ARTHROPOD BIOLOGY

Adaptations for Symbiont-Mediated External Digestion in *Sirex noctilio* (Hymenoptera: Siricidae)

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ABSTRACT Wood-feeding insects face a host of physical, chemical, and nutritional barriers in their food resource. Wood-boring Hymenoptera in the family Siricidae are associated with mutualistic basidiomycete wood-rot fungi, which assist colonization and provide nutrition for their insect partner, though functional properties of this symbiosis are poorly described. In this study, we document the behavioral and morphological adaptations of the globally invasive woodwasp, *Sirex noctilio* F., for foraging using its fungal symbiont *Amylostereum areolatum* (Chaillet ex Fries) Boidin. Larvae concentrated foraging near the border of fungal symbiont growth in pine xylem. Foraging larvae do not ingest bulk xylem tissue, but rather use specialized asymmetric mandibles to press xylem shavings and extract liquid fractions. Fluids drain toward the oral cavity via a sulcus on the occlusal surface of the left mandible. Processed shavings are expelled from the oral cavity without ingestion and passed along the underside of the larvae via peristaltic undulation to the rear of the feeding gallery. Larval midguts lack elaborated chambers typical in insects reliant on microbial fermentation of cellulose, and no xylem tissue was recovered from gut dissections. Larval behavior and functional morphology indicate larval *S. noctilio* do not ingest xylem, but instead use the fungus as an external gut for digestion of recalcitrant lignocellulosic compounds.

KEY WORDS European woodwasp, external rumen, wood-boring, insect-fungal mutualism, mandible

The xylem tissue of woody plants is one of the most abundant and longest-lasting biological materials on Earth (Ryan et al. 2010). Recalcitrant lignocellulosic polymers limit wood decay to specialized microorganisms, which as a group metabolize some or all components of wood, including lignin, cellulose, and hemicellulose (Breznak 1982, Klepzig et al. 2009, Watanabe and Tokuda 2010). Digestion of wood and its subsequent conversion to more nutritious or comparatively more assimilable compounds creates an opportunity for other organisms in the local environment of wood-decay microbes. As such, the majority of described wood-feeding insects feed in close association with one or more microorganisms (Kukor and Martin 1983, Jonsell et al. 1999, Boddy 2001, Waldrop and Firestone 2004). Wood-feeding insects may derive nutrients from consumption of wood-decay microorganisms, but may also benefit from extracellular enzymes involved in digestion or degradation of plant defensive chemistry (Kukor and Martin 1983, Kukor et al. 1988, Blanchette 1991, Friesen and Faris 2012). Wood-feeding and association with microorganisms have important implications for the ecology and evolution of forest ecosystems, as wood-boring insects play integral roles in tree mortality and biomass decay (Haack 2006, Watanabe and Tokuda 2010). Wood-feeding insects typically possess specialized adaptations for feeding and maintenance of associations (Klepzig et al. 2004, Warnecke et al. 2007). Descriptions of functional morphological and behavioral characteristics are essential for understanding evolutionary adaptations to feeding on wood and microbial symbiosis in wood-feeding insects.

Wood-feeding insects obtain nutrition using microbial associations in a number of ways. Direct consumption of one or more microbial associates may provide most, if not all, required nutrients (e.g., "fungus farming;" Mueller and Gerardo 2002), although additional microbial associations may be required to supplement essential nutrients, such as nitrogen (Gomathi et al. 2005, Morales-Jiménez et al. 2009, Pinto-Tomás et al. 2009). Alternatively, wood-boring insects may key-in on specific functional components of microbial pathways as required by the physical and nutritional demands of feeding on their preferred host (Kukor and Martin 1983, Kukor et al. 1988). Microbial digestive enzymes may be involved in neutralizing plant defensive chemistry and breakdown of wood polymers (e.g., "external rumen" and "exogenous enzyme" hypotheses, respectively; Swift et al. 1979, Kukor and Martin 1983). Microbial pathogenicity may be used by wood-feeding insects that are otherwise un-

