CARRION BEETLES IN ORCHARDS AND RANCHES IN OKLAHOMA AND INVESTIGATION OF GENES ASSOCIATED WITH ANTIMICROBIAL PRODUCTION

By

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FULL CARRION BEETLES IN ORCHARDS AND RANCHES IN OKLAHOMA AND INVESTIGATION OF GENES ASSOCIATED WITH ANTIMICROBIAL PRODUCTION

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Abstract:

Family Silphidae, the carrion beetles, is a highly evolved family of Coleoptera that has members with unique social behaviors and physical abilities that allow them to utilize decaying vertebrate matter for sustenance and larval rearing. Two investigations were conducted to elaborate on the habitat association and gene expression of certain members of in the subfamily Nicrophorinae (burying beetles). The first investigation tested the effect of two forms of agricultural production on populations of Silphidae in eastern Oklahoma, with particular focus on the endangered American Burying Beetle (*Nicrophorus americanus* Olivier/ABB). Between 2017 and 2018, 10 weeks of field sampling was conducted in pecan orchards and cattle pastures of eastern Oklahoma, capturing a total of 2,338 Silphidae. Ultimately, no statistically significant differences were found between median capture values of Silphidae in either land usage (pecan orchard: [8]; cattle pasture [4]) in a majority of examined species, including the ABB. This finding suggests an overall trend toward generalism of habitat use by Silphidae in Oklahoma.

The second investigation tested the nature of antimicrobial secretions used by Subfamily Nicrophorinae (burying beetles) in the preservation of carrion and the expression of genes in response to food availability. Using two species of burying beetle, *Nicrophorus orbicollis* Say, which has a typical life cycle, and *Nicrophorus pustulatus* Herschel, a brood parasite, gene expression of excised salivary glands was analyzed through RNA sequencing of beetles exposed to and denied ground beef as food. *N. orbicollis* had higher expression of innate immunity compounds when fed (6.58% of characterized sequences) than did *N. pustulatus* (5.19%) and a higher percentage of expression overall (7.14%/4.22%). These findings suggest the active bactericidal compounds in burying beetle secretions to be antimicrobial proteins produced by individual beetles in response to feeding.

Together, these findings suggest that ABB can use areas of Oklahoma that are converted for pecan and ranching operations and that burying beetles have the potential to provide novel compounds for antibiotics or preservation of meat at room temperature.

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CHAPTER I

INTRODUCTION

Despite including the well-studied American burying beetle, *Nicrophorus americanus* Olivier, there is still much not yet studied about family Silphidae, also called the Carrion beetles. I first reviewed the literature and then conducted two investigations, one in the field to examine the effects of habitat change on Silphidae, and one in the laboratory to investigate production of antimicrobial proteins. I first reviewed the literature and then conducted two investigations, one in the field to examine the effects of habitat change on Silphidae, and one in the laboratory to investigate production of antimicrobial proteins.

The habitat association of many species of burying beetle is at least partially understood, with some preferring the presence of water (*Necrophila americana* Linnaeus), trees (*Nicrophorus orbicollis* Say), or sandy rangeland (*Nicrophorus carolinus* Linnaeus). Additionally, it is understood that human development and conversion of land from its natural state has a detrimental effect on Silphidae populations and the vertebrates that they rely upon. However, because of increasing demands for food, it is important to understand how Silphidae react to agriculture other than pivot-irrigated croplands. This is especially important because the ideal habitat association of the endangered American burying beetle is still largely unclear. In reviewing the literature, the effects of fruit orchards on the American burying beetle have not been investigated, and the effect of rangeland ranges from mixed reactions in Nebraska populations of American burying beetle to

not providing support for Oklahoma populations. Therefore, the goal of my first investigation was to conduct field surveys to determine the effects of pecan orchards and cattle grazing in Oklahoma on the occurrence of carrion beetles, including the American burying beetle.

Because members of the subfamily Nicrophorinae utilize small vertebrate carcasses for reproduction and because they must protect these resources from decomposition by soil microbes, there is growing interest in antimicrobial protection by burying beetles. These compounds, produced by most burying beetles, are necessary to successfully utilize carrion for food and for brood rearing. However, there is substantial debate in the literature concerning the form of antimicrobials and whether the beetles themselves are responsible or if the microbiota of their guts are responsible for carcass preservation. In the second part of my research, I tested the genetic response of two species of genus *Nicrophorus*, one that exhibits a typical life cycle and another which is a brood parasite, to the presence of food. Specifically, I examined RNA expression to determine if genes were differentially expressed between the species and feeding conditions. This research can lead to greater insights into how antimicrobial compounds are produced by burying beetles.

CHAPTER II

REVIEW OF LITERATURE

Silphidae

Silphidae is a small family of Coleoptera commonly referred to as the "carrion beetles". They were originally described by Linnaeus in 1758 (Sikes et al. 2002). Carrion beetles are known primarily for using carcasses of small deceased vertebrates, with differing preferences and behaviors employed by two subfamilies, Silphinae and Nicrophorinae (Aleksandrowicz et al. 2005). The family Silphidae contains 30 genera and 208 species worldwide, of which 8 genera and 30 species are found in North America (Bedick, et al. 1999). While Silphinae primarily feed on larvae of other scavengers found in carrion, most Nicrophorinae (burying beetles) feed directly on flesh (Hoback et al., 2004). This difference is represented evolutionarily, as some members of Silphinae have moved toward a flightless or flight-dimorphic state, allowing for more predatory lifestyle, while all known Nicrophorinae retain flight ability to facilitate the search for carrion resources (Ikeda et al. 2008).

This source of food is high in nutrients, but its availability is unpredictable in the environment and its reliability as a food source is dependent on the activity of other necrophores (Eggert and Muller 1997). Burying beetles have a limited window of opportunity to secure and

prepare a carcass, because any carrion in the environment is the target of other invertebrates, including necrophagous, omnivorous, adventious arthropod species, vertebrate scavengers, bacteria and fungi (Campobasso et al. 2001). When vertebrates die during the day, they are rapidly colonized by scavenging flies (primarily families Calliphoridae and Sarcophagidae) attempting to deposit eggs. Any Silphidae attempting to secure a carcass must compete with anaerobic bacteria already inside the carrion that breaking down essential carbohydrates, lipids, and proteins, other necrophagous families of beetles such as Staphylinidae and Histeridae, and face both competition and predation from ant colonies (Eubanks et al. 2002; Scott et al. 1987; Shayya et al., 2018).

Between consumption by fly maggots and different microbes, the carcass will rapidly lose mass (a period referred to as "advanced decay") and will become unsuitable for reproduction by burying beetles (Carter et al., 2007). *Nicrophorus tomentosus* Weber was found to produce fewer offspring when in competition with flies (Scott 1994). In addition, a carcass may be overtaken by other Silphidae, and males will compete for both carrion and mating opportunities (Beeler et al.. 1999). While smaller burying beetles may be able to colonize more carcasses, larger beetles tend to be better at overtaking and defending a carcass, showing an interesting tradeoff that may work in either party's favor depending on the environment (Suzuki 2000).

Large clubbed antennae equipped with advanced chemoreceptors allow Silphidae species to detect decaying flesh from a great distance, as well as allowing females to detect males that release pheromone signals to attract mates and aid in securing the carcass (Anduaga 2009; Beeler et al. 1999). Through electroantennography, *N. vespilloides* was found to be sensitive to sulfur-containing compounds released from mouse carcasses following death. Dimethyl sulphide, dimethyl disulphide and dimethyl trisulphide were found to be particularly attractive, and traps baited with the two latter compounds yielded higher captures than traps with solely dimethyl sulfide (Dekeirsschieter et al. 2013; Kalinova et al. 2009; Podskalska et al. 2009). Interestingly, shaved mice carcasses produced less odorous chemicals than those with fur intact, which may explain the additional behavior of "shaving" a carcass performed by *Nicrophorus*, as doing so reduces the

likelihood of a carcass being discovered by other organisms attempting to utilize it (Woodard. 2006). In a laboratory setting, females exposed to a fresh carcass laid more eggs than females exposed to an aged carcass, suggesting the ability of females to judge carcass condition (Jacobs et al. 2014).

Upon securing a carcass, the beetles need to sequester it from flies and scavengers. When a carcass is found, the pair will sequester it by creating a "brood ball," a spherical mass of de-furred flesh buried underground. *Nicrophorus* also has the capability to opportunistically utilize the abandoned burrows of subterranean rodents to sequester their carcasses (Smith et al. 2000). Burial slows loss of weight and controls temperature variations, due to the absence of larval masses from Diptera and other insects normally colonizing exposed carrion (Correa et al. 2014).

Burying beetles have evolved powerful and specialized physical capabilities and advanced social behaviors, including behavioral plasticity and nurturing of offspring (Beeler et al. 1999). These are one of the few Coleoptera, or insects in general, where a monogamous mated pair will raise and protect their young (biparental care) to increase chances of survival in their brood. Biparental care may have evolved to ward off infanticide by invading Silphidae (Trumbo 2006). There are species-specific maternal behaviors, which influence the growth of larvae in unique ways (Benowitz et al. 2015). Parents are capable of making advanced choices during brooding, including discrimination between individual mates, infanticide, and defense against unrecognized beetles (Steiger and Muller 2010; Trumbo 2006). Once brooding begins, parents must provide almost constant effort to ensure that the prepared carcass remains secure and nutritious for growing larvae. The young are altricial, and parents will provide care regardless of delays in their own nourishment in order to ensure broad success (Hopwood et al. 2013). In an experimental setup where larvae were allowed and denied parental intervention, the presence of parental care increased larval size and successful maturations than broods developing without parental involvement in *Nicrophorus vespilloides* Herbst. This level of behavioral plasticity is due to the high competition needed to survive on a prepared carcass, which creates a resource requirement bottle-neck that leads to heavy

niche specialization in an environment where resources for survival are so unpredictable (Royle and Hopwood 2017). The female will deposit fertilized eggs in adjacent soil, and the hatched larvae move into the brood ball for food and shelter. There, they will be nurtured with regurgitated carrion until they are capable of feeding themselves. The parents may provide an excision in the carcass' integument to provide easier access to its biomass for when the larvae reach a self-sustaining instar (Eggert et al. 1998). Larvae remain guarded by their parents and nourished using the brood ball, until growth is complete. Larvae then disperse into the soil where pupation occurs and new beetles emerge in approximately six weeks (Suzuki, 2013). Many species in northern climates overwinter utilizing antifreeze proteins, a rare strategy among northern temperate insects (Duman et al. 2004).

An additional feature of burying beetles is their abundant accompanying phoretic mites (*Poecilochirus carabi* Canestrini), which breed alongside their hosts. These mites have been observed to exhibit a range of host associations. Some have shown host generalism and opportunistic host shifting while other populations exhibit specificity to individual species within *Nicrophorus* (Brown and Wilson. 1992; Duarte et al. 2017; Nehring et al. 2017; Salona-Bordas and Perotti. 2014; Schwarz et al.. 1998). The mites breed on decomposing corpses or fly eggs, and use scavenger insects for transport (phoresy), a practice shared with other species of insects, nematodes, fungi, and microbes that rely on ephemeral resources that are unpredictable (Wilson and Knollenberg 1987). The mites engage in a symbiotic relationship with burying beetles, providing services including cleaning for the host (Perotti and Braig 2009). The timing varies by species, but mites will attach to their host at a species-specific point in their host's life cycle, usually targeting hosts of larger body weight, and disembark with the host to colonize a new carrion resource.

The mites that are specific to burying beetles reproduce alongside the hosts, often within the same brood chamber, and prepare the next generation (Grossman and Smith 2008; Schwarz and Koulianos. 1998; Schwarz et al. 1998; Wilson and Knollenberg 1987). Wilson and Knollenberg (1987) found *P. carabi* to alternate between mutualistic and parasitic interactions with *Nicrophorus orbicollis* Say. The mites contributed in the short term reduction of fly competition, but also preyed

on their host's brood when population density was too high. Ultimately, after 2-3 broods, the absence of mites decreased fitness in *N. orbicollis*, suggesting an overall positive benefit to Silphidae from their presence (Wilson and Knollenberg 1987). In contrast, laboratory experiments analyzing the relationship between *P. carabi* and *N. vespilloides* found that mites increased bacterial diversity on the carcass while causing host beetles to reduce the antibacterial activity of their secretions, leading to no benefit to the beetle or reduction of bacterial abundance (Duarte et al. 2017). An additional study found increased *P. carabi* density negatively affected burying beetle success by preventing *N. vespilloides* larvae from attaining optimum weight (De Gasperin and Kilner 2016). Oophagy of *N. vespilloides* was noted by the mite species *Poecilochirus davydovae* Hyatt, adding yet another potential reason for reduced clutch sizes (Blackman and Evans 1994).

Antimicrobial Peptides

Unlike the acquired immunity found in vertebrates, where exposure to specific antigens produces a recognition and a subsequent immune response, insects rely on innate immunity (Boman. 1995; Lavine and Strand 2002). Innate immunity, found in all organisms, is a nonspecific set of immune factors coded uniformly, and provides broad protection against a wide array of pathogens. Compounds created by a particular species' innate immunity may correspond to the endemic flora of their environment, and may not recognize foreign pathogens (Boman 1995). In insects, immunity consists of a cellular and humoral response. Inside the haemolymph, hemocytes are present to provide cellular immune responses, protecting the insect through phagocytic destruction of damaged cells, wound clotting, and healing (Urbanski et al. 2016). The humoral response consists of chemical, protein, and enzyme responses (Lavine and Strand 2002). Included in the humoral response is the production of antimicrobial peptides (AMPs), produced in the fat body (Hoffmann et al. 1996). AMPs are numerous and a crucial part of innate immunity by multicellular organisms against gram-positive and gram-negative bacteria, protozoa, fungi, and viral infection (Bulet et al. 1999; Okorochenkov et al. 2011). Of the AMPs, the only category

utilized externally by insects is defensins, which primarily impact gram-positive bacteria (Bulet et al. 1999; Hoback et al. 2004). By attacking the bacterial membrane, the cytoplasm loses control over permeability and hemorrhages crucial molecules, resulting in cell lysis. Depending upon the defensins, bacteria may be killed immediately or over the course of a few hours (Bulet et al. 1999). A compound associated with cellular and humoral response is prophenoloxidase, a defense against invading pathogens linked to the recognition and immobilization of bacteria (Marmaras et al. 1996).

Burying beetles must produce antimicrobial compounds to preserve the carcass, thereby allowing survival and growth of larvae. Parental beetles coat the carcass with secretions produced both orally and anally. In doing so, the pair is able to limit growth of bacteria, which helps them successfully maintain a carcass for personal nourishment and brood rearing, and to protect their young (Arce et al. 2012). If parents are removed, the buried carcass will lose mass brood size will decline (Jacobs et al., 2014). In order Blattodea, *Pseudacanthotermes spiniger* Sjoestedt utilizes a similar mechanism to protect its eggs from the fungi it uses for nutrition, producing an antifungal and mildly antibacterial solution containing termicin (Da Silva et al. 2003). In order Hymenoptera, *Philanthus triangulum* Fabricius also preserves its prey with glandular secretions (Jacques et al. 2009).

Arce et al. (2012) successfully isolated anal secretions from *N. vespilloides*, and attempted to identify them as a possible antimicrobial agent. Subsequently, they analyzed the pathway of the substance, tested its efficacy as a bactericide, and attempted to quantify its impact upon fitness. Using plates of *Escherichia coli* Migula and *Bacillus subtilis* Ehrenberg, secretions taken from female *N. vespilloides* under different environmental conditions were tested. When secretions were taken from a beetle without access to a carcass, there was no notable effect. When the beetle was given access to a carcass, secretions produced at the time of egg laying had significantly reduced bacteria populations through lysis and they determined that the acting component is an insect lysozyme (Arce et al., 2012).

Comparative studies have found burying beetles vary in both the strength and administration of antimicrobial secretions. For example, the oral secretions of *Nicrophorus marginatus* Fabricius showed higher antimicrobial property than anal secretions, while the opposite was found in *N. vespilloides*. Other species were found to utilize both anal and oral secretions (*N. tomentosus, N. orbicollis*) to secure a carcass. This corresponds with differences in soil and habitats preferred among species. While most *Nicrophorus* are capable of secreting antimicrobial compounds, some have greater capacity (*N. tomentosus, N. orbicollis*), and some appear to lack the capacity entirely (*N. pustulatus* Herschel, *N. carolinus* Linnaeus) (Hoback et al. 2004). However, follow up studies by Jacques et al. (2009) showed that *N. carolinus* produces antimicrobial secretions at warmer temperatures, highlighting the importance of experimental conditions and their influence on antimicrobial activity. *N. tomentosus* and *N. orbicollis* may compensate for the comparatively reduced activity of their secretions by utilizing by using both anal and oral secretions to provide carcass preservation. *N. orbicollis* also cares for its larvae longer, and produces larger larvae compared to *N. vespilloides* (Benowitz et al. 2015), which could explain the need for additional production of antimicrobial secretions. Trumbo (1994) hypothesized that *N. pustulatus* does not have need for antimicrobial secretions because it has been shown to be a brood parasite (Trumbo, 1994). This species has also been recorded acting as a "parasitoid" of oviparous snakes, targeting the eggs of black rat snakes, *Elaphe obsoleta* Say (Blouin-Demers and Weatherhead 2016; Smith et al. 2007). In at least one species (*N. vespilloides*), production of antimicrobial compounds in anal exudate only occurred when the species was presented with a carcass (Cotter and Kilner 2010). The primary active compound in antimicrobial secretions is *lysozyme*, an antibacterial protein associated with innate immunity that causes cell lysis by mechanically degrading the peptidoglycan layer of a bacterial cell wall (Boman 1995). In monitoring gene expression of *N. vespilloides*, the Lysosome-coding gene Lys6 was the 14th most transcribed compared to 5,967th in a nonbreeding females and was upregulated 1,409 times more in breeding females. This is only

one of multiple genes coding for multiple lysosomes, furthering evidence that lysosomes play a key role in the *Nicrophorus* breeding process (Palmer et al. 2016).

The production of antimicrobial compounds is both sex-specific and triggered by the presence of a carcass or offspring, because of energy-expense in generating these enzymes (Jacobs et al. 2016; Steiger et al. 2011). Arce et al. (2012) reared *N. vespilloides* larvae on chicken liver inoculated with a buffer control, parental anal secretion, or two concentrations of lysozyme. Results indicated a strong increase in survival of larvae given chicken liver with either the anal secretion or high lysozyme concentration, and in turn, an increase in survival and dispersal of larvae that successfully made it to last instar (Arce et al. 2012). This supports the conclusion that lysozyme-laden anal secretions are the source of *N. vespilloides* antimicrobial activity, and demonstrates their positive effect on brood success.

Other enzymes may function for both digestion and antimicrobial activity. The oral secretions of *N. marginatus* contain large quantities of Phospholipase A2 (PLA2), which reacts with fatty acids and is suggested to hydrolyze bacterial membranes (Jacques et al. 2009; Rana et al. 1997). Anal secretions are used defensively by many beetles. *Necrodes surinamensis* Fabricius, the only member of Silphinae to produce antimicrobial secretions, produces a mixture of aliphatic acids and terpene alcohols for defense. These chemicals reduce bacterial growth and show that defense chemicals may serve as the original source of antimicrobial peptides (Roach et al.. 1990).

In addition to antimicrobial proteins in oral secretions, the anal secretions of *N. vespilloides* contain digestive and detoxifying enzymes, as well as antimicrobial gut microbiome species including *Yarrowia* ascomycetous yeasts, which are part of extraoral digestion and bacterial control (Vogel et al. 2017). These produced proteins and symbiont microorganisms contribute to allowing *Nicrophorus* to utilize vertebrate carrion. These helpful microorganisms may be shared via vertical transmission in anal secretions, but Kaltenpoth and Steiger (2014) conclude that these colonies are acquired from the environment or horizontal exchange. It has been suggested that the beetles do not actually “preserve’ the carcass at all, and they instead overwhelm bacteria on the carcass with

the contents of their own gut biome, which increases the bacterial load and eliminates colonies from the environment. This hypothesis is at least partially supported by studies that found that bacterial colonies on a prepared carcass align more closely with those of *N. vespilloides'* oral and anal secretions than what would be found on an unmaintained corpse (Shukla et al. 2018).

This interaction between *Nicrophorus* parents and larvae is referred to as "social immunity", defined as immune responses that increase the fitness of multiple individuals besides the individual that mounts the response (Cotter et al. 2010; Duarte et al. 2016). In cases of "personal immunity" (where the challenged individual is the main beneficiary of the response), invertebrates' innate response includes the production of antimicrobial peptides and lysozymes to kill or impede the spread of bacteria (Cotter and Kilner 2010). These responses with antimicrobial secretions are essentially the humoral immune response, projected outward from the body to affect the environment, leading to a benefit to the larvae. This social immunity occurs at cost to the parents. Limited availability of resources means that the beetle must balance its own personal immunity with the protection of the brood. Any compromising of the beetle's own immunity results in decreased reproductive investment (Reavey et al. 2015). During periods of induced production of antimicrobial secretions, *N. vespilloides* had spikes in concentration of juvenile hormone (JH), a possible indicator (or even regulator) of this activity (Cotter and Kilner 2010). These spikes occur upon the discovery of a carcass and the appearance of larvae (Reavey et al. 2014). These fluctuating JH levels, and the necessary redirection of energy toward antimicrobial creation and lytic activity, have been linked to shortened lifespan. Energy, nutrients, and amino acids that would be utilized for egg production or tissue repair are diverted to brood ball maintenance (Cotter et al. 2010). Reavey et al. (2014) found evidence that parents suppressed personal immunity during breeding, only reducing egg laying when immune upregulation occurred post-breeding (Reavey et al.. 2014).

Whether the brood itself contributes to this social immunity is still in question. In an experiment to determine whether *N. vespilloides* eggs contribute to their own survival, Jacobs et al. (2014) separated fresh eggs from the mother and inoculated them with bacteria (concentrated *E.*

coli and *M. luteus*). Additional eggs were probed with similarly infected and uninfected tungsten needles to mimic "septic" and "sterile" wounds. Both sets of eggs were incubated to allow bacterial growth. The exposure to bacteria caused fatality equivalent to a ~30% decline in potential brood size, and the researchers found no indication that the eggs themselves produced any antibacterial compounds. There was also little expenditure by the parents to protect the eggs directly. It is hypothesized that, during this stage, the parent's efforts are focused upon securing and preparing the carcass, so as to ensure their brood has a usable shelter when they hatch (Jacobs et al. 2014). Moreover, females lay eggs in a separate chamber near the carcass, rather on the carcass itself (Milne and Milne 1976).

N. vespilloides larvae are capable of producing antimicrobial compounds. Riley (2014) showed larvae to secrete antimicrobial agents independent of parental involvement, maintaining lysozyme-like activity (LLA) in oral secretions in response to a microbial signal. Further evidence that the active antimicrobial compound is an insect lysozyme from the larvae's inability to endure exposure to lysozyme-resistant *S. aureus* compared to a lysozyme-susceptible strain (Arce et al. 2013).

Reavey et al. (2014) found that *N. vespilloides* larval antibacterial secretion production was maximal immediately after birth and that the rate decreased with age. They hypothesized this to be a protection by the larvae to survive being orphaned, or to divert energy to development and self-feeding. This could also correspond with the parents' initial efforts to inoculate the carcass with their gut flora, and wane as the immunity has been established.

During brood rearing, the roles taken by the parents may be sex-specific. Female *N. vespilloides* produce greater concentrations of antimicrobial agents than the males. Males take a greater role in physical defense of the brood. When the circumstances were artificially altered, however, the beetles displayed a limited flexibility (Cotter and Kilner 2010). When pairs were separated, "widowed" males left to care for the brood increased their production of antimicrobial agents to compensate, though they were not able to fully replace the female's production.

Experimentally widowed females, did not increase their production but instead reduced the antimicrobial concentrations, which were then comparable to widowed males. Neither widowed sex was able to fully replicate the productivity of a mated pair, affirming the need for biparental care to maintain social immunity in this species (Cotter and Kilner. 2010). For *N. orbicollis* a pair of caretaking females had four times the reproductive success of single females (Trumbo 1994).

In a laboratory setting, *N. tomentosus* had increased reproductive success while in competition with maggots when engaging in group rearing, with groups of four faring better than pairs alone (Scott 1994). In cases of interspecies conflict with individuals being excluded, or in cases of large enough carrion to support multiple broods, hybrid couplings of subordinate and dominant females and males can form and tend to rear individual or joint broods, with the same essential roles being filled (Eggert and Muller 1997).

Urbanski et al. (2017) examined the effects of temperature and season on personal immunity, artificially exposing two groups of *N. vespilloides* to conditions imitating the different seasons in Poland during development. During the experiment, haemolymph was extracted and tested for cellular and humoral response activity. Cellular phagocytic activity was high in summer, but decreased considerably in autumn and winter. Humoral phenoloxidase activity was relatively unaffected, staying consistent throughout summer and showing a slight dip in autumn followed by a high point in the middle of winter. Jacques et al. (2009) examined the effects of temperature and food type on oral secretions of two *Nicrophorus* species. They found that the protein content of *N. carolinus* was negatively affected by low temperature, with 25°C producing much higher antimicrobial activity than 4°C. *N. marginatus*, however, had high activity at 4°C and 10°C. These results suggest different species have ideal temperatures for antimicrobial activity. In analyzing food choice, both species' secretions had an increase in protein content when presented with carrion (rat) than when presented with non-carrion meat (ground beef). The proteins secreted would be likely used in the production of a brood ball, showing the beetles to be flexible in response to food and reproductive resources (Jacques et al. 2009). Further experimentation with a wider variety of

variables surrounding burying beetle's food selection and brood rearing would garner a better understanding of conditions to which they are adapted.

There are potential medical and industrial applications of our understanding of antimicrobial substances derived from burying beetles. Given that AMPs do not attack microbes metabolically, but physically, they offer a potentially different mode of action against Multidrug-resistant bacteria. Insect AMPS, in general, are multipotent and not cytotoxic (Bulet et al. 1999). Ntsawa et al. (2012) suggested that AMPs are a viable resource in developing new clinical and veterinary therapeutics due to the lower likelihood of subsequent pathogen resistance and because they are generally fast acting and effective against susceptible pathogens. There are already peptide-based medications on the market as antibiotics including Polymyxin-B, which is used for the treatment of Gram-negative bacterial infections (Ntwasa et al. 2012).

The greatest hurdles to overcome in exploring this resource are cost, AMPs' potentially damaging hemolytic ability, and their reduced survival with proteolytic enzymes found inside a living body. Alternatively, these compounds also have potential use in the meat industry, as a tool for inhibiting bacterial growth on surfaces. *Nisin*, a bacteria-created antimicrobial peptide (*bacteriocin*), inhibits Gram-positive bacterial pathogens such as *Listeria* on beef when applied in a liquid form and when incorporated into a Polyethylene film wrapping (Siragusa et al. 1999).

American Burying Beetle

The best known North American Silphid is *Nicrophorus americanus* Olivier, the American Burying beetle or "ABB" (USFWS 2008). It is the largest of family Silphidae, weighing up to 2g and measuring 5-6 cm in length. The species is highly mobile, able to fly 0.10-18.14 miles (1-29.19km) through their range when weather conditions are favorable (Jurzenski 2012). As with any other Nicrophorinae, adult ABB will locate a suitable carcass, between 100-200g in size, and a pair will form a brood ball after working together to bury a suitable carcass underground. In the spring, when nightly temperatures exceed 15.5°C (60ºF), ABB activity begins. In autumn, activity

ends when nighttime temperatures fall below this same threshold. During this time, young ABB move underground to overwinter (USFWS, 2015). This results in an active season beginning as early as April and as late as October. Depending on the region, survival improves with the availability of carrion near the end of the active season, with size and gender not affecting survival rates in areas where soils never freezes (Schnell et al. 2008). When a carcass is used for breeding larvae reach maximum size in approximately 14 days. Pupating larvae remain in nearby soil for 48-65 days until Teneral adults emerge (Ratcliffe 1996; Schnell et al. 2007). The older, Senescent parents will die within the season, and their offspring will continue the cycle by establishing a brood of their own in the following year (Peck and Anderson 1985).

Decline

Historically, ABB was commonly found and ranged across 35 United States and 3 adjacent Canadian provinces, accounting for the entire eastern half of the country (Davis 1980; Peck and Kaulbars 1987). During the last century, that former range has since dwindled to only 6 states on the outer edges of ABB's historic range. Currently, the known occurrence of ABB include a handful of counties in South Dakota, Nebraska, Kansas, Oklahoma, Arkansas, and Rhode Island (Miller and McDonald 1997). This change represents up to a 90% decline in range (Lomolino et al.. 1995). Three geographically isolated populations are centered in Nebraska, Oklahoma, and on Block Island, Rhode Island (Bedick et al. 1999; USFWS 2015). When ABB was added to the Federal Endangered Species list on August 14, 1989, it was only known in Block Island, RI and Latimer County, OK, but subsequent surveys expanded its range to include populations in Arkansas, Kansas, Nebraska, and South Dakota. One county of Texas was recorded, though this population has been subsequently extirpated. In the U.S. Fish and Wildlife's 5-Year Review of the ABB (2008), it was reported to occur in 21 counties in Oklahoma, with a particularly notable concentration located in Muskogee County (Lomolino et al. 1995; USFWS 2008). Many factors contribute both to the species' decline and being able to effectively sample for its occurrence,

including its ability to travel great distances and its low detectability (Leasure, 2017). In particular, there is a lack of understanding of the demography and habitat preferences of ABB, which hinder conservation efforts and have resulted in no critical habitat being identified for the species.

