Galante, E., and Marcos-García, M. A. 2014.** Facilitation among saproxylic insects inhabiting tree hollows in a Mediterranean forest: the case of cetonids (Coleoptera: Cetoniidae) and syrphids (Diptera: Syrphidae). *Environ. Entomol.* 43: 336-343.
- Schlyter, F., and Anderbrant, O. 1993.** Competition and niche separation between two bark beetles: existence and mechanisms. *Oikos.* 68: 437-447.
- Schoeller, E. N., Husseneder, C., and Allison, J. D. 2012.** Molecular evidence of facultative intraguild predation by *Monochamus titillator* larvae (Coleoptera: Cerambycidae) on members of the southern pine guild. *Naturwissenschaften.* 99: 913-924.
- Schroeder, L. M., and Weslien, J. 1994.** Interactions between the phloem-feeding species *Tomicus piniperda* (Col: Scolytidae) and *Acanthocinus aedilis* (Col: Cerambycidae), and the predator *Thanasimus formicarius* (Col: Cleridae) with special reference to brood production. *Entomophaga.* 39: 149-157.
- Schroeder, L. M. 1997.** Oviposition behavior and reproductive success of the cerambycid *Acanthocinus aedilis* in the presence and absence of the bark beetle *Tomicus piniperda*. *Entomol. Exp. Appl.* 82, 9-17.
- Scully, E. D., Hoover, K., Carlson, J., Tien, M., and Geib, S. M. 2012.** Proteomic analysis of *Fusarium solani* isolated from the Asian longhorned beetle, *Anoplophora glabripennis*. *PLoS ONE*, 7: e32990.
- Seibold, S., Bäessler, C., Brandl, R., Büche, B., Szallies, A., Thorn, S., Ulyshen, M. D., and Müller, J. 2016.** Microclimate and habitat heterogeneity as the major drivers of beetle diversity in dead wood. *J. Appl. Ecol.* 53: 934-943.

- Stachowicz, J. J. 2001.** Mutualism, facilitation, and the structure of ecological communities. *Biosci.* 51: 235-246.
- Stokland, J. N. and Siitonen, J. N. 2012.** Mortality factors and decay succession, pp. 110-149. In J. N. Stokland, J. Siitonen, and B. G. Jonsson (eds.), *Biodiversity in Dead Wood*. Cambridge University Press, New York, NY.
- Sydenham, M. A. K., Häusler, L. D., Moe, S. R., and Eldegard, K. 2016.** Inter-assembly facilitation, the functional diversity of cavity-producing beetles drives the size diversity of cavity-nesting bees. *Ecol. Evol.* 6: 412-425.
- Takahashi, K. H., Tuno, N., and Kagaya, T. 2005.** The relative importance of spatial aggregation and resource partitioning on the coexistence of mycophagous insects. *OIKOS.* 109: 125-134.
- Tavakilian, G., Berkov, A., Meurer-Grimes, B., and Mori, S. 1997.** Neotropical tree species and their faunas of xylophagous longicorns (Coleoptera: Cerambycidae) in French Guiana. *Bot. Rev.* 63: 303-355.
- Togashi, K. 2007.** Lifetime fecundity and female body size in *Paraglenea fortune* (Coleoptera: Cerambycidae). *Appl. Entomol. Zool.* 42: 549-556.
- Torres-Vila, L. M. 2017.** Reproductive biology of the great capricorn beetle, *Cerambyx cerdo* (Coleoptera: Cerambycidae): a protected but occasionally harmful species. *Bull. Entomol. Res.* 107: 1-13.
- Torres 2018.** Wood trait preferences of Neotropical xylophagous beetles (Coleoptera: Cerambycidae). CUNY Academic Works. [https://academicworks.cuny.edu/cc\\_etds\\_theses/736](https://academicworks.cuny.edu/cc_etds_theses/736)
- Ulyshen, M. D., and Šobotnik, J. 2018.** An introduction to the diversity, ecology, and conservation of saproxylic insects, pp. 1-50. In M. D. Ulyshen (ed.), *Saproxylic insects: diversity, ecology, and conservation*. Springer, Cham, Switzerland.
- Valladares, F., Bastias, C. C., Godoy, O., Granda, E., and Escudero, A. 2015.** Species coexistence in a changing world. *Fron. Plant Sci.* 6: 866.
- Veech, J. A. 2013.** A probabilistic model for analyzing species co-occurrence. *Glob. Ecol. Biogeogr.* 22: 252-260.
- Victorsson, J. 2012.** Semi-field experiments investigating facilitation: arrival order decides the interrelationship between two saproxylic beetle species. *Ecol. Entomol.* 37: 395-401.
- Wertheim, B., Sevenster, J. G., Eijs, I. E. M., and Alphen, J. J. M. van. 2000.** Species diversity in a mycophagous insect community: the case of spatial aggregation vs. resource partitioning. *J. Anim. Ecol.* 69: 335-351.