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able to access host tissues (Coutts 1969a, Christiansen et al. 1987, Friesen and Faris 2012). Association with microbial symbionts differs depending on nutritional and functional demands of the food resource and traits of the microbial associates (Moran et al. 2005). Wood-feeding and association with microbial associates require specific morphological, behavioral, and associative adaptations for persistence through time. Association with wood-rot fungi by wood-feeding insects is well established (Jonsell et al. 1999, Chiappini and Aldini 2012). However, association between insects and wood-rot fungi is not evidence of functional relationships. The vast majority of wood-feeding insects may merely co-colonize with wood-rot fungi, but functional attributes of these associations are poorly understood (Jonsell and Nordlander 2004).

The invasive woodwasp, *Sirex noctilio* F., maintains an obligate mutualism with the wood-rot fungus *Amylostereum areolatum* (Chaillet ex Fries) Boidin. *A. areolatum* is a weak pine pathogen, but possess enzymes capable of digesting all components of pine wood (Coutts 1969b). Little is known about nutrient relations between *S. noctilio* and its fungal symbiont. It has long been assumed that the fungal partner was the major direct source of nutrition; however, recent studies suggest a more complex nutritional interaction (Thompson et al. 2013). Wood digestion is facilitated by both the physical and enzymatic disruption of cellular contents, which manifest in specializations of both the mouthparts and the gut of wood-feeding insects. Symbioses between wood-feeding insects and microbes often result in specialized behaviors and morphological structures. For example, termites have specialized mouthparts to preprocess wood that is then digested in gut chambers modified to facilitate microbial fermentative digestion (Deligne 1999, Brune and Friedrich 2000). Likewise, in wood-feeding Coleoptera, modifications of the gut and mandibles are indicative of feeding on fungal decayed or intact wood (Grünwald et al. 2010, Chiappini and Aldini 2012). This research describes characteristics of a wood-feeding mutualism between a woodwasp and its wood-decay white-rot fungal symbiont using functional morphology and feeding behavior.

Characteristics of diet and foraging preferences manifest in the biochemistry and morphology (e.g., mandibles, gut, salivary glands, etc.) of the forager (Schmidt et al. 2000, Hochuli 2001). Insect mouthparts are the first point of interaction with food substrates and are under direct selection for functional traits. Mandibles of wood-feeding insects provide important clues to the manner in which insects extract nutrients from the refractory food resource, that is, wood, and the role of microbial associates (Acorn and Ball 1991, Deligne 1999, Chiappini and Aldini 2012). In wood-feeding Coleoptera, "wedge-" and "chisel-shaped" mandibles correlate with feeding upon deteriorating and sound wood, respectively (Chiappini and Aldini 2012). The condition of wood fed upon has drastic differences in available nutrients. Sound wood retains stores of easily assimilable nutrients, such as sugars and starches, whereas decayed wood presents a nutritionally and structurally altered profile depending on the extent of enzymatic degradation and duration of fungal habitation (Martínez-Inigo et al. 1999). Wood digestion in insects is facilitated by both physical disruption of lignocellulose and diverse enzymes specific to the components of lignocellulose (e.g., lignase, cellulase, etc.). Morphological features of mandibles typical to this mode of feeding include the raised mola on the occlusal surface (OS) of mandibles in cerambycid beetles for grinding woody material and the presence of expanded invaginations of the gut for housing microbial symbionts for fermentative digestion (Stehr 1987, Delalibera et al. 2005, Grünwald et al. 2010). In contrast, wood-feeding Lyctidae do not consume cellulosic material and presumably practice extra-oral digestion, which again is reflected in the mandibles and streamlined morphology of the gut canal (Parkin 1940, Crowson 1981, Chiappini and Aldini 2012). Fermentative digestion of wood manifests with characteristic expanded chambers, which slow the passage of food and increase interaction with gut-associated fungi and bacteria, some of which are involved in nutrient supplementation (Brune and Friedrich 2000, Grünwald et al. 2010). Analysis of mandibles, gut, and feeding patterns of larvae have the potential to reveal how *S. noctilio* overcomes physical and nutritional barriers to feeding on pine wood and has important implications for understanding the ecology of this globally invasive insect.