Creighton et al. (1993) hypothesized that one of the greatest factors contributing to the struggle for survival is its size compared to other members of *Nicrophorus*, as the ABB is the largest of North America's burying beetles. The large size of the beetle leads to the need for larger carcasses, which are comparatively rare compared to smaller carrion. The decline or loss of species of suitable size within recent history, including the now-extinct Passenger Pigeon and declining prairie dog and quail populations likely impacted some portions of the ABB's range (Sikes and Raithel 2002). The loss of ABB has also likely impacted other parts of the food chain. Field observations have shown the species to be eaten by larger vertebrates, with Jurzenski and Hoback (2011) finding evidence of ABB being consumed by *Didelphis virginiana* Kerr (American opossum) and *Lithobates* Fitzinger (leopard frogs).

Sikes and Raithel (2002) reviewed proposed explanations for ABB's decline and lack of recovery. DDT usage had been argued against, due to the species' eventual absence from areas never treated with the pesticide (Raithel 1991). Modern-day uses of pesticide for control of rangeland grasshoppers may have some effect. Jurzenski (2012) found direct mortality of *N. marginatus* from Malathion and detriment to brood success in *N. orbicollis*, but given the nocturnal nature of ABB it is unlikely pesticide control poses a direct threat to surviving populations (Jurzenski 2012). In tandem with other effects of agriculture and land conversion, pesticides may limit reproductive success for the species, though insufficient research has been conducted to fully test this hypothesis.

Expansion of human populations and increases in the amount of artificial light has also been explored. ABB and other Silphids have varied reactions to artificial light, though it has been suggested to be only a minor hindrance because of continued abundance of *N. pustulatus* (Sikes and Raithel 2002). Recent research by Wormington et al. (2017), however, revealed that ABB

captures declined with increased moonlight and that cloud cover near cities produced the same effect by reflecting city lights. In contrast to the response of ABB, *N. orbicollis* was unaffected.

Lastly, the phylogenetic placement of ABB coupled with its distribution at the edges of its former range has given rise to the hypothesis that a pathogen uniquely deleterious to the species has spread, leaving the remainder of Nicrophorinae unaffected (Peck and Anderson 1985). To date, no data has been found to support this hypothesis.

Ultimately, the cause of ABB decline is considered anthropogenic in origin (Creighton et al. 2007). The hypothesis presented as the "best explanation" for ABB decline is habitat fragmentation and its effects on community species composition (Sikes and Raithel 2002). Gibbs and Stanton (2001) determined that total carrion beetle abundance was much higher in contiguous forests than fragmented forests, noting that vertebrate species thriving in fragmented forests were generally smaller generalists. They also suggested a link between fragmented habitat and the prevalence of vertebrate scavengers that compete with carrion beetles for ground-nesting songbirds (Gibbs and Stanton 2001). Trumbo and Bloch (2000) quantified the effects of habitat size adjustment with non-endangered burying beetles, showing that *N. marginatus* was never present in fields of <5ha, but was the only species found in areas of 25ha. Leasure and Hoback (2017) found ABB occurrence to be negatively influenced by human populations. A study on the effect of habitat fragmentation of forest-dwelling Silphidae in New York found decreased carrion beetle diversity, increased fly populations, and inconsistent phoretic mite populations in forest fragments compared to contiguous forests. The Silphidae found in these fragments were small-bodied habitat generalists rather than larger species (Gibbs and Stanton 2001). In similar studies, larger body size has been linked to a greater susceptibility to fragmentation in Carabidae (ground beetles) and Staphylinidae (rove beetles) (Jennings and Tallamy 2006).

A study on Japanese Silphidae linked loss of forest to increased removal of useable carcasses by vertebrate scavengers, and decreased burial by burying beetles. Creighton et al. (2007) concluded that ABB did not occur areas of tree removal, while they occurred in adjacent areas

where the forest was not impacted (Creighton et al. 2007). In contrast, ABB in the loess canyons of Nebraska decrease in the presence of invasive Eastern Red Cedar forests and instead favor open grassland (Walker and Hoback 2007).

If the breeding pair of burying beetles is unable to secure a suitable carcass, their cache of food is disturbed, or their pupae are prematurely uncovered, they will not successfully reproduce. Because most burying beetle species are univoltine and active only during warm periods, there is limited opportunity for the species to breed.

Throughout the central US, prairie has been tilled for cropland or converted for livestock grazing. As with any activity that changes the flora of an environment, the effects on biodiversity depend on the capability of endemic species to adapt, and those unable to do so will be extirpated. Any disruption of the beetles or their caches of carrion upsets the cycle, and may prevent a successful new generation. The very specific needs of the ABB requiring the largest carcasses, means that changes in carrion resources along with an increase in competition from vertebrate scavengers, and disruption of their underground stores by agriculture will causes losses of the species.

Habitat Association

Given the decline in range and subsequent listing as endangered of the ABB, much research has been devoted to preserving suitable land for the species to inhabit. This is a difficult task, as the species has been classified as a habitat generalist, showing different habitats preferences among isolated populations, with possible factors including availability of food, climate, absence of human disturbance, and types of vegetation (Leasure and Hoback 2017). Holloway and Schnell (1997), along with Lomolino and Creighton (1996) suggest that ABB remain in ecosystems with appropriately sized (100-200g) carrion (Sikes and Raithel 2002). This hypothesis is supported by Schnell et al. (2014), who found no significant association between ABB numbers and those of avians or mammals at their sampling site in Oklahoma, leading them to suggest that ABB uses

environments based on the presence of potential food sources, rather than the environment itself. Similar results were reported by Dobesh (2017) in the Nebraska National Forest, a hand planted forest established in 1907.

In the two largest remaining populations, Nebraska ABB are associated with prairies in the Sandhills and loess canyons. In Eastern Oklahoma, it is associated with mature forests with deep soil (Lomolino and Creighton 1996; Schweitzer and Master 1987). These differing hypotheses are concerning, for if the species is a specialist for the prairie, then this could suggest that the return of or planting of forests could cause the species' decline, and vice versa (Sikes and Raithel 2002). These contrasting opinions have also prevented the designation of critical habitat and the ABB remains classified as a habitat generalist (Schweitzer and Master 1987). This may be supported by the species' wide historic range over multiple distinct environments. At its peak, the species was found from "the Laurentian mixed forests of Maine and Nova Scotia" to "the prairie parklands of eastern Oklahoma" (USFWS 2008). It is possible that vegetation plays less a role in determining ideal habitat, as anthropogenic change plays a role in determining where ABB cannot survive. The US Fish and Wildlife service considers the following conditions unsuitable for the species' survival; regular tilling, nonnative vegetation, frequently mowed or grazed land with vegetation under 20cm in height, land developed until the upper layer is destroyed, maintained roadways, stockpiled soil, and saturated soils or standing water (USFWS 2015).

Jurzenski et al. (2014) summarized factors that contribute to ABB's presence in the Sandhills as "loamy sand, variable soil textures" and "wetland" and factors contributing to their absence as "loam soil, agriculture, woodland, and development". A study on the effect of artificial forests in Nebraska found that ABB occur in the Nebraska Sandhill prairie over adjacent hand-planted pine forests in Cherry county, Nebraska (Farriester et al. 2018). In contrast, ABB in Oklahoma appear to occur across a range of habitats. Studies at two National Guard Bases in Oklahoma (Camp Gruber, near Braggs) and Arkansas (Fort Chaffee, near Fort Smith) found ABB occurring in "open grassland to bottomland forest" habitats (Creighton et al. 1993; Creighton et al.

2007; Lomolino et al. 1995). Another study in Oklahoma found that ABB in the Tiak District of the Ouachita National Forest preferred mature forest landscapes (Lomolino and Creighton 1996). However, analysis of survey data at Camp Gruber has also found a positive correlation of ABB populations with areas of recent burning and grassland recovery, indicating a possible prairie preference (Howard 2007). Leasure (2017) also found positive correlation with recently burned sites and ABB population, as well as with grassland and open-canopy woodlands at Fort Chaffee. A study at Camp Gruber found that, during their active season, there is no apparent difference in ABB habitat association across savannah, grassland, or forest (Freeman 2018), although capture rates were always higher in open habitats than in forests.

In addition to primary vegetation cover, other factors appear to strongly influence occurrence of ABB. Soil is particularly important, as the success of the beetles' to bury a vertebrate carcass and their ability to overwinter depends on their capability to dig into the earth. An analysis of overwintering ABB in Arkansas recovered beetles buried between 0-20cm underground (Schnell et al. 2007). In analyzing the Sandhills of Nebraska, Jurzenski et al. (2014) found a positive association between ABB and loamy sand soil, wetlands land cover, and precipitation, while noting a negative association with woodland land cover, developed land cover, and maximum temperature (Jurzenski et al. 2014). These may also explain the dissonance in habitat association between ABB in Nebraska versus Oklahoma, given the large differences between the loose sandy soils in the north and the thick clays of the south. Soils and availability of water also influence conversion to agriculture along with other land usage in ABB's range. Soil compaction, caused from the use of machinery, short crop rotations, and intensive grazing, which leads to decreased soil fertility, may further limit the success of ABB in construction of a brood chamber and successful rearing of young (Hamza and Anderson 2005). Willemssens (2015) concluded that soil compaction caused by a standard pickup truck going off-road does not cause significant mortality (<5%) of buried *Nicrophorus* .

One type of habitat conversion that has received little attention is the planting of orchards. The United States is the leading world producer of pecans, and Oklahoma is home to an extensive industry, with pecan orchards accounting for approximately 34802.965 hectares of land in 2017 (Herrera 1993; USDA-NASS 2018). Of the 41 Oklahoma counties included in the USFWS's 2016 Range alone, there were 31,894.49 hectares of pecan orchards in 2012 (USDA-NASS 2018). Despite being a widespread crop, pecans are native to Oklahoma. In 2016, Oklahoma ranked fifth in the nation in pecans, producing 4.46% of the United States' total yield. That same year, 12,000,000 pounds of Pecan (in shell) were harvested and utilized, and at $2.06 per pound represented a $25 million industry (USDA-NASS and ODAFF 2017). Production increased in 2017, jumping to 15 million pounds (Brus 2017). When establishing an orchard, farmers are advised to seek out land not prone to frequent flooding with deep alluvial soils that have good sub-irrigation and aeration and are capable of permeability (Carroll and Smith 2015). None of these traits preclude ABB's habitation.

Landowners have multiple issues they face during the seven months of pecan maturation. A notable pathogen is pecan scab (*Fusicladium effusum* Seyran), and notable arthropod pests include pecan weevil (*Curculio caryae* Horn), pecan nut casebearer (*Acrobasis nuxvorella* Neunzig) and hickory Shuckworm (*Cydia caryana* Fitch). In particular, pecan weevils pose the greatest arthropod threat of economic injury (Mulder et al. 2012). To control these, pecans are subject to rigorous and thorough spraying of pesticides throughout season. Beneficial species that provide biological control in pecan orchards include members of the insect families Chrysopidae, Coccinellidae, and Aphelinidae, as well as *Parus bicolar* Linnaeus (Tufted Titmouse) (Mizell and Schiffhauer 1990; Tedders 1983; Whitcomb 1971). However, improper or ill-advised use of certain pesticides can cause detriment to beneficial species. Certain carbamates and organophosphates were found to cause notable toxicity in most of the beneficial arthropods previously mentioned, with some species impacted by a majority of pesticide variations. The reproductive success of the Tufted Titmouse was also found to suffer from these compounds (Mizell and Schiffhauer 1990;

Patnode and White 1991). To avoid undue damage to the ecosystem, landowners are advice to monitor pesticide drift though analyzing weather conditions, further integrate biological control agents such as *Bt* (*Bacillus thuringiensis*), and avoid controlling "sub-economic injury levels of aphids in early seasons" to avoid killing beneficial insects (Lee et al. 2013). Aside from pests and diseases, landowners must fertilize their crops, control ground floor cover and invasive flora, and limit overcrowding (Lee et al. 2013). Farmers will often allow cattle to graze in the orchard to keep plant cover low (Carroll and Smith 2015). The level of conversion and upkeep needed may have unintended consequences for endemic populations of Silphidae.

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CHAPTER III

OCCURRENCE OF CARRION BEETLES, INCLUDING THE AMERICAN BURYING BEETLE (NICROPHORUS AMERICANUS), IN OKLAHOMA RANCHES AND PECAN ORCHARDS

Abstract

The American Burying Beetle, *Nicrophorus americanus* (ABB) Olivier, is classified as a habitat generalist, which remains in areas that have not been developed to row crop agriculture. There is comparatively little data on how the species reacts to grazing or plantings of managed trees. ABB is present in eastern Oklahoma, where natural grasslands have been converted for agriculture primarily for cattle and pecan production. Between 2017 and 2018, sampling for carrion beetles was conducted on land used as cattle pastures and pecan orchards. Sampling produced 807 successful trap nights across 10 locations for five continuous nights using pitfall traps baited with decomposed rats. Trapping efforts resulted in 2,338 Silphidae being captured belonging to nine different species. *120* ABB were collected, of which *67* captures came from habitats with cattle grazing, *two* came from pecan orchards, and *five* came from areas containing both factors. Traps set on cattle pastures caught approximately *8* times as many ABB as those deployed in pecan orchards. However, Kruskal-Wallis H-Test analysis found no statistically significant difference in ABB or Silphidae presence between habitat designations. These findings suggest that overall that a *Nicrophorus*, and ABB, display habitat generalism. Although previous studies demonstrate that

managed lands may still impact the species, and additional studies may attempt to highlight the effect of managed versus unmanaged pecan agriculture, grazing and pecan orchards in Oklahoma do not appear to exclude occurrence.

Introduction

Burying beetles (Silphidae: *Nicrophorinae*) are important to a productive ecosystem. While most carrion-utilizing beetles (Staphylinidae, Histeridae, subfamily Silphinae) either survive by feeding on Diptera larvae the overwhelming majority of Nicrophorinae must sustain themselves and their brood by locating an appropriately-sized, decomposing carcass and sequestering it underground. They form the carrion into a brood ball, protected from surface scavengers and maintained until the brood is successfully raised (Campobasso et al. 2001; Mullins et al. 2013). As decomposers, they contribute to the release of nitrogen, phosphorous, and carbon back into the soil (Carter et al. 2007). Burying beetles also preserve the carrion they utilize and thus limit the spread of pathogens from microbial organisms to other species. Burying beetles improve soil fertility during the process of rearing their broods and hold importance in forensics (Carvalho et al. 2000; Conley 2014; Freeman 2018; Souza et al. 2008).

Due to competition and habitat overlap by multiple burying beetles, different species occupy different niches. The largest carcass mass that is usable by Nicrophorinae is utilized by *Nicrophorus americanus* Olivier, the American burying beetle (ABB). This species can utilize carcasses between 80-200g in size, which is necessary to support adults weighing up to 2g. Limited to North America, the ABB also holds the distinction of being the only endangered species of Silphidae, and the only endangered beetle in Oklahoma. Since its 1989 endangered listing, much of the research on this species has focused on range and habitat usage.

ABB populations are centered in eastern Oklahoma and northern Nebraska. In Nebraska, it appears that the ABB prefers mixed grass prairie with minimal tree cover. However, in Oklahoma

the species has exhibited “vegetation generalism”, with no notable trend between capture data and vegetation composition (Farriester et al. 2018; Freeman 2018; Walker and Hoback 2007). Habitat fragmentation and habitat conversion has affected many populations of ABB, but there is comparatively little understanding of how the species reacts to land already converted from its natural landscape for livestock or for monocultures of native trees. While an understanding of the species’ natural habitat is crucial to preserving remaining populations, it is also important to explore ABB’s potential survival on land already converted from its natural landscape to help determine if these land use changes in the future can support ABB. Silphidae were found to endure in forested patches inside the heavily urbanized New York City, suggesting that local survival is possible despite large habitat changes (Fusco et al. 2017).

Two types of land usage, grazed pasture and pecan orchards, were considered for this study. Owners of cattle often introduce nonnative plants, and pastures frequently have shortened vegetation and are subject to high soil compaction (Redfearn and Bidwell 2003). In pecan orchards, native grasses are removed and trees are maintained using various chemicals and fertilizers, and these modifications can attract different forms of new wildlife (Mulder et al. 2008). Given the presence of cattle and pecans around eastern Oklahoma and within ABB’s current range, the surveys conducted in this experiment intend to expand upon our understanding of how ABB reacts to a wider range of environments. In addition, by looking in locations on the outskirts of currently established ABB range, the aim is to contribute to an updated understanding of the species’ current range. The purpose of this study was to determine in the effects of land use on the presence or absence of ABB and other Silphidae on areas in the species’ range in eastern Oklahoma. This information can be used to assess if types of agriculture can function in maintaining current populations and help to determine which form of grassland conversion, if any, allows ABB to maintain presence.

Methodology

Trapping

Sampling was conducted in accordance with the US Fish and Wildlife Department's established ABB protocol, (Bedick et al. 2004). Each location was surveyed for a minimum of five consecutive trap nights (which cannot be broken up by three consecutive nights of unsuitable weather conditions) with traps checked no later than 10:00AM to prevent desiccation of captured beetles. Traps utilized were a modification of the 5-gallon above-ground bucket trap design proposed by Leasure et al. (2012). The pitfall trap consists of a 5-gallon bucket with ~7cm of peat moss and topped with a lid with a funnel, sealed to prevent escape, with holes drilled in the bucket to prevent flooding, and attached to a tree or post (Cavallaro et al. 2017). The bait consisted of a previously frozen rat carcass (RodentPro.com, IN) allowed to thaw and rot for 3-7 days prior to sampling. Each trap was rebaited with a similarly rotted rat halfway through sampling. In the event of the presence of Red Imported Fire Ants, *Solenopsis invicta* Buren, which are prevalent in southern Oklahoma, traps were emptied out, the soil replaced, and the trap relocated nearby. (Bedick et al. 2004).

Site Selection

The locations for sampling weeks were determined by availability of pecan orchards in proximity to cattle pastures, within or on the edge of the 2016 ABB Range provided by the USFWS (USFWS 2016). Prior to sampling, private landowners who maintained pecan orchards were contacted and permission for sampling was requested. When granted approval from landowners, traps were placed within the property. In all other cases, traps were set along county roads and highways within public right of way. USFWS protocol suggests a distance of 1.0 mile (1.6 km) between each trap to account for the most effective survey radius. Each sampling week to a maximum of 20 traps set to ensure taps could be investigated within the daily time limit.

In addition to sampling *cattle pastures* and *pecan orchards*, traps sites were noted for their placement or proximity to land designated as native plants and non-agricultural vegetation (forest, prairie, grassland devoid of agricultural use). These designations were confirmed using an aerial view from Google Earth®, and consolidated into the additional habitat designation. This additional designation was labelled *control* in data analysis, operating on the hypothesis that these locations would provide the most ideal refuge for Silphidae. Additional trap site data, including proximity to highways, human residence, and bodies of water were also recorded, but this data was not utilized during analysis. In some cases, a trap was close to both a pecan orchard and a cattle pasture, or was set in an orchard where landowners allowed cattle to graze, and was designated as a location that utilized both cattle and pecan production (*both*).

Sampling and Statistical Analysis

Upon checking each trap, all Silphidae were counted and recorded, before being returned, with individual numbers recorded by species, location, and day. In the event of the capture of an ABB, the beetle's sex, age (teneral/senescent), and size of pronotum was recorded, utilizing a set of digital calipers. Each ABB was marked by using a surgical cauterizer (Bovie Aaron Disposable High Temp Cautery Tip, Fine, 10/bx) to permanently darken one of the beetle's elytral spots before release. Any recaptured beetles were given an additional brand. By marking spots in clockwise order, beginning with upper right and ending with pronotum, the day of capture was also indicated. Data for total Silphidae caught across 2017 and 2018 failed the Normality Test ($P < 0.050$) and was analyzed using the non-parametric Kruskal-Wallis test with SigmaScan®, comparing the median captures of ABB and Silphids by habitat designation.

Prior to sampling, because cattle pastures represent low input agricultural systems, it was hypothesized that burying beetles, including ABB, would occur more often in this land usage than pecan orchards. Additionally, it was hypothesized that, overall, there would be higher Silphidae capture rates in locations when agriculture is not present.

Results

Beginning on May 30, 2017, and ending on August 1, 2018, 167 locations were sampled in seven regions of eastern Oklahoma across 10 5-day sampling events (Figure 1). These traps covered 12 counties: Marshall, Pushmataha, Le Flore, McCurtain, Payne, Cherokee, Muskogee, Pontotoc, Pottawatomie, Tulsa, Wagoner, and Washington (Table 1). These 50 total days of sampling yielded 802 successful trap nights (Table 2). Trap nights were missed in some locations, with traps either disturbed by overabundance of ants or vandalized. One trap was not recovered in Washington County (Week 7) and was not reset. Two supplemental traps were set in Pushmataha County in 2018 to reinvestigate sites set in 2017 (Week 10).

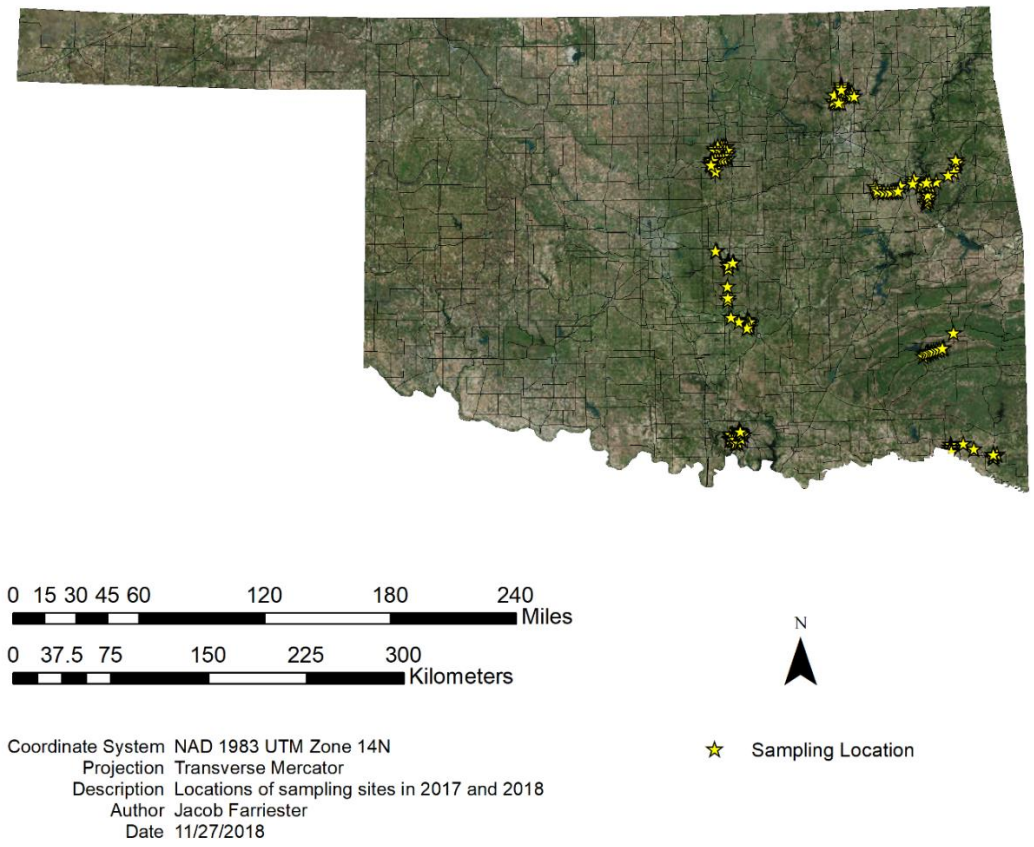

Figure 1: Map of all sampled locations in Eastern Oklahoma (2017 and 2018).

Of the 167 traps, 27 were categorized as pecan orchard, 74 were categorized as cattle pastures, and 21 were classified as both (Table 1). Trapping yielded 2,334 Silphidae belonging to 9 species in total. In addition to ABB, *Nicrophorus orbicollis* Say, *Nicrophorus carolinus* Linnaeus, *Nicrophorus tomentosus* Weber, *Nicrophorus pustulatus* Herschel, *Nicrophorus marginatus* Fabricius, *Necrophila americana* Linnaeus, and *Necrodes surinamensis* Fabricius were recovered. *Oiceoptoma novaboracense* Forster was also captured in one location, but was not included in analysis. Additional families of insect found in these traps included; Blattidae, Calliphoridae, Dermestidae, Elateridae, Formicidae, Histeridae, Mutillidae, Nymphalidae, Panorpidae, Pentatomidae, Reduviidae, Rhaphidophoridae, Scarabaeidae, Sphingidae, Staphylinidae, and Tabanidae.

Table 1: Trap site designation by location of sampling conducted between 2017 and 2018.

Week	Counties Investigated	Total	Pecan Orchard	Cattle Pasture	Both	Control
1	Marshall	20	10	8	1	10
2	Pushmataha, Leflore	10	0	3	0	9
3	McCurtain	15	1	6	3	9
4	Payne, Lincoln	20	3	10	1	12
5	Muskogee, Cherokee	20	3	12	1	9
6	Pontotoc, Pottawatomie	20	3	5	7	12
7	Tulsa, Washington	20	3	13	2	9
8	Payne, Lincoln	20	5	8	2	15
9	Muskogee, Wagoner	20	1	5	4	15
10	Pushmataha	2	0	2	0	2
		167	**29**	**72**	**21**	**102**

Table 2: Weekly capture data of all Silphidae caught between 2017 and 2018.

Week	County Investigated	Start Date	Stop Date	Traps	Trap Nights	*N. americanus*	*N. carolinus*	*N. marginatus*	*N. orbicollis*	*N. pustulatus*	*N. tomentosus*	*Necrodes surinamensis*	*Necrophila americana*
1	Marshall	5/30/2017	6/3/2017	20	91	0	0	49	125	163	20	34	3
2	Leflore	6/14/2017	6/18/2017	1	5	0	0	0	0	1	0	0	0
	Pushmataha	6/14/2017	6/18/2017	9	43	0	0	1	26	31	7	64	12
3	McCurtain	6/26/2017	6/30/2017	15	71	0	305	2	20	25	0	18	1
4	Payne	7/6/2017	7/10/2017	20	97	0	57	20	124	128	1	44	8
5	Cherokee	7/15/2017	7/19/2017	5	25	0	0	0	3	2	0	14	7
	Muskogee	7/15/2017	7/19/2017	15	75	53	0	16	15	9	0	20	2
6	Pottawatomie	8/18/2017	8/22/2017	12	52	0	-6	0	61	45	1	25	0
	Pontotoc	8/18/2017	8/22/2017	8	47	0	28	4	6	12	0	68	2
7	Washington	8/16/2017	8/20/2017	13	57	0	0	10	0	0	0	21	1
	Tulsa	8/16/2017	8/20/2017	7	35	0	0	1	1	7	0	10	3
8	Lincoln	7/1/2018	7/5/2018	2	10	0	0	0	1	0	1	0	0
	Payne	7/1/2018	7/5/2018	18	90	0	31	6	29	150	0	15	5
9	Wagoner	7/14/2018	7/18/2018	1	5	1	0	0	0	1	0	3	0
	Muskogee	7/14/2018	7/18/2018	19	94	66	2	8	52	148	1	56	28
10	Pushmataha	7/28/2018	8/1/2018	2	10	0	0	0	0	1	0	0	0
				167	**807**	**120**	**417**	**117**	**463**	**723**	**31**	**392**	**72**

ABB was captured 120 times during sampling (Table 2). From this, there were three recaptures, resulting in 117 unique ABB captures. Twenty-four out of 167 traps yielded ABB, all within Muskogee, Wagoner, and Cherokee counties. Three traps designated as *pecan orchards* yielded two ABB, 12 traps designated as *cattle pastures* yielded 67, and 3 traps designated as *both* yielded five ABB. Of *Nicrophorinae*, the highest captures were of *n. pustulatus*, while the lowest was *n. tomentosus*. Of Silphinae, the highest captures were of *Necrodes surinamensis*, found during nine of the 10 total weeks (table 1).

There was no statistically significant difference in median values of all Silphidae captures by habitat designation (H=3.642, df=3, P=0.303) (Figure 2). Similarly, Nicrophorinae captures among habitat designations (H=2.079, df=3, P=0.557) and Silphinae captures among habitat designations were not statistically significant (H=4.507, df=3, P=0.212) (Figure 3,4).

The difference in capture rates of ABB among habitats was not significant (H=3.159, df=3, P=0.368) (Figure 5). In examining the closely related *N. orbicollis*, which is often considered as a proxy for the presence of ABB, more were caught in sites involving trees and prairie (pecans, both, and control) than those solely associated with cattle (H=8.963, df=3, P=0.030) (Figure 6).

