BACTERIAL COMMUNITIES IN CARRION AND BURYING BEETLE (SILPHIDAE) SECRETIONS

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Bachelor of Science in Biology

University of North Dakota

Grand Forks, ND

2018

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
May, 2021

BACTERIAL COMMUNITIES IN CARRION AND BURYING BEETLE (SILPHIDAE) SECRETIONS

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ACKNOWLEDGEMENTS

I'd like to first express my gratitude to Dr. Hoback for his support during the past two years and for encouraging me to pursue my interests studying silphid microbiomes. Thank you for sharing your enthusiasm and knowledge of burying beetle ecology with me and encouraging me throughout the writing process. I never would have anticipated enjoying digging through rat buckets but I'm glad I got the chance.

I'd like to acknowledge the Army National Guard and OSU Vice President of Research for providing funding, and the Oklahoma Animal Disease Diagnostic Laboratory for enabling the identification of my cultured isolates.

I'd also like to acknowledge Dr. Gustafson and his lab, particularly Dr. William Johnson, for aiding me during the culturing phase of my project and for continuing to investigate the isolates I identified.

I'm extremely grateful to committee members, Drs. Hoback, Gustafson, Ma, and Ochoa Corona for their guidance, suggestions, and critiques of my work and for their support and confidence in my ability.

I'm deeply indebted to Dr. Noha Youssef for funding the Illumina iSeq-100 sequencing of my culture-independent samples and providing me with her expertise when analyzing the results. Without her input I would have a far more limited view of the silphid secretion microbiome. I'm excited to transition to a doctoral program under her guidance.

To my friends and family, particularly my partner Alex Harman and my dog Potato, I'd like to thank you for your support and encouragement throughout the past two years.

Name: CARRIE J. PRATT

Date of Degree: MAY, 2021

Title of Study: BACTERIAL COMMUNITIES IN CARRION AND BURYING BEETLE (SILPHIDAE) SECRETIONS

Major Field: ENTOMOLOGY AND PLANT PATHOLOGY

Abstract: The family Silphidae contains about two hundred species of carrion and burying beetles divided into two subfamilies: Silphinae and Nicrophorinae. While both groups exhibit necrophagous feeding patterns, they are differentiated by their reproductive behaviors. Silphinae, known as carrion beetles, oviposit on or near a carcass, hatching free-living larvae that receive no parental care and feed on the carcass and fly larvae. Nicrophorinae, known as burying beetles, exhibit a unique reproductive strategy, preparing a carcass into a brood ball and providing biparental care to their offspring. Adult nicrophorines coat the brood ball in antimicrobial oral and anal secretions that prevent the normal microbial succession of soil and carcass microbes that would cause the carcass to rot. The community of microbes present in these secretions makes up the secretion microbiome, which is distinct from the gut microbiome, the microbiome of carcasses, and the microbiome of prepared carcasses.

Characterizing the silphid microbiome is important to understanding how a specialized group of invertebrates interact with microbes to utilize a carcass and in the case of nicrophorines, efficiently preserve and digest a carcass in order to successfully rear offspring. Studying silphid secretion microbiomes may also provide insights to developing novel antibiotics and methods of meat preservation. The goal of this research was to characterize the secretion microbiomes of silphid species from the two families of Silphidae. First, secretions from *Nicrophorus americanus* were cultured and bacterial isolates were identified using MALDI-TOF MS and 16S rRNA gene sequencing. Following this, secretions from *Necrodes surinamensis, Necrophila americana,* and five *Nicrophorus* spp*., N. pustulatus, N. americanus, N. marginatus, N. orbicollis,* and *N. tomentosus* were characterized using culture-independent 16S rRNA gene sequencing.

I cultured and identified thirteen bacterial isolates in nine genera from *Nicrophorus americanus* secretions and identified 694 bacterial genera from seven species of silphid. I identified a core group of 89 bacterial genera shared across the seven silphid species I sampled. I was unable to find strong evidence that the two subfamilies of Silphidae have different secretion microbiomes or that silphids with differing reproductive styles had significantly different secretion microbiomes. Instead, it appears that silphids share a core group of bacteria that differ in abundance across subfamilies and reproductive styles, and that many of these bacterial genera are associated with other necrophagous insect microbiomes and carcass microbiomes.

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CHAPTER I

LITERATURE REVIEW

Silphidae

The family Silphidae contains 15 genera and 183 extant species of carrion and burying beetles (Sikes, 2008). Silphids are amphipolar, meaning they are predominantly restricted to temperate zones, although most species are Holarctic (Sikes, 2008). This preference for the northern continents is likely due to the increased presence of competitors, particularly ants, further south (Scott et al., 1987). Most silphids are semelparous, meaning they undergo one reproductive episode before death, and are predatory carrion feeders, eating both carrion and other insects, primarily dipteran larvae (Steele, 1927, Pukowski, 1933, Sikes, 2008). The fossil record reveals Silphidae as early as 165mya during the middle Jurassic, indicating that they may have played a role in the decomposition of early mammals and dinosaurs (Lane et al., 2020).

Beetles in the family Silphidae are divided into two subfamilies: Silphinae and Nicrophorinae (Sikes, 2008). While both groups exhibit necrophagous feeding patterns, they are differentiated by the size of carcass they utilize and their reproductive behaviors. The subfamily Silphinae contains 12 genera and 111 species that prefer large carcasses (>300 g) which they share with other necrophagous insects and vertebrate scavengers (Sikes, 2008). Adults feed primarily on dipteran larvae at carcasses, but will also feed on carrion (Ratcliffe, 1996). Females oviposit on the carcass or in the soil nearby and the larvae that hatch are free-living, feeding

primarily on carrion, but occasionally on fly larvae (Ratcliffe, 1996). Compared to other silphids, carrion beetles in the subfamily Silphinae are poorly studied.

The subfamily Nicrophorinae contains 3 genera and 72 species that are generally referred to as burying beetles because they monopolize small vertebrate carcasses (up to 300 g, but usually less than 50 g), by burying them underground (Sikes et al., 2005, Sikes, 2008). The genus *Nicrophorus* dominates Nicrophorinae with 70 species worldwide (Lane et al., 2020). Distribution, phenology, and habitat preference vary by species, but in general, nicrophorines are nocturnal, semelparous, reproductively active in summer months, and present in a variety of habitats, ranging from forests to fields and marshes (Scott, 1998). Burying beetle diversity is highest at northern latitudes, likely increasing congeneric competition (Scott et al., 1987), and the beetles are less abundant, less diverse, and less reproductively successful at southern latitudes (Trumbo, 1990b). More than 200 behavioral ecology studies have been performed on nicrophorines because of their unique reproductive strategy preparing a carcass into a brood ball and providing biparental care to offspring (Sikes, 2008). *Nicrophorus vespilloides* in particular has become a model organism for studying parental care (Sikes, 2008).

Brood Ball Preparation

The reproductive cycle of *Nicrophorus* spp. burying beetles has been well documented by Pukowski (1933) and elaborated upon by Milne and Milne (1976) and others. Beetles begin their reproductive cycle by finding a carcass and determining if it is suitable. Carrion is an ephemeral resource and there is strong competition for it, not only from vertebrate scavengers, but also from insect competitors like necrophagous flies and ants, and a wide variety of microbes (Trumbo, 1990b, Burkepile et al., 2006). Wilson and Fudge (1984a) found that the majority of carcasses are found by beetles within 24 hours after death. Burying beetles assess a carcass with their mouthparts and antennae, walk along the length and width, and lift the carcass to gauge its size

and suitability. If the carcass is found by a single male beetle, the male enters into a posture Pukowski (1933) called “sterzeln” after the apiological term, raising their abdomen and releasing female attracting pheromones. If several males arrive at a carcass before a female is present, they will work together to bury it, then compete amongst themselves when the female arrives (Bartlett, 1988). If the carcass is found simultaneously by multiple beetles of both sexes, consexuals will fight until the largest male and female remain. The winning beetles then work together to bury the carcass underground by excavating the soil from beneath it. This process typically takes 5-8 hours but may take considerably longer if there are obstacles (Milne & Milne, 1976).

Once the carcass is buried, the beetles create a chamber and roll the carcass into a ball. Pushing on the carcass to roll it into a ball minimizes surface area and compacts both the carcass and the surrounding soil, creating the chamber. Throughout the burial process, the fur or feathers are removed and used to line the chamber. The beetles then coat the carcass in a layer of antimicrobial oral and anal secretions that prevent the growth of soil and carcass-borne microbes that would cause the carcass to rot (Hall et al., 2011, Arce et al., 2012). This in turn alters the emissions of microbe-produced sulfur volatiles, reducing carcass discovery and use by congeners and necrophagous flies (Trumbo et al., 2021). At this point, the carcass is considered “prepared” and is referred to as a brood ball.

The behaviors of brood ball preparation trigger female ovarian development (Scott & Traniello, 1987), and oviposition occurs in a chamber above the brood ball (Milne & Milne, 1976). Females lay more eggs than will survive, correlating with her size, and then limit the number of offspring based on the size of the carcass through cannibalism (Wilson & Fudge, 1984a). Heavier carcasses result in heavier broods with more offspring surviving to the teneral stage immediately after emerging from the pupa (Kozol et al., 1988). Kozol et al. (1988) hypothesized that *Nicrophorus* spp. make a tradeoff between many small offspring or a few large

offspring. “Extra” larvae are killed by parent beetles and consumed to yield fewer but larger offspring (Sikes, 2008).

After oviposition, the beetles create a depression in the top of the brood ball into which they regurgitate partially digested carrion to feed the larvae when they hatch (Milne & Milne, 1976). Upon hatching, the larvae move to the brood ball and use stridulation to beg for food, which is regurgitated by the parents. While larvae are able to feed themselves, it is initially inefficient, and so they beg for food from their parents (Smiseth et al., 2003). The larvae are fed by their parents for approximately 72 hours, after which they are nutritionally independent and able to efficiently feed themselves (Smiseth et al., 2003). However, the parent beetles remain with the brood for an additional 48 hours to defend them from conspecifics and predators (Smiseth et al., 2012). The larvae develop through three instars, then disperse and form pupal cells in the soil around the brood ball chamber where they pupate and remain until they emerge. Teneral adults leave the brood ball chamber and seek out their own food sources. This process takes approximately four weeks (Lane et al., 2020). Burying beetles overwinter as either adults or as pre-pupal final instars (Sikes, 2008).