As a group, sawflies exhibit a diversity of behavioral, anatomical, and symbiotic adaptations. Endophagous wood-feeding occurs primarily within the superfamilies Siricoidea and Xyphidroidea (Haack and Mattson 1993). The evolution to wood-feeding in Siricidae necessitated overcoming both physical and nutritional constraints of their preferred host plant tissue. Wood is enzymatically refractory and exceedingly low in available nutrients, such as nitrogen (Mattson 1980). The enzymatic and nutritional constraints of wood represent a major evolutionary hurdle to herbivores. Adaptations within the Siricidae to overcome barriers to wood-feeding opened up a ubiquitous, yet little used resource. It is currently unknown how sawflies managed the evolutionary transition into wood-feeding. In this study, we show that the constraints of wood-feeding for *S. noctilio* are coupled with adaptations associated with a wood-rot fungal mutualist for external digestion of wood and assimilation of ubiquitous energy-rich plant compounds.

Materials and Methods

Sample Collection. Tree sections colonized by *S. noctilio* were collected from red pines (*Pinus resinosa* Solander ex Aiton) from May to August in 2010 and 2011. Trees showing signs of female oviposition (e.g., resin beads, thinning crown, etc.; $n = 30$) were felled and trunks were sectioned to ≈60 cm lengths (bolts). Representative subsamples (5–6 bolts per tree) were taken from each tree for extraction of larvae. Sampled pines ranged in diameter from 13 to 24 cm at breast height (≈1.3 m). Larvae were extracted from pine xylem by tracking larval galleries and sequentially splitting bolts (<1 cm in width). Bolts were haphazardly selected from all possible bolts. All samples were collected in Tioga County, PA (41°44′54″ N; 77° 18′04″ W).

Foraging Pattern. We examined the location or pattern of larval foraging chambers relative to the fungal symbiont and its lignocellulosic enzymes using the methods described by Thompson et al. (2013) for staining laccase, a characteristic enzyme of white-rot fungi. Briefly, 1.5 ml of the enzyme stain ABTS (2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (1.175 mg/ml dH_2O) was drenched over a cross-section of xylem containing the feeding chamber of individual larva. ABTS is colorless until exposed to oxidase enzymes, such as the multicopper or phenol oxidase family of enzymes associated with white-rot fungi (Niku-Paavola et al. 1990). Laccase oxidizes ABTS solution turning it blue. Because of the limited permeability of wood and the molecular size of laccase, laccase is only found relatively close to the fungal hyphae from which it was excreted (Leonowicz et al. 2001). The close association between laccase and fungal hyphae yield a reliable assay for detection of white-rot fungal hyphae in wood (Niku-Paavola et al. 1990). Laccase is primarily associated with basidiomycete white-rot fungi (Baldrian 2004), but not all fungi have been tested for enzyme activities. The efficacy of the ABTS assay at differentiating *A. areolatum* from heterospecific fungi in trees naturally infested by *S. noctilio* was evaluated using a pure culture of *Ophiostoma ips*, a fungal symbiont of *Ips pini*, and the most common fungus encountered in pine trees colonized by *S. noctilio* in nature.

Foraging patterns of larvae relative to the fungal symbiont were evaluated by measuring the nearest distance (millimeter) from the anterior (head) end of the feeding cavity of larvae to the border between wood containing the symbiont and wood yet to be colonized by the fungal symbiont (n = 113). Wood bolts were cut from naturally infested red pines, split to reveal larvae, cross-sectioned and drenched with ABTS solution. The position of larvae relative to the nearest growth of the fungal symbiont was taken as a proxy for foraging tendencies in wood relative to new and old growth of the fungal symbiont in wood.