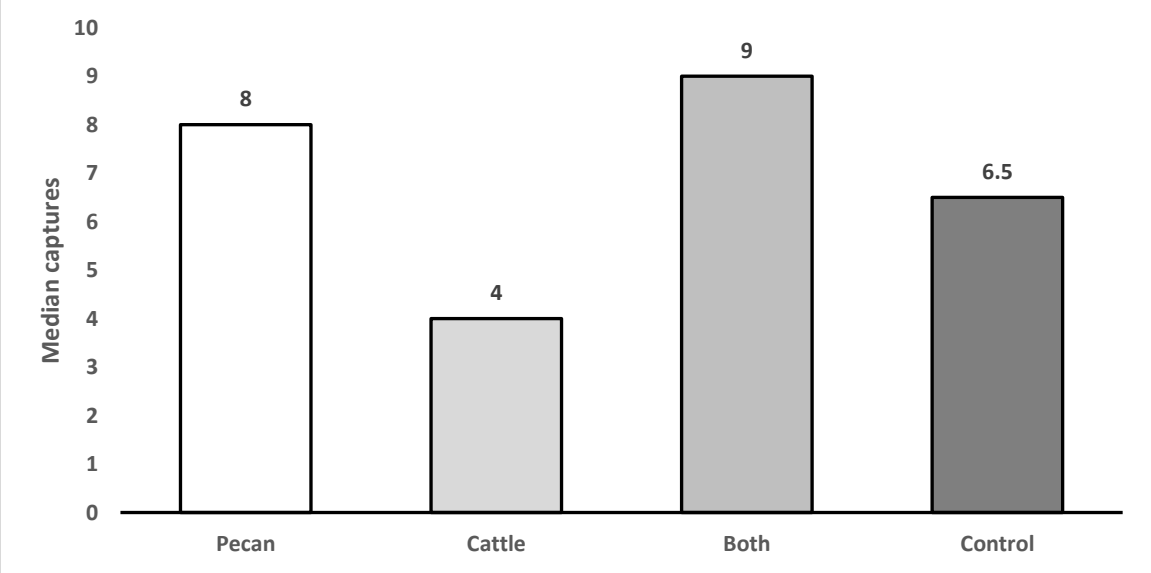

Figure 2: Median captures of Silphidae caught during 2017 and 2018 in eastern Oklahoma by habitat designation.

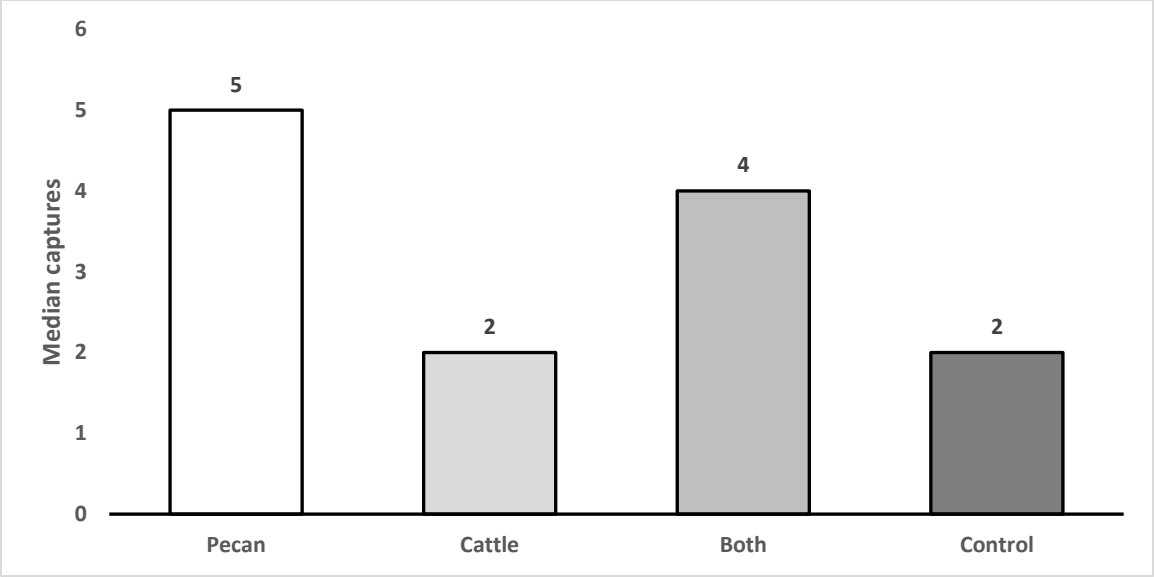

Figure 3: Median captures of Nicrophorinae caught during 2017-2018 sampling by habitat designation

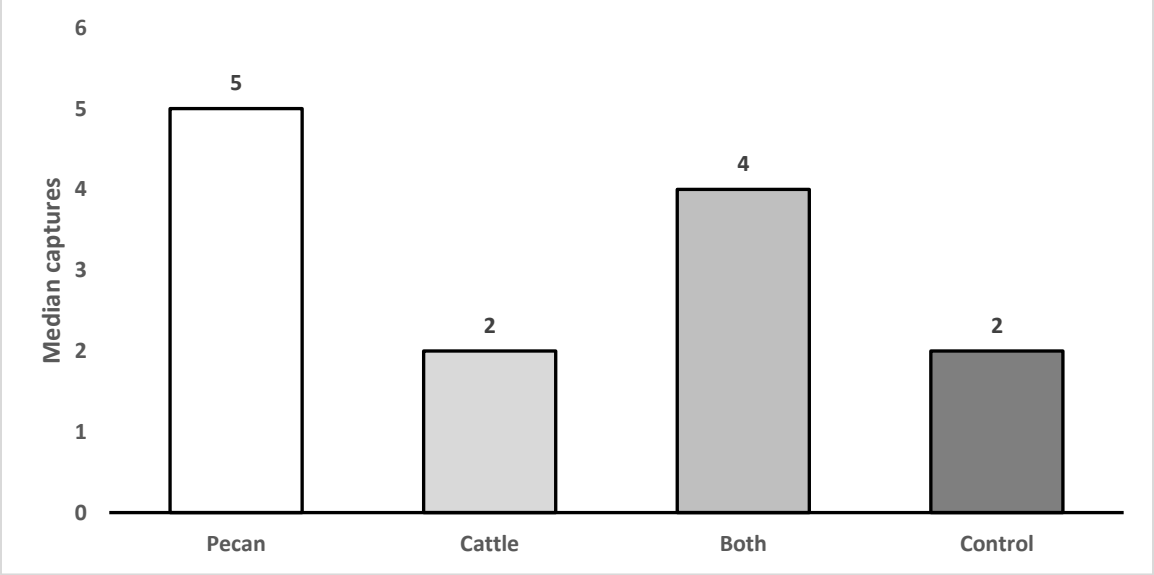

Figure 4: Median captures of Silphinae caught during 2017-2018 sampling by habitat designation

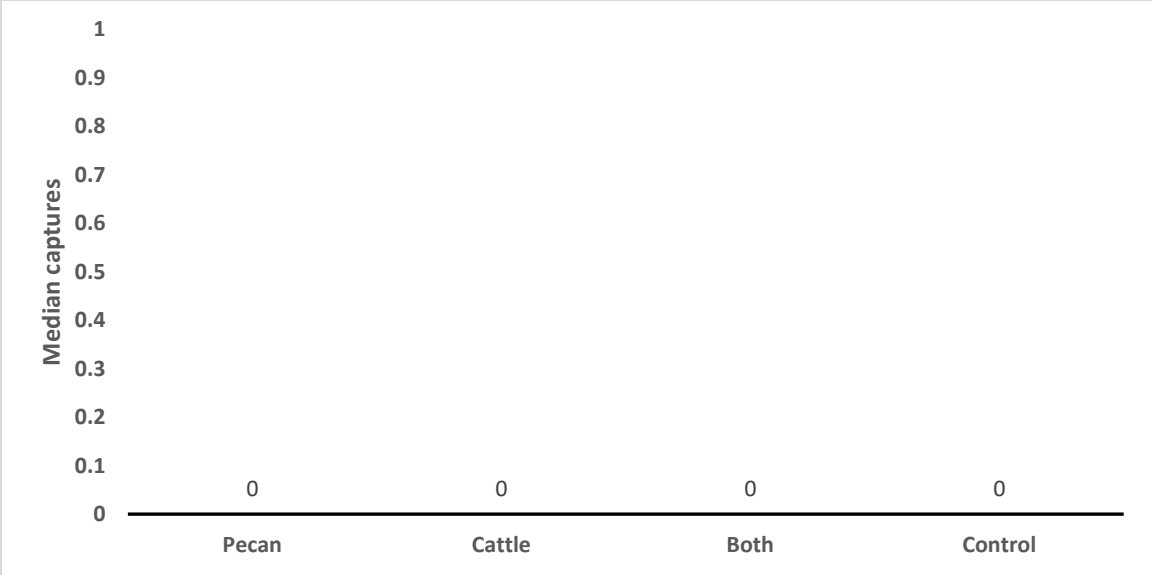

Figure 5: Median captures of N. americanus (ABB) caught during 2017-2018 sampling by habitat designation.

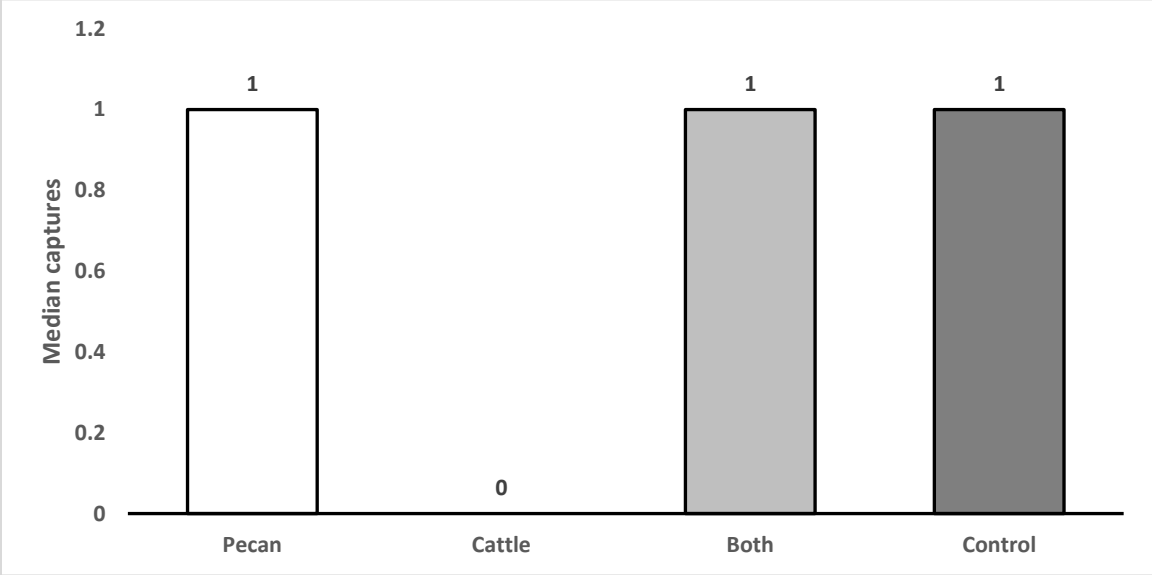

Figure 6: Median captures of N. orbicollis caught during 2017-2018 sampling by habitat designation.

While size, gender, and age data acquired for captured ABB was not utilized in this experiment, this data is included in Appendix 2.

Discussion

Most ABB sampling in Oklahoma is limited to development surveys for energy projects, where upon the discovery of a beetle, the traps are pulled and sampling ends. This technique is useful for surveys to establish presence, but is not useful for updating the species' range or for gauging population size. Bedick et al. (2004) first proposed the 5-day sampling protocol for the ABB, a timeline supported by Butler et al. (2013) to maximize accurate capture of *Nicrophorus* spp. and allow for increased detection of small American burying beetle populations. Leasure et al. (2012) developed a trap design that ensures carrion beetles can detect the scent of bait and are captured alive, and has been noted for its ease and efficiency in the field while minimizing potential for disturbance compared to Nebraska below-ground pitfall traps (Leasure et al. 2012). By setting traps no closer than 1 mile apart, traps provide independent samples because ABB can fly more than 0.5 miles in a single night in search of food (USFWS 2015). This value was confirmed by Leasure (2017), who found a radius of 800m (~0.5 mi) to be most effective when setting traps. The technique of elytral branding ensures a permanent indication of the beetle's capture that extends past the end of the 5-day sampling period, and may be used in future surveys without impeding the beetle's reproductive (Hall et al. 2015; Jenkins et al. 2016). By utilizing a whole, intact carcass, the conditions of the experiment most closely mimic natural conditions and help ensure the beetles can successfully detect the oligosulphides released during decomposition (Kalinova et al. 2009; Woodard 2006). Openly decomposing carrion leads to the exposure and propagation of zoonotic bacteria, either through spread by Diptera or through scavenging by vertebrates, which pose a health hazard to both fauna in the environment and any human populations nearby (van Essen and van Leeuwen 2000). Suzuki (2000) found that carcasses were subsequently less attractive to various necrophagous Diptera when *N. vespilloides* was given access to the carcass and allowed to roll, bury, and chemically treat the carcass.

The Kruskal-Wallis H-Test was used to determine any statistically significant difference between captures by habitat designation. Overall analysis of all Silphidae caught in 2017-2018 showed the following average numbers: Pecan (15.8), cattle (7.5), both (13.9), and Control (12.3) (Figure 7). A similar trend in values was found when comparing all Nicrophorinae caught (Figure 8). It was anticipated that sites containing only non-agricultural vegetation would provide a positive control; however, the only time when control traps had greater numbers was in the case of Silphinae (Figure 9). This could be explained by prevalence of *Necrophila americana*, which is associated with water. The very high Standard Deviation in the results across all comparisons of ABB capture suggests that this may have been less to do with the habitat itself and more the higher number of trap sites associated with cattle pastures (Figure 10). Analysis of captures associated with *N. orbicollis* suggested an equal occurrence among habitat types (Figure 11). Previously, *N. orbicollis* has been found to associate with forests (Bishop et al. 2002, Walker and Hoback 2007). The possible forest specialization of this species, at least in comparison to the ABB, may preclude its use as an indicator for the presence of ABB in field studies.

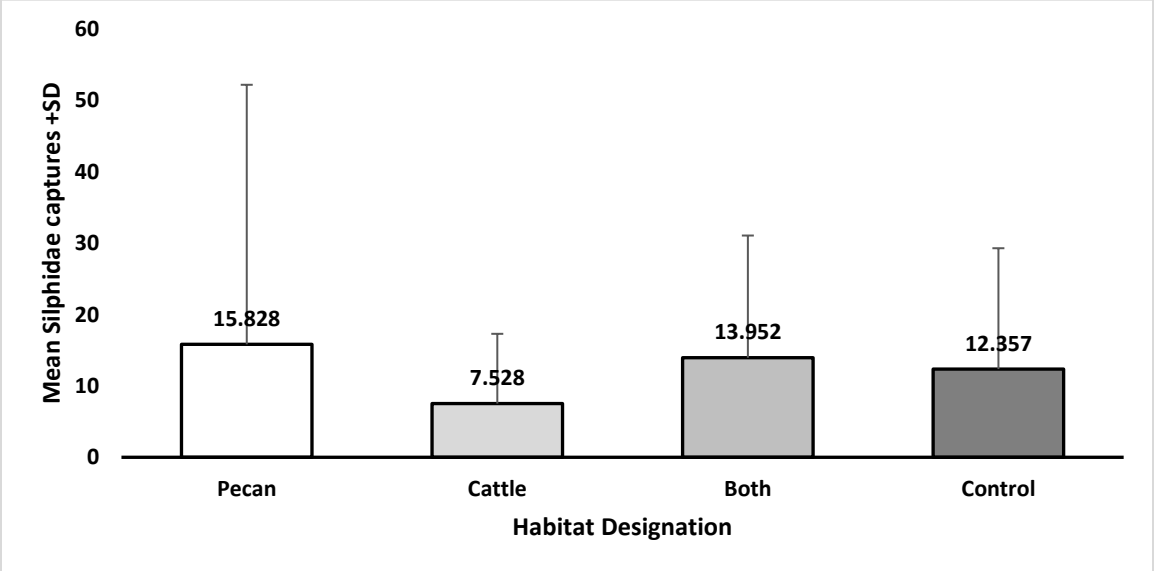

Figure 7: Mean captures +SD of all Silphidae caught during 2017 and 2018 in eastern Oklahoma by habitat designation.

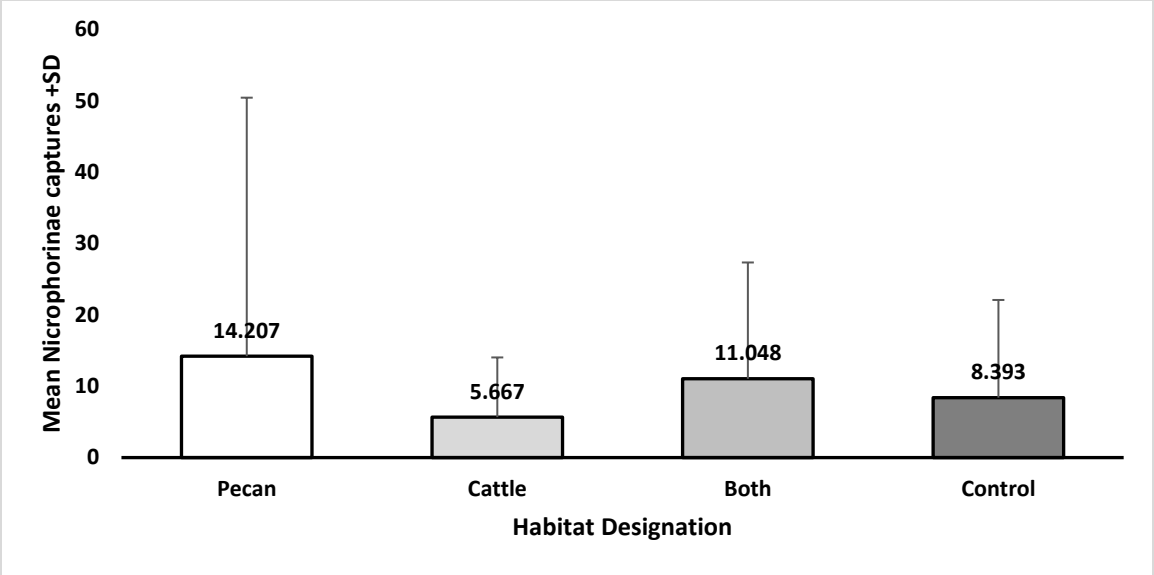

Figure 8: Mean captures +SD of all Nicrophorinae (burying beetles) caught during 2017 and 2018 in eastern Oklahoma by habitat designation.

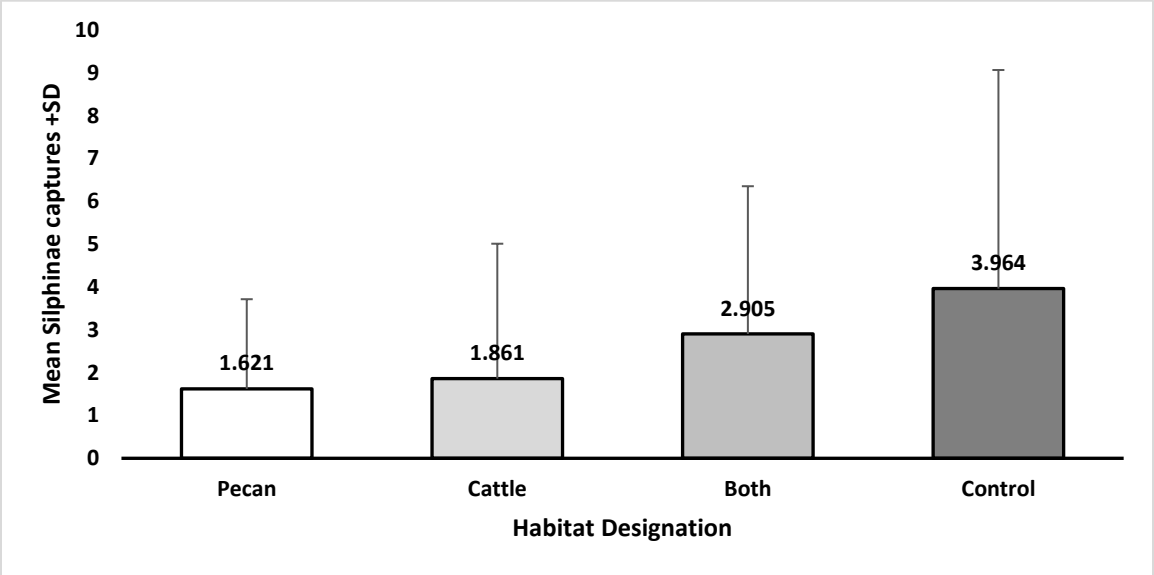

Figure 9: Mean captures +SD of all Silphinae (carrion beetles) caught during 2017 and 2018 in eastern Oklahoma by habitat designation.

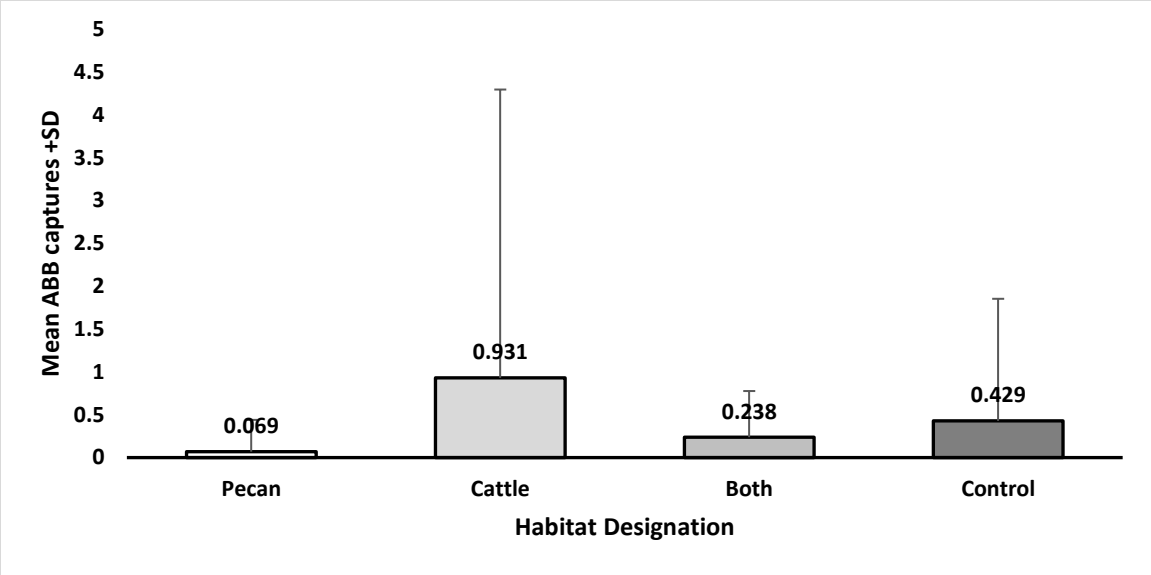

Figure 10: Mean captures +SD of all N. americanus (ABB) caught during 2017 and 2018 in eastern Oklahoma by habitat designation.

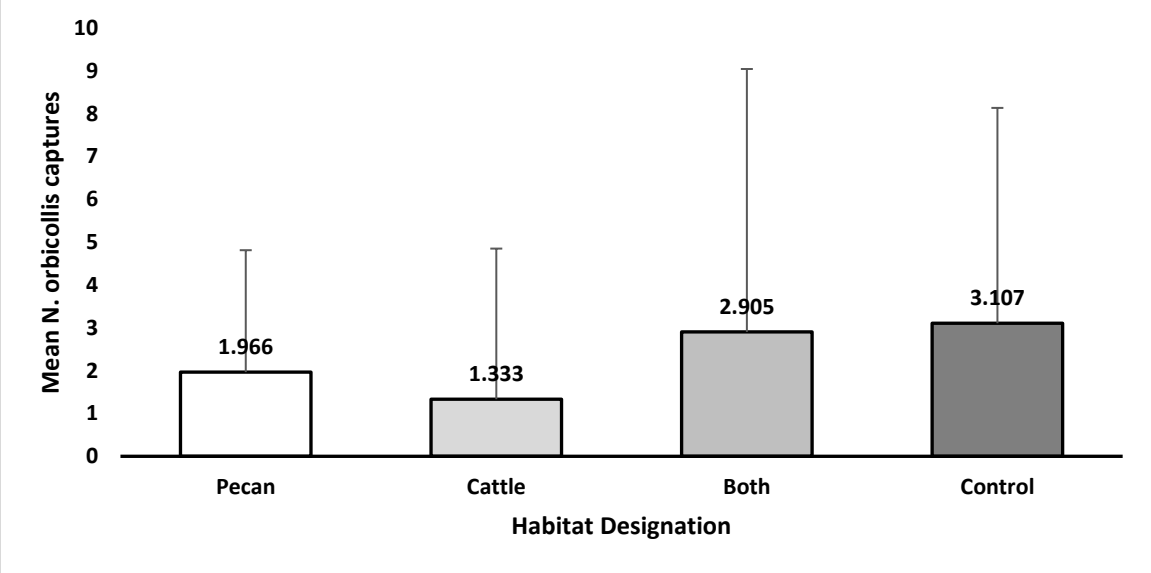

Figure 11: Mean captures +SD of all N. orbicollis caught during 2017 and 2018 in eastern Oklahoma by habitat designation.

The results of this study are likely influenced by management. Pecan growers attempt to control squirrels (*Sciurus carolinensis* Gmelin) and various avians (Crows, Blue Jays, etc.) to increase yield. When interviewed, producers stated that they actively killed squirrels in their orchards, but did not allow carcasses to remain in the orchard, which may limit carrion beetles.

Pesticides used by pecan growers that are absent on cattle pastures may also impact carrion beetles. Of note, one trap from Week 3 was placed on a mature pecan tree within the OSU Mac Lindley Research Station near Valiant, OK, and yielded 160 *N. carolinus* and 63 *N. pustulatus* over the 5-day period, substantially more than other traps set in or nearby the same station. Of note, this tree was reportedly not sprayed with any insecticidal compounds, and the understory vegetation received only a light herbicide application. Thus, it is possible that pesticides play a part, in addition to a lack of carrion resources, in rendering pecan orchards less suitable for Silphidae. However, the mobility of Silphids suggests that follow-up studies may be required to further validate this point. Creighton et al (2009) determined ABB in eastern Oklahoma were negatively affected by deforestation, while Walker and Hoback (2007) suggested the encroachment of invasive *Juniperus virginiana* L. (eastern red cedar) in Nebraska limits the species. Both studies were conducted in the same year. Previous OSU research in Oklahoma using light traps in pecan farms yielded an unexpected presence of ABB that inspired research to further investigate such findings (Mulder 2017).

Despite the equivalent of nearly 2 months of sampling, ABB was only found in one of the seven parts of the state that were sampled. Traps set near the cities of Braggs, Muskogee, Fort Gibson, and Haskell yielded ABB, while traps farther east near Tahlequah did not. During both sampling trips, separate OSU sampling excursions occurred at nearby Camp Gruber, an established area of high ABB occurrence. A 2-year survey on that military base concluded that ABB associated with all habitat types inside the base, regardless of vegetation composition, and yielded 1,870 individual ABB captured (Freeman 2018). OSU researchers caught 449 ABB between 7/12/2017 and 7/16/2017, compared to my 51 captures during overlapping sampling. In 2018, researchers caught 796 ABB during the same timeframe when this study caught 67. Some traps for this survey were set barely one mile from traps set inside Camp Gruber, and yet the overall captures were less than a tenth. The remarkable dissonance between capture rates of both separate studies does suggest that land conversion may still impact the species. However, until more data can be gathered

to elucidate the findings from this study, then it can be assumed that pecan orchards and cattle grazing do not factor into the presence or absence of ABB or Silphidae in Oklahoma. Other factors such as soil and the presence or absence of usable carrion pose a greater likelihood of serving as the limiting factor of populations of ABB.

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CHAPTER IV

COMPARATIVE GENE EXPRESSION AND UPREGULATION OF POSSIBLE ANTIMICROBIAL PEPTIDE-PRODUCING GENES OF *NICROPHORUS ORBICOLLIS* AND *NICROPHORUS PUSTULATUS*

Abstract

Burying beetles (Silphidae: Nicrophorinae), are unique among insects in their method of necrophagy and brood rearing, working in biparental tandem to sequester suitably-sized carrion underground and limiting decomposition through the production of antimicrobial protein secretions. This study compares two burying beetles with different life histories; one that engages in typical burying beetle behavior (*Nicrophorus orbicollis* Say) and a brood parasite which does not produce antimicrobial secretions (*Nicrophorus pustulatus* Herschel). Through next generation sequencing of cDNAs synthesized from RNA isolated from excised salivary gland tissue from both species, I investigated differences in gene expression when both species were given access to and denied ground beef as food. Both species exhibited higher expression in sequences coding for innate immune response proteins when exposed to food (6.58%/5.19% of overall characterized sequences) than when starved. Between both species, *N. orbicollis* had higher expression of innate immunity proteins than *N. pustulatus* (7.14%/4.22%). This suggests that these compounds are linked to the feeding process and supporting the hypothesis that active compounds in burying beetle secretions are antimicrobial peptides, rather than gut bacteria.

Introduction

Beetles in the family Silphidae are reliant on carrion, but are radically divided between two subfamilies in their ability to utilize it as a resource. A method utilized by subfamily Nicrophorinae (burying beetles) is coating the buried carcass with oral and anal secretions that delay decomposition and inhibit bacterial growth (Ratcliffe 1996). The nature of this protection is still only moderately understood. The case for the active agent being a protein comes from an experiment which found that the secretions of *Nicrophorus marginatus* Fabricius reduced in antimicrobial effectiveness when exposed to heat and when exposed to a proteinase (Hoback et al. 2004). Alternatively, it has been suggested that this effect being produced or bolstered by symbiont bacterial and fungal colonies in Nicrophorinae's microbiome (Kaltenpoth and Steiger 2014).