Parental Care

During the approximately two-week period where *Nicrophorus* parent beetles stay with their larvae (Sikes, 2008), they exhibit a high degree of care. Parental care is defined as when parents exhibit behaviors that increase offspring growth and survival, sometimes at a cost to their own fitness (Smiseth et al., 2012). This care protects offspring from environmental threats including predators, desiccation, and starvation, and is observed in behaviors like gamete or food provisioning, oviposition-site selection, nest building or burrowing, and egg or offspring care (Smiseth et al., 2012). Among insects, only fifty families have been documented to exhibit parental care (Costa, 2006 as cited by Trumbo, 2012). In burying beetles, this care is clearly

displayed in food provisioning, nest building via burial and preparation of a brood ball, and offspring attendance and care after nutritional independence (Smiseth et al., 2012). Several studies have found that carcass preparation and parental care improve larval mass, growth rates, and survival (Eggert et al., 1998, Rauter & Moore, 2002, and Smiseth et al., 2003).

Although both male and female beetles are individually capable of carcass burial and raising offspring (Kozol et al., 1988) the majority of carcasses are occupied by a pair of beetles that exhibit biparental care (Pukowski, 1933). This form of care, where male and female parents work together, is extremely rare in insects (Pilakouta et al., 2018). The selection for biparental care in burying beetles may be because carrion, a nutrient rich and ephemeral resource, leads to strong competition and the need for resource guarding which sets the stage for offspring protection (Trumbo, 2012). Modifying the microenvironment of a carcass by burying it underground also works to create a favorable space, incentivizing parent beetles to stay and it is likely these processes collectively supported the evolution of biparental care (Nowak et al., 2010 as cited by Trumbo, 2012).

Although biparental care is shown by burying beetles, individual parents have differing roles. Both parents exhibit the same tasks, but the time spent on each task differs between the sexes (Smiseth & Moore, 2004). Female parents provide care longer than males and spend more time providing direct care to offspring by provisioning and processing carrion (Scott & Traniello, 1990, Smiseth & Moore, 2004). Male parents assist in burying and preparing a carcass which reduces detection by congeners and other competitors, but later shift their focus to guarding the carcass, brood, and female from conspecifics (Scott, 1998). Scott (1989) hypothesized that paternal care likely originated due to the benefits of guarding, rather than assisting with brood care. These benefits are clearly shown by the fact that the presence of a male greatly reduces the likelihood of brood takeover by conspecific intruders, which results in infanticide and replacement (Trumbo, 1990a, 1991, Scott, 1990, Ratcliffe, 1996).

Despite a good deal of investigative effort, research on the possible benefits of paternal presence remains controversial. A number of studies have found no differences in offspring success between broods raised by a single female, a single male, or a pair of beetles (Wilson & Fudge, 1984a, Bartlett, 1988, Scott, 1989, Trumbo, 1990a, Müller et al., 1998, Smiseth & Moore, 2004). Pilakouta et al. (2018) found that broods with two parents produce larger offspring that were more likely to survive to adulthood then offspring raised by a single parent**,** showing the only evidence that biparental care has a synergistic effect and improves offspring fitness. In contrast, Scott (1989) found that broods with two parents exhibited reduced offspring survival, smaller larvae with lower total weights, and broods with a higher failure rates when males were present after hatching. Fetherston et al. (1994) found that single parent beetles provide more care to compensate for a lost mate, while Smiseth & Moore (2004) found that females do not compensate for the loss of male care.

Other factors also affect offspring. Carcass size positively correlates with the number of larvae, their total mass, and the duration of maternal care (Kozol et al., 1988, Scott & Traniello, 1990, Scott, 1998). Older carcasses with better established microbial communities decrease reproductive success and negatively impact larval growth (Rozen, 2008). Larvae reared on older carcasses that had been thawed seven days prior were smaller, begged more, and dispersed later than those grown on freshly thawed carcasses (Rozen et al., 2008). However, as Trumbo (2016) noted, this does not reflect realistic field conditions, where carcasses would have already been used by other animals including vertebrate scavengers and dipterans by the time a week had passed. Trumbo (2016) redefined old carcasses as thawed 80 hours based on studies suggesting that most breeding occurs 1-4 days postmortem and found that age of a carcass did not affect reproductive success (total number of larvae, mean mass of larvae, total brood mass).

Antimicrobial Secretions

One of the most important aspects of brood ball preparation is the coating of the carcass in antimicrobial oral and anal secretions. Several authors hypothesized that the secretions were antimicrobial (Trumbo, 1994, Ratcliffe, 1996, Scott, 1998) before the activity was quantified by Hoback et al. (2004), who also identified differences in antimicrobial activity between nicrophorines and silphines. Most Nicrophorinae they tested demonstrated antimicrobial activity in secretions, with oral secretions being the most active, while the only Silphinae with antimicrobial activity detected in anal secretions was *Necrodes surinimensis* (Hoback et al., 2004). Hoback et al. (2004) hypothesized that defensive anal secretions may represent the first evolutionary steps toward the development of antimicrobial secretions for carcass preservation.

The antimicrobial activities of *Nicrophorus* sp. secretions are more studied than those of the silphines. Jacques et al. (2009) found that temperature and food type affect the antimicrobial activities of *Nicrophorus* spp. oral secretions. Cotter & Kilner (2010) observed that female *Nicrophorus* spp. display higher antimicrobial activity compared to males and that both parents adjust their antibacterial activity in response to their partners, strengthening observations that parents usually play different care roles. Several recent studies have found that antibacterial (lytic) activities in *Nicrophorus* spp. anal secretions increase upon carcass discovery, peak during reproduction, and decline following brood dispersal (Cotter & Kilner, 2010, Steiger et al., 2011, Arce et al., 2012). Rana et al. (1977) found that *Nicrophorus* spp. oral secretions, but not midguts, contain phospholipase A_2, an enzyme that hydrolyzes polyunsaturated fatty acids and may aid in pre-oral digestion to provide beetle larvae with polyunsaturated fatty acids or work to preserve carcasses by attacking bacterial membranes.

The antimicrobial activity of nicrophorine secretions originates from lysozymes (Arce et al., 2012) and small antimicrobial peptides (Rana et al., 1997, Hoback et al., 2004, Hall et al.,

2011). Arce et al. (2012) found that in the absence of parental care, *Nicrophorus* spp. larvae raised on either anal secretion or hen egg-white lysozyme coated chicken livers survived at twice the rate of control larvae. Degenkolb et al. (2011) identified a number of metabolites in secretions with anti-putrefactive, antimicrobial activity. However, it is unclear whether the critical antimicrobial compounds in *Nicrophorus* spp. are produced exclusively by the beetles themselves or are supplemented by bacterial symbionts and expressed in secretions to reduce carcass microbes (Hall et al., 2011).

Microbiomes

Symbiosis describes the cohabitation of different organisms and includes the relationship between a host and its bacterial symbionts (de Bary, 1879, as translated by Oulhen et al., 2016). The term microbiome describes the community of microbes occupying a defined habitat that possess distinct physio-chemical properties (Whipps et al., 1988). The community of microbes present in the secretions of *Nicrophorus* spp. makes up the secretion microbiome. This microbial community is distinct from the gut microbiome, the microbiome of carcasses, and the microbiome of prepared carcasses (Miller et al., 2019).

The microbiome of carcasses is referred to as the necrobiome and plays an important role in enhancing microbe competition with larger organisms by decreasing carcass attractiveness to animal scavengers (Janzen, 1977, Burkepile et al., 2006). Microbe-colonized carcasses are more likely than fresh carcasses to be either undetected or unused, and attract fewer scavenging animals (Burkepile et al., 2006). The necrobiome also changes over time throughout decomposition following a pattern of bacterial succession (Pechal, 2012, Burkepile et al., 2006). Fresh marine carcasses for example, are dominated by Proteobacteria, but after two days bacterial density increases >250 times and the community shifts to Firmicutes and Bacteroidetes (Burkepile et al., 2006). Terrestrial swine carcasses are initially dominated by Proteobacteria and

Firmicutes populations that fluctuate and increase as decomposition progresses (Pechal, 2012, Weatherbee et al., 2017). In the presence of insects, the richness of bacterial genera decreases over time, as insects either directly (e.g., consumption) or indirectly (e.g., excretions) modify carcass bacterial communities (Pechal, 2012). Important genera associated with carcasses throughout decomposition include *Psychrobacter, Acinetobacter, Moraxella, Aerococcus, Jeotgalicoccus, Micrococcus, Ignatzschineria, Proteus, Psychrobacillus,* and *Clostridium* (Pechal, 2012). These bacteria colonize animal carcasses and produce unpleasant toxic volatiles that lead to spoilage and can cause illness (vomiting, diarrhea, and gut flora reorganization) in exposed mammals (Burkepile et al., 2006).

Solter et al. (1989) and Berdela et al. (1994) were the first to analyze silphid microbiomes and identified 23 and 39 bacterial taxa respectively from cultured midguts, hindguts, and hemolymph using analytical profile index identification, a technique for identifying known bacteria based on biochemical tests. Since then, advancements in technology have allowed for culture independent characterization of various silphid microbiomes. These characterizations of silphid microbiomes have mainly centered on *Nicrophorus* spp. gut communities and the cooperative metabolism that aids carcass preservation and larval development (Miller et al., 2019 and Vogel et al., 2017). Burying beetles preserve carrion in part by inoculating carcasses with their own gut microbiota, preventing normal microbial succession, and eliminating microbial competitors (Miller et al., 2019, Shukla et al., 2018). Inoculation with beetle microbiota reduces bacterial diversity on carcasses compared to unprepared carcasses (Miller et al., 2019), lowers the levels of putrescine and cadaverine, volatiles that would reveal carcass location, and lowers the levels of proteases, which are involved with protein breakdown (Shukla et al., 2018). Inoculation causes the exterior of the carcass to form a biofilm-like matrix that acts as a site for microbial symbiont nutrient processing (Shukla et al., 2018). This matrix supports larval development by breaking down carcass tissues for nutrient acquisition and detoxification, improving biomass

conversion, and inoculating the larval gut (Miller et al., 2019, Shukla et al., 2018, and Vogel et al., 2017). Anal secretions and the carcass matrix have been shown to be critical to the vertical transmission of endogenous bacteria originating from parent beetles and to the inoculation of the larval gut that protect larvae from colonization by carcass microbes (Duarte et al., 2017 and Miller et al., 2019, and Heise et al., 2019). Endogenous bacteria from parent beetles colonize larval guts better than carcass bacteria and if established first, provide colonization resistance against *Serratia*, an insect pathogen linked to larval mortality (Wang & Rozen, 2018). When raised to adulthood, beetles colonized with bacteria originating from their parents also produce heavier broods than beetles colonized by carcass bacteria (Wang & Rozen, 2018).