Larval Siricidae leave characteristic linear feeding galleries in pine wood (Rafes 1960, Madden 1981). Characteristic foraging patterns are indicative of active choice in foraging location (Scheirs and De Bruyn 2002). Larval ontogeny may affect foraging behavior (Hochuli 2001); therefore, we estimated larval instar as the diameter of the larval feeding cavity before staining with ABTS. Madden (1981) demonstrated that, depending on conditions within the tree, larvae go through 8–12 larval instars and that larval tunnel diameter reasonably approximates larval instar for trees of a similar condition. Larval spatial relationships to their fungal symbiont were plotted to visualize the relationship between symbiont and larvae in trees and was statistically analyzed by constructing linear models for size and spatial position data. Competing linear models were evaluated using multimodel selection (see Statistical Analysis for details).

Adaptive Morphology and Foraging Mechanics. Observations of larval feeding mechanics were made opportunistically on larvae that were apparently undisturbed during splitting of the tree trunk (n = 5). Splitting of bolts is highly invasive, but when possible, larvae were observed foraging in situ for up to 15 min. Special attention was paid to excavation mechanics and the fate of chewed wood within the oral cavity. After observation, larvae were fully excavated and placed in 70% ethanol for dissection. Samples of frass packed in galleries were inspected for indications of manipulations in the mandibles or gut. The term frass is commonly used to refer to insect feces, but has multiple meanings in the literature (Weiss 2006). In this study, system frass refers to the wood particles that are tightly packed into the trailing tunnel (gallery) left by foraging larva. Larval guts were dissected to examine ingested material in larvae and were supplemented by additional larvae extracted at the same time as larvae-observed foraging (n = 20 total). Internal organs were removed using fine tipped forceps and documented photographically using a Leica MZ APO stereo microscope (Leica Microsystems, Buffalo Grove, IL). Images of internal anatomical features were photo documented and analyzed using Nikon Digital Sight DS Fi1 digital camera and Nikon NIS-Elements BR imaging software (Nikon, Melville, NY). Images were annotated using Adobe Illustrator CS6 (Adobe System Inc., San Jose, CA). Scanning electron micrographs (SEM) of the head, mandibles, and frass were captured with a Hitachi TM3000.

Statistical Analysis. Linear models were fit to the foraging data describing larval size or instar relative to their spatial relationship with *A. areolatum* in the xylem. Distance of foraging larvae from the border of fungal symbiont and the size of the larvae at the time of collection were used as response and predictor variables, respectively. Linear, exponential, and piecewise regression models were fit to the foraging data and evaluated for explanatory power using akaike information criterion (AIC) criteria in the R package "AICcmodavg" (R Development Core Team 2009, Mazerolle 2012). Plots of the data revealed a possible threshold effect in late instar larvae. Therefore, we used piecewise regression and maximum likelihood to test for and locate a breakpoint response in larval behavior using the R package "segmented" (Muggeo 2008). Linear models for the separate segments in the threshold model were fit using the lm command in the base package and supplied as a single model for model selection (R Development Core Team 2009).

Results

Foraging Pattern. ABTS stains of laccase were specific to *A. areolatum* in laboratory cultures growing on sterilized wood and in red pine tissues naturally infested by *S. noctilio.* The ubiquitous bark beetle symbiont, *Ophiostoma ips*, did not exhibit oxidase activity against ABTS. The great majority of larvae (85%, n =

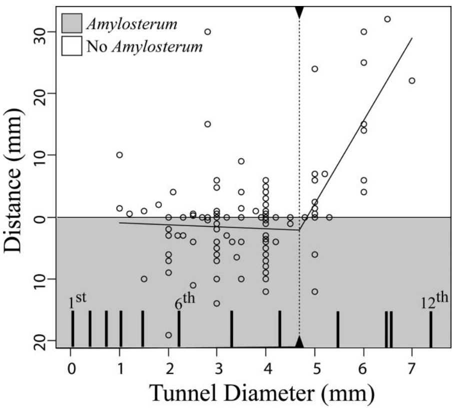

Fig. 1. *Sirex* larvae (n = 113) feeding in pine xylem colonized by the symbiont *Amylostereum* (gray) and uncolonized (white). Larva (○); line of best fit from piecewise regression model (solid black line); maximum likelihood estimate for behavioral shift to pupation (vertical —); and larval instar (solid bar x-axis, size or instar described in Madden [1981]).