While it is understood that the lifestyle and production of antimicrobial compounds are not uniform among Nicrophorinae, it is yet unknown whether there is a notable difference in genetic sequences and expression of genes encoding these compounds between species. Species in this group are members of the same family, even the same genus, and occupy the same environment, but behave in radically different ways. Hoback et al. (2004) attempted to investigate the antimicrobial activity of multiple carrion beetles, of both subfamilies (Nicrophorinae and Silphidae). Specifically, it aimed to determine the comparative power of each species' secretions to control bacterial growth and differentiate between oral and anal exudate. By measuring the net change in bioluminescence of colonies of *Aliivibrio fischerii* Urbanczyk inoculated with an array of different exudates, they determined that, while a majority of Nicrophorinae (and one species of Silphinae) are capable of causing a drop in bacterial bioactivity (bioluminescence in the experiment) at a significant level, two species of burying beetle were incapable of doing so (Hoback et al. 2004: Urbanczyk et al. 2007). While one of the two has since been found to possess antimicrobial oral secretions (*N. carolinus*), another species continues to display no such activity (Jacques et al. 2009). In a subfamily full of beetles engaging in antimicrobial activity, *Nicrophorus pustulatus* Herschel is unique. While the species rears its larvae with the same biparental care,

brood protection, offspring attendance and food provisioning as other burying beetles, itis primarily a brood parasite (Smiseth et al. 2014; Smith et al. 2007; Trumbo 2007). This was demonstrated in particular when *N. pustulatus* females were denied carrion then engaged in parasitism of carcasses controlled by *N. orbicollis* Say (Trumbo, 1994). In addition, the species has been suggested that this species has undergone a rare host shift, and is emerging as a parasitoid of the eggs of black snake (*Elaphe obsoleta* Say) of ecological and conservational note (Smith et al. 2007). Given the specificity of members of Nicrophorinae with carrion selection and habitat association, *N. pustulatus* may have found a unique niche as a member of the most socially advanced family of Coleoptera (Wilson 1971). A carcass may be utilized by numerous beetles, though pairs must compete to utilize the carcass to ensure their own reproductive success, driving off invading beetles and preventing infanticide (Wilson and Knollenberg 1987). By comparison, *N. orbicollis* has no such hindrances. While their secretions are weak compared to other burying beetles, they compensate by utilizing oral and anal exudate to control microbe populations (Hoback et al. 2004). Phylogenetically, *N. pustulatus* developed after *N. orbicollis* and yet is close in relation to *N. marginatus* and *N. tomentosus*, which produce antimicrobial exudate (Hoback et al. 2004; Sikes and Venables 2013). It can be hypothesized that the unique behavior by *N. pustulatus* was a result of the change in antimicrobial capability, or if it was genetically abandoned as the species developed its niche. Either way, we are interested to see if any genetic sequences coding for any potential antimicrobial peptides (AMP) used by their counterparts may still be found in *N. pustulatus*, and are simply not expressed. We hypothesize that the salivary glands of burying beetles hold the best potential for the presence of any protein-coding sequences of antimicrobial activity, and attempted to determine what, if any, overlap or difference was found when comparing *N. pustulatus* to *N. orbicollis.* In addition, we are interested in the possible differences in gene expression of beetles exposed to or denied access to food. The extreme difference in feeding behavior of these two closely related species suggests that differences may be found in the same subfamily. I also hypothesize that the salivary glands of fed burying beetles demonstrate

differential expression of genes compared to that of starved beetles, which I propose are necessary for the production of antimicrobial secretions.

Methodology

Trapping and RNA Extraction

Beetles were collected between May 26-27, 2018, during sampling for the endangered *Nicrophorus americanus* Olivier conducted on Camp Gruber Training Center in Muskogee County OK. Trapping followed the US Fish and Wildlife Department's ABB protocol (Bedick et al. 2004). Both mornings, before 10:00AM, 5-gallon above-ground Silphidae bucket traps filled with ~7cm of peat moss and baited with a rotted rat carcass (RodentPro.com, IN) were checked (USFWS 2016). Captured beetles of both species were kept alive in moist soil, with half deprived of food and half provided high-fat (30%) ground beef as sustenance. Upon return to the lab, the beetles were additionally divided by sex. Additional *N. orbicollis* were collected on July 29, 2018 and handled following the same procedure. Surviving beetles were anesthetized in an ice bath before we extracted salivary gland tissue. The combined tissue of five individual beetles was immersed in a tube containing RNAzol RT (Molecular Research Center, Inc. Cincinnati, Ohio), frozen with liquid nitrogen, and stored in -80ºC. Total RNA was isolated from salivary gland tissue using the Trizol according to the manufacturers protocol (ThermoFisher Scientific). Initially, tissues were homogenized in a microfuge tube with a plastic pestle in 500 µl Trizol and allowed to stand at 25°C for 5 min. After chloroform addition (200 µl), samples were vigorously mixed for 2 min followed by centrifugation (13,000 X g for 10 min). The aqueous layer was then placed in a microfuge tube and an equal volume of isopropanol was added, and after mixing, the samples were placed at -20°C (16 h). The samples were then centrifuged (13,000 X g for 30 min) and pellets washed once with 75 % ethanol and once with 70% ethanol, and then re-pelleted (13,000 X g for 5 min). The

supernatant was removed, and dried pellets were re-suspended in nuclease-free water. RNA samples were quantified with a NanoDrop (ThermoFisher, MA) and size distributions were analyzed by BioAnalyzer 2100 (Agilent, CA) using a RNA Nano chip. RNA samples (1 µg of each) were then used to make Illumina libraries using the TruSeq RNA Sample Preparation Kit v2 following the manufacturer's instructions (Illumina Inc., San Diego, California), except only 13 PCR amplification cycles were conducted. The Illumina libraries were then quantified and the quality was checked with the BioAnalyzer 2100 using a DNA 100 chip. All the libraries had similar size distributions between 200-500 bp with a ~260 bp peak. The libraries were then sent to Macrogen (Macrogen Corporation, Rockville, Maryland) for sequencing.

RNA Sequence Analysis

All generated RNAseq reads were *de novo* assembled using the RNA assembly program Trinity (Grabherr et al. 2011). Peptide sequences were called from the Trinity assembly using the reading frame prediction program Transdecoder (Haas et al. 2013). All final gene models and predicted transcript peptides were annotated using the Trinotate platform (Bryant et al. 2017) with a combination of homology-based search using NCBI Blast+ (Camacho et al. 2009), conserved protein domain identification using HMMER's hmmscan using the PFAM 30.0 database (Finn et al. 2015; Finn et al. 2016), and cellular localization with signal P 4.0 (Hoff and Stanke 2013; Petersen et al. 2011). Comparative blast analysis was conducted against the Uniprot TReBML database for additional functional annotation (Fusco et al., 2017). Homology was suggested by a sequence's E value of e-10 and were included in top-hit species based analysis. Transcriptional abundance/expression levels were calculated using mapping of transcriptional reads to the assemblies using the short read aligner bowtie2 (Langmead and Salzberg 2012) along with the quantification program RSEM using the recommended protocol for *de novo* transcriptome assemblies (Langmead et al. 2012; Li and Dewey 2011). Differential expression between conditions for each species was calculated using the Bioconductor EdgeR (Robinson et al. 2010)

suite with a dispersion value of 0.2. Completed sequences were then assembled by their comparative expression between Fed and Starved, P-Value, and FDR. Selected sequences were analyzed using NCBI blastp and categorized by function using definitions provided by the UniProt Consortium (The UniProt Consortium 2017).

Results

Sixty *N. pustulatus* and 76 *N. orbicollis* were utilized, though some *N. orbicollis* captured were lost to cannibalism. This produced 28 total samples of processed tissue, 12 *N. pustulatus* and 16 *N. orbicollis*. Limitations in numbers of *N. orbicollis*, particularly male *N. orbicollis*, meant that two samples from July only utilized tissue from three beetles each. One sample utilized salivary tissue recovered from the head of a starved cannibalized male *N. orbicollis* carcass in order to meet uniform numbers. Sequenced RNA-Seq libraries from the salivary glands of both species of Silphidae generated an average of 289.38 million reads. Of a total of 62,476/64,484 predicted peptides for both species, 29,985/33,754 were complete, and measured an average length of 337.6/326.9 (*N. orbicollis*/*N. pustulatus,* respectively). Transcriptome completeness estimation using phylogenic marker gene identification with gVolante and Busco estimated a completion rate of 99.44%/99.34% respectively, using conserved Arthropoda single copy genes (Hara et al. 2015; Nishimura et al. 2017; Simao et al. 2015). Estimated completion rate 95.31%/97.00% of these genes were having both a sequenced start and stop codon. In *N. orbicollis*, 1,066 total core genes were queried, with 1012 completely detected. In *N. pustulatus*, 1,066 total core genes were queried, with 1,023 completely detected. RNAseq produced 71,210 total sequences between both species and 895 total sequences had a false discovery rate under 0.05. Four hundred sequences (100 per category) of these 895 were analyzed further in total (Appendix 3-6). Using the transcriptome data, BLAST analysis was utilized to assign individual transcripts in gene functional categories (Table 2). In the case of *N. orbicollis*, the fed beetles' transcriptome exhibited a variety of gene functional groups, with a number of transcripts encoding products involved with immunity, and reproduction.

The starved *N. pustulatus* transcriptome demonstrated the upregulation genes involved with amino acid synthesis, metabolism, and immune response/feeding behavior.

	N. orbicollis	
Designation	Fed	Starved
Amino Acid Synthesis	3	10
Behavior and Physical Structure	5	2
Biological Signaling	7	6
Cell Function and Metabolism	13	21
Cell Structure and Division	13	6
Immunity and Defense	5	7
Protein Synthesis	0	3
Reproduction and Life Cycle	12	2
Ribosomal Proteins	0	31
Transcription and Translation	8	1
Transportation	10	3
Uncharacterized Protein	24	8

	N. pustulatus	
Designation	Fed	Starved
Amino Acid Synthesis	12	2
Behavior and Physical Structure	5	1
Biological Signaling	7	7
Cell Function and Metabolism	21	15
Cell Structure and Division	6	14
Immunity and Defense	4	3
Protein Synthesis	0	5
Reproduction and Life Cycle	6	3
Ribosomal Proteins	0	8
Transcription and Translation	8	16
Transportation	8	15
Uncharacterized Protein	23	11

Table 3: Number of BLASTp-predicted sequences in each designation for fed and starved beetles of both species.

Discussion

A majority of genetic comparisons came from *N. vespilloides*, whose genome and methylome were assembled by Cunningham et al. (2015). Other species found during BLAST analysis of *N. orbicollis* were *Agrilus planipennis* Fairmaire, *Tribolium castaneum* Herbst, and *Leptinotarsa decemlineata* Say. Other species found during BLAST analysis of *N. pustulatus* were *Onthophagus taurus* Schreber, *Anoplophora glabripennis* Motschulsky, and *Tribolium castaneum* Herbst. The presence of genetics predicted as lass Arachnida species *Varroa destructor* Anderson

& Trueman and *Varroa jacobsoni* Oudemans may point toward contamination from the carapace and *Nicrophorus'* phoretic mites, though both species are parasites of *Apis cerana* Fabricicus (Asian Honey Bee). The matching of *N. pustulatus* with *Pelodiscus sinensis* Wiegmann (Chinese softshell turtle**)** is likely an error. Potential sources of error come from accidental inclusion of the beetles' associated phonetic mites, their relatively esoteric gut flora, and any microorganisms on their external carapace. The overwhelming majority of sequences that were predicted to match with *N. vespilloides* supports the methodology used, and with further genomic sequences made available for Nicrophorus species, the results would only improve.

Both species of Nicrophorinae displayed a significantly wider range (Increased Functional Groups) of genes expressed when given access to sustenance (Table 3). Overall, *N. orbicollis* displayed a higher number of immune response-related genes than *N. pustulatus*, with immunity-related genes making up 7.14% of all characterized sequences of *N. orbicollis* compared to 4.22% in *N. pustulatus* (Figure 12). When these genes were present in both species, they were upregulated in the presence of sustenance. In looking at the sequences expressed, starved *N. orbicollis* expressed sequences possibly coding for endotoxin-sensitive serine protease and UDP-glucuronosyltransferases, used in detoxifications of various substrates as well as physical structural development (Huang et al. 2008; Muta et al. 1990). Fed *N. orbicollis*, by comparison, expressed sequences possibly coding for proteins that regulate macrophages to provide for pathogen-specific host defense, control digestion, relate to larval innate tracheal immune response, recognize invading microorganisms and activate the prophenoloxidase cascade, and acts as a receptor for microorganism and disease (Figure 13) (Arrese et al. 2006; Finn et al. 2016; The UniProt Consortium 2017). Similarly, starved *N. pustulatus* expressed UDP-glucuronosyltransferases, as well as a hydrolytic enzyme linked to defense against bacteria and fungi (Angelino et al., 2015). ±logFC refers to the degrees of magnification of gene upregulation, with positive or negative direction indicating the degree to which it is upregulated by either category. Fed *N. pustulatus* exhibited sequences coding for multiple serine proteases and serine

protease inhibitors, which are important in insect immunity (Zou et al. 2006). An additional sequence of interest in fed *N. pustulatus* include a protein involved with taste (protein Malvolio-like isoform X2) that is a gene included in the Nramp family (natural resistance-associated macrophage protein) (Figure 14) (Consortium 2018; Mehlferber et al. 2017).

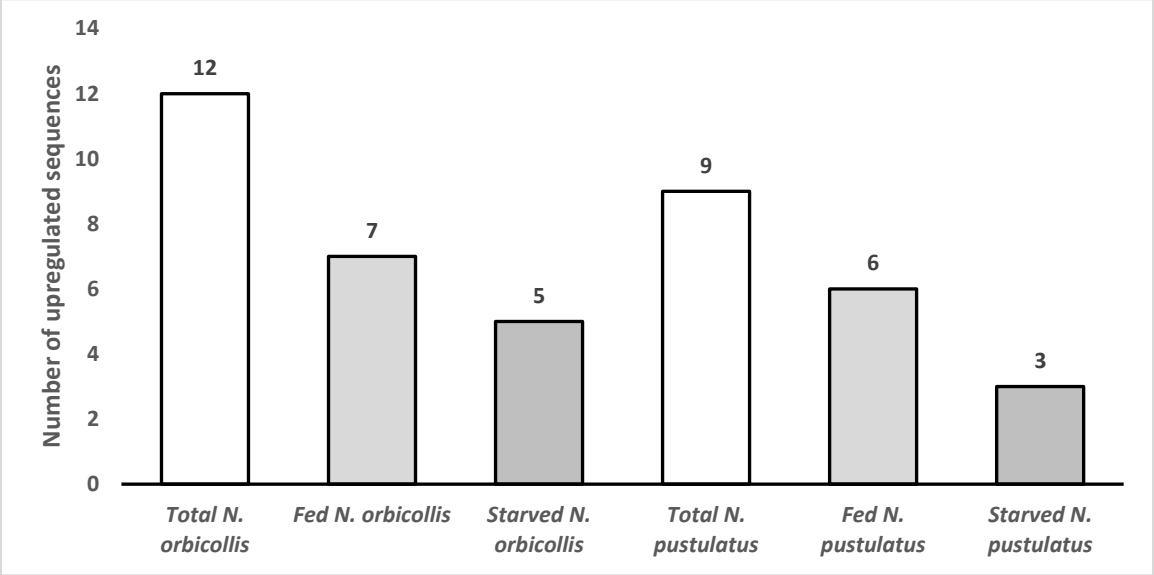

Figure 12: Immune and Defense Sequences by species category

These results suggest several critical interpretations. First, that the increased expression of immunity-related genes is linked to the presence of food, indicating that these genes are linked to the process of feeding. Whether it is to protect the beetle from the microbes they are about to face in the carcass or to secure it for consumption, this tells us that the beetle participates in the sanitation of carrion, which supports the theory that the active compounds in Nicrophorinae secretions are proteins created by the beetles themselves and that this protection is an extension of the burying beetle's innate immune response. Second, the reason for the comparative impotence of *N. pustulatus* in producing these secretions is due to a lack of presence or viability of the same immune response genes that *N. orbicollis* (and potentially all other similar species of Nicrophorinae) possess. These genes are not present like a vestigial organ in the species' genome, but appear to

have been weeded out phylogenetically as the species has adapted to its unique niche within the environment.

During the field work component of this research, of surprise was the disproportionate representation of sexes of *N. orbicollis* caught at Camp Gruber, with females showing dramatically higher captures. Of the 76 *N. orbicollis* utilized in this experiment, 50 were female, and 26 were male. In neither sampling attempt did males exceed females in number. This could potentially indicate as-yet unknown environmental factors favoring females, or a potential infestation of Wolbachia in Camp Gruber Silphidae (Jiggins 2003).

The development of these transcriptomes will aid in future understanding of this unique and esoteric family of Coleoptera, possibly aiding in the study of the endangered American Burying Beetle. By understanding the nature of this chemical protection, we hope to unlock what could be the next great tool in antibiotic defense. With antimicrobial peptides, capable of killing bacteria at the physical level, we can circumvent even the hardiest multi-drug resistant bacteria. Compounds like these have potential in medicine, in food safety, and in multiple other fields. With such a unique family of insects, there is much more still that we can discover.

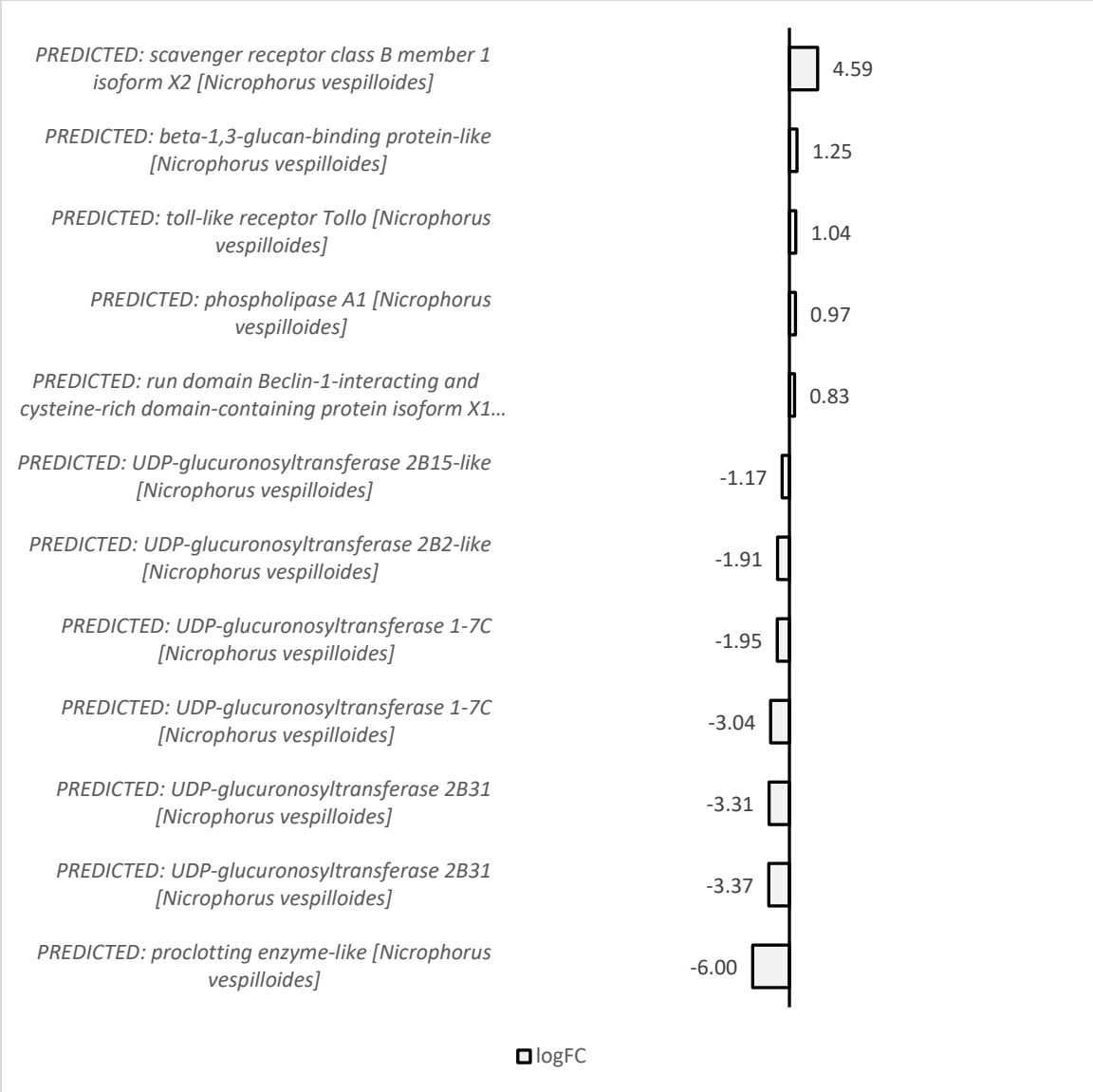

Figure 13: ±logFC of BLASTp-predicted RNA sequences pertaining to immune response expressed between fed (+logFC) and starved (-logFC) N. orbicollis.

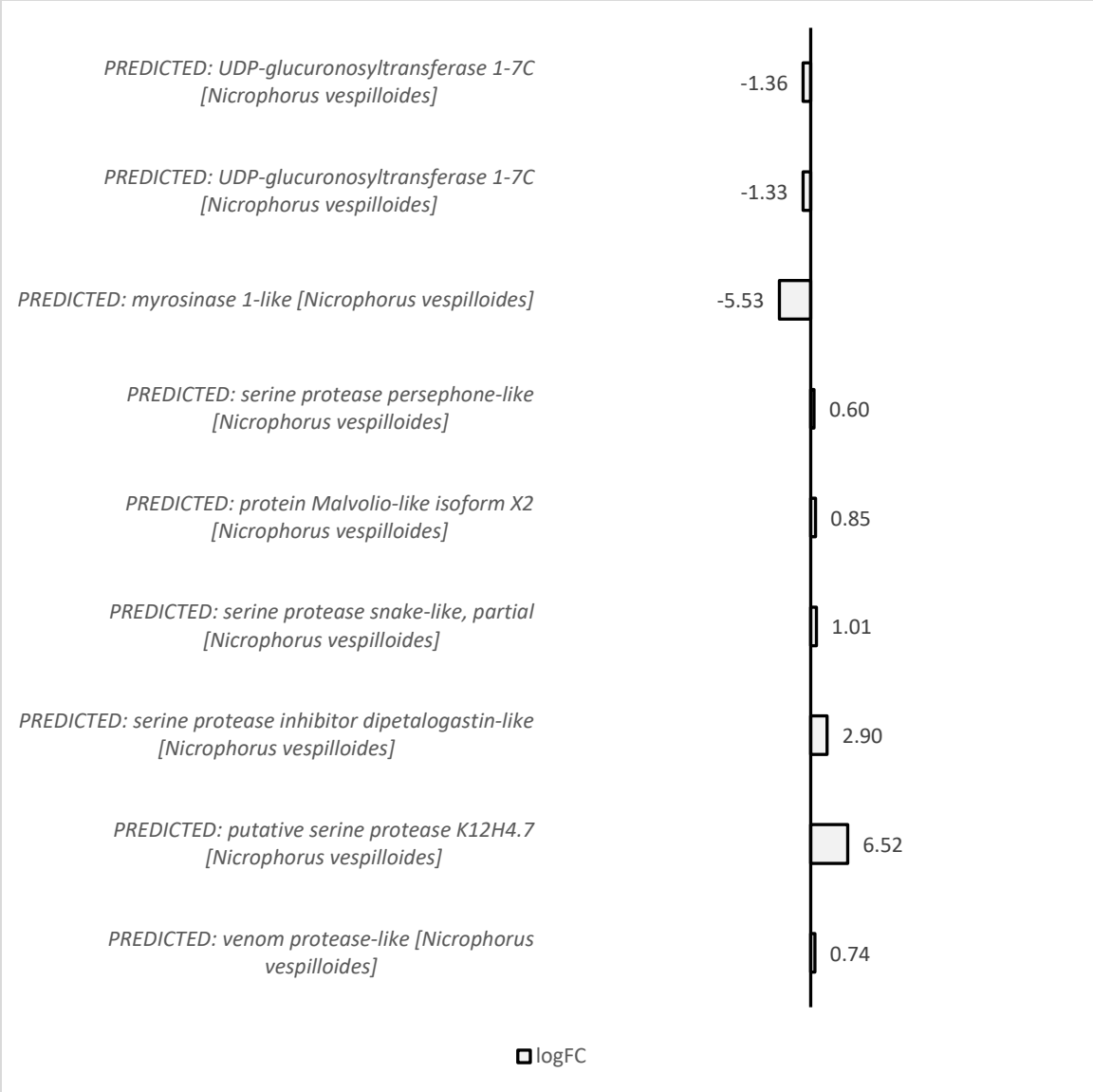

Figure 14: ±logFC of BLASTp-predicted RNA sequences pertaining to immune response expressed between fed (+logFC) and starved (-logFC) *N. pustulatus*.

Acknowledgements

I thank Mason Taylor, Thomas Hess, and Melissa Reed for their help in gathering Silphidae for this study. I also thank Anna Goldkamp, Dr. Brian Couger, Dr. Darren Hagen, and Dr. John E. Gustafson for their aid in the processing of samples and interpretation of data. Resources for this study came indirectly from the US Fish and Wildlife Service.

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CHAPTER V

CONCLUSION

The first goal of this research was to test the effects of pecan orchards and cattle ranches in Oklahoma on the occurrence of carrion beetles, including the American burying beetle. After 50 nights of sampling, the habitat association of the ABB appears to be generalist and ABB were found on both pecan orchards and cattle pastures, but in only one part of the state. The research showed that burying beetles remain in areas altered by conversion to ranching or pecan production at rates similar to those found in natural vegetation. These results suggest that a majority of burying beetles do not have a preference of habitat, at least not based on vegetation. The presence of these types of agriculture may support ABB occurrence as long as suitable carrion resources and soil remains in the area. While these findings potentially help in conservation decisions and creation of options for habitat management for both agricultural production and an endangered species, there is still more to be studied. The conclusions of this study were limited due to extremely low capture numbers of the American burying beetle, and inconsistent captures of other Silphidae, so further sampling would help confirm results found in 2017 and 2018. Additional surveys for Silphidae in different types of agriculture and pecan and ranches with different management strategies would further evaluate whether the species is compatible with habitat changes associated with these practices. In particular, a follow up study to compare organic orchards with conventional ones may

may explain the observed variability. In addition, vertebrate control measures at pecan orchards probably vary among growers and should be further investigated. For ranches, more information on stocking rates and grazing regimes could improve the interpretation of results.

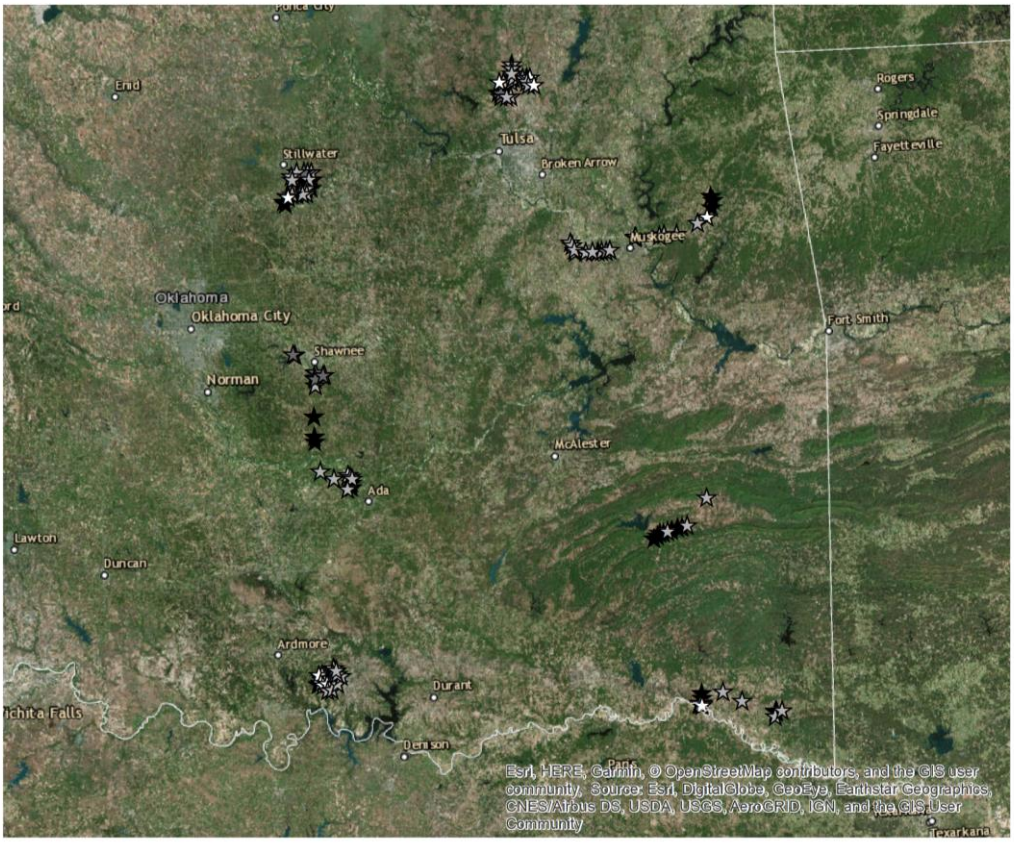
In the second part of my thesis, gene expression when fed meat in two species of burying beetle, *Nicrophorus orbicollis* and *Nicrophorus pustulatus* was investigated. My goal was to determine whether encountering food causes burying beetles to activate genes to secure the carcass for their use. When fed, both species reacted accordingly, with a greater variety of immune response-related proteins coded for in analyzed sequences. Between the two, *N. orbicollis* expressed higher numbers of immune-related genes compared to *N. pustulatus*, confirming that these burying beetles have different life histories.