Identifying the core microbiome, or the shared members between two or more microbial assemblages, is an important task when studying microbiomes (Turnbaugh et al., 2007 and Hamady & Knight, 2009 as cited by Shade & Handelsman, 2012). Identifying core microbiomes can help explain the relationships between habitats, including host organisms, by enabling further research an understanding of their metabolic capabilities. Several studies have identified a number of bacterial taxa repeatedly associated with *Nicrophorus* spp., indicating a core microbiome shared across *Nicrophorus* spp. (Miller et al., 2019 (*N. defodiens*), Olmstead, 2018 (*N. marginatus*, *orbicollis*, *tomentosus*, *americanus*), Shukla et al., 2018 (*N. vespilloides*), Duarte et al., 2017(*N. vespilloides*), Shukla et al., 2017(*N. vespilloides*), Vogel et al., 2017(*N. vespilloides*), and Kaltenpoth & Steiger, 2014(*N. humator, vespillo, orbicollis, pustulatus, tomentosus, Necrophila americana, Oiceoptoma noveboracense*).

Nicrophorus spp. core microbiomes are similar to other necrophagous and flesh-eating insects (Vogel et al., 2017) like calliphorid (Weatherbee et al., 2017) and sarcophagid flies (Gupta et al., 2014). Flies likely acquire a portion of their microbiomes from the carcasses they feed on as the relative abundances of dominant microbial phyla are similar across multigeneric maggot masses, gut microbiomes, and carcass surfaces (Weatherbee et al., 2017). Thus, it is likely that

carcasses and the insects that feed on them colonize one another and share a group of core taxa that play a role in carcass nutrient processing.

Rationale

The main rationale for studying silphid microbiomes is to further research and understanding of ecosystem ecology and how insects and bacteria interact to recycle carcass nutrients. Carrion represents an ephemeral and nutrient-rich resource that changes rapidly and facilitates a high degree of competition between microbes, invertebrates, and vertebrates. Studying silphid microbiomes, specifically the microbiome contained within their secretions, will further our understanding of how they interact with microbes to utilize a carcass and efficiently digest the resource to support biomass conversion and rapid nutrient assimilation (Vogel et al., 2017).

Studying bacterial strains isolated from silphids may also reveal the presence of spe3cies capable of producing novel antimicrobials. For example, Heise et al. (2019) identified an antimicrobial compound produced by the Gram-negative bacterium *Serratia marcescens* isolated from *N. vespilloides,* which demonstrated activity against the human pathogen *Staphylococcus aureus*. Antibiotic resistance is a worldwide crisis as resistant bacteria emerge and reduce antibiotic efficacy (Ventola, 2015). The overuse and misuse of antibiotics selects for bacteria expressing antibiotic resistance. In addition, the pipeline for new systemic antimicrobials has been slowed due to low financial returns and regulatory requirements, even though developing new antibiotics is critical for combating infections caused by extant antibiotic resistance pathogens (Ventola, 2015).

Finally, because burying beetles preserve the brood ball by coating it in secretions with antimicrobial activity and an associated microbial community that prevents brood ball rotting for weeks, it is possible to identify factors that may contribute to the development of novel meat

preservation methods. Studying the antimicrobial genome encoded factors produced by both burying beetles themselves and their secretion microbiomes, may identify nontoxic compounds which can act as meat preservatives.

Thesis Objectives

The goal of this research was to characterize the secretion microbiomes of seven silphid species among Silphinae and Nicrophorinae with the underlying hypothesis that the two subfamilies would have significantly different secretion microbiomes. Characterization was achieved utilizing two approaches. In the first approach, secretions from *Nicrophorus americanus* were cultured and thirteen bacterial isolates were identified using MALDI-TOF MS and 16S rRNA gene sequencing. In the second approach, secretions from *Necrodes surinamensis, Necrophila americana,* and five *Nicrophorus* spp*., N. pustulatus, N. americanus, N. marginatus, N. orbicollis,* and *N. tomentosus* were characterized using culture-independent 16S rRNA gene sequencing. This research made it possible to characterize the secretion microbiomes of seven species of Silphidae and compare the secretion microbiomes among carrion and burying beetles.

CHAPTER II

CULTURE DEPENDANT ANALYSIS OF BACTERIAL COMMUNITIES OF THE *NICROPHORUS AMERICANUS* SECRETION MICROBIOME

ABSTRACT

The American burying beetle (*Nicrophorus americanus*) is a federally threatened necrophagous insect that provides biparental care to its offspring using vertebrate carrion. *Nicrophorus* beetles prepare a brood ball by first burying a carcass, then removing the fur or feathers and digestive tract, and finish by covering the carcass in oral and anal secretions. Both the beetles and the bacteria present in their secretions produce antimicrobial agents that prevent the carcass from decomposing and help preserve it for consumption throughout the duration of larval development. In order to identify the bacteria present in *N. americanus* secretions, bacterial colonies were isolated from a pair of laboratory-bred beetles. Using Gram-staining, matrix assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS), and 16S rRNA gene sequencing, thirteen isolates of aerobic bacteria were identified. Although many of the identified isolates are ubiquitous, several have previously been linked to carrion or other necrophagous insects. Characterizing the burying beetle secretion microbiome is critical to further understanding bacterial metabolism and gaining insight into how the bacteria associated with *N. americanus* recycle the nutrients stored in carrion.

INTRODUCTION

Nicrophorus americanus is the largest silphid in North America, averaging lengths of 30-35mm, considerably larger than any other Nicrophorus spp. (USFWS, 1989 and Anderson, 1982). Known by its common name, the American burying beetle is a federally threated species, distinguished by its predominantly orange pronotum (Ratcliffe, 1996). Originally listed as endangered in 1989 by the United States Fish and Wildlife Service, *N. americanus* were known at the time to be reduced to populations in Rhode Island and eastern Oklahoma. Modern surveys have identified populations in the Red River, Arkansas River, and Flint Hills regions in Texas, Oklahoma, Kansas, and Arkansas, the Loess Canyons, Sandhills, and Niobrara regions in Nebraska and South Dakota, Block Island, Rhode Island, and a reintroduced population on Nantucket Island, Massachusetts (USFWS, 2020). Although the known range has expanded since 1989, *N. americanus* still faces threats from changes in land use associated with urbanization and agriculture, and threats from climate change, primarily increasing temperatures (USFWS, 2020). Under projected climate conditions, 59% of the existing range and at least three populations will be lost in the next 50 years (USFWS, 2019). As of 2020, *N. americanus* has been down listed to threatened, because although it is still likely to become endangered in the future, it is apparently not in immediate danger of extinction throughout its range (USFWS, 2020). It remains listed as critically endangered on the International Union for Conservation of Nature (IUCN) Red List of Threatened Species (World Conservation Monitoring Centre, 1996).

Nicrophorus americanus utilizes the unique *Nicrophorus* reproductive strategy described in Chapter 1. This involves finding a carcass and burying it, then creating a chamber and brood ball (Pukowski, 1933). The brood ball is coated with antimicrobial oral and anal secretions that inhibit exogenous microbe growth. (Hall et al., 2011, Arce et al., 2012). Larvae are cared and fed prepared carrion by the parent beetles for several weeks until they disperse and form pupal cells in the surrounding soil where they pupate and later emerge as adults (Pukowski, 1933).

Previous research has revealed that streaking *N. americanus* secretions on soil-specific Reasoner's 2A agar resulted in the growth of small colonies (Hall et al., 2011). However, the microbes present in these colonies failed to be identified (Hall, 2011). The goal of this research was to culture and isolate *N. americanus* secretion bacteria in order to identify them using Gram staining, MALDI-TOF MS, and 16S rRNA gene sequencing, opening avenues for further study.

METHODS

Nicrophorus americanus and Secretion Collection

As part of ongoing research efforts, a colony of laboratory-bred *N. americanus* is maintained at Oklahoma State University. The parents of these laboratory-bred beetles were collected in July 2019 at the Camp Gruber Oklahoma Army National Guard training facility in western Muskogee County, Oklahoma using aboveground pitfall traps (Leasure et al., 2012) baited with rotten rat.

Captured beetles were brought to the Oklahoma State University and bred within a week of collection. After development and maturation, offspring were separated into individual containers and fed weekly on aged ground beef, mealworms, and wax worms. At the time of secretion collection, offspring beetles were approximately four months old.

In order to stimulate secretion production, two lab bred beetles, one male and one female, were placed together into a container (13.97 x 29.21 x 34.29 cm) containing moist sphagnum peat moss. A thawed large rat (275-375 g) was placed on top of the soil and the container was kept in a darkened laboratory room at 23°C. The beetles belonged to two separate broods from wild-caught parents and had similar pronotal widths (10.75 mm). One week later, oral and anal secretions were collected from the pair using sterile cotton swabs. Beetles were first briefly submerged in water to remove any soil that might contaminate secretions and then lightly

palpated to induce secretion behavior (Hoback et al., 2004). Secretions are dark brown and can be easily visualized (Figure 1).

Culturing

Cotton swabs were streaked onto nutrient agar plates maintained at room temperature (21°C) for three days. After the initial stage of growth, individual colonies were picked for isolation based on morphology. From the mixed plates (Figure 2), colonies with different color, shape, and consistency were chosen in order to isolate a variety of bacteria. Three to four different colonies were picked from each secretion type and plated using the four-quadrant streak method to isolate single colonies, generating a total of fifteen isolates (Figure 3). These isolates were grown for an additional two days at room temperature (21°C).

Following isolation, individual colonies were then transferred to liquid nutrient broth and grown overnight at room temperature (21°C) in a Thermo Scientific™ MaxQ™ 4000 shaker incubator. Two of the liquid cultures did not grow, leaving a total of thirteen isolates which were then stored in triplicate as 20% glycerol stocks at -80°C.

Gram Staining

One of the first steps when attempting to identify bacterial colonies is to Gram stain. For over a century, Gram staining has been used to divide bacteria into two categories, Gram-positive bacteria and Gram-negative bacteria. Gram-positive bacteria have a thick peptidoglycan layer that retains crystal violet-iodide complexes and stains the bacteria purple, while Gram-negative bacteria have thin peptidoglycan layers, and decolorize to pink (Beveridge & Davies, 1983). Bacteria can also be broadly divided by their morphology, generally falling into either cocci (spheres) or bacilli (rods), although there are a multitude of other shapes (Young, 2006). Identifying both the Gram status and general morphology of bacteria can be a fast and inexpensive first step towards identification, especially for experienced bacteriologists. For this

research, liquid cultures were Gram stained using a BD BBL™ kit following standard protocol. All images were collected with a compound microscope (Fisher Science Education™ S90008) at 1000X 1.25 NA.