**Wilson, J. B. 1990.** Mechanisms of species coexistence: twelve explanations for Hutchinson's 'paradox of the plankton': evidence from New Zealand plant communities. *N. Z. J. Ecol.* 13: 17-42.

**Wissinger, S., Steinmetz, J., Alexander, J. S., Brown, W. 2004.** Larval cannibalism, time constraints, and adult fitness in caddisflies that inhabit temporary wetlands. *Oecologia.* 138: 39-47.

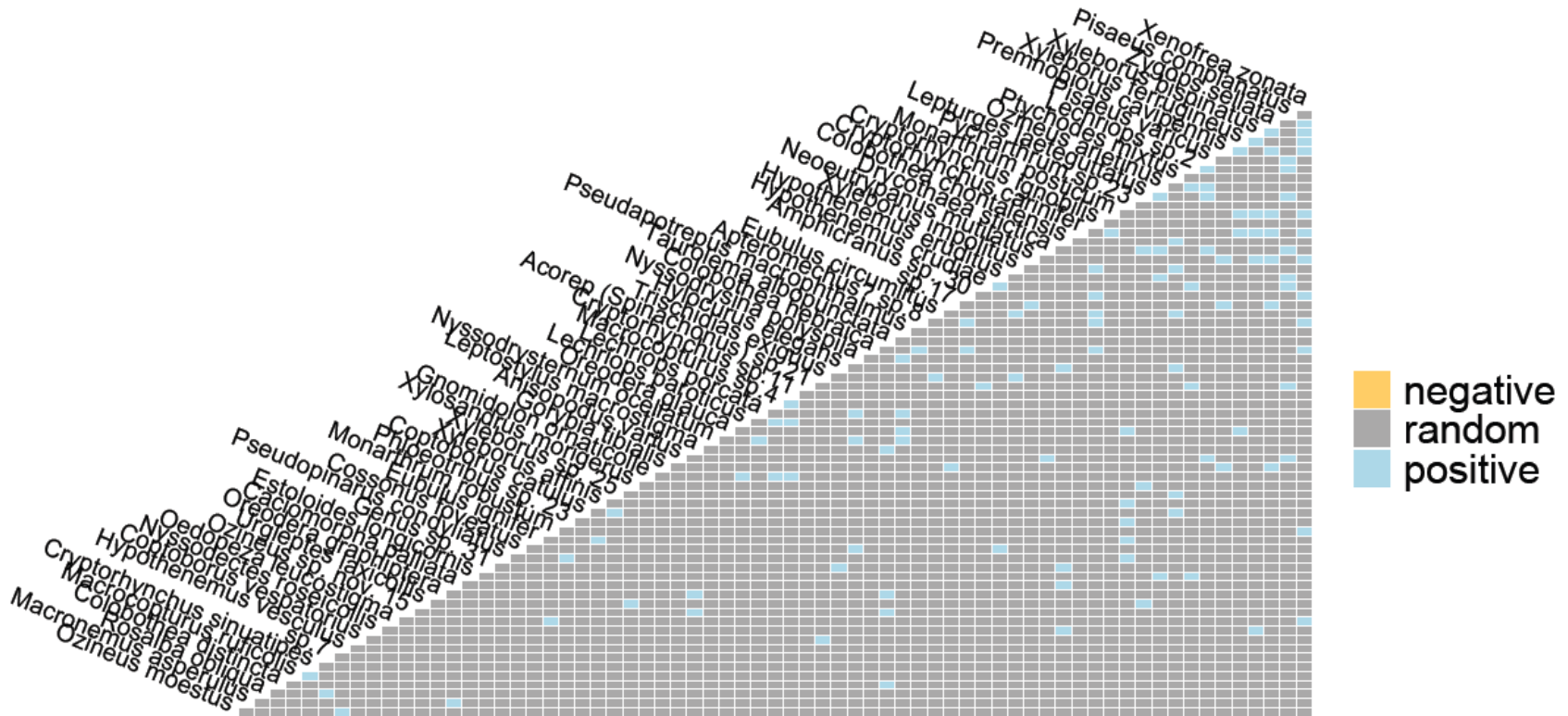
**Xiao, S., Zhao, L., Zhang, J., Wang, X., and Chen, S. 2013.** The integration of facilitation into the neutral theory of community assembly. *Ecol. Modell.* 215: 127-134.