This study and the methodology used would be improved if conducted along with a Silphidae breeding project. Genetic analysis would be improved with samples from inbred lines, as assembling a genome or transcriptome from wild-caught leads to extra variables and decreased accuracy. From an inbred larva, I could even assemble the genome of the species, which generates many possible investigations including adding details to the search for application of Silphidae antimicrobial secretions to industry. The antimicrobial peptides have the potential to successfully control growth of even the hardiest multidrug resistant bacteria, which has exciting possibilities in everything from medicine to food science.

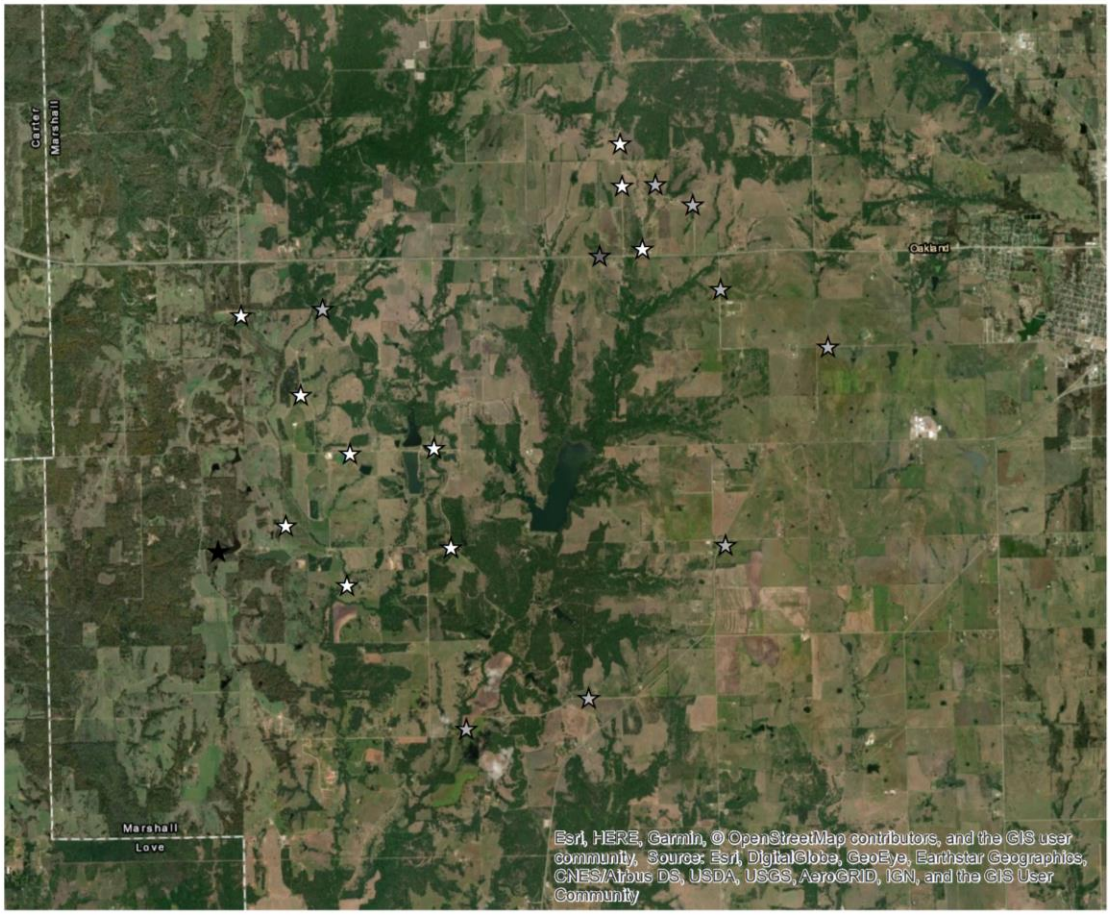
Insects are the most diverse class of animal on earth, and beetles represent the most diverse type. The Silphidae has relatively few species but stands out from the rest with advanced social behaviors, extreme strength, and by performing important and unique ecological services. There is still so much to learn and to be learned about family Silphidae, for their benefit as well as our own.

APPENDICES

Appendix 1: Maps of sampling locations and habitat designations of trapping sites investigated between 2017 and 2018 in eastern Oklahoma. All maps were assembled using ArcMap 10.6 ®.



Coordinate System NAD 1983 UTM Zone 14N
Projection Transverse Mercator
Description Locations of sampling sites in 2017 by habitat designation
Author Jacob Farriester
Date 11/28/2018



0 1.25 2.5 5 Miles

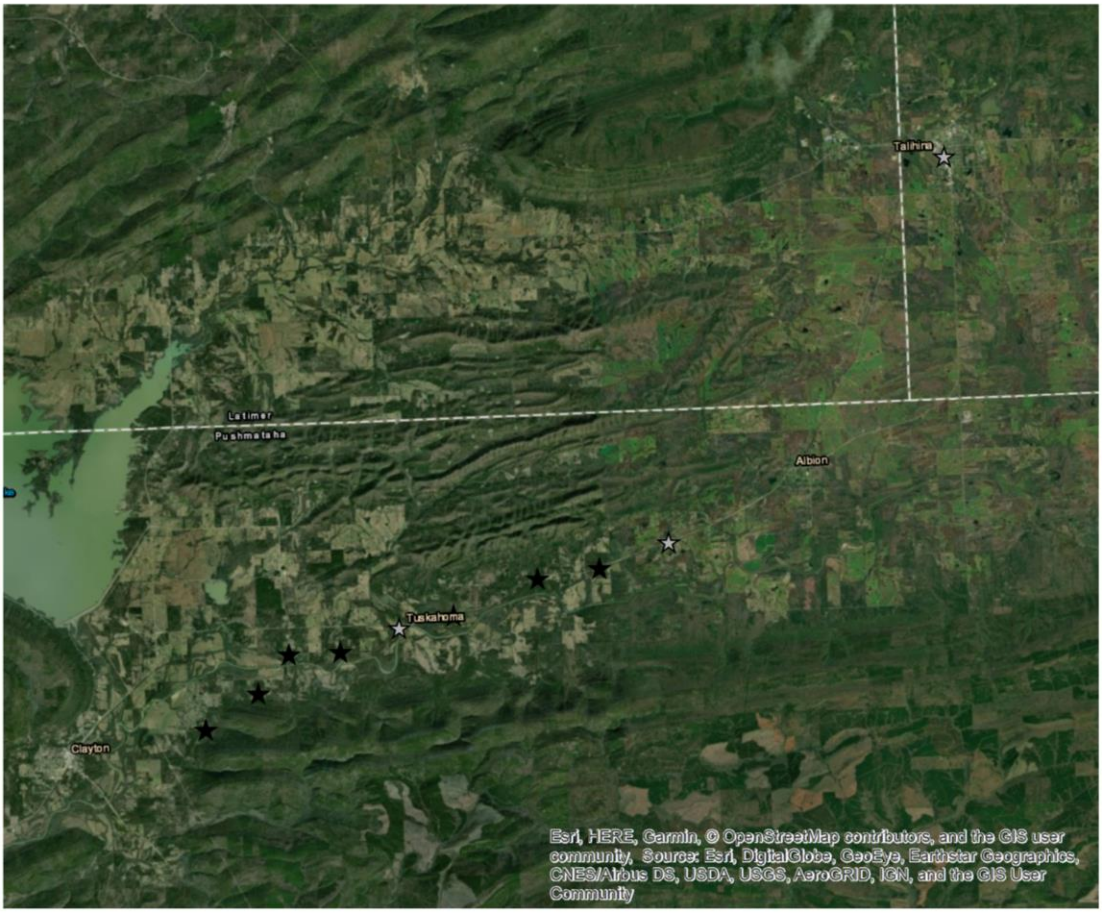
0 1.25 2.5 5 Kilometers

N

Designation

- Both
- Cattle
- Pecan
- Control and Undefined

Coordinate System NAD 1983 UTM Zone 14N
Projection Transverse Mercator
Description Locations of sampling sites in 2017 (Week 1)
Author Jacob Farriester
Date 11/28/2018



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0 2.5 5 10 Kilometers

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Designation

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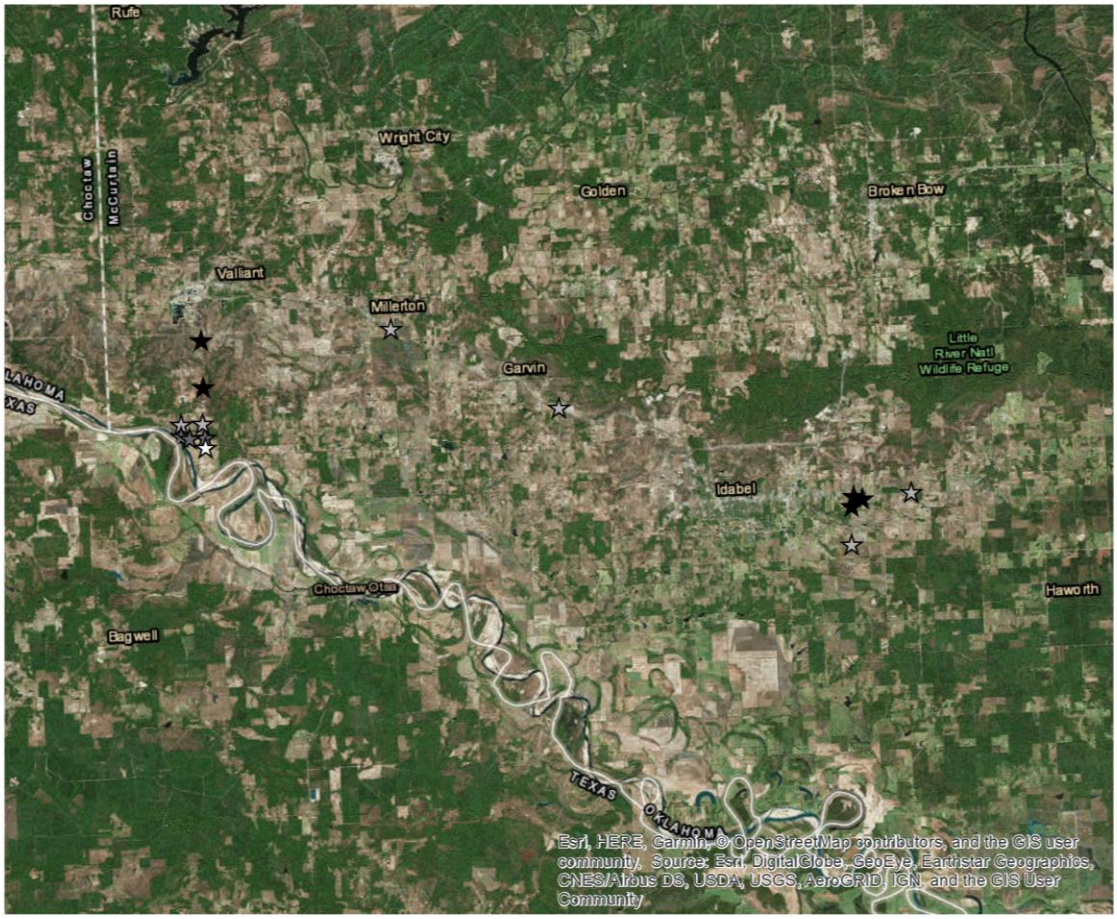
- ☆ Cattle
- ☆ Pecan
- ★ Control and Undefined

Coordinate System NAD 1983 UTM Zone 14N
Projection Transverse Mercator
Description Locations of sampling sites in 2017 (Week 2)
Author Jacob Farriester
Date 11/28/2018



0 3.75 7.5 15 Miles

0 3.75 7.5 15 Kilometers

N

Designation

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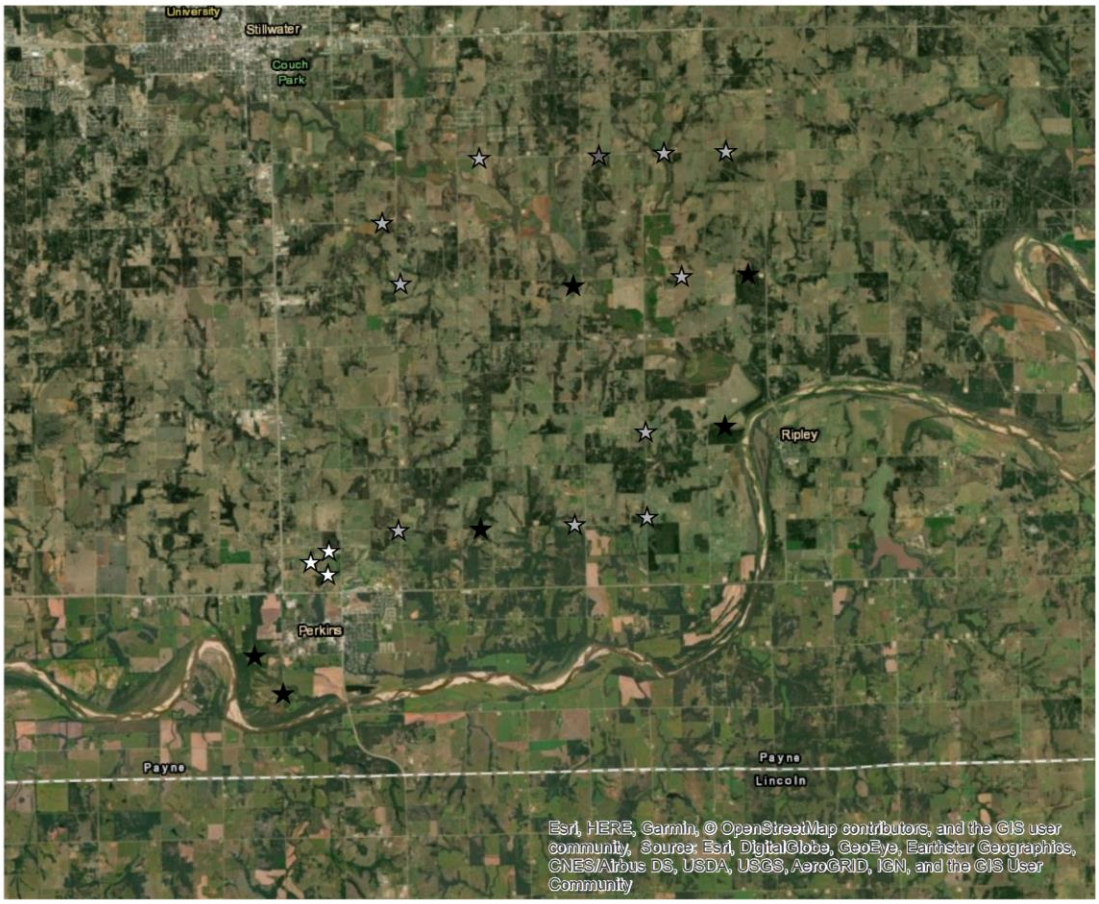
- ☆ Cattle
- ☆ Pecan
- ★ Control and Undefined

Coordinate System NAD 1983 UTM Zone 14N
Projection Transverse Mercator
Description Locations of sampling sites in 2017 (Week 3)
Author Jacob Farriester
Date 11/28/2018



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0 2.5 5 10 Kilometers

N

Designation

- Both
- Cattle
- Pecan
- Control and Undefined

Coordinate System NAD 1983 UTM Zone 14N
Projection Transverse Mercator
Description Locations of sampling sites in 2017 (Week 4)
Author Jacob Farriester
Date 11/28/2018

0 5 10 20 Miles

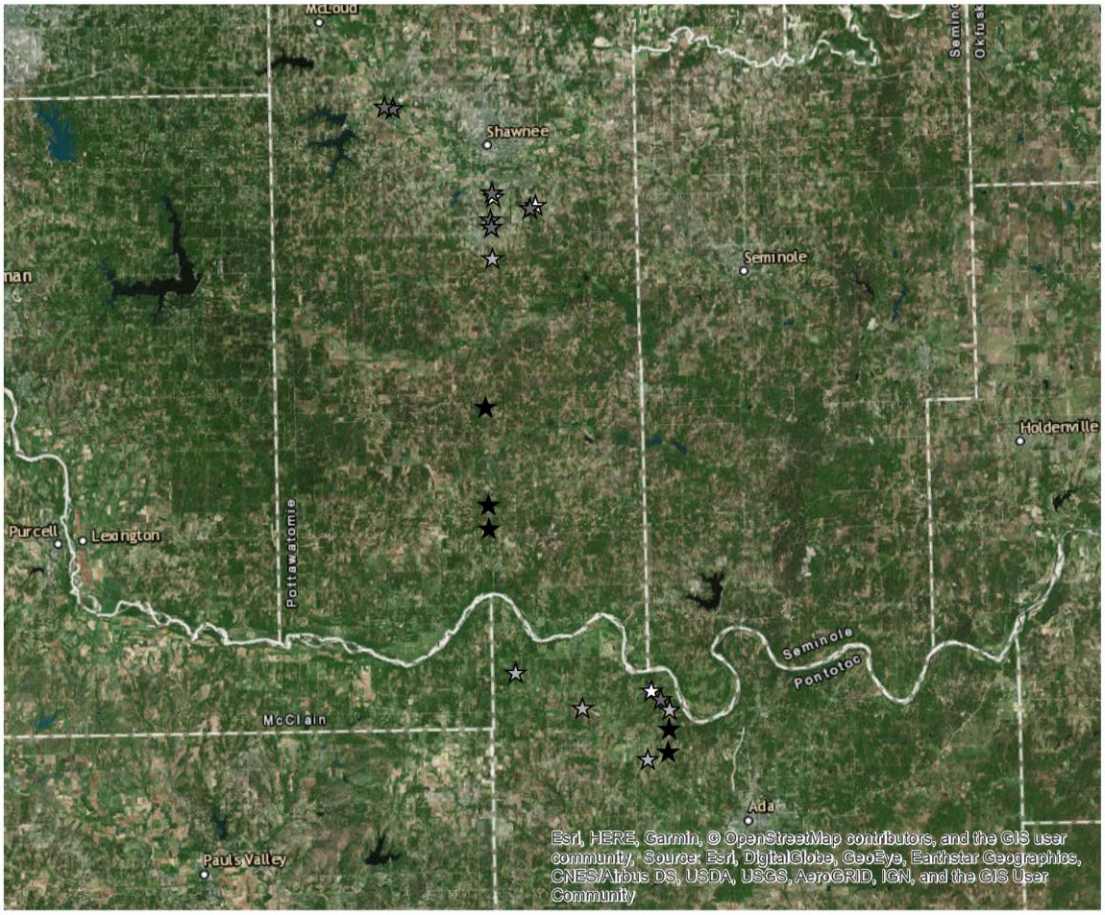
0 5 10 20 Kilometers

N

Designation

- ★ Both
- ☆ Cattle
- ☆ Pecan
- ★ Control and Undefined

Coordinate System NAD 1983 UTM Zone 14N
Projection Transverse Mercator
Description Locations of sampling sites in 2017 (Week 5)
Author Jacob Farriester
Date 11/28/2018



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0 5 10 20 Kilometers

N

Designation

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- ☆ Cattle
- ☆ Pecan
- ★ Control and Undefined

Coordinate System NAD 1983 UTM Zone 14N
Projection Transverse Mercator
Description Locations of sampling sites in 2017 (Week 6)
Author Jacob Farriester
Date 11/28/2018

0 2.5 5 10 Miles

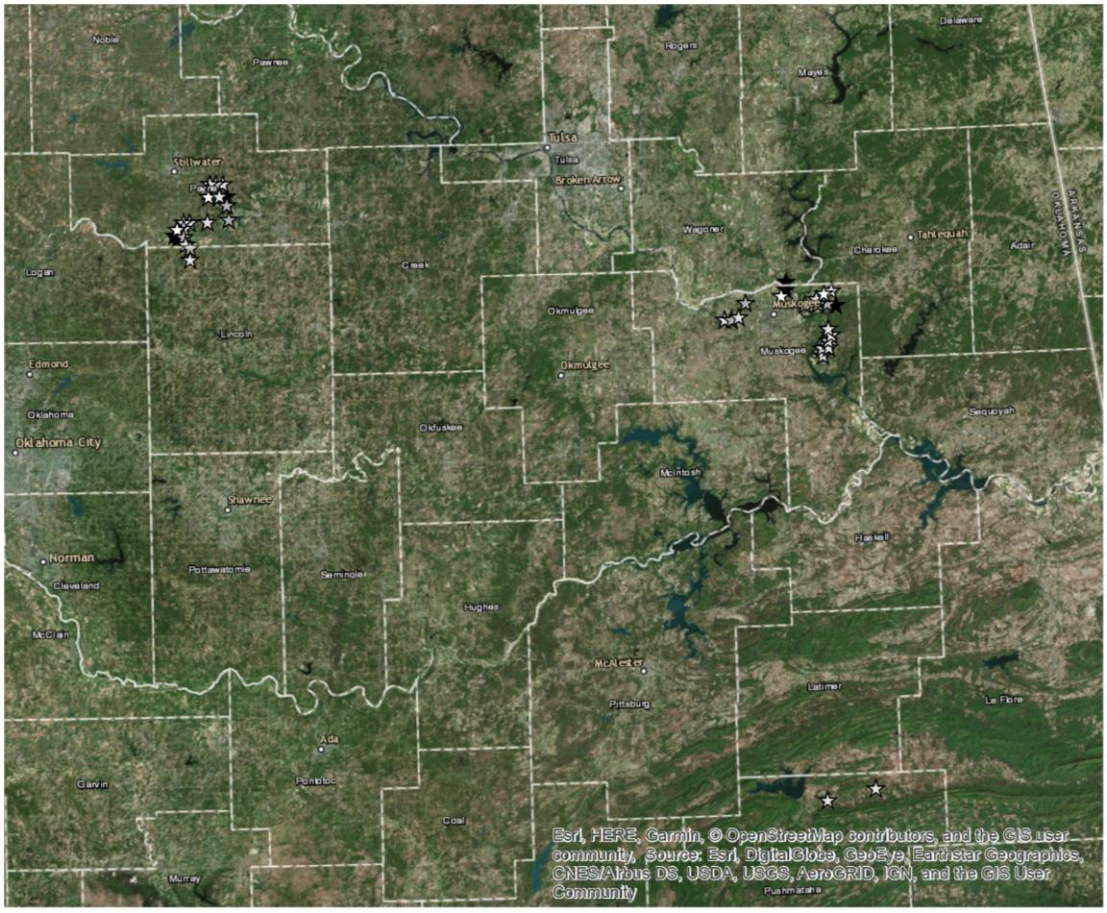
0 2.5 5 10 Kilometers

N

Designation

- ★ Both
- ☆ Cattle
- ☆ Pecan
- ★ Control and Undefined

Coordinate System NAD 1983 UTM Zone 14N
Projection Transverse Mercator
Description Locations of sampling sites in 2017 (Week 7)
Author Jacob Farriester
Date 11/28/2018



0 25 50 100 Miles

0 25 50 100 Kilometers

N

Designation

- ☆ Both
- ☆ Cattle
- ☆ Pecan
- ★ Control and Undefined

Coordinate System NAD 1983 UTM Zone 14N
Projection Transverse Mercator
Description Locations of sampling sites in 2018 by habitat designation
Author Jacob Farriester
Date 11/28/2018

0 3.75 7.5 15 Miles

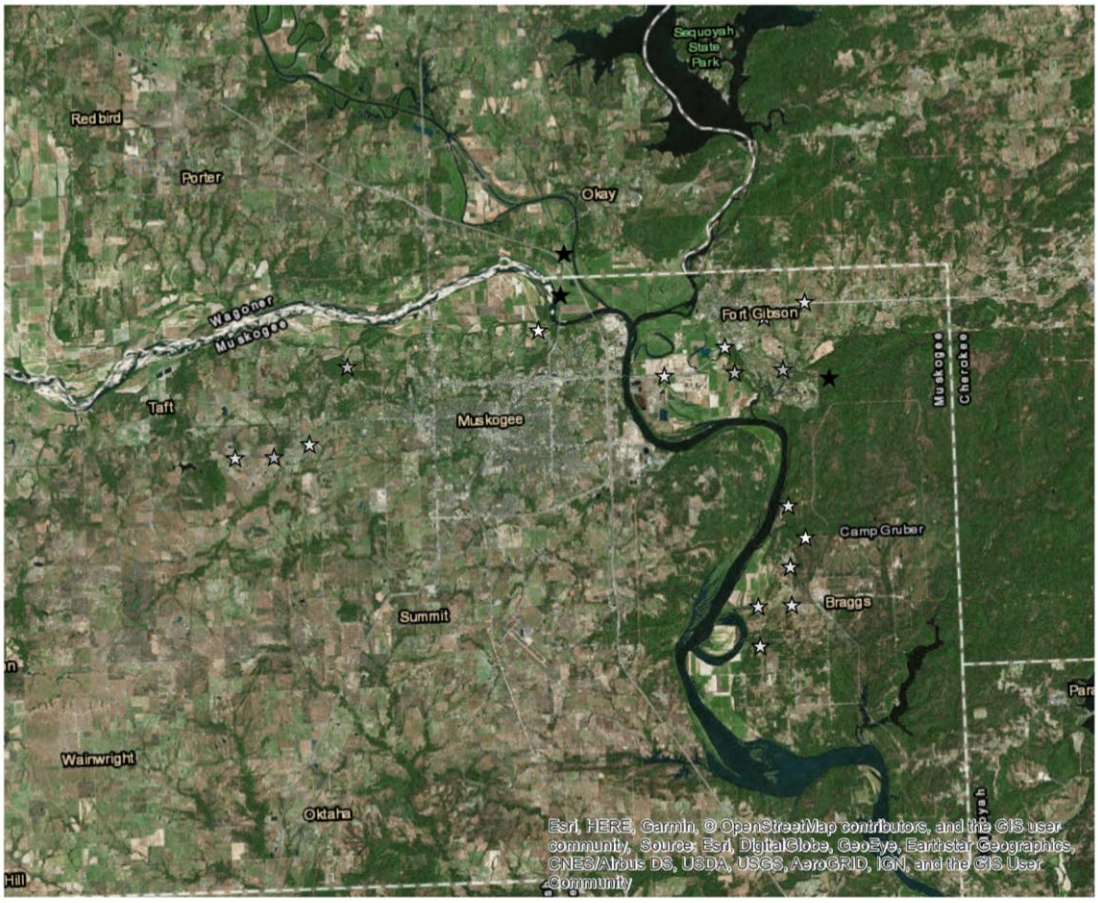
0 3.75 7.5 15 Kilometers

N

Designation

- ☆ Both
- ☆ Cattle
- ☆ Pecan
- ★ Control and Undefined

Coordinate System NAD 1983 UTM Zone 14N
Projection Transverse Mercator
Description Locations of sampling sites in 2018 (Week 8)
Author Jacob Farriester
Date 11/28/2018



0 3.75 7.5 15 Miles

0 3.75 7.5 15 Kilometers

N

Designation

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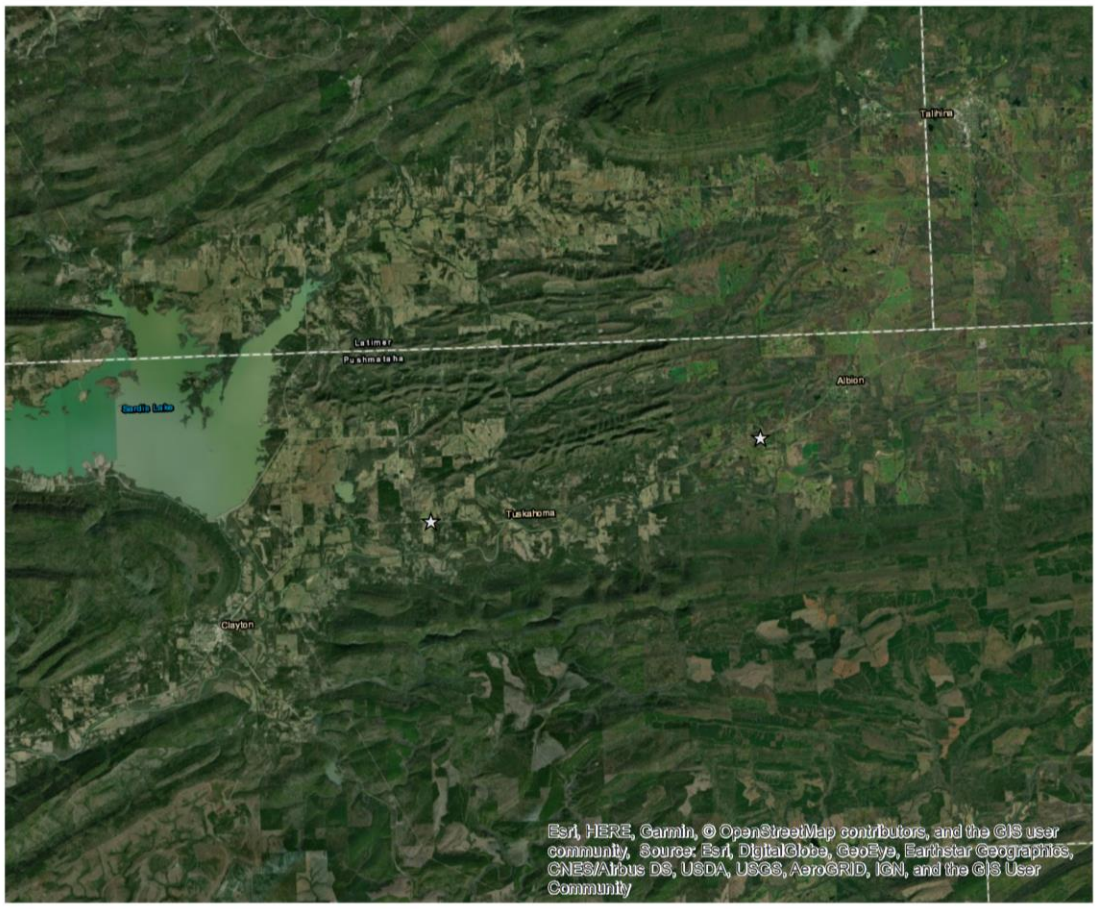
- ☆ Cattle
- ☆ Pecan
- ★ Control and Undefined

Coordinate System NAD 1983 UTM Zone 14N
Projection Transverse Mercator
Description Locations of sampling sites in 2018 (Week 9)
Author Jacob Farriester
Date 11/28/2018



0 2.5 5 10 Miles

0 2.5 5 10 Kilometers

N

Designation

- ☆ Both
- ☆ Cattle
- ☆ Pecan
- ★ Control and Undefined

Coordinate System NAD 1983 UTM Zone 14N
Projection Transverse Mercator
Description Locations of sampling sites in 2018 (Week 10)
Author Jacob Farriester
Date 11/28/2018

Appendix 2: Capture data recorded for all Nicrophorus americanus Olivier (ABB) caught during 2017-2018 sampling.