113) were found within 1 cm of the edge of fungal symbiont growth in wood (Fig. 1). The area immediately adjacent to new fungal growth is expected to have the highest concentration of wood-degrading enzymes (Blanchette 1991). Larvae exhibited the characteristic feeding pattern up to a larval tunnel diameter of 4.7 ± 0.2 mm (mean ± SE), after which larvae appeared to leave laccase and other fungal enzymes (Fig. 1). Model selection results for linear models describing the relationship between larvae and the fungal symbiont in red pine indicated a threshold effect with respect to foraging behavior and larval instar (Table 1). Maximum likelihood analysis revealed a potential breakpoint in typical foraging behavior that was punctuated by a distinct shift in foraging pattern, estimated at a larval tunnel diameter 4.7 ± 0.2 mm (mean ± SE). Based on prior work showing strong positive correlations of larval tunnel diameter with larval size and instar (Madden 1981), we estimate this threshold corresponds to the eighth- to ninth-larval instar. The threshold model was marginally better than the exponential model, with both models predicting separation of larvae from the fungal symbiont at late larval instars.

Table 1. AICc model selection of linear models predictive of larval foraging patterns in red pine relative to larval size and distance outside or within wood colonized by the fungal mutualist *Amylostereum*

Model	K	AICc	Δ AICc	AICc Wt.	LL
Piecewise	4	757.31	0.00	0.85	−374.47
Exponential	4	760.77	3.46	0.15	−376.20
Linear	3	789.96	32.66	0.00	−391.87
Null	2	807.99	50.68	0.00	−401.94

K is the number of model parameters. AICc is the model likelihood. Δ AICc is the AICc scaled so the lowest value is zero (for ease of interpretation). AICc Wt is the model wt. LL is the log-likelihood of model.

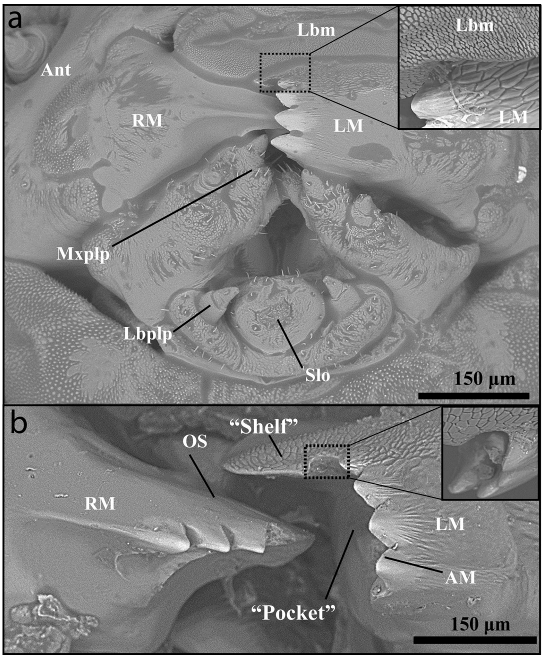

Fig. 2. Scanning electron micrograph of the feeding appendages of larval *Sirex* with mandibles occluded (a) and open (b). Right and left mandible (RM and LM, respectively) are asymmetrically aligned along the mesal margin. The mandibles are bordered by the maxilary (Mxplp) and labial (Lbplp) palps and the salivary oriface (Slo) from below and the a highly textured labium (Lbm; inset a). The top of the left mandible extends as a "shelf" above the OS of the right mandible. The "pocket" created by the "shelf" of the left mandible holds wood cut by the AM and the "carpenter's plane" (inset b), where it is pressed, releasing fluid fractions.