**Yi, D., Kuprin, A. V., Lee, Y. H., and Bae, Y. J. 2017.** Newly developed fungal diet for artificial rearing of the endangered long-horned beetle *Callipogon relictus* (Coleoptera: Cerambycidae). *Entomol. Res.* 47: 373-379.

**Zuo, J., Conelissen, J. H. C., Hefting, M. M., Sass-Klaassen, U., Van Logtestijn, R. S. P., Van Hal, J., Goudzwaard, L., Liu, J. C., and Berg, M. P. 2016.** The (w)hole story: facilitation of dead wood fauna by bark beetles? *Soil Biol. Biochem.* 95: 70-77.

## Appendices

**Appendix 1.** Results of co-occurrence analysis across all canopy branches. Species that co-occurred significantly more often than would be expected by chance in blue, and species that did not co-occur more or less often than would be expected by chance in grey.



- negative
- random
- positive



**Appendix 2.** Co-occurrence analysis results for canopy branches.

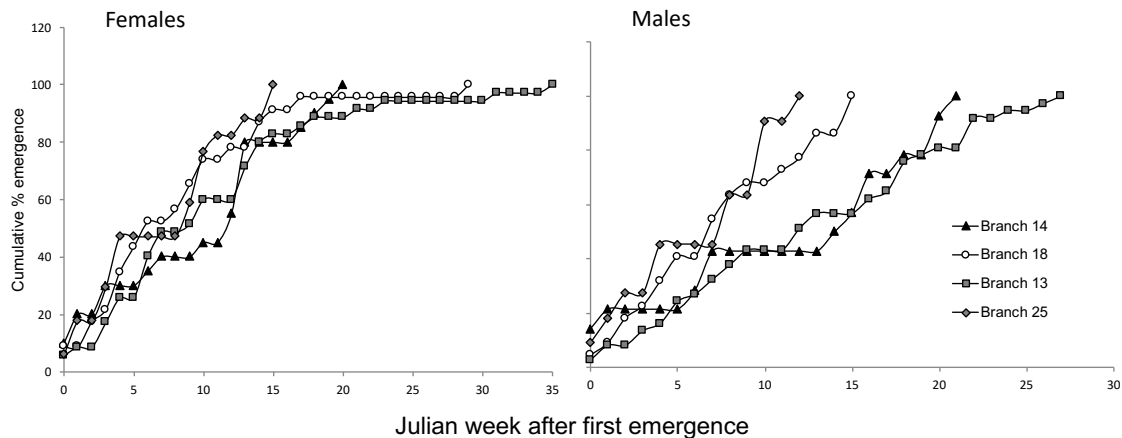
Species 1	Species 2	sp1	sp2	obs	prob	exp	p_co
Gasterocercini sp.17	<i>G. tibialis</i>	4	6	3	0.012	0.5	0.00571
<i>H. elegans</i>	<i>C. chontalensis</i>	3	4	3	0.006	0.3	0.00030
<i>P. cavipennis</i>	<i>N. mutilatus</i>	7	9	5	0.033	1.4	0.00203

Sp1 = number of canopy branches that yielded species 1, sp2 = number of canopy branches that yielded species 2. Obs = number of canopy branches where both species occurred, prob = probability of coexistence, exp = expected number of sites where both species occur, p\_co = probability that the observed species co-occurrence is higher than expected.

**Appendix 3.** *Gorybia tibialis* generalized regression results

Parameter	Estimate	SE	Wald X <sup>2</sup>	p > X <sup>2</sup>
Plant species ( <i>T. tessmannii</i> )	2.009	0.671	8.948	0.003
Plant species ( <i>T. tessmannii</i> ) * Partition (canopy)	1.007	0.507	3.939	0.047
Partition (L ground) *Gasterocercini sp. 17	0.009	0.008	1.270	0.260
Intercept	-0.453	0.404	1.257	0.262
Plant species ( <i>Tachigali tessmannii</i> )*Partition (L ground)	0.581	0.709	0.672	0.412
Plant species ( <i>Apeiba tibourbou</i> )*Partition (L ground)	-0.125	0.855	0.021	0.884
Plant species ( <i>Apeiba tibourbou</i> )*Partition (canopy)	-0.016	0.948	0.000	0.986
Plant species ( <i>Apeiba tibourbou</i> )	-0.006	0.565	0.000	0.992
Plant species ( <i>T. tessmannii</i> )*Gasterocercini sp. 17	-0.001	0.154	0.000	0.994
Forest type (Primary)* Gasterocercini sp. 17	0.000	0.154	0.000	0.999