Week	Capture Date	Site ID	Sex	Pronotal Width (mm)	Age (Teneral / Senescent)	Elytral Brand	Recapture and Marking	Comments
5	7/15/2017	11	M	7.31	T	UR		
5	7/15/2017	13	F	7.71	T	UR		
5	7/15/2017	16	M	7.11	T	UR		
5	7/15/2017	16	F	10.44	T	UR		
5	7/16/2017	11	M	8.11	T	BR		
5	7/16/2017	11	M	9.12	S	BR		
5	7/16/2017	12	F	9.94	S	BR		
5	7/16/2017	16	F	7.15	T	BR	UR	
5	7/16/2017	16	F	8.32	S	BR		
5	7/16/2017	17	M	8.21	T	BR		
5	7/16/2017	17	M	7.97	S	BR		
5	7/17/2017	15	F	10.13	S	BL		
5	7/17/2017	15	M	9.03	S	BL		
5	7/17/2017	16	M	8.25	S	BL		
5	7/17/2017	16	F	9.26	S	BL		
5	7/17/2017	16	M	9.24	T	BL		
5	7/17/2017	16	F	7.41	S	BL		
5	7/17/2017	17	F	10.01	T	BL		
5	7/18/2017	7	M	6.75	T	UL		
5	7/18/2017	11	M	10.25	S	UL		
5	7/18/2017	13	M	10.09	S	UL		
5	7/18/2017	15	M	9.77	S	UL		
5	7/18/2017	16	M	8.9	S	UL		
5	7/18/2017	16	M	11.38	T	UL		
5	7/18/2017	16	M	10.26	T	UL		
5	7/18/2017	16	M	9.23	S	UL		
5	7/18/2017	16	M	9.96	T	UL		
5	7/18/2017	16	F	10.29	T	UL		
5	7/18/2017	17	M	10.2	T	UL		
5	7/18/2017	17	F	9.72	S	UL		
5	7/18/2017	17	F	8.52	S	UL		
5	7/18/2017	17	M	9.57	T	UL		
5	7/18/2017	17	M	9.67	T	UL		
5	7/18/2017	17	M	9.38	T	UL		
5	7/18/2017	17	M	10.32	T	UL		
5	7/18/2017	17	M	9.56	T	UL		
5	7/19/2017	8	F	10.83	S	P		

Week	Capture Date	Site ID	Sex	Pronotal Width (mm)	Age (Teneral / Senescent)	Elytral Brand	Recapture and Marking	Comments
5	7/19/2017	12	M	9.37	S	P		
5	7/19/2017	13	F	8.76	T	P		
5	7/19/2017	13	M	9.99	T	P		
5	7/19/2017	15	M	9.35	S	P		
5	7/19/2017	16	M	9.59	S	P		
5	7/19/2017	16	M	9.63	T	P		
5	7/19/2017	16	F	9.26	T	P		
5	7/19/2017	16	F	9.3	T	P		
5	7/19/2017	16	M	9.99	T	P		
5	7/19/2017	16	F	11.71	S	P		
5	7/19/2017	16	F	7.57	T	P		
5	7/19/2017	16	M	9.24	S	P		
5	7/19/2017	16	F	7.6	S	P		
5	7/19/2017	17	M	9.18	S	P	UL	
5	7/19/2017	18	M	9.73	S	P		
5	7/19/2017	19	M	9.25	T	P		
9	7/14/2018	2	F	9.31	S	UR		
9	7/14/2018	2	M	12.07	T	UR		
9	7/14/2018	2	F	10.3	S	UR		
9	7/14/2018	2	F	10.29	T	UR		
9	7/14/2018	3	F	8.91	S	UR		
9	7/14/2018	6	F	8.86	T	UR		
9	7/14/2018	6	M	11.91	S	UR		
9	7/14/2018	6	M	9.45	T	UR		
9	7/14/2018	7	M	9.56	S	UR		
9	7/14/2018	7	M	10.59	S	UR		
9	7/14/2018	7	F	10.27	S	UR		
9	7/14/2018	8	M	11.07	S	UR		
9	7/14/2018	8	F	10.85	S	UR		
9	7/14/2018	8	M	10.8	S	UR		
9	7/14/2018	8	F	7.14	T	UR		
9	7/14/2018	8	F	8.6	S	UR		
9	7/14/2018	8	M	10.39	S	UR		
9	7/14/2018	8	F	8.25	S	UR		
9	7/14/2018	16	M	10.59	T	UR		
9	7/15/2018	1	F	7.93	T	BR		
9	7/15/2018	1	F	8.21	S	BR		
9	7/15/2018	5	F	9.89	T	BR		
9	7/15/2018	5	M	9.87	T	BR		

Week	Capture Date	Site ID	Sex	Pronotal Width (mm)	Age (Teneral / Senescent)	Elytral Brand	Recapture and Marking	Comments
9	7/15/2018	5	F	9.13	T	BR		
9	7/15/2018	5	M	8.68	T	BR		
9	7/15/2018	5	F	10.53	T	BR		
9	7/15/2018	6	F	9.1	T	BR		
9	7/15/2018	7	M	9.9	S	BR		
9	7/15/2018	7	M	9.43	T	BR		
9	7/15/2018	7	M	10.33	S	BR		
9	7/15/2018	15	M	8.8	S	BR		
9	7/15/2018	19	F	10.25	T	BR		
9	7/16/2018	4	M	10.68	T	BL		
9	7/16/2018	20	F	9.91	S	BL		
9	7/16/2018	20	M	11.71	S	BL		
9	7/17/2018	2	F	7.4	T	UL		
9	7/17/2018	3	F	8.86	T	UL		
9	7/17/2018	4	F	9.8	T	UL		
9	7/17/2018	4	M	9.75	T	UL		
9	7/17/2018	4	M	8.93	S	UL	P?	Possible Pronotal Branding
9	7/17/2018	5	M	10.05	T	UL		
9	7/17/2018	6	M	8.73	T	UL		
9	7/17/2018	6	F	8.29	S	UL		
9	7/17/2018	7	F	10.37	S	UL		
9	7/17/2018	7	M	9.43	S	UL		
9	7/17/2018	7	F	9.68	T	UL		
9	7/17/2018	7	F	9.18	S	UL		
9	7/17/2018	7	F	7.98	S	UL		
9	7/17/2018	7	F	9.46	S	UL	UR?	Possible UR Recap
9	7/17/2018	7	M	8.11	S	UL		
9	7/17/2018	9	M	11.23	S	UL		
9	7/17/2018	15	M	10.99	T	UL		
9	7/17/2018	16	F	10.44	S	UL		
9	7/17/2018	18	F	8.8	S	UL		
9	7/17/2018	20	M	9.23	S	UL		
9	7/18/2018	1	F	8.92	S	P		
9	7/18/2018	4	F	8.13	S	P		
9	7/18/2018	6	F	9.22	T	P		
9	7/18/2018	7	F	9.24	S	P		
9	7/18/2018	7	M	8.94	S	P		

Week	Capture Date	Site ID	Sex	Pronotal Width (mm)	Age (Teneral / Senescent)	Elytral Brand	Recapture and Marking	Comments
9	7/18/2018	7	M	10.47	S	P		
9	7/18/2018	7	F	9.03	S	P		
9	7/18/2018	14	F	10.13	T	P		
9	7/18/2018	14	M	10.33	T	P		
9	7/18/2018	17	F	9.5	S	P		
9	7/18/2018	19	F	9.14	S	P		
9	7/18/2018	20	M	12.63	S	P		

Appendix 3: RNA sequences of "Starved" N. orbicollis with BLAST hits.

Sequence	BLAST Hits	logFC	logCPM	PValue	FDR
TRINITY_DN30416_c1_g1_i7	PREDICTED: thioredoxin domain-containing protein 5 [Nicrophorus vespilloides]	-11.852	4.220171	3.76E-08	6.98E-05
TRINITY_DN31684_c1_g1_i2	PREDICTED: thioredoxin domain-containing protein 5 [Nicrophorus vespilloides]	-10.1956	2.604738	9.88E-09	2.22E-05
TRINITY_DN31761_c1_g1_i25	PREDICTED: fibrous sheath CABYR-binding protein-like [Nicrophorus vespilloides]	-9.22183	3.313804	0.000254	0.038798
TRINITY_DN31852_c1_g1_i10	PREDICTED: talin-2 isoform X2 [Nicrophorus vespilloides]	-8.82125	1.258747	1.67E-07	0.000192
TRINITY_DN28434_c2_g1_i2	PREDICTED: microtubule-associated protein futsch-like isoform X3 [Nicrophorus vespilloides]	-7.01726	-0.18887	0.00025	0.038477
TRINITY_DN31960_c1_g1_i7	PREDICTED: receptor-type guanylate cyclase Gyc76C-like isoform X3 [Nicrophorus vespilloides]	-7.00367	-0.29149	0.000267	0.03964
TRINITY_DN30136_c0_g3_i1	PREDICTED: protein l(2)37Cc [Nicrophorus vespilloides]	-6.43318	-0.78472	1.39E-05	0.005988
TRINITY_DN24059_c0_g1_i1	PREDICTED: proclotting enzyme-like [Nicrophorus vespilloides]	-5.99831	4.782382	0.000265	0.03964
TRINITY_DN32095_c2_g1_i3	PREDICTED: bifunctional purine biosynthesis protein PURH [Nicrophorus vespilloides]	-5.90324	6.587372	2.65E-09	8.70E-06
TRINITY_DN31145_c1_g3_i4	PREDICTED: aspartate aminotransferase, cytoplasmic [Nicrophorus vespilloides]	-4.35133	4.062006	1.70E-06	0.001069
TRINITY_DN25965_c0_g1_i1	PREDICTED: phosphoglycolate phosphatase 2-like [Nicrophorus vespilloides]	-4.13058	-0.78917	6.68E-05	0.016854
TRINITY_DN28929_c2_g1_i10	PREDICTED: C-type lectin 37Db-like [Nicrophorus vespilloides]	-3.63166	0.42755	0.000132	0.026085
TRINITY_DN29693_c0_g1_i1	PREDICTED: UDP-glucuronosyltransferase 2B31 [Nicrophorus vespilloides]	-3.36536	4.38379	2.46E-05	0.009278
TRINITY_DN29693_c0_g3_i2	PREDICTED: UDP-glucuronosyltransferase 2B31 [Nicrophorus vespilloides]	-3.31346	2.797338	1.93E-05	0.00764
TRINITY_DN29424_c0_g1_i1	PREDICTED: uncharacterized protein LOC108569479 [Nicrophorus vespilloides]	-3.30139	3.945254	8.29E-05	0.019353
TRINITY_DN31145_c1_g3_i2	PREDICTED: aspartate aminotransferase, cytoplasmic [Nicrophorus vespilloides]	-3.0827	3.165211	2.94E-06	0.001672
TRINITY_DN29564_c0_g5_i1	PREDICTED: aminomethyltransferase, mitochondrial [Nicrophorus vespilloides]	-3.07923	3.14681	2.43E-07	0.000241
TRINITY_DN25376_c0_g1_i2	PREDICTED: C-type lectin 37Db-like [Nicrophorus vespilloides]	-3.06092	4.137534	5.48E-05	0.015381
TRINITY_DN29424_c0_g2_i3	PREDICTED: uncharacterized protein LOC108569479 [Nicrophorus vespilloides]	-3.04229	3.933057	0.000124	0.025228
TRINITY_DN27970_c0_g2_i2	PREDICTED: UDP-glucuronosyltransferase 1-7C [Nicrophorus vespilloides]	-3.04019	0.553884	2.29E-06	0.001376
TRINITY_DN29564_c0_g4_i2	PREDICTED: aminomethyltransferase, mitochondrial [Nicrophorus vespilloides]	-2.88335	2.564252	9.35E-07	0.000701
TRINITY_DN30931_c0_g2_i1	PREDICTED: glycine dehydrogenase (decarboxylating), mitochondrial [Nicrophorus vespilloides]	-2.54765	1.776197	6.01E-11	5.12E-07
TRINITY_DN63290_c0_g1_i1	PREDICTED: probable methylmalonate-semialdehyde dehydrogenase [acylating], mitochondrial [Nicrophorus vespilloides]	-2.49838	3.221705	1.28E-10	6.81E-07
TRINITY_DN22971_c0_g1_i2	PREDICTED: trifunctional purine biosynthetic protein adenosine-3 [Nicrophorus vespilloides]	-2.48007	-0.09386	6.08E-05	0.016077
TRINITY_DN27467_c0_g1_i1	PREDICTED: 4-coumarate--CoA ligase 1-like [Nicrophorus vespilloides]	-2.46974	2.535118	1.51E-07	0.000184
TRINITY_DN31936_c4_g1_i2	PREDICTED: phosphoribosylformylglycinamidine synthase [Nicrophorus vespilloides]	-2.43265	5.528761	1.43E-06	0.000909
TRINITY_DN29306_c1_g1_i6	PREDICTED: uncharacterized protein LOC108561775 isoform X1 [Nicrophorus vespilloides]	-2.33255	6.853037	6.67E-07	0.000592
TRINITY_DN31911_c2_g1_i2	PREDICTED: alpha-aminoadipic semialdehyde synthase, mitochondrial-like [Nicrophorus vespilloides]	-2.31054	6.441084	3.66E-06	0.00205
TRINITY_DN31911_c2_g3_i1	PREDICTED: alpha-aminoadipic semialdehyde synthase, mitochondrial-like, partial [Nicrophorus vespilloides]	-2.16716	6.686973	7.62E-07	0.000638
TRINITY_DN28567_c2_g3_i1	PREDICTED: acyl-CoA Delta(11) desaturase-like [Nicrophorus vespilloides]	-2.08904	6.498283	8.21E-05	0.019251
TRINITY_DN31501_c1_g1_i1	PREDICTED: hornerin-like [Nicrophorus vespilloides]	-1.98041	3.363671	3.55E-05	0.012123
TRINITY_DN27970_c0_g1_i1	PREDICTED: UDP-glucuronosyltransferase 1-7C [Nicrophorus vespilloides]	-1.95358	1.972921	9.13E-05	0.020513
TRINITY_DN28577_c0_g1_i3	PREDICTED: low density lipoprotein receptor adapter protein 1-like [Nicrophorus vespilloides]	-1.90674	8.217249	0.000348	0.045744
TRINITY_DN28979_c1_g1_i1	PREDICTED: UDP-glucuronosyltransferase 2B2-like [Nicrophorus vespilloides]	-1.90537	5.324168	7.19E-11	5.12E-07
TRINITY_DN31928_c6_g1_i6	PREDICTED: putative fatty acyl-CoA reductase CG5065 isoform X4 [Nicrophorus vespilloides]	-1.87254	-0.32283	9.78E-05	0.021519
TRINITY_DN26888_c1_g1_i4	PREDICTED: glycine N-methyltransferase isoform X1 [Nicrophorus vespilloides]	-1.86987	10.8104	2.91E-09	8.87E-06
TRINITY_DN28386_c1_g4_i1	PREDICTED: uncharacterized protein LOC108569400 isoform X1 [Nicrophorus vespilloides]	-1.86576	3.875197	3.99E-08	7.09E-05
TRINITY_DN28071_c0_g1_i1	PREDICTED: tryptophan 2,3-dioxygenase [Nicrophorus vespilloides]	-1.84491	2.804069	0.000149	0.027495
TRINITY_DN27437_c0_g3_i1	PREDICTED: acyl-CoA Delta(11) desaturase-like [Nicrophorus vespilloides]	-1.83689	6.007411	0.000122	0.025092
TRINITY_DN31673_c2_g2_i1	PREDICTED: uncharacterized protein LOC108566841 [Nicrophorus vespilloides]	-1.82735	6.304287	1.62E-07	0.000192
TRINITY_DN30817_c0_g1_i6	PREDICTED: serine protease easter-like [Nicrophorus vespilloides]	-1.80495	6.304492	0.000335	0.044613
TRINITY_DN26681_c0_g1_i6	PREDICTED: leucine-rich repeat-containing protein 20 isoform X1 [Nicrophorus vespilloides]	-1.80306	4.646876	0.00023	0.036318
TRINITY_DN30634_c1_g1_i3	PREDICTED: cAMP-specific 3',5'-cyclic phosphodiesterase isoform X7 [Nicrophorus vespilloides]	-1.68501	4.825178	0.000264	0.039627
TRINITY_DN28869_c1_g1_i2	PREDICTED: secretin receptor-like [Nicrophorus vespilloides]	-1.6141	4.513792	8.92E-09	2.12E-05
TRINITY_DN29115_c0_g1_i1	PREDICTED: facilitated trehalose transporter Tret1-like [Nicrophorus vespilloides]	-1.54838	2.144397	1.35E-06	0.000876
TRINITY_DN30947_c1_g1_i3	PREDICTED: organic cation transporter protein [Nicrophorus vespilloides]	-1.52587	4.927826	9.87E-11	6.02E-07
TRINITY_DN26396_c0_g2_i2	40S ribosomal protein S5 [Varroa destructor]	-1.505	0.728679	0.000142	0.027055
TRINITY_DN31673_c2_g2_i4	PREDICTED: uncharacterized protein LOC108566841 [Nicrophorus vespilloides]	-1.45733	5.628435	1.24E-05	0.005558
TRINITY_DN31653_c2_g3_i1	PREDICTED: 40S ribosomal protein S12 [Nicrophorus vespilloides]	-1.45005	7.642601	3.40E-07	0.000323
TRINITY_DN30947_c1_g3_i1	PREDICTED: organic cation transporter protein [Nicrophorus vespilloides]	-1.44895	6.14746	9.89E-08	0.000128
TRINITY_DN28147_c0_g1_i1	PREDICTED: 5-formyltetrahydrofolate cyclo-ligase [Nicrophorus vespilloides]	-1.38985	2.041314	6.05E-05	0.016077
TRINITY_DN22885_c0_g1_i1	PREDICTED: 40S ribosomal protein S20 [Nicrophorus vespilloides]	-1.34277	9.030341	6.11E-05	0.016077
TRINITY_DN30967_c0_g2_i2	PREDICTED: angiotensin-converting enzyme-like [Nicrophorus vespilloides]	-1.33605	2.255023	2.92E-05	0.010798
TRINITY_DN27955_c0_g2_i2	PREDICTED: NAD-dependent L-serine dehydrogenase [Nicrophorus vespilloides]	-1.33322	8.943376	1.24E-06	0.000842
TRINITY_DN31673_c2_g1_i1	PREDICTED: uncharacterized protein LOC108566841 [Nicrophorus vespilloides]	-1.30607	6.217967	0.00017	0.029116
TRINITY_DN31650_c1_g1_i3	PREDICTED: 40S ribosomal protein SA [Nicrophorus vespilloides]	-1.30054	7.134596	8.97E-05	0.020364
TRINITY_DN28545_c0_g1_i10	PREDICTED: 40S ribosomal protein S10 [Nicrophorus vespilloides]	-1.26979	3.634714	7.44E-06	0.003653
TRINITY_DN26396_c0_g1_i1	PREDICTED: 40S ribosomal protein S5 [Nicrophorus vespilloides]	-1.26268	9.850813	0.000194	0.032375
TRINITY_DN25227_c1_g2_i1	PREDICTED: 60S ribosomal protein L12 [Nicrophorus vespilloides]	-1.25652	8.879822	1.76E-05	0.007151
TRINITY_DN29505_c0_g2_i1	PREDICTED: cytochrome P450 6k1-like [Nicrophorus vespilloides]	-1.25139	3.001201	4.50E-05	0.013627
TRINITY_DN30134_c0_g1_i7	PREDICTED: 60S ribosomal protein L28 [Nicrophorus vespilloides]	-1.24802	6.81602	3.24E-05	0.011524
TRINITY_DN26400_c0_g1_i5	PREDICTED: 40S ribosomal protein S14 [Nicrophorus vespilloides]	-1.2415	8.231184	0.000313	0.043111
TRINITY_DN27502_c1_g1_i1	PREDICTED: 40S ribosomal protein S3a [Nicrophorus vespilloides]	-1.24062	8.529508	5.20E-06	0.002776
TRINITY_DN31650_c1_g1_i2	PREDICTED: 40S ribosomal protein SA [Nicrophorus vespilloides]	-1.22784	5.614219	0.000379	0.048741
TRINITY_DN26432_c1_g1_i1	PREDICTED: 60S ribosomal protein L27a [Nicrophorus vespilloides]	-1.22466	8.650459	0.000309	0.042767
TRINITY_DN25131_c0_g1_i3	PREDICTED: ubiquitin-60S ribosomal protein L40-like [Rhagoletis zephyria]	-1.21121	2.029735	0.000118	0.024628
TRINITY_DN30674_c1_g4_i2	PREDICTED: guanine nucleotide-binding protein subunit beta-like protein isoform X1 [Nicrophorus vespilloides]	-1.20805	9.801626	0.00019	0.032006
TRINITY_DN26061_c2_g1_i11	PREDICTED: 40S ribosomal protein S2 [Nicrophorus vespilloides]	-1.19253	9.107017	8.40E-05	0.019493
TRINITY_DN31650_c1_g2_i1	PREDICTED: 40S ribosomal protein SA [Nicrophorus vespilloides]	-1.18715	8.602712	0.000116	0.02459
TRINITY_DN31650_c1_g2_i2	PREDICTED: 40S ribosomal protein SA [Nicrophorus vespilloides]	-1.1758	7.216777	0.000197	0.0328
TRINITY_DN28614_c0_g1_i2	PREDICTED: UDP-glucuronosyltransferase 2B15-like [Nicrophorus vespilloides]	-1.17431	2.133234	5.78E-05	0.015626
TRINITY_DN26479_c0_g1_i2	PREDICTED: 40S ribosomal protein S8 [Nicrophorus vespilloides]	-1.15968	8.944414	3.75E-05	0.012284
TRINITY_DN27809_c0_g1_i1	PREDICTED: glycine cleavage system H protein [Nicrophorus vespilloides]	-1.13955	3.91979	6.58E-06	0.003428
TRINITY_DN27526_c0_g1_i1	PREDICTED: 60S acidic ribosomal protein P1 [Nicrophorus vespilloides]	-1.12997	7.331749	4.92E-05	0.014348
TRINITY_DN26149_c0_g1_i6	PREDICTED: 40S ribosomal protein S24 isoform X3 [Nicrophorus vespilloides]	-1.12798	7.67568	4.68E-05	0.013982

Sequence	BLAST Hits	logFC	logCPM	PValue	FDR
TRINITY_DN26396_c0_g1_i3	PREDICTED: 40S ribosomal protein S5 [Nicrophorus vespilloides]	-1.12527	8.227753	0.000166	0.02903
TRINITY_DN24183_c0_g1_i3	PREDICTED: 40S ribosomal protein S5 [Nicrophorus vespilloides]	-1.12078	9.171231	0.00027	0.03964
TRINITY_DN26149_c0_g1_i4	PREDICTED: 40S ribosomal protein S24 isoform X1 [Nicrophorus vespilloides]	-1.11492	4.991674	0.000299	0.042145
TRINITY_DN21306_c2_g1_i2	PREDICTED: 60S ribosomal protein L19 [Nicrophorus vespilloides]	-1.10434	5.295774	0.000149	0.027495
TRINITY_DN30691_c0_g3_i3	PREDICTED: 1,5-anhydro-D-fructose reductase-like [Nicrophorus vespilloides]	-1.10427	3.564726	5.75E-05	0.015626
TRINITY_DN30518_c1_g1_i2	PREDICTED: 60S ribosomal protein L21 [Nicrophorus vespilloides]	-1.09893	8.69787	6.71E-05	0.016854
TRINITY_DN26331_c2_g1_i5	elongation factor 1-alpha, partial [Nicrophorus tomentosus]	-1.09501	9.604547	0.000254	0.038798
TRINITY_DN29448_c0_g1_i1	PREDICTED: uridine 5'-monophosphate synthase [Nicrophorus vespilloides]	-1.08166	3.376407	3.23E-05	0.011524
TRINITY_DN28355_c0_g3_i1	PREDICTED: uncharacterized protein LOC108564571, partial [Nicrophorus vespilloides]	-1.06396	0.728571	0.000166	0.02903
TRINITY_DN24526_c0_g1_i1	PREDICTED: 60S ribosomal protein L31 [Nicrophorus vespilloides]	-1.03999	8.686354	0.000358	0.04673
TRINITY_DN29256_c0_g1_i2	PREDICTED: THO complex subunit 5 homolog [Nicrophorus vespilloides]	-1.02413	3.047473	6.57E-05	0.016694
TRINITY_DN29474_c1_g1_i2	PREDICTED: 40S ribosomal protein S4 [Nicrophorus vespilloides]	-1.02284	8.456977	0.000321	0.043429
TRINITY_DN23136_c2_g1_i1	PREDICTED: 60S ribosomal protein L18a-like [Nicrophorus vespilloides]	-1.01461	9.955418	0.000164	0.028896
TRINITY_DN25496_c0_g1_i1	PREDICTED: mitochondrial ornithine transporter 1 [Nicrophorus vespilloides]	-0.99564	3.701811	7.02E-06	0.003596
TRINITY_DN25918_c0_g1_i1	PREDICTED: 60S ribosomal protein L11 isoform X2 [Nicrophorus vespilloides]	-0.97405	7.35388	8.45E-05	0.019503
TRINITY_DN27862_c0_g1_i8	PREDICTED: glutaryl-CoA dehydrogenase, mitochondrial [Nicrophorus vespilloides]	-0.9545	6.63453	0.000229	0.036292
TRINITY_DN21306_c2_g1_i6	PREDICTED: 60S ribosomal protein L19 [Nicrophorus vespilloides]	-0.95446	9.407948	0.000153	0.027621
TRINITY_DN31609_c0_g3_i3	PREDICTED: peroxidase-like [Nicrophorus vespilloides]	-0.94781	3.586275	0.000189	0.031878
TRINITY_DN28705_c0_g3_i1	PREDICTED: cytochrome P450 9e2-like [Nicrophorus vespilloides]	-0.9355	3.556568	0.000293	0.041702
TRINITY_DN22500_c0_g1_i1	PREDICTED: protein LLP homolog [Nicrophorus vespilloides]	-0.9158	5.383765	0.000331	0.04413
TRINITY_DN31991_c3_g1_i2	PREDICTED: dnaJ homolog subfamily B member 11 [Nicrophorus vespilloides]	-0.89233	5.18296	0.000202	0.033223
TRINITY_DN25794_c1_g3_i2	PREDICTED: 40S ribosomal protein S7 [Nicrophorus vespilloides]	-0.86982	6.223965	0.000326	0.043719
TRINITY_DN26950_c1_g1_i2	PREDICTED: elongation factor 1-gamma [Nicrophorus vespilloides]	-0.86967	9.216607	0.000142	0.027055
TRINITY_DN30149_c0_g1_i1	PREDICTED: 60S ribosomal protein L26 [Nicrophorus vespilloides]	-0.86618	9.086762	4.80E-05	0.014199
TRINITY_DN30996_c0_g2_i4	PREDICTED: methylcrotonoyl-CoA carboxylase subunit alpha, mitochondrial [Nicrophorus vespilloides]	-0.71708	4.165159	0.000173	0.029456

Appendix 4: RNA Sequences of "Fed" N. orbicollis with BLAST hits.