MALDI-TOF MS

Mass spectrometry (MS) is a technique for chemical compound analysis based on the mass to charge ratio of ionized compounds. Developed in the 1980's, matrix assisted laser desorption ionization (MALDI) allowed MS to be applied to large biological molecules (Singhal et al., 2015). Samples are mixed with an organic compound matrix that crystallizes when dry, and the matrix-sample crystal is ionized with a laser, generating singly protonated ions (Singhal et al., 2015). These ions are detected by time of flight (TOF) analyzers that measure their mass to charge ratios and create a characteristic spectrum called a peptide mass fingerprint that is compared against a database of spectra to identify the sample (Singhal et al., 2015). When identifying bacteria, a mass range of 2-20kDa is used, representing housekeeping and ribosomal proteins, allowing for the identification of bacteria down to genus, and in some cases species and strain (Fagerquist et al., 2010 as cited by Singhal et al., 2015). Fast, reliable, and cost-effective, whole cell MALDI-TOF MS can be an effective tool for identifying bacterial isolates, (Singhal et al., 2015). Bacterial isolates were streaked from 20% glycerol stocks on new agar plates and grown at room temperature (21°C) for two days before being brought to the Oklahoma Animal Disease Diagnostic Laboratory (OADDL) for MALDI-TOF MS.

16S rRNA Gene Sequencing

The 16S rRNA gene is the most commonly used region of DNA for determining phylogenetic relationships between bacteria (Clarridge, 2004). Sequencing this region is a popular bacterial identification method because it is present in nearly all bacteria, has not changed in function, indicating that sequence changes accurately measure time, and is a suitable size for

informatics (Patel, 2001 as cited by Janda & Abbot, 2007). Sequencing of the 16S rRNA gene is reliable, cost-effective, and provides sequence data that can be easily transferred and analyzed on multiple platforms. New agar plates were streaked from 20% glycerol stocks and grown at room temperature (21°C) overnight. A single colony was then transferred to 1μL of deionized water and then brought to OADDL for 16S rRNA gene sequencing services through Eurofins Genomics LLC.

RESULTS

After Gram staining, two of the thirteen isolates were Gram-positive. MALDI-TOF MS identified ten of the thirteen isolates to genus, with the remaining three identifications going no further than Gram-positive or Gram-negative and denoting cell morphology. 16S rRNA gene sequencing identified all thirteen isolates to genus (Figure 4). The ten isolates identified to genus by MALDI-TOF MS aligned with the 16S rRNA gene sequencing identifications, and the three isolates unidentified by MALDI-TOF MS were identified by 16S rRNA gene sequencing (Table 1). One isolate from male *N. americanus* anal secretions was identified as *Glutamicibacter* sp./ *Arthrobacter* sp. It should be noted that this identification follows the current taxonomy, which reclassified selected species of the genus *Arthrobacter* into the novel genera *Glutamicibacter* (Busse, 2016). The final identifications for these isolates included the following genera: *Gordonia, Proteus, Acinetobacter, Myroides, Pseudochrobactrum, Corynebacterium, Vitreoscilla, Paracoccus,* and *Glutamicibacter.*

A *Proteus* sp. and a *Myroides* sp. were each identified in two secretion samples (Table 1). Interestingly, these organisms were the only isolates identified by MALDTI-TOF to the species level and were identified as *Proteus hauseri* and *Myroides odoratimimus*. An *Acinetobacter* sp. was isolated from three secretion samples (Table 1).

To further the isolate identifications from 16S rRNA gene sequencing, the raw sequences were submitted to BLAST, with most resulting in confident identification to species (Table 2). The sequences had an average query cover of 77.85% and average percent identity of 99.20%, indicating generally good species identifications.

DISCUSSION

Some interesting discrepancies arose between identification methods (Table 3). One isolate from male anal secretions stained Gram-positive and was identified as a Gram-positive cocci-rod by MALDI-TOF MS, but identified as *Paracoccus alimentarius*, a Gram-negative bacterium by 16S rRNA gene sequencing. A literature search revealed that when Gram-stained this species can appear under decolorized and be inadvertently reported as Gram-positive (Dyer & Harris, 2020). An isolate from female anal secretions showed the inverse scenario. It stained Gram-negative but was identified by MALDI-TOF MS as a Gram-positive rod and by 16S rRNA gene sequencing as a *Gordonia* sp., a Gram-positive bacterium. It is unclear why this isolate decolorized. A final isolate from male anal secretions also decolorized similarly. While it initially appeared to stain Gram-negative, MALDI-TOF MS and 16S rRNA gene sequencing identified the isolate as a *Glutamicibacter* sp./*Arthrobacter* sp., a Gram-positive bacterium. The genus description of *Arthrobacter* explains that this bacterium appears as Gram-negative rods in young cultures and transitions to Gram-positive coccus in old cultures (Conn & Dimmick, 1947). At the time of Gram staining, cultures were no more than 24 hours old, classifying them as young cultures, and likely resulting in the initial identification as Gram-negative.

For the purposes of discussion, I'll be addressing each of the bacterial isolates and some relevant information. Some of the bacteria are ubiquitous while others are common in carrion necrobiomes and associated necrophagous insects. These descriptions are meant to be a brief

overview of each isolate in order to give a general idea of what the bacteria is, why they might have been found in *N. americanus*, and potential benefit to *N. americanus*.

Gordonia spp. are abundant environmental bacteria, frequently isolated from soil and water (Ramanan et al., 2013). Able to degrade pollutants and xenobiotics, or chemicals not naturally produced or expected within an ecological system, *Gordonia* spp. are often isolated for use in environmental and industrial biotechnologies (Arensk̈otter et al., 2004). However, they have also been documented as human opportunistic pathogens (Ramanan et al., 2013). In insects, *Gordonia* spp. have been isolated from domestic silk moth *Bombyx mori* ovarian tissue derived cell lines (Kondo et al., 2000) and colony housed vegetable fed black soldier flies *Hermetia illucens* (Zheng et al., 2013), indicating no specific link between the bacteria and vertebrate decomposition. My *N. americanus* isolate was identified as *G. sihwensis* through a BLAST search. This species was first isolated from a wastewater treatment reactor at the artificial Lake Sihwa, South Korea (Kim et al., 2003). This isolate also appeared to have hyphae when imaged, a trait that has been described in *Gordonia* spp. (Murray, 2015).

Proteus spp. are found in a variety of natural environments, including wild and domestic animals, soil and water, where they indicate fecal pollution, and human clinical sources, where they act as opportunistic pathogens (Drzewiecka, 2016). The bacteria attract adult blowflies *Lucilia sericata* and increase their frequency of oviposition (Ma et al., 2012), and are found in the guts of larval and adult flesh flies *Sarcophaga* spp., where they are hypothesized to increase oviposition by producing volatiles that attract carrion-breeding species (Gupta et al., 2014). In carrion beetles, the presence of these *Proteus* spp. produced volatiles may be a detriment, as they can reveal the location of the carcass to both predators and competitors and may increase the likelihood of carcass takeover (Trumbo, 1990a and Ma et al., 2012 as cited by Shukla et al., 2018). *Proteus* spp. have previously been identified in *N. vespilloides* (Vogel et al., 2017), and our research found *Proteus* spp. in seven additional carrion beetle species (Chapter 3).

Acinetobacter spp. are ubiquitous and found in plants, animals, humans, and the environment (Al Atrouni et al., 2016). Considered "microbial weeds", because they successfully dominate a variety of ecological niches, *Acinetobacter* spp. inhibit competitor growth by producing organic acids and acidifying the environment (Cray et al., 2013). They also produce biofilms and enzymes that degrade vertebrate tissue (Bergogne-Bérézin & Towner, 1996).

Myroides spp. are yellow aerobic bacteria found across a variety of environments including a number of insect guts (Dharne et al., 2008). Capable of secreting antimicrobial substances (Dharne et al., 2008), *Myroides* spp. may serve to protect their insect hosts against other pathogenic bacteria (Deguenon et al., 2019). However, in black blow flies *Phormia regina*, *Myroides odoratimimus* was identified almost exclusively on the outside of the insects, indicating that it was likely acquired from feeding and breeding sites and is a normal component of the necrobiome (Deguenon et al., 2019). This is confirmed by the high abundance of *Myroides* spp. on swine carcasses (Pechal, 2012). Interestingly, *Myroides odoratimimus* strains from adult flesh flies demonstrated a multiple drug resistance phenotype and may possess a yet unknown resistance mechanism (Dharne et al., 2008).

The genus *Pseudochrobactrum* was first described in 2006 and contained two species, *P. asaccharolyticum*, and *P. saccharolyticum*, isolated in Sweden from knee aspirate and an industrial glue (Kämpfer et al., 2006). Upon culturing, colonies were beige, translucent, and shiny, with entire (smooth) edges, matching the type description (Kämpfer et al., 2006). *Pseudochrobactrum* spp. have been found to dominate the bacterial communities of carcasses after they rupture (Dowell-Curby, 2017, Tomberlin et al., 2017), and have been identified in studies of coffin flies *Conicera similis* (Iancu et al., 2018), parasitic rove beetles *Aleochara bipustulata,* wasps *Trybliographa rapae* (Bili et al., 2016), and entomopathogenic nematodes *Rhabditis regina* (Jiménez-Cortés et al., 2016).

Corynebacterium spp. are Gram-positive rod-shaped bacteria that appear white when initially cultured but discolor to yellow or tan after 48 hours (Bernard et al., 2010). Of the 88 published species, 53 are opportunistic human pathogens (*C. diphtheriae* causes diphtheria), and the remaining 35 can be isolated from animals, the environment, water, foodstuffs, or synthetic materials (Bernard et al., 2012). The species identified in our female *N. americanus* oral secretions, *C. stationis,* was reclassified from the *Brevibacterium* in 2010 (Bernard et al., 2010). *Corynebacterium* spp. have been isolated from honey bee *Apis mellifera* brood combs and hive floors (Piccini et al., 2004), are a prominent genus in kissing bugs *Triatominae* spp. (Gumiel et al., 2015), and were cultured from the ovaries and mycetome of the bed bug *Cimex lectularius*. (Steinhaus, 1941).

Vitreoscilla spp. are obligate aerobes found in hypoxic habitats (Joshi et al., 1998). Isolated from cow dung, the genus was described in 1949 (Pringsheim, 1949) and is currently represented by three species (Euzéby, 1997). *Vitreoscilla* spp. have been described in the hindguts of burying beetles *N. vespilloides* (Vogel et al., 2017) and are abundant in the microbial communities of beetle tended carcasses (Shukla et al., 2018).

Paracoccus spp. have been isolated from a multitude of environmental sources like soil, sludge, water, air, and organisms, including insects (Kim et al., 2018). They are notably present on both mouse (Shukla et al., 2018) and swine carcasses (Pechal, 2012), and at low abundances within the digestive tracts of the burying beetle *N. defodiens* (Miller et al., 2021). *Paracoccus alimentarius*, the species identified in our male *N. americanus* anal secretions, was first isolated from salted pollack, a type of fish and Korean foodstuff (Kim et al., 2018).

Glutamicibacter spp. are ubiquitous bacteria found in a variety of sources including soil, air, water, human and veterinary clinical specimens (Busse, 2016), In arthropods, *Glutimicibacter* spp. have been identified in the guts of 28-spotted potato ladybird beetles *Henosepilachna*

vigintioctopunctata, which feeds on solanaceous plants (Lü et al., 2019), spider mites *Tetranychus phaselus* feeding on beans (Zhu et al., 2020), and the blue-bottle fly *Protophormia terraenovae* (Lysenko, 1959).