Effects of plant species, partition, forest type, and Gasterocercini sp. 17 and combined factors on abundance of *Gorybia tibialis*. L ground = large ground sections. SE = standard error. R<sup>2</sup> = 0.934

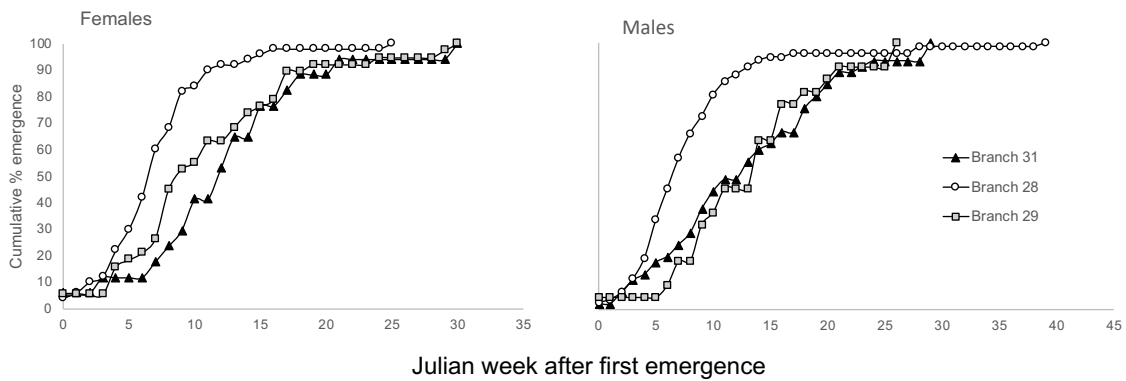


**Appendix 4.** Cumulative percent emergence by Julian week after first emergence for females and males of *G. tibialis* that emerged from canopy branches of *T. tessmannii*.

**Appendix 5.** *Neoeutrypanus mutilatus* generalized regression results

Parameter	Estimate	SE	Wald X <sup>2</sup>	p > X <sup>2</sup>
Zero inflation	0.845	0.044	365.740	<0.0001
Plant species ( <i>E. biflava</i> )	2.636	0.502	27.595	<0.0001
Partition (L ground) * <i>P. cavipennis</i>	-0.031	0.016	3.897	0.0484
<i>P. cavipennis</i>	0.003	0.003	1.253	0.263
Partition (canopy)	-0.547	0.661	0.685	0.408
Intercept	0.540	0.755	0.512	0.474
Partition (L ground)	0.244	0.744	0.108	0.743

Effects of plant species, partition, forest type, and *P. cavipennis* and combined factors on abundance of *Neoeutrypanus mutilatus*. L ground = large ground sections. SE = standard error. R<sup>2</sup> = 0.999



**Appendix 6.** Cumulative percent emergence by Julian week after first emergence for females and males of *N. mutilatus* emerging from branches of *E. biflava*.

**Appendix 7:** Summary of results.

<b>Cerambycid + Curculionid</b>	<b>Co- occur</b>	<b>GR</b>	<b>H<sub>1</sub>: Elytral length</b>	<b>H<sub>1</sub>: Result</b>	<b>H<sub>2</sub>: Emergence week</b>	<b>H<sub>2</sub>: Result</b>
<i>G. tibialis</i> + <i>Gasterocercini</i> sp. 17	Pos	None	Branch 25>13=18>14	F: 14>13&25 M: 14&18>13&25	Branch 25<13=18<14	F: 13,18,25<14 M: 13,18,25<14
<i>C. chontalensis</i> + <i>H. elegans</i>	Pos	Pos	Branch 37>38>36	F: 36>38>37 M: 38>36>37	Branch 37<38<36	F: 37<36,38 M: 37<38< 36
<i>N. mutilatus</i> + <i>P. cavipennis</i>	Pos	Neg *ground	Branch 31=28>29	F: 31=28=29 M: 31=28>29	Branch 31=28<29	F: 28<31&29 M: 28<31&29

Co-occur = results of co-occurrence analysis, GR = Generalized regression, H = hypothesis, none = no significant effect, pos = significantly positive, neg = significantly negative, F = female, M = male