Sequence	BLAST Hits	logFC	logCPM	PValue	FDR
TRINITY_DN30496_c1_g1_i12	PREDICTED: nucleoporin NSP1-like isoform X10 [Nicrophorus vespilloides]	0.757438	6.896445	9.89E-05	0.02165
TRINITY_DN26973_c0_g1_i1	PREDICTED: septin-2 isoform X2 [Nicrophorus vespilloides]	0.764849	5.331915	0.000145	0.02731
TRINITY_DN29721_c2_g2_i1	PREDICTED: activating signal cointegrator 1 [Nicrophorus vespilloides]	0.797584	2.651544	0.000152	0.027621
TRINITY_DN24031_c0_g1_i3	PREDICTED: SH3 domain-binding glutamic acid-rich protein homolog [Nicrophorus vespilloides]	0.808941	5.156011	4.21E-05	0.01293
TRINITY_DN25626_c1_g1_i6	PREDICTED: cyclin G [Nicrophorus vespilloides]	0.828339	6.735093	8.03E-05	0.019077
TRINITY_DN31053_c0_g2_i2	PREDICTED: run domain Beclin-1-interacting and cysteine-rich domain-containing protein isoform X1 [Nicrophorus vespilloides]	0.831885	3.220494	0.000308	0.042732
TRINITY_DN30452_c1_g1_i1	PREDICTED: heat shock protein beta-1 isoform X1 [Nicrophorus vespilloides]	0.853457	7.948938	0.000384	0.049107
TRINITY_DN27345_c0_g1_i2	PREDICTED: TPPP family protein CG45057-like [Nicrophorus vespilloides]	0.870937	8.13556	1.92E-06	0.00117
TRINITY_DN29454_c0_g1_i1	PREDICTED: cysteine--tRNA ligase, cytoplasmic [Nicrophorus vespilloides]	0.919916	4.060732	3.80E-05	0.012284
TRINITY_DN29769_c0_g1_i2	PREDICTED: transmembrane protein 245 [Nicrophorus vespilloides]	0.94119	5.479275	3.79E-05	0.012284
TRINITY_DN30766_c0_g1_i3	PREDICTED: dnaJ homolog subfamily C member 5 homolog isoform X3 [Nicrophorus vespilloides]	0.960667	0.612798	0.000121	0.025092
TRINITY_DN31731_c3_g4_i2	PREDICTED: phospholipase A1 [Nicrophorus vespilloides]	0.965605	3.563684	0.00022	0.035523
TRINITY_DN29051_c0_g1_i1	PREDICTED: toll-like receptor Tollo [Nicrophorus vespilloides]	1.043812	3.666871	0.000386	0.049235
TRINITY_DN28649_c1_g1_i2	PREDICTED: uncharacterized protein LOC108556691 [Nicrophorus vespilloides]	1.068848	5.521181	0.000221	0.035523
TRINITY_DN31587_c0_g2_i3	PREDICTED: protein diaphanous isoform X2 [Nicrophorus vespilloides]	1.069385	5.793833	0.00025	0.038477
TRINITY_DN31518_c2_g1_i1	PREDICTED: uncharacterized protein LOC108568551 [Nicrophorus vespilloides]	1.080917	4.957565	0.000116	0.02459
TRINITY_DN31395_c0_g1_i1	PREDICTED: myosin heavy chain, non-muscle isoform X1 [Nicrophorus vespilloides]	1.109698	2.931981	0.00027	0.03964
TRINITY_DN30255_c3_g1_i1	PREDICTED: transferrin [Nicrophorus vespilloides]	1.109792	10.91811	0.0002	0.033078
TRINITY_DN29700_c1_g2_i1	PREDICTED: uncharacterized protein LOC108560885 [Nicrophorus vespilloides]	1.154639	6.484904	0.000245	0.038251
TRINITY_DN28323_c3_g3_i1	PREDICTED: uncharacterized protein LOC108557985, partial [Nicrophorus vespilloides]	1.202675	5.263102	0.000246	0.038251
TRINITY_DN31395_c0_g1_i2	PREDICTED: myosin heavy chain, non-muscle isoform X3 [Nicrophorus vespilloides]	1.236661	3.026332	5.24E-05	0.014825
TRINITY_DN26083_c0_g1_i1	PREDICTED: uncharacterized protein LOC108569241 [Nicrophorus vespilloides]	1.241491	3.319756	0.000342	0.045263
TRINITY_DN28440_c0_g5_i3	PREDICTED: caskin-2 isoform X1 [Nicrophorus vespilloides]	1.242819	6.550971	0.000223	0.035666
TRINITY_DN28770_c0_g1_i2	PREDICTED: beta-1,3-glucan-binding protein-like [Nicrophorus vespilloides]	1.245573	2.589316	5.75E-06	0.003029
TRINITY_DN28166_c0_g1_i2	PREDICTED: monocarboxylate transporter 1 isoform X2 [Nicrophorus vespilloides]	1.256048	3.247742	0.000157	0.027984
TRINITY_DN25632_c0_g1_i2	PREDICTED: nucleoporin NSP1-like isoform X7 [Nicrophorus vespilloides]	1.272692	5.392152	7.51E-05	0.018427
TRINITY_DN27585_c3_g1_i6	PREDICTED: protein FAM214A isoform X1 [Nicrophorus vespilloides]	1.276433	4.163814	3.94E-05	0.01249
TRINITY_DN29713_c0_g2_i2	PREDICTED: rab11 family-interacting protein 4 isoform X4 [Nicrophorus vespilloides]	1.30217	5.502114	3.00E-05	0.010952
TRINITY_DN29518_c1_g2_i2	PREDICTED: moesin/ezrin/radixin homolog 1 isoform X3 [Nicrophorus vespilloides]	1.307342	7.994714	0.000135	0.026244
TRINITY_DN30251_c1_g2_i2	PREDICTED: protein yellow [Nicrophorus vespilloides]	1.321044	5.35706	8.04E-05	0.019077
TRINITY_DN29942_c1_g2_i1	PREDICTED: probable multidrug resistance-associated protein lethal(2)03659 [Nicrophorus vespilloides]	1.327554	3.426456	2.09E-07	0.000223
TRINITY_DN29002_c1_g1_i1	PREDICTED: uncharacterized protein LOC108561395 [Nicrophorus vespilloides]	1.331985	2.807529	3.39E-05	0.011666
TRINITY_DN30588_c3_g1_i1	PREDICTED: exostosin-1-like [Nicrophorus vespilloides]	1.333834	4.336528	5.06E-05	0.014572
TRINITY_DN28975_c0_g1_i1	PREDICTED: uncharacterized protein LOC108568132 [Nicrophorus vespilloides]	1.345488	6.595405	0.000193	0.032287
TRINITY_DN30923_c0_g4_i2	PREDICTED: serine protease snake-like [Nicrophorus vespilloides]	1.379463	1.999229	0.000293	0.041702
TRINITY_DN26770_c0_g1_i2	PREDICTED: peroxidasin [Nicrophorus vespilloides]	1.386231	6.979697	0.00027	0.03964
TRINITY_DN28119_c0_g1_i12	PREDICTED: coiled-coil domain-containing protein AGAP005037 isoform X3 [Nicrophorus vespilloides]	1.417115	1.439333	9.10E-07	0.000701
TRINITY_DN31518_c2_g1_i3	PREDICTED: uncharacterized protein LOC108568551 [Nicrophorus vespilloides]	1.446228	4.771198	3.78E-05	0.012284
TRINITY_DN31182_c1_g2_i1	PREDICTED: LIM and SH3 domain protein F42H10.3 [Nicrophorus vespilloides]	1.455936	3.56346	0.000278	0.04041
TRINITY_DN26428_c0_g3_i2	PREDICTED: reticulon-1 isoform X2 [Nicrophorus vespilloides]	1.54722	5.611279	4.01E-05	0.012593
TRINITY_DN29556_c0_g2_i4	PREDICTED: spermatogenesis associated 6-like protein [Nicrophorus vespilloides]	1.582157	-0.06531	7.87E-05	0.019077
TRINITY_DN27770_c0_g1_i1	PREDICTED: nose resistant to fluoxetine protein 6-like [Nicrophorus vespilloides]	1.595773	5.109201	9.42E-05	0.02095
TRINITY_DN23824_c0_g1_i2	PREDICTED: uncharacterized protein LOC108568551 [Nicrophorus vespilloides]	1.663207	2.725949	7.69E-10	3.29E-06
TRINITY_DN30139_c2_g2_i2	PREDICTED: YY1-associated factor 2 [Nicrophorus vespilloides]	1.685918	3.31122	6.27E-05	0.0162
TRINITY_DN26534_c0_g1_i2	PREDICTED: uncharacterized protein LOC108566776 isoform X1 [Nicrophorus vespilloides]	1.715619	2.966207	1.12E-06	0.000807
TRINITY_DN24140_c0_g1_i1	PREDICTED: uncharacterized protein LOC108564009 [Nicrophorus vespilloides]	1.735878	6.789351	0.000221	0.035523
TRINITY_DN29905_c0_g2_i2	PREDICTED: plexin-B [Nicrophorus vespilloides]	1.742293	3.110337	8.03E-05	0.019077
TRINITY_DN29659_c1_g1_i6	PREDICTED: voltage-dependent anion-selective channel-like [Nicrophorus vespilloides]	1.74833	-0.38062	7.54E-08	0.000101
TRINITY_DN29598_c1_g2_i2	PREDICTED: uncharacterized protein LOC108567882 isoform X3 [Nicrophorus vespilloides]	1.759853	1.925433	4.48E-05	0.013627
TRINITY_DN28975_c0_g1_i3	PREDICTED: uncharacterized protein LOC108568132 [Nicrophorus vespilloides]	1.85718	6.484038	1.76E-05	0.007151
TRINITY_DN26226_c3_g1_i3	PREDICTED: ion transport peptide-like isoform X1 [Nicrophorus vespilloides]	2.200228	0.608954	1.35E-05	0.005898
TRINITY_DN31888_c23_g4_i1	PREDICTED: vitellogenin-5 [Nicrophorus vespilloides]	2.277242	11.51842	4.26E-06	0.002334
TRINITY_DN27662_c0_g2_i10	PREDICTED: DNA topoisomerase 3-beta-1 isoform X1 [Nicrophorus vespilloides]	2.38762	1.091887	9.01E-14	1.28E-09
TRINITY_DN26081_c4_g1_i1	PREDICTED: general odorant-binding protein 56d-like [Nicrophorus vespilloides]	2.537999	4.750175	8.89E-07	0.000701
TRINITY_DN30447_c0_g2_i1	PREDICTED: putative mediator of RNA polymerase II transcription subunit 26 isoform X1 [Nicrophorus vespilloides]	2.567072	5.716877	1.79E-06	0.001106
TRINITY_DN22164_c0_g2_i1	PREDICTED: vitellogenin-5 [Nicrophorus vespilloides]	2.61792	8.320145	2.23E-05	0.008561
TRINITY_DN25902_c0_g2_i2	PREDICTED: titin-like, partial [Nicrophorus vespilloides]	2.6318	-0.02322	0.000282	0.040646
TRINITY_DN26751_c6_g1_i3	PREDICTED: pair-rule protein odd-paired-like [Nicrophorus vespilloides]	2.859051	3.693832	1.31E-06	0.000858
TRINITY_DN31622_c0_g2_i7	complexin isoform X1 [Agrilus planipennis]	2.876777	0.421364	3.34E-05	0.011602
TRINITY_DN30511_c1_g1_i4	PREDICTED: protein lethal(3)malignant blood neoplasm 1 [Nicrophorus vespilloides]	2.903147	3.050167	4.82E-05	0.014199
TRINITY_DN30447_c0_g1_i2	PREDICTED: putative mediator of RNA polymerase II transcription subunit 26 isoform X2 [Nicrophorus vespilloides]	2.950436	4.251352	0.000295	0.04177
TRINITY_DN30511_c1_g2_i2	PREDICTED: protein lethal(3)malignant blood neoplasm 1 [Nicrophorus vespilloides]	3.117838	1.967273	0.000143	0.027132
TRINITY_DN25808_c0_g4_i1	PREDICTED: hexamerin-like [Nicrophorus vespilloides]	3.136074	10.04914	0.000314	0.043125
TRINITY_DN26152_c1_g1_i6	PREDICTED: uncharacterized protein LOC108564802 isoform X1 [Nicrophorus vespilloides]	3.184309	4.566791	0.000276	0.040389
TRINITY_DN30607_c0_g1_i2	PREDICTED: SNF-related serine/threonine-protein kinase-like isoform X1 [Nicrophorus vespilloides]	3.195214	-0.44835	1.34E-08	2.86E-05
TRINITY_DN28070_c1_g2_i1	PREDICTED: early growth response protein 4 [Nicrophorus vespilloides]	3.289764	-0.29826	2.37E-06	0.001404
TRINITY_DN25420_c1_g2_i1	PREDICTED: pupal cuticle protein C1B-like [Nicrophorus vespilloides]	3.504467	1.527713	0.000316	0.043156
TRINITY_DN29300_c0_g1_i8	PREDICTED: cytochrome P450 4C1-like [Nicrophorus vespilloides]	4.09063	1.599291	2.93E-05	0.010798
TRINITY_DN31296_c1_g1_i3	PREDICTED: hexamerin-like, partial [Nicrophorus vespilloides]	4.131218	-0.14116	0.000127	0.025672
TRINITY_DN27597_c0_g1_i15	PREDICTED: aryl hydrocarbon receptor nuclear translocator-like protein 1 isoform X3 [Nicrophorus vespilloides]	4.255885	-0.21156	0.000118	0.024628
TRINITY_DN24166_c0_g1_i2	PREDICTED: LOW QUALITY PROTEIN: uncharacterized protein LOC108568173 [Nicrophorus vespilloides]	4.343861	6.406877	1.66E-05	0.006869
TRINITY_DN26136_c0_g1_i2	PREDICTED: scavenger receptor class B member 1 isoform X2 [Nicrophorus vespilloides]	4.586056	3.045607	2.76E-06	0.001594
TRINITY_DN27495_c0_g2_i9	PREDICTED: uncharacterized protein LOC108562918 isoform X1 [Nicrophorus vespilloides]	4.746895	4.139153	3.34E-05	0.011602
TRINITY_DN27495_c0_g2_i6	PREDICTED: uncharacterized protein LOC108562918 isoform X1 [Nicrophorus vespilloides]	4.939751	3.424603	8.33E-06	0.003997
TRINITY_DN30541_c0_g2_i21	PREDICTED: type I inositol 1,4,5-trisphosphate 5-phosphatase isoform X4 [Nicrophorus vespilloides]	4.956456	-0.41284	0.000316	0.043156

Sequence	BLAST Hits	logFC	logCPM	PValue	FDR
TRINITY_DN27495_c0_g2_i12	PREDICTED: uncharacterized protein LOC108562918 isoform X1 [Nicrophorus vespilloides]	5.07472	6.746419	5.41E-07	0.000492
TRINITY_DN30795_c1_g3_i4	PREDICTED: cytochrome P450 4d2-like [Nicrophorus vespilloides]	5.154981	4.55122	5.92E-05	0.015884
TRINITY_DN27495_c0_g2_i1	PREDICTED: uncharacterized protein LOC108562918 isoform X2 [Nicrophorus vespilloides]	5.173826	6.17791	4.82E-06	0.002603
TRINITY_DN26687_c0_g3_i2	PREDICTED: hexamerin-like [Nicrophorus vespilloides]	5.723667	10.24366	1.90E-07	0.000213
TRINITY_DN27118_c0_g1_i23	PREDICTED: splicing factor 1 isoform X2 [Nicrophorus vespilloides]	6.03586	-0.93961	0.000391	0.049601
TRINITY_DN30511_c1_g2_i3	PREDICTED: protein lethal(3)malignant blood neoplasm 1 [Nicrophorus vespilloides]	6.160399	2.226881	0.000125	0.025334
TRINITY_DN28625_c0_g1_i2	PREDICTED: protein EFR3 homolog cmp44E isoform X3 [Nicrophorus vespilloides]	6.686496	-0.48795	0.000187	0.031603
TRINITY_DN24166_c0_g1_i5	PREDICTED: LOW QUALITY PROTEIN: uncharacterized protein LOC108568173 [Nicrophorus vespilloides]	6.702805	2.000158	5.73E-05	0.015626
TRINITY_DN27495_c0_g2_i7	PREDICTED: uncharacterized protein LOC108562918 isoform X1 [Nicrophorus vespilloides]	6.703667	3.928615	6.30E-05	0.0162
TRINITY_DN29217_c0_g1_i1	PREDICTED: proton-coupled amino acid transporter-like protein CG1139 [Nicrophorus vespilloides]	6.928165	-0.41376	0.000286	0.041073
TRINITY_DN30857_c0_g1_i7	PREDICTED: acyl-CoA synthetase family member 4 homolog [Nicrophorus vespilloides]	6.991161	-0.36441	0.000317	0.043156
TRINITY_DN25487_c0_g1_i8	PREDICTED: transmembrane protein 138 [Nicrophorus vespilloides]	7.008256	-0.31601	0.000246	0.038251
TRINITY_DN28476_c0_g1_i17	PREDICTED: probable E3 ubiquitin-protein ligase HERC4 isoform X2 [Nicrophorus vespilloides]	7.177074	-0.13131	0.000254	0.038798
TRINITY_DN25275_c0_g2_i13	PREDICTED: uncharacterized protein F13E6.1 isoform X2 [Tribolium castaneum]	7.179837	-0.16648	1.80E-05	0.007236
TRINITY_DN26952_c0_g2_i5	neuroglobin-like [Leptinotarsa decemlineata]	7.364894	-0.08966	4.94E-05	0.014348
TRINITY_DN31763_c3_g3_i2	PREDICTED: sodium/potassium-transporting ATPase subunit beta-1-like [Nicrophorus vespilloides]	7.396478	0.064673	9.97E-05	0.02172
TRINITY_DN30915_c2_g1_i6	PREDICTED: dual specificity mitogen-activated protein kinase 7-like [Nicrophorus vespilloides]	7.512143	0.160917	8.09E-05	0.019077
TRINITY_DN28382_c1_g1_i5	PREDICTED: polycomb group protein Psc-like isoform X2 [Nicrophorus vespilloides]	7.554052	0.214433	1.27E-06	0.000849
TRINITY_DN25492_c3_g1_i6	PREDICTED: wee1-like protein kinase [Nicrophorus vespilloides]	7.666162	0.261844	1.04E-06	0.000766
TRINITY_DN26401_c0_g1_i2	perilipin-3-like isoform X4 [Varroa jacobsoni]	7.700621	0.341408	8.85E-07	0.000701
TRINITY_DN29958_c0_g1_i24	PREDICTED: CLIP-associating protein isoform X6 [Nicrophorus vespilloides]	8.535256	2.215348	0.000132	0.026085
TRINITY_DN24605_c0_g1_i6	PREDICTED: protein LSM12 homolog [Nicrophorus vespilloides]	8.970112	1.491811	3.27E-05	0.011524
TRINITY_DN28765_c0_g3_i10	PREDICTED: proton-coupled amino acid transporter-like protein pathetic isoform X3 [Nicrophorus vespilloides]	9.687939	2.176544	0.000378	0.048741
TRINITY_DN27612_c0_g1_i5	PREDICTED: uncharacterized protein LOC108559507 [Nicrophorus vespilloides]	10.41808	2.859219	0.00028	0.040499
TRINITY_DN29800_c0_g3_i14	PREDICTED: activating transcription factor of chaperone isoform X1 [Nicrophorus vespilloides]	10.53262	2.979385	3.76E-05	0.012284

Appendix 5: RNA Sequences of "Starved" N. pustulatus with BLAST hits.

Sequence	BLAST Hits	logFC	logCPM	PValue	FDR
TRINITY_DN24552_c1_g2_i4	PREDICTED: methionine aminopeptidase 1 isoform X1 [Nicrophorus vespilloides]	-10.1231	2.113247	0.000622	0.035771
TRINITY_DN23761_c3_g1_i2	PREDICTED: lysine-specific histone demethylase 1A-like [Nicrophorus vespilloides]	-8.59892	0.694074	9.50E-17	5.42E-13
TRINITY_DN22206_c0_g1_i4	PREDICTED: monocarboxylate transporter 12 [Nicrophorus vespilloides]	-8.37454	2.649825	4.26E-07	0.000109
TRINITY_DN27133_c2_g1_i10	PREDICTED: protein cueball [Nicrophorus vespilloides]	-8.27916	0.401807	4.75E-06	0.000815
TRINITY_DN22877_c0_g1_i7	PREDICTED: double-stranded RNA-binding protein Staufen homolog 2 isoform X2 [Nicrophorus vespilloides]	-8.09574	0.215541	1.45E-05	0.001918
TRINITY_DN21535_c0_g1_i6	PREDICTED: ELAV-like protein 3 isoform X3 [Nicrophorus vespilloides]	-7.9712	0.1243	4.46E-05	0.004872
TRINITY_DN26447_c0_g1_i1	PREDICTED: LOW QUALITY PROTEIN: A-kinase anchor protein 9-like [Nicrophorus vespilloides]	-7.72067	-0.1125	0.000797	0.042752
TRINITY_DN26601_c0_g1_i25	PREDICTED: ribose-phosphate pyrophosphokinase 1 isoform X2 [Nicrophorus vespilloides]	-7.28486	-0.48054	0.000242	0.017644
TRINITY_DN26700_c2_g1_i6	PREDICTED: transcription factor RFX3 isoform X1 [Nicrophorus vespilloides]	-7.09051	-0.64559	0.000583	0.034162
TRINITY_DN23156_c1_g1_i14	PREDICTED: aryl hydrocarbon receptor nuclear translocator homolog isoform X2 [Nicrophorus vespilloides]	-6.97008	-0.73918	3.22E-07	8.76E-05
TRINITY_DN19137_c0_g1_i2	PREDICTED: uncharacterized protein LOC108567257 [Nicrophorus vespilloides]	-6.44216	3.1881	0.000937	0.048315
TRINITY_DN23852_c0_g1_i7	PREDICTED: myrosinase 1-like [Nicrophorus vespilloides]	-5.52768	-0.88891	9.70E-08	3.29E-05
TRINITY_DN25539_c4_g1_i2	PREDICTED: dopamine N-acetyltransferase-like [Nicrophorus vespilloides]	-5.33117	-0.12269	1.74E-07	5.23E-05
TRINITY_DN20340_c0_g1_i2	PREDICTED: sodium- and chloride-dependent GABA transporter 1 [Nicrophorus vespilloides]	-4.82214	2.404717	0.000791	0.042546
TRINITY_DN24033_c0_g1_i14	PREDICTED: uncharacterized protein LOC108560911 isoform X1 [Nicrophorus vespilloides]	-4.04102	-0.87979	0.0009	0.046896
TRINITY_DN16859_c0_g1_i1	PREDICTED: nose resistant to fluoxetine protein 6-like [Nicrophorus vespilloides]	-3.66167	1.642572	7.09E-05	0.00692
TRINITY_DN26689_c0_g1_i6	PREDICTED: hornerin-like [Nicrophorus vespilloides]	-3.51056	2.68604	0.000119	0.010322
TRINITY_DN24195_c0_g1_i5	PREDICTED: monoacylglycerol lipase ABHD12-like [Nicrophorus vespilloides]	-3.45907	-1.06492	5.07E-05	0.0054
TRINITY_DN34710_c0_g1_i1	PREDICTED: nose resistant to fluoxetine protein 6-like [Nicrophorus vespilloides]	-3.32484	0.41189	2.37E-07	6.68E-05
TRINITY_DN21503_c0_g1_i5	PREDICTED: poly(A)-specific ribonuclease PARN-like domain-containing protein 1 [Nicrophorus vespilloides]	-3.24026	-0.22858	0.000808	0.043062
TRINITY_DN26910_c3_g5_i1	uncharacterized protein LOC112544562 [Pelodiscus sinensis]	-3.19721	1.793374	0.000586	0.034258
TRINITY_DN26901_c1_g1_i11	PREDICTED: F-box/LRR-repeat protein 21 [Nicrophorus vespilloides]	-2.94731	1.887524	0.000454	0.028268
TRINITY_DN24786_c2_g1_i2	PREDICTED: arrestin homolog [Nicrophorus vespilloides]	-2.56337	3.431493	0.000286	0.019991
TRINITY_DN24535_c0_g1_i3	PREDICTED: small nuclear ribonucleoprotein Sm D3 [Nicrophorus vespilloides]	-2.43973	1.798365	9.54E-08	3.28E-05
TRINITY_DN25969_c3_g2_i1	PREDICTED: phosphatidate phosphatase LPIN3 isoform X2 [Nicrophorus vespilloides]	-2.3667	0.58781	9.44E-05	0.008652
TRINITY_DN25961_c2_g1_i12	PREDICTED: uncharacterized protein LOC108558232 isoform X3 [Nicrophorus vespilloides]	-2.21139	0.673603	3.49E-05	0.00401
TRINITY_DN24546_c0_g1_i1	PREDICTED: probable G-protein coupled receptor 52 [Nicrophorus vespilloides]	-2.20348	4.715691	1.65E-10	1.52E-07
TRINITY_DN25881_c0_g1_i1	PREDICTED: titin [Nicrophorus vespilloides]	-2.1372	5.708518	3.50E-06	0.000636
TRINITY_DN26294_c1_g1_i12	PREDICTED: pre-mRNA-splicing factor ISY1 homolog [Nicrophorus vespilloides]	-2.0914	-0.11664	2.12E-05	0.002636
TRINITY_DN26014_c1_g2_i5	PREDICTED: uncharacterized protein LOC108569472 [Nicrophorus vespilloides]	-2.0768	2.511617	0.000199	0.015143
TRINITY_DN25789_c1_g1_i12	PREDICTED: fibrous sheath CABYR-binding protein-like [Nicrophorus vespilloides]	-2.05853	5.332461	0.000552	0.032589
TRINITY_DN25961_c3_g1_i1	PREDICTED: TBC1 domain family member 16 [Nicrophorus vespilloides]	-1.98944	2.983354	5.94E-07	0.000145
TRINITY_DN21615_c0_g1_i3	PREDICTED: replication factor C subunit 4 [Nicrophorus vespilloides]	-1.98493	0.627686	3.80E-05	0.00433
TRINITY_DN21430_c0_g2_i4	PREDICTED: uncharacterized protein LOC108567300 [Nicrophorus vespilloides]	-1.96877	4.866064	0.000605	0.035037
TRINITY_DN26149_c2_g1_i1	PREDICTED: transcription initiation factor IIA subunit 1-like isoform X1 [Nicrophorus vespilloides]	-1.95362	-0.03563	0.000496	0.030275
TRINITY_DN25429_c0_g2_i1	PREDICTED: organic cation transporter 1-like isoform X1 [Nicrophorus vespilloides]	-1.92177	0.711309	2.22E-07	6.33E-05
TRINITY_DN26334_c0_g1_i2	PREDICTED: phosphoribosylformylglycinamidine synthase [Nicrophorus vespilloides]	-1.79826	6.642294	5.88E-13	9.31E-10
TRINITY_DN24401_c1_g1_i7	PREDICTED: uncharacterized protein LOC108559189 [Nicrophorus vespilloides]	-1.77867	3.942925	7.97E-08	2.84E-05
TRINITY_DN25789_c1_g1_i10	PREDICTED: fibrous sheath CABYR-binding protein-like [Nicrophorus vespilloides]	-1.76688	6.074859	0.000638	0.036334
TRINITY_DN23846_c1_g2_i4	carbonic anhydrase-related protein 10 [Anoplophora glabripennis]	-1.61275	7.041752	5.02E-07	0.000127
TRINITY_DN16720_c0_g1_i1	PREDICTED: coiled-coil domain-containing protein 170 isoform X2 [Nicrophorus vespilloides]	-1.58322	0.363915	0.000538	0.032255
TRINITY_DN23293_c0_g1_i7	PREDICTED: uncharacterized protein LOC108565890 isoform X1 [Nicrophorus vespilloides]	-1.51328	2.37502	8.62E-05	0.008128
TRINITY_DN24767_c0_g1_i2	PREDICTED: uncharacterized protein LOC108566830 [Nicrophorus vespilloides]	-1.43157	3.689851	1.35E-05	0.001832
TRINITY_DN24555_c0_g1_i2	PREDICTED: uncharacterized protein LOC108559594 [Nicrophorus vespilloides]	-1.40391	3.954373	0.000923	0.047837
TRINITY_DN23392_c0_g1_i1	PREDICTED: glutamate receptor ionotropic, kainate 2-like isoform X1 [Nicrophorus vespilloides]	-1.37717	2.022054	0.000141	0.011926
TRINITY_DN26090_c1_g1_i1	PREDICTED: ankyrin-3 [Nicrophorus vespilloides]	-1.37231	5.325421	2.46E-07	6.87E-05
TRINITY_DN25655_c0_g2_i2	PREDICTED: UDP-glucuronosyltransferase 1-7C [Nicrophorus vespilloides]	-1.36037	3.993651	0.000289	0.020101
TRINITY_DN25655_c0_g2_i1	PREDICTED: UDP-glucuronosyltransferase 1-7C [Nicrophorus vespilloides]	-1.32763	2.193356	0.000221	0.016471
TRINITY_DN26971_c1_g1_i3	PREDICTED: ubiquitin carboxyl-terminal hydrolase calypso isoform X1 [Nicrophorus vespilloides]	-1.31772	2.449075	3.61E-09	2.15E-06
TRINITY_DN25429_c0_g3_i1	PREDICTED: organic cation transporter 1-like [Nicrophorus vespilloides]	-1.305	3.671495	0.000215	0.016157
TRINITY_DN26631_c3_g2_i6	PREDICTED: longitudinals lacking protein, isoforms F/I/K/T-like isoform X8 [Nicrophorus vespilloides]	-1.01587	3.024991	3.59E-08	1.46E-05
TRINITY_DN25862_c3_g2_i1	RNA-binding protein Musashi homolog Rbp6 isoform X1 [Onthophagus taurus]	-0.99954	1.387528	7.85E-05	0.007592
TRINITY_DN24618_c3_g3_i4	PREDICTED: methyl-CpG-binding domain protein 5-like, partial [Nicrophorus vespilloides]	-0.98531	5.78822	6.68E-07	0.000159
TRINITY_DN23377_c0_g1_i1	PREDICTED: H/ACA ribonucleoprotein complex subunit 2-like protein [Nicrophorus vespilloides]	-0.96284	2.234135	0.000102	0.009166
TRINITY_DN26101_c2_g5_i1	PREDICTED: methyl-CpG-binding domain protein 5-like, partial [Nicrophorus vespilloides]	-0.94586	4.146038	1.41E-05	0.00189
TRINITY_DN26388_c0_g2_i1	PREDICTED: tryptophan--tRNA ligase, mitochondrial [Nicrophorus vespilloides]	-0.93059	8.385847	0.000798	0.042752
TRINITY_DN26075_c1_g2_i4	PREDICTED: H/ACA ribonucleoprotein complex subunit 4 [Nicrophorus vespilloides]	-0.92273	5.125853	5.77E-07	0.000142
TRINITY_DN22630_c6_g1_i1	PREDICTED: nucleolar GTP-binding protein 1 [Nicrophorus vespilloides]	-0.92035	7.851147	0.000934	0.048242
TRINITY_DN24449_c1_g1_i3	PREDICTED: uncharacterized protein LOC108560112 isoform X4 [Nicrophorus vespilloides]	-0.91406	3.842991	5.18E-10	3.99E-07
TRINITY_DN25365_c5_g5_i1	PREDICTED: non-canonical poly(A) RNA polymerase PAPD5-like [Nicrophorus vespilloides]	-0.90545	4.676476	1.68E-05	0.002158
TRINITY_DN23347_c0_g1_i7	PREDICTED: probable elongation factor 1-beta isoform X2 [Nicrophorus vespilloides]	-0.89501	7.389432	7.61E-06	0.00116
TRINITY_DN26388_c0_g1_i1	PREDICTED: 60S acidic ribosomal protein P0 [Nicrophorus vespilloides]	-0.88919	9.902379	0.000618	0.035573
TRINITY_DN26388_c0_g2_i2	PREDICTED: tryptophan--tRNA ligase, mitochondrial [Nicrophorus vespilloides]	-0.8852	-0.04623	0.00035	0.023452
TRINITY_DN26423_c0_g1_i8	PREDICTED: splicing factor 3B subunit 3 isoform X1 [Nicrophorus vespilloides]	-0.88267	3.823532	7.80E-05	0.007564
TRINITY_DN26720_c0_g1_i5	PREDICTED: phosphomannomutase 2 [Nicrophorus vespilloides]	-0.87976	4.780459	5.71E-05	0.005861
TRINITY_DN26936_c1_g1_i1	PREDICTED: histone-lysine N-methyltransferase SMYD3 isoform X3 [Nicrophorus vespilloides]	-0.87405	5.430621	0.000145	0.012192
TRINITY_DN24571_c0_g4_i1	PREDICTED: inosine-5'-monophosphate dehydrogenase-like [Nicrophorus vespilloides]	-0.86044	2.552942	0.000242	0.017644
TRINITY_DN25828_c0_g2_i9	PREDICTED: mannosylglucosyl-3-phosphoglycerate phosphatase isoform X1 [Nicrophorus vespilloides]	-0.84218	4.103063	0.000481	0.029609
TRINITY_DN25592_c0_g1_i2	protein timeless homolog [Anoplophora glabripennis]	-0.83839	2.97267	9.39E-09	4.54E-06
TRINITY_DN25005_c0_g1_i4	PREDICTED: importin-5 [Nicrophorus vespilloides]	-0.81167	2.940173	0.000545	0.032399
TRINITY_DN25985_c0_g1_i1	PREDICTED: probable ATP-dependent RNA helicase kurz [Nicrophorus vespilloides]	-0.80946	3.920938	5.20E-06	0.000876
TRINITY_DN26774_c0_g3_i2	PREDICTED: dual specificity protein phosphatase 15 [Nicrophorus vespilloides]	-0.78122	1.076231	0.00076	0.041186
TRINITY_DN27105_c0_g1_i1	PREDICTED: vacuolar protein sorting-associated protein 13B [Nicrophorus vespilloides]	-0.78081	3.871606	0.000644	0.036508
TRINITY_DN26497_c0_g2_i1	PREDICTED: AMP deaminase 2 isoform X5 [Nicrophorus vespilloides]	-0.76675	2.991526	0.000251	0.018118