Of the nine different genera identified in the *N. americanus* isolates, seven genera appear to be widespread in the environment. The two others, *Pseudochrobactrum* and *Vitreoscilla*, are small genera with two and three species respectively. However, even within these genera, isolates come from a variety of sources, including knee aspirate, industrial glue, and cow dung. Because of their ubiquity, these bacteria are found associated with a number of arthropods, particularly insects. The fact that many of the *N. americanus* isolate genera have also been identified in insects like blow flies, flesh flies, coffin flies, and other burying beetles, all necrophagous insects, indicates that there is likely a shared community of bacteria present in most carrion feeding insects, likely obtained from the carrion itself. This conclusion is supported by some of these bacteria being identified in the necrobiomes of a number of vertebrate carcasses. Although no bacterial isolates were found to be unique to burying beetles, a shared bacterial community across necrophagous insects and carrion is of interest. Identifying microbial communities is the first step to further studying their metabolism and gaining insight into how they recycle the nutrients stored in carrion.

Because these isolates were cultured from a single pair of beetles and not a pool of individuals of the same sex due to limitations with beetles producing sufficient quantities of secretions, this data cannot be generalized. Although the bacteria were certainly isolated from *N. americanus*, it cannot be assumed that all individuals house these bacteria. The culturing of these isolates also indicates nothing about their abundance within the secretions of the beetle, which is another facet of how the secretion microbiome, the beetle, and the carcass interact. Further research to elucidate the relative abundances of each of the isolates and additional bacterial taxa

within secretions, guts, integuments, environments, and across populations will allow us to gain more understanding about the niche that burying beetles and their associated microbes occupy.

It is important to note that the cultured bacteria were limited to aerobic bacteria able to grow on nutrient agar at room temperature. It is likely that with different media, temperature, light, oxygen, and time, different bacteria would grow, and with repetition a variety of bacteria would be isolated.

I was able to successfully culture *N. americanus* secretion bacteria and identify each of the isolates. Using Gram staining and microscopy I was able to photograph and visualize each of the isolates, and through MALDI-TOF MS and 16S rRNA gene sequencing, I was able to identify each of the isolates to genus. These genera included *Gordonia, Proteus, Acinetobacter, Myroides, Pseudochrobactrum, Corynebacterium, Vitreoscilla, Paracoccus,* and *Glutamicibacter,* generally ubiquitous bacteria that have some associations with necrophagous insects and carrion necrobiomes.

FIGURES

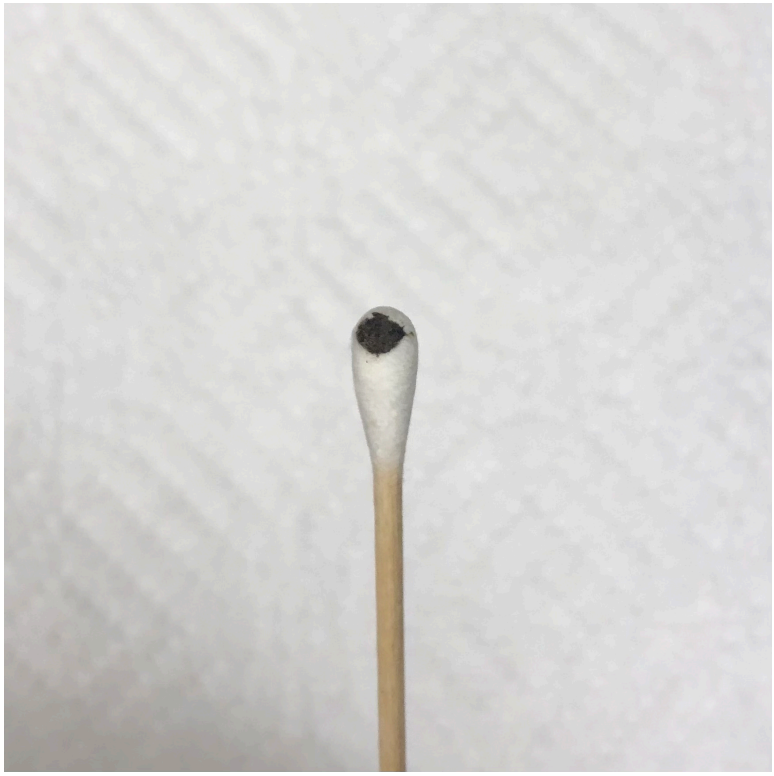


Figure 1. Secretion discoloration on a cotton swab. Dark brown coloring made collection visualization straightforward.

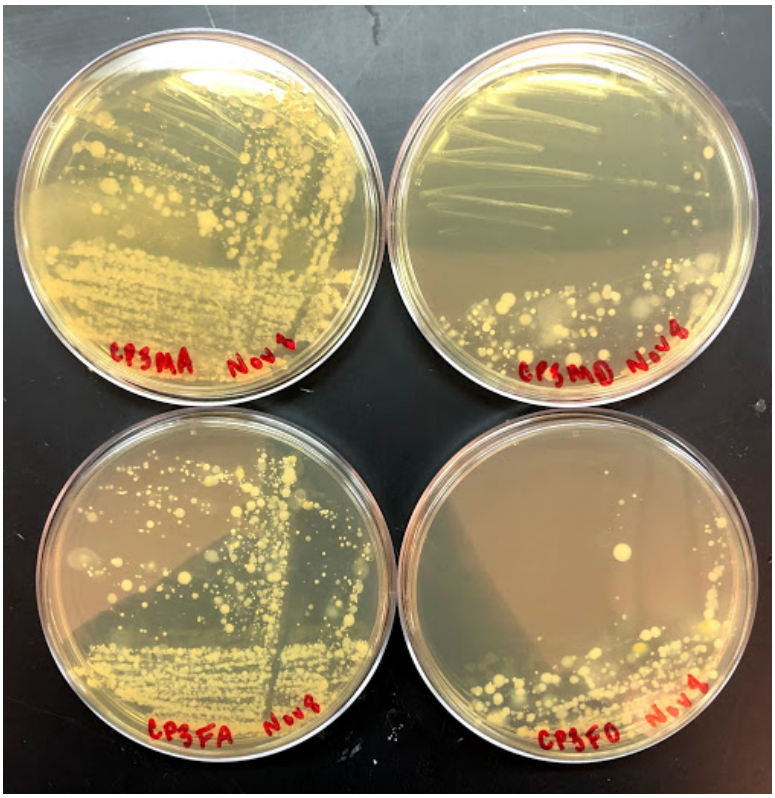


Figure 2. Mixed plates of secretion bacteria. Clockwise from top left, sample sources are male anal secretions, male oral secretions, female oral secretions, and female anal secretions.

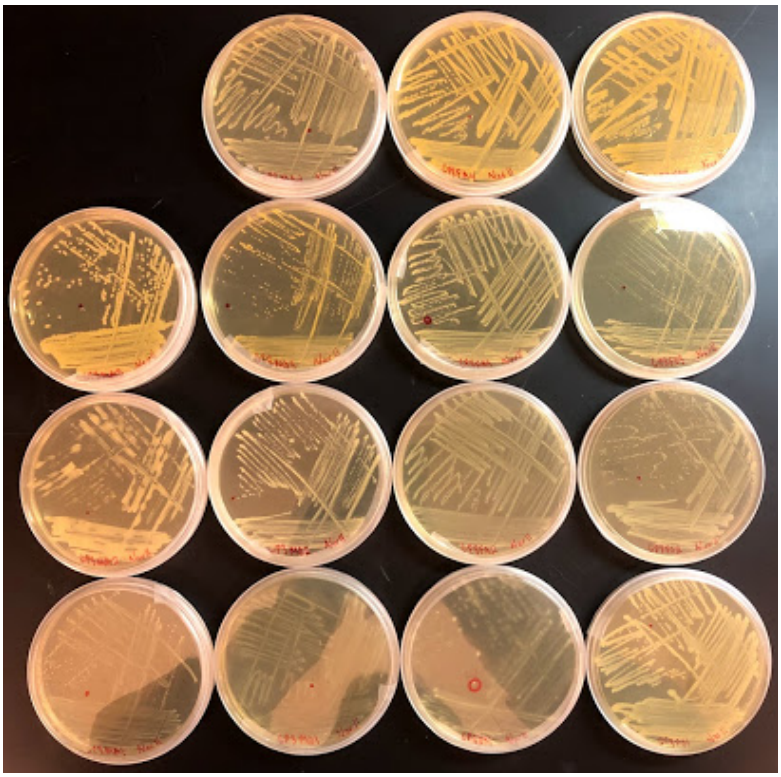


Figure 3. Fifteen isolates picked from mixed plates based on morphological characters. Three different colonies were picked from the male anal secretion mixed plate, and four different colonies were picked from each of male oral secretion, female oral secretion, and female anal secretion mixed plates.