Adaptive Morphology and Foraging Mechanics. *S. noctilio* larvae possess asymmetric mandibles with anterior margins aligned perpendicular along the mesial plane (Fig. 2). When occluded, the right mandible fits into a pocket created by apical margin (AM) of the left mandible and a "shelf" protruding from the dorsal surface of the left mandible. The dorsal shelf extends above the right mandible forming a pressing surface or OS against the dorsal surface of the right mandible (Fig. 2b). The OSs of the mandibles are smooth except with a single groove running through the "pocket" of the left mandible and toward the posterior preoral chamber. In contrast to the smooth surfaces of the OSs of the mandibles, the dorsal surface of the left mandible and the labrum are highly textured (Fig. 2a inset). The toothed AM of the left mandible contains an indented cleft at the right angle where it meets the dorsal shelf (Fig. 2b inset). The AM of the left mandible and the "cleft" form a cutting plane for the excavation of wood in thin shavings that are putatively pulled into the oral cavity by the posteriorly facing teeth of the right mandible. Excavated wood is then crushed between the "shelf" of the left mandible and the dorsal surface of the right mandible, pressing liquid fractions into groves within the pocket that direct flow toward the oral cavity.

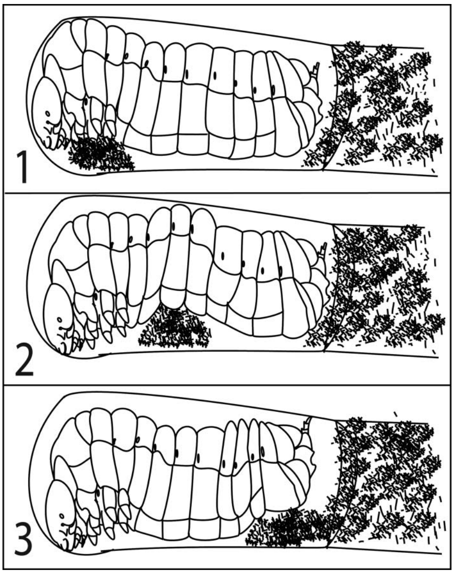

Fig. 3. Diagram depicting stages of feeding by *Sirex* larvae. After removal of liquid fractions by mandibles chewed wood (frass) accumulates under the head (1) before being positioned under the abdomen by the legs (2) and finally being pushed to the back of the feeding cavity via a peristaltic undulation of the abdomen (3).

Observations of larval feeding showed chewed wood dropped from mandibles under the head capsule where it accumulated in a pile. The accumulated pile of wood shavings was then passed externally along the ventral surface of the abdomen via peristaltic undulation of the body from head to tail (Fig. 3). The chewed or "pulped" xylem tissue (frass) was then packed tightly into the trailing gallery by the horn at the apex of the abdomen. Dissections failed to recover wood particles in the gut of larvae. Wood particles of <0.01 mm diameter were isolated from the proventriculus of one larva, but no wood was present in the midguts of any dissected larvae ($n = 20$).

The alimentary architecture of *S. noctilio* larvae is simple compared with that of the wood-feeding cerambycid beetle, *Xylotrechus saggitatus* (Britton) (Fig. 4). The guts of larvae lack expanded chambers typical for insects that employ microbial fermentation (Fig. 4a). Scanning electron micrographs of frass indicated minimal manipulation of xylem and are consistent with observations of foraging larvae and mandible functional morphology (Fig. 4b). In contrast, the wood-feeding cerambycid larvae had multiple chambers and produced frass that was highly processed and degraded (Fig. 4c and d).

Discussion

Wood-feeding is constrained by recalcitrant lignocellulose, nutrient deficiency, and chemical defenses of host plants. Wood-boring insects cope with these barriers through a combination of morphological, behavioral, and symbiotic adaptations (Klepzig et al. 2009, Chiappini and Aldini 2012). Here, we show that *S. noctilio* has multiple adaptations for xylophagy or wood-feeding that are influenced by interactions with its white-rot fungal symbiont, *A. areolatum.* We found support from foraging patterns, mandibular morphology, and internal anatomy for the hypothesis that the fungal symbiont acts as an "external rumen" for larvae foraging in pine wood.