Sequence	BLAST Hits	logFC	logCPM	PValue	FDR
TRINITY_DN24548_c1_g1_i2	PREDICTED: pescadillo homolog [Nicrophorus vespilloides]	-0.74708	5.357603	3.81E-06	0.000684
TRINITY_DN26889_c1_g1_i1	PREDICTED: protein kinase C, brain isozyme isoform X3 [Nicrophorus vespilloides]	-0.74456	3.55971	0.000947	0.04866
TRINITY_DN26312_c3_g3_i1	PREDICTED: transcription factor AP-4 isoform X1 [Nicrophorus vespilloides]	-0.73955	2.64678	0.000154	0.012583
TRINITY_DN26380_c0_g1_i1	PREDICTED: inosine-5'-monophosphate dehydrogenase-like [Nicrophorus vespilloides]	-0.73862	4.81359	0.000375	0.02445
TRINITY_DN25863_c1_g1_i3	PREDICTED: probable ATP-dependent RNA helicase DDX47 [Nicrophorus vespilloides]	-0.73794	5.135393	0.000597	0.034729
TRINITY_DN24023_c0_g1_i5	PREDICTED: dual specificity protein phosphatase CDC14B isoform X1 [Nicrophorus vespilloides]	-0.73566	2.235668	0.00031	0.021316
TRINITY_DN25511_c0_g1_i3	PREDICTED: U3 small nucleolar RNA-associated protein 14 homolog A [Nicrophorus vespilloides]	-0.73494	3.330651	0.000221	0.016471
TRINITY_DN24654_c1_g1_i1	PREDICTED: protein SDA1 homolog [Nicrophorus vespilloides]	-0.73258	4.481128	1.62E-05	0.002104
TRINITY_DN25875_c0_g1_i11	PREDICTED: MAP kinase-interacting serine/threonine-protein kinase 1-like isoform X1 [Nicrophorus vespilloides]	-0.72298	6.601506	5.00E-06	0.000848
TRINITY_DN25260_c0_g1_i2	PREDICTED: nucleolar complex protein 2 homolog [Nicrophorus vespilloides]	-0.71622	3.920844	7.91E-11	7.78E-08
TRINITY_DN26092_c1_g2_i2	PREDICTED: probable malonyl-CoA-acyl carrier protein transacylase, mitochondrial [Nicrophorus vespilloides]	-0.71027	3.609793	0.000494	0.030229
TRINITY_DN24095_c0_g1_i4	PREDICTED: nucleolin 1-like isoform X2 [Nicrophorus vespilloides]	-0.70847	6.051246	3.90E-05	0.004393
TRINITY_DN24168_c0_g2_i3	PREDICTED: ribosome biogenesis protein NSA2 homolog [Nicrophorus vespilloides]	-0.68921	6.518366	0.000129	0.011042
TRINITY_DN25092_c0_g1_i8	PREDICTED: serine--tRNA synthetase-like protein Slimp [Nicrophorus vespilloides]	-0.68217	6.06922	6.13E-05	0.006196
TRINITY_DN26715_c0_g2_i1	PREDICTED: receptor-type guanylate cyclase Gyc76C-like isoform X3 [Nicrophorus vespilloides]	-0.678	6.166907	1.69E-05	0.002158
TRINITY_DN25875_c0_g1_i3	PREDICTED: MAP kinase-interacting serine/threonine-protein kinase 1-like isoform X1 [Nicrophorus vespilloides]	-0.66162	8.528075	1.01E-05	0.001461
TRINITY_DN25995_c0_g2_i1	PREDICTED: PIH1 domain-containing protein 1 [Nicrophorus vespilloides]	-0.64242	1.011825	3.47E-05	0.00401
TRINITY_DN26114_c0_g2_i5	PREDICTED: zinc finger E-box-binding homeobox 1-like isoform X2 [Nicrophorus vespilloides]	-0.58601	7.90322	0.000171	0.013522
TRINITY_DN25712_c4_g2_i1	PREDICTED: endothelin-converting enzyme 1 isoform X1 [Nicrophorus vespilloides]	-0.5827	6.166736	0.000245	0.017783
TRINITY_DN25533_c1_g2_i1	PREDICTED: bystin [Nicrophorus vespilloides]	-0.57762	4.31943	1.29E-05	0.001768
TRINITY_DN25722_c4_g1_i3	PREDICTED: eukaryotic translation initiation factor 4E type 2 isoform X2 [Nicrophorus vespilloides]	-0.56728	1.998676	0.000777	0.042052
TRINITY_DN26431_c0_g4_i1	PREDICTED: tRNA (cytosine(38)-C(5))-methyltransferase [Nicrophorus vespilloides]	-0.53355	2.998181	0.000193	0.014923
TRINITY_DN24628_c0_g1_i5	PREDICTED: RNA-binding protein squid isoform X2 [Nicrophorus vespilloides]	-0.49105	6.590398	1.03E-08	4.87E-06
TRINITY_DN26425_c0_g1_i2	PREDICTED: trafficking protein particle complex subunit 11 [Nicrophorus vespilloides]	-0.46944	5.192915	0.000381	0.024718
TRINITY_DN26478_c0_g4_i2	PREDICTED: nucleolar MIF4G domain-containing protein 1 homolog [Nicrophorus vespilloides]	-0.42431	1.774275	0.000151	0.012404
TRINITY_DN24590_c0_g2_i8	PREDICTED: ubiquilin-1 [Nicrophorus vespilloides]	-0.39027	6.275375	0.000314	0.021554

Appendix 6: RNA sequences "Fed" N. pustulatus with BLAST hits.

Sequence	BLAST Hits	logFC	logCPM	PValue	FDR
TRINITY_DN24241_c0_g1_i3	PREDICTED: uncharacterized protein LOC108566533 [Nicrophorus vespilloides]	0.38453	7.647358	6.99E-05	0.006899
TRINITY_DN24701_c0_g1_i1	PREDICTED: NADPH:adrenodoxin oxidoreductase, mitochondrial [Nicrophorus vespilloides]	0.52629	3.662286	4.49E-06	0.000775
TRINITY_DN25893_c1_g1_i5	PREDICTED: serine protease persephone-like [Nicrophorus vespilloides]	0.596847	5.98616	1.04E-06	0.000231
TRINITY_DN26248_c0_g2_i6	PREDICTED: metal transporter CNNM4 isoform X2 [Nicrophorus vespilloides]	0.599798	5.721187	1.37E-05	0.001856
TRINITY_DN23563_c1_g1_i1	PREDICTED: uncharacterized protein LOC108560885 [Nicrophorus vespilloides]	0.618631	6.2897	0.000391	0.025173
TRINITY_DN26208_c0_g1_i2	PREDICTED: uncharacterized protein LOC108560920 [Nicrophorus vespilloides]	0.629104	8.097988	1.97E-05	0.00248
TRINITY_DN25370_c0_g2_i6	PREDICTED: probable ATP-dependent RNA helicase DHX35 [Nicrophorus vespilloides]	0.629428	4.735135	5.00E-09	2.79E-06
TRINITY_DN25860_c0_g1_i16	PREDICTED: uncharacterized protein LOC108567583 [Nicrophorus vespilloides]	0.669142	3.798114	0.000324	0.02198
TRINITY_DN25544_c0_g1_i3	PREDICTED: trifunctional enzyme subunit alpha, mitochondrial [Nicrophorus vespilloides]	0.673847	8.914094	0.000517	0.031235
TRINITY_DN24773_c3_g1_i4	PREDICTED: dehydrogenase/reductase SDR family member 7 [Nicrophorus vespilloides]	0.676262	6.835877	5.51E-05	0.005735
TRINITY_DN25024_c0_g1_i4	PREDICTED: insulin-like growth factor-binding protein complex acid labile subunit [Nicrophorus vespilloides]	0.67871	8.89132	5.94E-05	0.006075
TRINITY_DN26082_c2_g3_i2	PREDICTED: bcl-2-related ovarian killer protein homolog A-like [Nicrophorus vespilloides]	0.681597	2.92044	0.000115	0.010117
TRINITY_DN26770_c1_g2_i7	PREDICTED: very long-chain specific acyl-CoA dehydrogenase, mitochondrial [Nicrophorus vespilloides]	0.696396	6.214906	2.71E-06	0.000511
TRINITY_DN26549_c0_g1_i3	PREDICTED: filamin-B-like isoform X2 [Nicrophorus vespilloides]	0.702542	5.720092	0.000507	0.030822
TRINITY_DN26302_c1_g1_i2	PREDICTED: uncharacterized protein LOC108568564 [Nicrophorus vespilloides]	0.71	7.570744	0.000171	0.013522
TRINITY_DN26807_c1_g2_i1	PREDICTED: hemicentin-1 [Nicrophorus vespilloides]	0.716506	6.207008	0.000247	0.017874
TRINITY_DN23524_c2_g2_i1	PREDICTED: transcription factor Sox-11-B-like [Nicrophorus vespilloides]	0.719616	5.121265	3.84E-05	0.004363
TRINITY_DN23301_c3_g2_i5	PREDICTED: venom protease-like [Nicrophorus vespilloides]	0.739576	4.526856	0.000967	0.049542
TRINITY_DN24519_c3_g1_i2	PREDICTED: LIM and SH3 domain protein F42H10.3 [Nicrophorus vespilloides]	0.764397	5.068574	6.63E-07	0.000159
TRINITY_DN25263_c0_g1_i2	PREDICTED: motile sperm domain-containing protein 2-like [Nicrophorus vespilloides]	0.767016	5.184081	1.69E-09	1.15E-06
TRINITY_DN25973_c1_g1_i1	PREDICTED: formin-like protein CG32138 isoform X2 [Nicrophorus vespilloides]	0.776256	6.328166	0.0009	0.046896
TRINITY_DN24218_c0_g1_i5	PREDICTED: protein fem-1 homolog B [Nicrophorus vespilloides]	0.812773	3.772183	9.97E-05	0.00905
TRINITY_DN25626_c2_g1_i12	PREDICTED: glycerol-3-phosphate acyltransferase 1, mitochondrial isoform X1 [Nicrophorus vespilloides]	0.82735	7.427023	0.000186	0.014523
TRINITY_DN25878_c1_g1_i9	PREDICTED: transcription factor kayak [Nicrophorus vespilloides]	0.829942	3.525944	2.68E-05	0.003242
TRINITY_DN25650_c1_g2_i5	PREDICTED: transmembrane protein 214 [Nicrophorus vespilloides]	0.840423	5.878123	1.31E-07	4.21E-05
TRINITY_DN26797_c2_g2_i3	PREDICTED: uncharacterized protein LOC108568212 [Nicrophorus vespilloides]	0.84787	4.016652	0.000541	0.032311
TRINITY_DN26428_c0_g2_i4	PREDICTED: uncharacterized protein LOC108561148 [Nicrophorus vespilloides]	0.848617	4.103341	0.000123	0.010619
TRINITY_DN24768_c0_g1_i6	PREDICTED: protein Malvolio-like isoform X2 [Nicrophorus vespilloides]	0.851341	4.815622	0.000545	0.032399
TRINITY_DN26794_c2_g1_i2	PREDICTED: RNA-binding protein fusilli isoform X2 [Nicrophorus vespilloides]	0.85294	6.595166	9.09E-08	3.16E-05
TRINITY_DN24541_c0_g3_i1	PREDICTED: putative carbonic anhydrase 3 [Nicrophorus vespilloides]	0.853007	0.898478	0.000654	0.036928
TRINITY_DN24194_c0_g2_i2	PREDICTED: uncharacterized protein LOC108568564 [Nicrophorus vespilloides]	0.853094	4.140497	0.000593	0.034603
TRINITY_DN25359_c0_g1_i3	PREDICTED: insulin-like growth factor-binding protein complex acid labile subunit isoform X1 [Nicrophorus vespilloides]	0.854347	3.844952	0.000488	0.029935
TRINITY_DN25174_c0_g1_i18	PREDICTED: hydroxysteroid dehydrogenase-like protein 2 isoform X2 [Nicrophorus vespilloides]	0.86554	7.397244	0.000472	0.029211
TRINITY_DN25630_c0_g1_i1	PREDICTED: uncharacterized protein LOC108560419 [Nicrophorus vespilloides]	0.869645	5.433215	0.000552	0.032589
TRINITY_DN26301_c0_g1_i14	PREDICTED: facilitated trehalose transporter Tret1-2 homolog [Nicrophorus vespilloides]	0.877659	7.46169	9.90E-05	0.009022
TRINITY_DN25174_c0_g1_i19	PREDICTED: hydroxysteroid dehydrogenase-like protein 2 isoform X1 [Nicrophorus vespilloides]	0.881197	9.631605	0.000113	0.010012
TRINITY_DN26238_c1_g1_i6	PREDICTED: activating transcription factor of chaperone isoform X2 [Nicrophorus vespilloides]	0.890853	5.609269	2.53E-08	1.09E-05
TRINITY_DN26542_c0_g1_i4	PREDICTED: carbonic anhydrase 15-like [Nicrophorus vespilloides]	0.891805	5.107456	8.64E-05	0.008128
TRINITY_DN27161_c5_g2_i4	PREDICTED: NADPH--cytochrome P450 reductase [Nicrophorus vespilloides]	0.901714	7.226025	5.39E-05	0.005668
TRINITY_DN26021_c4_g1_i1	PREDICTED: latrophilin Cirl isoform X7 [Nicrophorus vespilloides]	0.919252	3.595741	4.18E-05	0.004672
TRINITY_DN27062_c4_g1_i8	PREDICTED: inter-alpha-trypsin inhibitor heavy chain H3-like isoform X1 [Nicrophorus vespilloides]	0.920534	7.059542	2.32E-05	0.002851
TRINITY_DN25174_c0_g1_i22	PREDICTED: hydroxysteroid dehydrogenase-like protein 2 isoform X1 [Nicrophorus vespilloides]	0.925809	5.111933	2.37E-05	0.002904
TRINITY_DN23222_c0_g1_i2	PREDICTED: protein yellow [Nicrophorus vespilloides]	0.948931	6.07546	0.000614	0.03543
TRINITY_DN24369_c0_g1_i5	PREDICTED: gonadotropin-releasing hormone II receptor-like [Nicrophorus vespilloides]	0.952492	7.212099	1.01E-06	0.000228
TRINITY_DN24269_c0_g1_i5	PREDICTED: monoacylglycerol lipase ABHD12-like [Nicrophorus vespilloides]	0.963914	4.657395	1.99E-06	0.000403
TRINITY_DN26557_c0_g2_i2	PREDICTED: uncharacterized protein LOC108563814 [Nicrophorus vespilloides]	0.971899	3.287504	0.000822	0.043466
TRINITY_DN26786_c1_g2_i4	PREDICTED: transport and Golgi organization protein 2 [Nicrophorus vespilloides]	1.00118	6.398342	1.72E-05	0.002181
TRINITY_DN24133_c1_g1_i4	PREDICTED: serine protease snake-like, partial [Nicrophorus vespilloides]	1.005986	8.722371	0.000947	0.04866
TRINITY_DN26542_c0_g3_i1	PREDICTED: uncharacterized protein LOC108564841 [Nicrophorus vespilloides]	1.025024	2.078285	1.08E-05	0.001548
TRINITY_DN26834_c0_g1_i1	PREDICTED: lipid storage droplets surface-binding protein 1-like isoform X3 [Nicrophorus vespilloides]	1.027696	2.522669	6.18E-07	0.000149
TRINITY_DN15644_c0_g1_i1	PREDICTED: general odorant-binding protein 99a-like [Nicrophorus vespilloides]	2.28633	1.364915	9.43E-05	0.008652
TRINITY_DN22206_c0_g1_i3	PREDICTED: myosin-1-like [Nicrophorus vespilloides]	2.287175	0.9063	0.000971	0.049633
TRINITY_DN25420_c0_g1_i1	PREDICTED: laccase-2-like isoform X2 [Nicrophorus vespilloides]	2.295537	0.35673	2.06E-08	9.16E-06
TRINITY_DN20868_c0_g1_i1	PREDICTED: 4-coumarate--CoA ligase 1-like isoform X1 [Nicrophorus vespilloides]	2.370061	3.627952	1.68E-06	0.000345
TRINITY_DN22689_c0_g6_i3	PREDICTED: LOW QUALITY PROTEIN: uncharacterized protein LOC108568173 [Nicrophorus vespilloides]	2.581225	3.746662	1.36E-08	6.24E-06
TRINITY_DN24109_c0_g1_i11	PREDICTED: hexamerin-like [Nicrophorus vespilloides]	2.583099	8.191604	1.70E-05	0.002163
TRINITY_DN24530_c0_g3_i5	PREDICTED: sulfotransferase family cytosolic 1B member 1-like [Nicrophorus vespilloides]	2.594092	2.092228	0.000818	0.043326
TRINITY_DN23688_c0_g1_i6	PREDICTED: uncharacterized protein LOC108567224 [Nicrophorus vespilloides]	2.682579	5.709001	7.57E-05	0.007365
TRINITY_DN24906_c0_g1_i4	PREDICTED: monocyte to macrophage differentiation factor 2 isoform X2 [Nicrophorus vespilloides]	2.85142	-0.35952	0.000748	0.040735
TRINITY_DN26898_c2_g1_i1	PREDICTED: RNA-directed DNA polymerase from mobile element jockey-like [Nicrophorus vespilloides]	2.894317	-0.62179	9.38E-07	0.000212
TRINITY_DN24126_c1_g5_i1	PREDICTED: serine protease inhibitor dipetalogastin-like [Nicrophorus vespilloides]	2.900675	2.240203	4.26E-05	0.004728
TRINITY_DN24109_c0_g1_i10	PREDICTED: hexamerin-like [Nicrophorus vespilloides]	2.903527	7.371668	3.99E-06	0.000701
TRINITY_DN20934_c0_g1_i1	PREDICTED: uncharacterized protein LOC108556537 [Nicrophorus vespilloides]	2.950679	0.94103	0.000326	0.02198
TRINITY_DN26885_c2_g1_i2	PREDICTED: lon protease homolog, mitochondrial isoform X2 [Nicrophorus vespilloides]	2.97025	-0.12313	4.06E-06	0.000705
TRINITY_DN33776_c0_g1_i1	PREDICTED: LOW QUALITY PROTEIN: uncharacterized protein LOC108568173 [Nicrophorus vespilloides]	2.991557	1.409247	5.39E-05	0.005668
TRINITY_DN21954_c0_g1_i7	PREDICTED: uncharacterized protein LOC108567213 [Nicrophorus vespilloides]	3.085171	8.171551	1.76E-06	0.000358
TRINITY_DN26430_c6_g1_i1	PREDICTED: hexamerin-like [Nicrophorus vespilloides]	3.093432	4.432824	0.000115	0.010117
TRINITY_DN15822_c0_g1_i1	PREDICTED: farnesol dehydrogenase-like isoform X1 [Nicrophorus vespilloides]	3.32548	1.994281	3.32E-06	0.000614
TRINITY_DN22689_c0_g6_i1	PREDICTED: LOW QUALITY PROTEIN: uncharacterized protein LOC108568173 [Nicrophorus vespilloides]	3.325579	9.76701	2.09E-07	6.07E-05
TRINITY_DN25687_c1_g1_i9	PREDICTED: uncharacterized protein LOC108567218 [Nicrophorus vespilloides]	3.33331	0.156227	4.73E-05	0.005073
TRINITY_DN26718_c4_g1_i2	PREDICTED: uncharacterized protein LOC108567218 [Nicrophorus vespilloides]	3.386898	4.759355	4.12E-05	0.00463
TRINITY_DN25687_c1_g1_i8	PREDICTED: uncharacterized protein LOC108561395 [Nicrophorus vespilloides]	3.586901	0.262758	0.000318	0.021763

Sequence	BLAST Hits	logFC	logCPM	PValue	FDR
TRINITY_DN23000_c0_g1_i2	PREDICTED: uncharacterized protein LOC108561175 [Nicrophorus vespilloides]	3.726382	4.264636	1.87E-09	1.24E-06
TRINITY_DN24109_c0_g1_i3	PREDICTED: hexamerin-like [Nicrophorus vespilloides]	3.864105	6.739872	3.53E-08	1.46E-05
TRINITY_DN26629_c2_g1_i13	PREDICTED: glutamine synthetase 2 cytoplasmic [Nicrophorus vespilloides]	4.047463	4.421439	2.06E-10	1.75E-07
TRINITY_DN23663_c0_g1_i4	PREDICTED: hexamerin-like [Nicrophorus vespilloides]	4.223451	7.933845	7.58E-09	3.93E-06
TRINITY_DN19487_c0_g1_i1	PREDICTED: protein takeout-like [Nicrophorus vespilloides]	4.296347	1.726776	1.54E-07	4.66E-05
TRINITY_DN26430_c7_g1_i2	PREDICTED: hexamerin-like [Nicrophorus vespilloides]	4.431207	5.099232	1.39E-11	1.52E-08
TRINITY_DN24969_c0_g1_i2	PREDICTED: venom carboxylesterase-6-like [Nicrophorus vespilloides]	4.653181	-0.78331	1.26E-06	0.000271
TRINITY_DN24978_c2_g2_i3	PREDICTED: hexamerin-like [Nicrophorus vespilloides]	4.751665	8.080827	1.21E-11	1.44E-08
TRINITY_DN23663_c0_g1_i3	PREDICTED: hexamerin-like [Nicrophorus vespilloides]	4.76192	9.526704	1.09E-09	7.96E-07
TRINITY_DN22193_c0_g4_i2	PREDICTED: hexamerin-like [Nicrophorus vespilloides]	5.151503	3.838338	3.07E-08	1.29E-05
TRINITY_DN26430_c8_g1_i2	PREDICTED: hexamerin-like [Nicrophorus vespilloides]	5.211495	6.762046	2.21E-14	7.01E-11
TRINITY_DN24109_c0_g1_i1	PREDICTED: hexamerin-like [Nicrophorus vespilloides]	5.265006	9.23425	4.92E-13	8.77E-10
TRINITY_DN23663_c0_g1_i2	PREDICTED: hexamerin-like [Nicrophorus vespilloides]	5.32343	4.188027	1.17E-06	0.000256
TRINITY_DN24219_c5_g1_i10	PREDICTED: uncharacterized protein LOC108557777 isoform X1 [Nicrophorus vespilloides]	6.056087	0.057465	5.35E-05	0.005668
TRINITY_DN19664_c0_g1_i7	PREDICTED: Kv channel-interacting protein 1 isoform X3 [Tribolium castaneum]	6.443147	-1.18759	6.36E-05	0.006399
TRINITY_DN25334_c0_g1_i9	PREDICTED: putative serine protease K12H4.7 [Nicrophorus vespilloides]	6.515344	-1.11361	0.000507	0.030822
TRINITY_DN21884_c0_g2_i3	PREDICTED: peroxiredoxin-2-like [Nicrophorus vespilloides]	6.70069	-0.9881	0.000732	0.039996
TRINITY_DN24058_c0_g1_i5	PREDICTED: zinc finger MYM-type protein 1-like isoform X2 [Nicrophorus vespilloides]	6.856186	-0.83633	0.000215	0.016169
TRINITY_DN21980_c0_g1_i6	PREDICTED: proliferation-associated protein 2G4 [Nicrophorus vespilloides]	6.864103	-0.93108	2.27E-05	0.002808
TRINITY_DN26533_c0_g2_i2	PREDICTED: ell-associated factor Eaf [Nicrophorus vespilloides]	7.036248	-0.70046	2.34E-06	0.000463
TRINITY_DN26119_c0_g1_i4	PREDICTED: AP-1 complex subunit gamma-1 isoform X2 [Nicrophorus vespilloides]	7.240562	-0.53501	0.000465	0.028896
TRINITY_DN26770_c1_g1_i7	PREDICTED: very long-chain specific acyl-CoA dehydrogenase, mitochondrial [Nicrophorus vespilloides]	7.300113	-0.55314	0.000781	0.042171
TRINITY_DN25103_c1_g1_i3	PREDICTED: Hermansky-Pudlak syndrome 3 protein homolog isoform X1 [Nicrophorus vespilloides]	7.43418	-0.38868	7.10E-06	0.001109
TRINITY_DN24594_c0_g2_i7	PREDICTED: G protein-activated inward rectifier potassium channel 3 isoform X1 [Nicrophorus vespilloides]	7.558605	-0.31757	2.75E-07	7.58E-05
TRINITY_DN23028_c0_g1_i1	PREDICTED: uncharacterized protein LOC108564610 [Nicrophorus vespilloides]	8.010227	0.161736	0.000625	0.035806
TRINITY_DN26570_c1_g2_i5	PREDICTED: RING finger protein nhl-1 [Nicrophorus vespilloides]	8.198337	0.216414	5.64E-05	0.005823
TRINITY_DN24553_c1_g1_i1	PREDICTED: zinc finger homeobox protein 3 isoform X1 [Nicrophorus vespilloides]	8.268403	0.381839	0.000164	0.013282
TRINITY_DN26650_c1_g1_i10	PREDICTED: protein diaphanous isoform X6 [Nicrophorus vespilloides]	9.547773	2.693768	3.86E-07	0.000102

VITA

Jacob William Farriester

Candidate for the Degree of

Master of Science

Thesis: CARRION BEETLES IN ORCHARDS AND RANCHES IN OKLAHOMA AND INVESTIGATION OF GENES ASSOCIATED WITH ANTIMICROBIAL PRODUCTION

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Biographical:

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Conducting RNA extraction and analysis from insect tissue

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