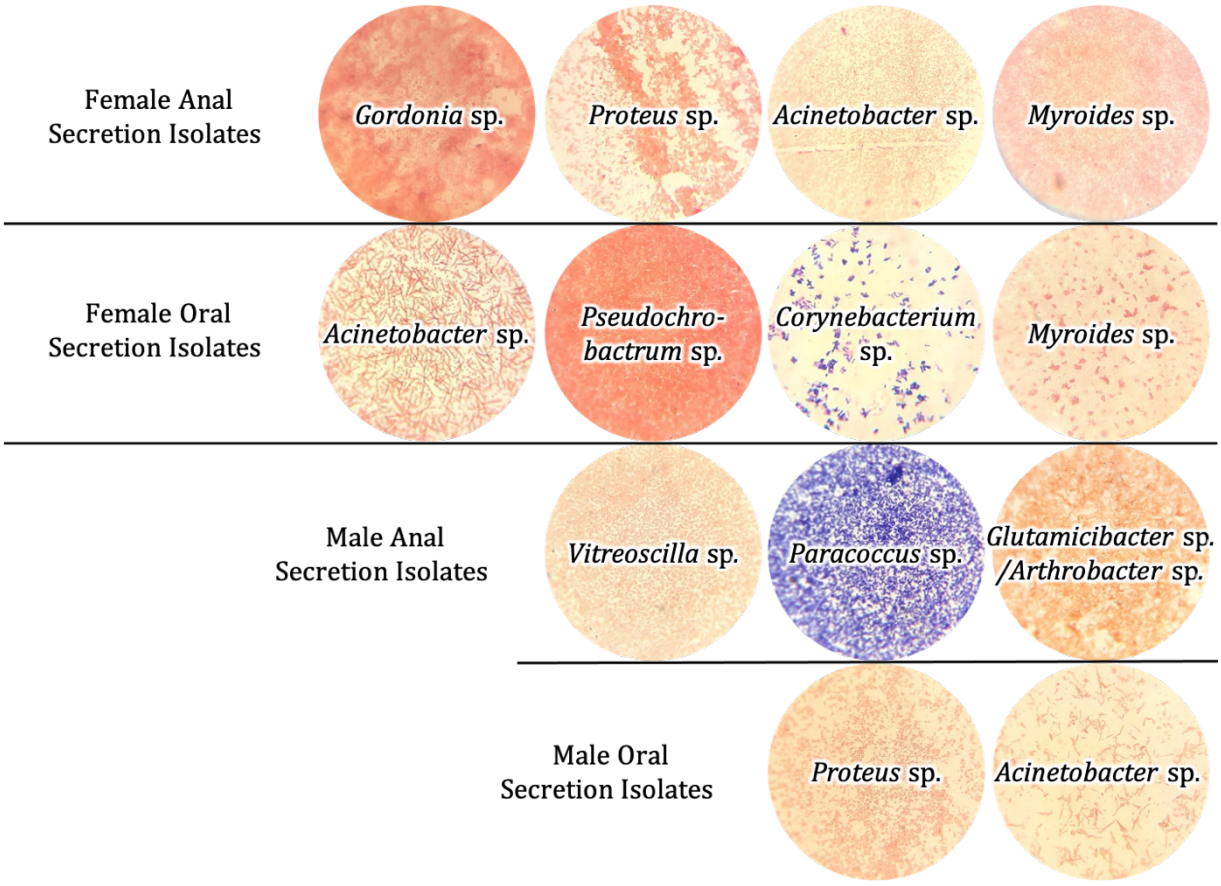


Figure 4. Bacterial isolates from *N. americanus* secretions Gram stained and photographed at 1000X. Images are overlaid with 16s rRNA gene sequencing identifications.

TABLES

Table 1. MALDI-TOF MS and 16S rRNA gene sequencing identifications of thirteen bacterial isolates from *N. americanus* secretions. Left columns indicate sex of the beetle and type of secretion.

Sex	Sample	MALDI-TOF ID	16S ID
Female	Anal	Gram-positive rod	*Gordonia* sp.
Female	Anal	*Proteus hauseri*	*Proteus* sp.
Female	Anal	*Acinetobacter* sp.	*Acinetobacter* sp.
Female	Anal	*Myroides odoratimimus*	*Myroides* sp.
Female	Oral	*Acinetobacter* sp.	*Acinetobacter* sp.
Female	Oral	*Pseudochrobactrum* sp.	*Pseudochrobactrum* sp.
Female	Oral	*Corynebacterium* sp.	*Corynebacterium* sp.
Female	Oral	*Myroides odoratimimus*	*Myroides* sp.
Male	Anal	Gram-negative rod	*Vitreoscilla* sp.
Male	Anal	Gram-positive cocci-rod	*Paracoccus* sp.
Male	Anal	*Glutamicibacter* sp.	*Glutamicibacter* sp./*Arthrobacter* sp.
Male	Oral	*Proteus hauseri*	*Proteus* sp.
Male	Oral	*Acinetobacter* sp.	*Acinetobacter* sp.

Table 2. BLAST identifications of thirteen bacterial isolates from *N. americanus* secretions. Left columns indicate sex of the beetle and type of secretion. Right columns indicate quality of the BLAST hit.

Sex	Sample	BLAST ID	Max Score	Query Cover	Per. Ident
Female	Anal	*Gordonia sihwensis*	1507	86%	98.93%
Female	Anal	*Proteus* nr. *vulgaris*	1489	83%	99.88%
Female	Anal	*Acinetobacter rudis*	1330	76%	98.28%
Female	Anal	*Myroides odoratimimus*	1421	65%	98.99%
Female	Oral	*Acinetobacter junii*	1487	90%	99.51%
Female	Oral	*Pseudochrobactrum asaccharolyticum*	1371	86%	98.45%
Female	Oral	*Corynebacterium stationis*	835	52%	98.09%
Female	Oral	*Myroides odoratimimus*	1426	65%	99.11%
Male	Anal	*Vitreoscilla* sp.	1476	75%	99.51%
Male	Anal	*Paracoccus alimentarius*	1435	80%	99.74%
Male	Anal	*Glutamicibacter* sp./*Arthrobacter* sp.	1495	83%	100.00%
Male	Oral	*Proteus* nr. *vulgaris*	1476	81%	99.50%
Male	Oral	*Acinetobacter gerneri*	1474	90%	99.63%

Table 3. Discrepancies found between Gram staining and true Gram stain classifications based on MALDI-TOF MS and 16S rRNA gene sequencing.

Sex	Sample	Gram Stain	MALDI-TOF ID	16S ID	True Gram Stain
Male	Anal	Gram-positive	Gram-positive cocci-rod	*Paracoccus alimentarius*	Gram-negative
Female	Anal	Gram-negative	Gram-positive rod	*Gordonia* sp.	Gram-positive
Male	Anal	Gram-negative	*Glutamibacter* sp.	*Glutamicibacter* sp./*Arthrobacter* sp.	Gram-positive

CHAPTER III

CULTURE INDEPENDENT ANALYSIS OF BACTERIAL COMMUNITIES OF SILPHID SECRETION MICROBIOMES

ABSTRACT

The family Silphidae is divided into two subfamilies, Silphinae and Nicrophorinae, differentiated by reproductive behaviors. Silphinae, known as carrion beetles, feed on carrion and fly larvae and the free-living larvae receive no parental care. Nicrophorinae, known as burying beetles, prepare a carcass into a brood ball and provide biparental care to their offspring. Preparation of a brood ball involves coating the carcass in antimicrobial oral and anal secretions. These secretions exhibit antimicrobial activities and contain a community of microbes, referred to as the secretion microbiome, which work to inhibit soil and carcass microbe succession, preventing normal decomposition and carcass spoiling. Culture-independent 16S rRNA gene sequencing was used to characterize the secretion microbiomes of five species of nicrophorine burying beetles and two species of related silphines from the Central Great Plains. Grouping species by subfamily and then by reproductive style (brood ball preparing vs non-brood ball preparing) allowed for the identification of shared bacterial taxa to determine if different groups had different microbial communities. A core microbiome of bacterial taxa across Silphidae included Lactobacillales, Enterobacterales, Bacillales, and Cardiobacteriales that differ in abundance across subfamily and reproductive style. Differing abundances in core genera and

accessory genera may be linked to the variance in antimicrobial activities of secretions across Silphidae and within Nicrophorinae.

INTRODUCTION

The secretion antimicrobial activities differ among members of the subfamilies Silphinae and Nicrophorinae (Hoback et al., 2004). Hoback et al. (2004) found that in general, nicrophorines produced secretions with antimicrobial activity while silphines did not. If the nicrophorines and silphines contain substantially different secretion microbiomes, it may be an indicator that some of the antimicrobial activity derives from endosymbiotic bacteria that may explain differences in antimicrobial activity. Previous studies of European and North American silphid gut microbiomes have found that bacterial communities are more congruent with sampling locality than host phylogeny (Kaltenpoth & Steiger, 2014). Thus, sampling beetles from the same locality may reveal bacterial communities that more strongly reflect the silphid host phylogeny.

In addition to grouping by phylogeny, I also chose to group the silphid species by reproductive styles in order to determine if species that prepare a brood ball harbor a different secretion microbiome than non-brood ball preparing species. Although the splitting of silphids into two subfamilies is easily recognized by the differentiation of reproductive behaviors, one nicrophorine I sampled, *Nicrophorus pustulatus*, prefers to breed on live snake eggs, a substantially different reproductive resource than used by other silphids. In order to emphasize how different this species is and rationalize the grouping of species based on reproductive style in addition to groupings based on phylogeny, it is important to discuss the relevant ecology of each species used.

Non-brood Ball Preparing Silphids

The first two beetles of this group, *Necrodes surinamensis* and *Necrophila americana,* belong to the subfamily Silphinae. These species are carrion beetles that provide little to no

parental care and do not prepare a brood ball. These species do not coat carcasses with their secretions and produce less secretion volume in the field than *Nicrophorus* spp. These beetles also prefer larger carcasses, making producing a large enough secretion quantity to coat a large carcass a significant challenge. It is also unlikely that these species inoculate a carcass with their own bacteria to alter the carcass microbial community.

Necrodes surinamensis and *Necrophila americana* are the only North American species within their respective genera (Ratcliffe, 1996). Adults feed primarily on dipteran larvae at carcasses, but will also feed on carrion (Ratcliffe, 1996). Females oviposit on or near the carcass and the larvae hatch after 2-4 days. Larvae are free-living and feed primarily on carrion, but occasionally feed on fly larvae (Ratcliffe, 1996). A complete life cycle from egg to adult takes approximately 10-12 weeks (Ratcliffe, 1996). *N. surinamensis* is unique amongst silphids in that it possesses antimicrobial defensive anal secretions hypothesized to be the first evolutionary steps towards the antimicrobial secretions for brood ball preparation found in *Nicrophorus* spp. (Hoback et al., 2004).

Nicrophorus pustulatus is a moderately sized (14-22 mm) silphid beetle unique among *Nicrophorus* spp. It produces approximately three times the number of offspring compared to congeners in captivity, giving it the largest brood size of nicrophorines (Trumbo, 1992). This large brood size led Trumbo (1992) to hypothesize that *N. pustulatus* preferred large carcasses. After experiments showed takeover of congener-controlled carcasses by *N. pustulatus* (Trumbo, 1994), and observations that the species has never buried any of 1,000+ research carcasses in the field (Wilson et al., 1984, Scott & Traniello, 1990, Trumbo, 1990b, Trumbo, 1991, as cited in Scott, 1998), it was hypothesized that *N. pustulatus* was a brood parasite.

However, in the early 2000's it was determined that *N. pustulatus* is actually a snake egg parasitoid after multiple findings of *N. pustulatus* adults in black rat snake (*Pantherophis*

obsoleta) nests and their larvae feeding on otherwise viable snake eggs (Blouin-Demers & Weatherhead, 2000, Keller & Heske, 2001). Parasitoids are a type of parasite which are parasitic only as larvae and feed on a single host, eventually killing it (Noble & Noble, 1971). It is hypothesized that *N. pustulatus* has undergone a host shift from carrion to snake eggs which they efficiently exploit as a resource while congeners do not (Smith et al., 2007). Preference for snake eggs may explain why the species has not been found burying research carcasses. Because rat snakes nest communally and have an average individual clutch size of 152g, snake nests represent a potentially large resource for reproducing *N. pustulatus* and potentially large broods (Blouin-Demers & Weatherhead, 2000). Additionally, ovipositing black rat snakes conceal their eggs and as a result *N. pustulatus* do not move them, bury them, or create a brood ball, treating snake eggs and carcasses differently (Smith et al., 2007). Finally, because *N. pustulatus* develop on living embryos, the need for antimicrobial secretions may be less for the species than other *Nicrophorus*.