In *S. noctilio,* the hypothesis for external digestion is supported by: 1) foraging in areas of greatest fungal symbiont enzyme activity, 2) specialization of mandibles for shearing xylem and squeezing out liquid extracts from wood, 3) limited manipulation of frass in feeding galleries, 4) limited gut lumen volume and lack of specialized fermentation chambers, and 5) and little to no wood tissue in the gut. This evidence

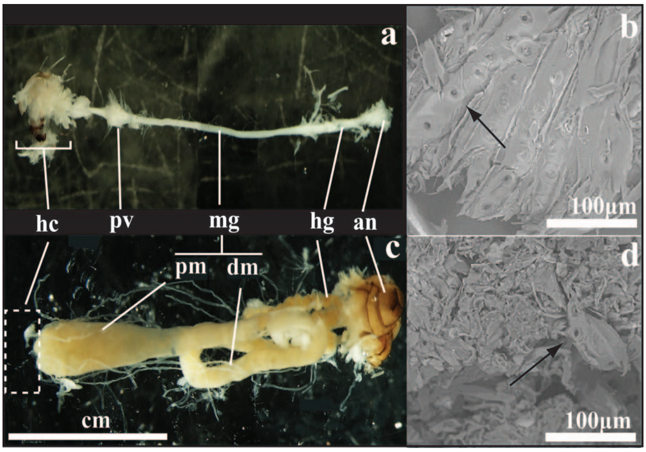

Fig. 4. Gut dissections showing the intestinal tract of larval *Sirex* (a) and a xylophagous red pine associate *Xylotrechus saggitatus* (c) and the morphology of their frass as observed under (600× magnification by SEM) (b and d, respectively). Fat bodies and malphigian tubules have been removed. Head capsule (hc); proventriculus (pv); midgut (mg); proximal midgut (pm); distal midgut (dm); hindgut (hg); anus (an); and tracheids (arrows). (Online figure in color.)

suggests larvae primarily ingest liberated organic compounds and monomers of fungal digestive reactions in the xylem (e.g., glucose, mannose, galactose, acetic acid, xylose, etc.; see review by Kirk and Cullen 1998). Outsourcing of digestive capability onto the fungal mutualist is supported by multiple evolutionary adaptations within the mandibles and gut for processing externally digested xylem. The Siricidae as a group are variously associated with *Amylostereum* spp. as internal feeders on woody tissue (Cartwright 1938). The association observed here between *S. noctilio* and *A. areolatum* may be representative of the Siricidae in general and may be a common theme among early colonizing wood-feeding Hymenoptera.

***S. noctilio* Foraging in the Presence of Its Symbiont.** Association with microorganisms benefit xylophagous insects through degradation of plant defensive compounds, digestion of recalcitrant plant polymers and through synthesis or assimilation of essential nutrients (Dowd 1992, Klepzig et al. 2009). Early instar larvae of *S. noctilio* fed in a pattern related to the presence of *A. areolatum* in pine xylem. Larvae were always located near the edge of fungal growth. However, later instar larvae (nearing pupation) were always found outside of fungal symbiont growth in trees. This pattern in feeding can be explained by the secretion of lignocellulosic enzymes that digest xylem tissue making nutrients accessible to the insect. Later instar larvae nearing pupation may benefit from disassociating from their fungal symbiont. *A. areolatum* excretes lignocellulosic enzymes into the xylem as it externally digests xylem tissue (Kirk and Cullen 1998, Leonowicz et al. 1999). Larval *S. noctilio* fed where new fungal growth and thus enzyme production were greatest (Blanchette 1991). Organic nutrients, such as nitrogen, may be concentrated in this area as fungi translocate limited nutrients to areas of active growth (Levi and Cowling 1969, Boswell et al. 2002). Translocation of nitrogen to areas of larval feeding may be an important role for *A. areolatum* in this symbiosis; however, trace levels of fungal-derived sterols in larval and adult tissues suggest that *S. noctilio* derives insubstantial direct nutrition from fungal sources (Thompson et al. 2013). Evidence from this study supports the hypothesis for external digestion or external rumen through selective feeding and morphological adaptations for consumption of liquid diet.