Because of this specialization as a snake egg parasitoid, lack of brood ball preparation, and lack of secretions with antimicrobial activity (Hoback et al, 2004), I grouped *N. pustulatus* with the non-brood ball preparing silphines, *Necrodes surinamensis* and *Necrophila americana.* Grouping these three species allowed me to compare the secretion microbiomes of carrion beetles that do not prepare brood balls to the secretion microbiomes of brood ball preparing nicrophorines.

Brood Ball Preparing Nicrophorines

Four species of brood ball preparing *Nicrophorus* spp. were included in this research: *N. americanus, N. orbicollis, N. marginatus,* and *N. tomentosus.* Each of these species prepare a brood ball for their offspring and exhibit parental care throughout larval development. Preparation of a brood ball includes coating it in antimicrobial secretions that contain bacteria that

modify the microbial communities of the carcass, making it useable by the beetles and their offspring.

The first three species represent typical burying beetle behavior. *N. americanus* is the largest North American silphid (30-35 mm), while *N. orbicollis* and *N. marginatus* are of an average size (14-23 mm) (Ratcliffe, 1996). These beetles reproduce in late May and June, appearing as teneral adults in late July and August, and overwintering as adults (Anderson & Peck, 1985). *N. americanus* and *N. orbicollis* are nocturnal and have a preference for fields and forested areas respectively (Scott, 1998). *N. margin*atus is a diurnal species with a preference for open grassy habitats (Ratcliffe, 1996). *N. americanus* was once widely distributed across the eastern United States (Anderson & Peck, 1985), but is now limited to populations in the Red River, Arkansas River, and Flint Hills regions in Texas, Oklahoma, Kansas, and Arkansas, the Loess Canyons, Sandhills, and Niobrara regions in Nebraska and South Dakota, Block Island, Rhode Island, and a reintroduced population on Nantucket Island, Massachusetts (USFWS, 2020). *N. orbicollis* are most prevalent across the eastern United States and southeastern Canada (Anderson & Peck, 1985), and *N. marginatus* is the most widely distributed *Nicrophorus* spp. in North America, extending across most of the United States, southern Canada, and northern Mexico (Ratcliffe, 1996).

The last species in this group, *N. tomentosus,* is the smallest species included in this research at 11-19 mm (Ratcliffe, 1996). Distributed across much of the United States, they do not completely bury a carcass in soil, but instead dig a shallow pit and cover the carcass with litter (Anderson & Peck, 1985). While I could not find literature specifically describing *N. tomentosus* covering a carcass in secretions, both its oral and anal secretions have antimicrobial activity (Hoback et al., 2004). In fact, of the seven *Nicrophorus* spp. analyzed by Hoback et al. (2004) *N. tomentosus* was one of only two species that had both antimicrobial oral and anal secretions. They hypothesized that this may be because the small beetles do not bury their carcasses deeply or

because their secretion production may be limited, requiring both oral and anal secretions to be used (Hoback et al., 2004).

Characterizing the previously undescribed secretion microbiomes of several species within Silphidae will allow the identification of similarities and differences between Silphinae and Nicrophorinae and beetles that do or do not prepare a brood ball. This may reveal phylogenetic patterns of composition that align with patterns of secretion antimicrobial activity or identify a specific microbiome shared only by brood ball preparing nicrophorines. This would support the hypothesis that brood ball preparing nicrophorines contain a community of bacteria that aid in the preservation of a brood ball by influencing the carcass microbiome via competition and antimicrobial compound production. I hypothesized that the microbiomes of the Silphinae and the Nicrophorinae and the microbiomes of silphids that do or do not prepare a brood ball would have major differences in taxa present that would reflect either the phylogenies of the subfamily split or the differences in reproductive styles.

METHODS

Beetle and Secretion Collection

Oral and anal secretions were separately collected from seven silphid species. One male and one female *Nicrophorus americanus* (n=2), and mixed sex *N. tomentosus* (n=4), *N. orbicollis* (n=4), *N. pustulatus* (n=4), *Necrodes surinamensis* (n=5), and *Necrophila americana* (n=5) were collected from the same above-ground pitfall trap (Leasure et al., 2012) baited with rotten rat in June 2020 at Camp Gruber, Oklahoma. Secretions were collected on site before release. Mixed sex *N. marginatus* (n=5) were trapped in August 2020 near O'Neill, Nebraska and brought back to Oklahoma State University where their secretions were collected. All secretions were collected on cotton swabs that were then broken off at the tip into 1.5 mL microcentrifuge tubes and frozen until DNA extraction.

DNA Extraction, PCR Amplification, and Illumina Sequencing

DNA was extracted from secretions using a DNeasy Plant Pro kit (Qiagen) according to manufacturer instructions. Resulting DNA concentrations were quantified using a Qubit® fluorometer (Life Technologies®, Carlsbad, CA). Isolated DNA was then used as a template to PCR amplify the 16S rRNA V4 hypervariable region using the 515F and 806R prokaryotic-specific primer pair (Wang & Qian, 2009). Products were then sequenced using the paired-end Illumina iSeq-100 sequencing system. Analysis of this sequence data then allowed for the culture-independent identification of the V4 hypervariable region of the secretion microbiome.

Sequence analysis

The software package Mothur was used for sequence processing and analysis, with most steps derived from the iSeq-100 SOP available from the Mothur website (Schloss, 2019). The 515F and 806R prokaryotic-specific primer pair were used to amplify a ~293 bp PCR amplicon from the V4 hypervariable region of the 16S rRNA molecule of the bacteria within the secretions. Forward and reverse sequence pairs were assembled into contigs that were further processed to eliminate sequences with ambiguous bases (N), sequences longer than 300bp, and sequences shorter than 260bp. This resulted in high quality sequences from all samples that were then aligned in Mothur using the recreated Silva SEED alignment database as a template. Alignments were pre-clustered and de-noised using a pseudo-single linkage algorithm (Huse et al., 2010), and misaligned and possible chimeric sequences were removed using chimera.slayer in Mothur. The remaining sequences were clustered into operational taxonomic units (OTUs) at 6% sequence divergence cutoff (the putative genus level) (Schloss & Handelmann, 2005) using the vsearch clustering method through Mothur. Sequence taxonomy was identified according to the Silva taxonomic outline.

Analyses

The seven species of silphids were divided first into subfamilies for analysis and then into two groups based on reproductive strategy, brood ball makers (*Nicrophorus americanus, N. tomentosus*, *N. orbicollis*, and *N. marginatus*) and non-brood ball makers *(N. pustulatus*, *Necrodes surinamensis,* and *Necrophila americana).* The R package vegan was used to analyze the data and create a constrained correspondence analysis (CCA) to graphically depict relationships between the different reproductive strategies. Mothur software was used to create Venn diagrams to compare shared genera. Sequences from the Chapter 2 cultured *N. americanus* secretion isolates were compared to the iSeq-100 data to compare culture-dependent and independent methods. Finally, I looked at data from nine previous studies of silphid associated microbiomes (Solter et al. 1989, Berdela et al. 1994, Kaltenpoth & Steiger, 2014, Duarte et al., 2017, Shukla et al., 2017, Vogel et al., 2017, Omstead, 2018, Shukla et al. 2018, Miller et al., 2019) to contextualize my findings.

RESULTS

Thirty-three bacterial phyla were identified from all sampled secretion microbiomes. The most abundant phyla were Firmicutes (53.7%), Proteobacteria (33.6%), Bacteroidetes (7.6%), Actinobacteriota (2.9%), Bacteria unclassified (1.3%), and Spirochaetota (0.2%) (Figure 5). Other phyla made up 0.7% of all sequences. Notably, the majority of Spirochaetota sequences were collected from *N. pustulatus* (89.9%).

I was able to identify 694 bacterial genera from the secretions of the seven sampled species of Silphidae. Each species had a number of unique accessory genera, genera that were identified in only that species (Figure 6). Analysis revealed that my sampled *N. americanus* secretions contained the largest number of genera (422) and the largest number of unique accessory genera (89). From the beetles analyzed, I identified 89 (12.8%) genera that were shared

across all seven species (Appendix A), and the genera present within each subfamily or reproductive style (Figure 7).

The most abundant taxa in my data set were from the classes Bacilli, Clostridia, and Bacteroidia, and the orders Lactobacillales, Bacillales, Enterobacterales, and Cardiobacterales (Table 4). Interestingly, sequences belonging to an unclassified Bacilli order within the Firmicutes, the family Wohlfahrtiimonadaceae within Gammaproteobacteria, and the class Bacteroidia within the family Bacteroidetes were most abundant in Silphinae species, and Clostridia were especially abundant in *N. pustulatus*. Clostridia taxa represented by a large number of V4 region amplicons in *N. pustulatus* were *Peptostreptococcus*, *Clostridium* sensu stricto 15, *Ruminococcus*, and an unclassified Lachnospiraceae genus.

Subfamilies

Analysis of the V4 region amplicons revealed 109 genera present in all five sampled Nicrophorinae species, while 136 genera were identified in the two sampled Silphinae species. Only 89 genera were shared between the subfamilies, resulting in 20 genera that were only identified in the nicrophorines (Appendix B), 11 of which were present at 50% or more in one species. Notably, *Thermus* was present at 96.5% in *N. tomentosus,* and *Paeniclostridium* was present at 95.8% in *N. americanus*.

By excluding unique genera, I identified 355 bacterial genera shared between two or more *Nicrophorus* spp. Analysis of the V4 region amplicons revealed that *N. pustulatus* had fewer shared genera compared to the datasets generated for the other *Nicrophorus* spp. Of the 355 bacterial genera shared by two or more *Nicrophorus* spp., 314 genera (88.5%) included *N. americanus*, while only 212 genera (59.7%) included *N. pustulatus* (Figure 8). The data indicated that *N. pustulatus* had a secretion microbiome that was more different from the other *Nicrophorus* spp. Based on these results, in the remaining analysis I included *N. pustulatus* with the two non-

brood ball preparing silphids*, Necrodes surinamensis,* and *Necrophila americana,* with the other group being the brood ball preparing nicrophorines including *N. americanus*, *N. marginatus*, *N. orbicollis*, and *N. tomentosus*.

Reproductive Styles

The constrained correspondence analysis (Figure 9) shows how the microbiomes of each silphid species relate to one another and where general microbiomes of brood ball preparing and non-brood ball preparing groups would be located. Brood ball preparing *N. americanus, N. tomentosus, N. marginatus,* and *N. orbicollis* appear to have similar secretion microbiomes to one another and share a number of bacterial genera. Additionally, while *N. pustulatus* secretions contain a community of bacteria that are similar to brood ball preparing *Nicrophorus* spp., analysis of the V4 region amplicons suggest that they were also more similar to non-brood ball preparing silphines than any other nicrophorine. The CCA also shows that *Necrodes surinamensis* has a secretion microbiome that is more closely related to *Nicrophorus* spp. than *Necrophila americana*.