Advantages to Feeding on Wood. Sound wood has higher proportions of easily digestible polysaccharides than fungal degraded wood (Kirk and Cullen 1998). Wood-rot fungi preferentially assimilate nutrients across a compound digestibility gradient (e.g., starch and sugar preceding more recalcitrant polymers, such as cellulose and lignin; Kirk and Cullen 1998). Insects feeding on dead or dying timber face a dynamic nutrient profile that shifts from plant to fungal reservoirs. Insects specializing on dead trees are adapted to particular stages of decay depending on fungal communities (Jonsell et al. 1999). *S. noctilio* deposits eggs and its fungal associate *A. areolatum* in living trees (Madden 1974). Deposition of eggs into living trees presents particular challenges for colonization, but also for insect nutrition. Previous research indicated a plant diet (Thompson et al. 2013). The predominance of plant compounds in *S. noctilio* and colonization patterns suggest easily digestible plant compounds may be a driving force in host plant selection and is easily supported by location of larval feeding relative to *A. areolatum.*

Insect Phenology and Symbiont or Resource Quality. Separation from fungal mycelia of *A. areolatum* in wood may have important implications for symbiont transfer. Late instar larvae carry concentrated packets of the fungal symbiont in external mycangia that are believed to act in the ultimate colonization of the symbiont in internal mycangia of pupated adults (Morgan 1968). Late instar larvae of this study separated from their fungal symbiont before pupation. It is unknown why larvae separate from *A. areolatum.* Separation is suggestive of a cessation of feeding, but may be related to declining resource quality, avoidance of late instar predators, and parasitoids or avoidance of factors affecting successful transfer of the fungal symbiont. The separation observed in this study accurately follows descriptions of feeding paths in wood for other siricids (Rafes 1960). Cues within the tree, such as moisture, fungal age, and nutrient content, that trigger initiation of pupation have important implications on *Sirex,* as body size in adults is highly variable and is correlated to both fitness and fecundity (Madden 1974, Bruzzone et al. 2009).

Adaptations for Xylophagy in *S. noctilio*. Evolutionary modifications of mandibles to exploit specific food resources are widespread in insects (Acorn and Ball 1991, Chiappini and Aldini 2012). In the sawfly families Pergidae and Argidae, specialized sponge-like modifications of mandibles remove toxic eucalypt oils from the leaves they feed upon (Schmidt et al. 2000). In *S. noctilio,* modifications including chiseled teeth for shaving wood, overlapping pressing surfaces, and a basal sulcus within the cupped left mandible represent specialized features for feeding on wood in the presence of their fungal symbiont. Symbioses often impart adaptations that strengthen partnerships. In this wood-feeding symbiosis, fungal digestion has apparently supplanted insect digestion. *Sirex* specializes on predigested polymers resulting in a low volume gut, ill-equipped for digestion of wood. Adaptations of the mandibles support this mode of feeding. Each results in *Sirex* being dependent on its fungal partner for sustenance. In turn, *A. areolatum* relies on *S. noctilio* for transport to new hosts and has lost the ability to reproduce sexually (van der Nest 2013). *A. areolatum* is only weakly pathogenic to trees and cannot colonize wood in the absence of phytotoxins produced by *S. noctilio* and injected at oviposition (Coutts 1969a,b). It remains unclear how either larvae or their fungal symbiont overcomes nitrogen deficiencies of their host plant.

In conclusion, differences in feeding modes among wood-feeding insects reflect the various adaptions to food resources. Early colonizing insects are presented with different plant nutrients and fungal communities than later colonizing insects. The colonization patterns of xylophagous insects reflect their optimal foraging environment and are reflected in their morphological and behavioral adaptations (Aukema et al. 2004, Jonsell et al. 2005). In the case of *S. noctilio* and possibly other Siricidae, association with wood-rot fungi has mutual benefits of colonization and nutrition. In this study, we describe a suite of adaptations in larvae for external digestion and preferential extraction of easily assimilable plant compounds in association with a fungal symbiont.

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