Of the 694 genera identified in all silphids, 591 genera were present in at least one species of brood ball preparing nicrophorine. These 591 genera were then used to construct a Venn diagram (Figure 10). This Venn diagram identified a core microbiome of 130 bacterial genera present in all four brood ball preparing nicrophorines. Ignoring the 89 silphid core genera, 41 genera were unique to the brood ball preparing nicrophorines (Appendix C); however, only one genus, *Leucobacter*, was present in the four brood ball preparing nicrophorines but absent in the non-brood ball preparing silphids. Of the 64 *Leucobacter* sequences identified in my analysis, 39 (61%) were identified in *N. marginatus*. The Venn diagram also showed that even amongst brood ball preparing *Nicrophorus* spp., *N. americanus* has the largest number of unique accessory genera (143).

Of the 694 genera identified in all silphids, 417 genera were present in at least one species of non-brood ball preparing silphid. These 417 genera were then used to construct a Venn diagram (Figure 11). This Venn diagram demonstrated that 106 bacterial genera were present in all three non-brood ball preparing silphids. It also showed that amongst non-brood ball preparing silphids, *N. pustulatus* had the largest number of unique genera in my analysis (102).

Cultured N. americanus Isolates

Of the thirteen cultured *N. americanus* secretion isolates from Chapter 2, five were identified in the iSeq-100 samples (Table 5). Two *Proteus* spp. were identified in the iSeq-100 samples, one in *N. americanus* and the other in *Necrophila americana.* Three isolates were identified as *Acinetobacter* spp. One was identified in *Nicrophorus marginatus* secretions, and the other two, likely the same species (98.04% identity), were identified in *N. americanus*. Although only five of the isolate species were identified in the iSeq-100 samples, the majority of the isolate genera were identified in most silphid species with the exception of *Paracoccus* which was identified only in *N. marginatus* (Table 6).

Comparison to Previous Research

I constructed tables containing data from nine studies of silphid species and their associated microbiomes. I included only the data pertaining to the seven silphid species I sampled from four studies and data from an additional five studies analyzing *N. vespilloides* and *N. defodiens* (Table 7). The microbiomes characterized by these previous studies used samples predominantly taken from guts, but also included samples of hemolymph, ovaries, testes, salivary glands, anal secretions, and tended carcasses. Using what data was available, I constructed a table depicting the presence of genera in each of the publications (Table 8). Trends of abundant taxa were similar across all studies and although not all genera were identified in all studies, a core

group of genera were identified, with *Staphylococcus, Clostridium, Proteus, Providencia,* and *Acinetobacter* identified most often (Table 9) (Figure 12).

DISCUSSION

The first studies of silphid microbiomes cultured the guts and hemolymph of *N. orbicollis, N. tomentosus, Necrophila americana, Oiceoptoma inaequale, O. noveboracense*, and *Necrodes surinamensis* in order to identify medically important bacteria (Solter et al. 1989, Berdela et al. 1994). Two decades later, Kaltenpoth and Steiger (2014) performed the first culture-independent characterization of silphid associated microbiomes, studying the gut microbiomes from *Nicrophorus humator, N. orbicollis, N. pustulatus, N. tomentosus, N. vespillo, N. vespilloides, Necrophila americana,* and *O. noveboracense.* In the last five years, studies have been performed to characterize the secretion microbiomes of a number of other silphid species and their associated microbiomes (Duarte et al., 2017, Shukla et al., 2017, Vogel et al., 2017, Omstead, 2018, Shukla et al. 2018, Miller et al., 2019).

I was able to perform the first characterization of the secretion microbiomes of seven species of Silphidae. The most abundant phyla in my data set were Firmicutes and Proteobacteria, both of which are major phyla. Proteobacteria is currently the largest phylum within bacteria (Rizzatti et al., 2017). At the family level, I found Wohlfahrtiimonadaceae and unclassified families from Lactobacillales, Enterobacterales, Bacillales, and Bacilli to be the most abundant in my data set. I found that 89 genera (12.8%) identified across all silphid samples were shared and I propose that this represents a core microbiome that is generally consistent with previous research analyzing silphid associated microbiomes (Solter et al. 1989, Berdela et al. 1994, Kaltenpoth & Steiger, 2014, Duarte et al., 2017, Shukla et al., 2017, Vogel et al., 2017, Omstead, 2018, Shukla et al. 2018, Miller et al., 2019). I also found that Nicrophorinae shared fewer genera than

Silphinae. This may be because the nicrophorines possess a microbiome uniquely adapted to burying carcasses, giving rise to a less diverse but more specialized bacterial community. Notably, the most important genera associated with carcasses throughout decomposition identified by Pechal (2012) were all identified in the silphids sampled in this research.

My data showed that Firmicutes were more abundant in three of the five nicrophorines I sampled. This is similar to previous studies (e.g. Kaltenpoth and Steiger (2014)) that found Firmicutes to be generally present in higher abundances in Nicrophorinae compared to Silphinae and suggested that they may play a role in carcass preservation by producing antimicrobial compounds (Degenkolb et al., 2011). Many of the unique Nicrophorinae core genera fell within the class Clostridia (Firmicutes) and the order Enterobacterales (Gammaproteobacteria), taxa that produce bacteriolytic enzymes and antimicrobial compounds and were especially abundant (Degenkolb et al., 2011, Kaltenpoth & Steiger, 2014). Clostridiales, specifically *Tissierella*, ferment creatinine, which is abundant in animal tissues but cannot be used by insects, as a sole carbon and energy source (Kaltenpoth & Steiger, 2014). *Tisierella* was extremely abundant in *N. americanus,* but very low in *N. marginatus* and *N. pustulatus*

Members of Silphinae had additional taxa not present in nicrophorines in the orders Flavobacteriales (phylum Bacteroidetes) and Rhizobiales (Alphaproteobacteria), in accordance with Kaltenpoth and Steiger (2014). Across previous studies, unclassified Lactobacillales, Enterobacteriaceae, Enterococcaceae, Clostridiales, and Xanthomonadaceae have been identified as abundant families. Kaltenpoth and Steiger (2014) found that Xanthomonadaceae are more abundant in Silphinae. While I did find that Xanthomonadaceae were more abundant in Silphinae, they were 17.6 times more abundant in *Necrodes surinamensis* than *Necrophila americana,* and anywhere from 2 to 28 times more abundant than in any *Nicrophorus* spp.

Comparing the two silphid subfamilies, Kaltenpoth and Steiger (2014) found a high degree of consistency in microbial communities that they hypothesized came about as an adaptation to their carrion diet. They found weak inconclusive support for congruence between silphid phylogeny and gut microbial communities, highlighting instead geographical patterns in microbial communities of *Nicrophorus* spp. (Kaltenpoth & Steiger, 2014). Interestingly, they found that *N. pustulatus* did not differ from the other *Nicrophorus* spp. they analyzed, which they attribute to carrion feeding adults (Kaltenpoth & Steiger, 2014). In my anlysis, I found that *N. pustulatus* had fewer shared genera with other *Nicrophorus* spp. than any other congener, suggesting the species as an outlier and perhaps justifying its grouping with non-brood ball preparing beetles. This could be a result of characterizing the microbiomes of secretions versus guts, which are distinct (Miller et al., 2019). Miller et al. (2019) hypothesized that the antibiotic activities of anal secretions help eliminate nonessential gut bacteria from carcasses. It may also be that the microbial community associated with *N. pustulatus* is a secondary adaptation, explaining why it less closely resembles the microbiomes of other *Nicrophorus* spp.

I identified 591 bacterial genera unique V4 region amplicons from the anal and oral secretions of the four brood ball preparing *Nicrophorus* spp., 130 (22.0%) of which were shared across the four species. In comparison, characterizations of gut, ovary, teste, and salivary microbiomes by Olmstead (2018) identified 345 bacterial genera from the same four *Nicrophorus* spp., 59 (17.2%) of which were shared across the four species. Both my analysis and that of Olmstead (2018) suggested that *N. americanus* associated microbiomes were represented by the largest number of unique genera. Two unique core genera of brood ball preparing nicrophorines were particularly abundant in only one species, *Thermus* in *N. tomentosus,* and *Paeniclostridium* in *N. americanus*. The genus *Thermus* is perhaps known best for *T. aquaticus*, the thermophile from which Taq DNA polymerase was isolated. *Paeniclostridium* was formed in 2016 to accommodate two *Clostridium* spp. one of which is a putrefactive pathogenic anaerobe (Hall &

Scott, 1927, Sasi Jyothsna et al., 2016). Additionally, two uncultured genera from the families Planococcaceae and Burkholderiaceae were identified in the unique nicrophorine core. Unclassified Planococcaceae have been previously associated with breeding *Nicrophorus vespilloides* and their prepared carcasses (Duarte et al., 2017). *Burkholderia* have been associated with a number of insect taxa (Kaltenpoth & Flórez, 2020). In Lagriinae, a subfamily of darkling beetles (Tenebrionidae), *Burkholderia* are vertically transmitted to offspring and produce protective antifungal and antibacterial secondary metabolites (Kaltenpoth & Flórez, 2020).

Leucobacter, the only genus that was present in the four brood ball preparing beetles and none of the non-brood ball preparing beetles, contains a diverse set of species isolated from soil, activated sludge from chromium-contaminated wastewater, nematodes, and nonbiting midge, Chironomidae, egg masses (Sturm et al., 2011). It is unclear why this genus is the only one shared by all brood ball preparing beetles and missing from non-brood ball preparing beetles.

Comparing the thirteen cultured *N. americanus* secretion isolates from Chapter 2 to the iSeq-100 data emphasized that culture-dependent and independent studies identify different microbial communities. Culture-dependent methods are inherently limited by the selectivity of media and culture conditions that favor only a portion of the community, and underestimation of numbers and composition (Al-Awadhi et al., 2013). Although the culturing and isolating described in Chapter 2 were nowhere near exhaustive, I identified nine genera, compared to the 694 identified using Illumina iSeq-100. This is a clear demonstration that for the characterization of microbiomes, especially from multiple sample sources, culture-independent methods are superior in obtaining a comprehensive analysis of taxa present.

To date, several of the bacterial genera I identified by iSeq-100 from silphid secretion microbiomes have been associated with silphids. These fifteen genera are as follows. *Bacillus* spp. are ubiquitous, and are primarily harmless saprophytes, although a few species are pathogens

of vertebrates and insects (*B. anthracis, B. cereus, B. thuringiensis*) (Turnbull, 1996). *Staphylococcus* spp. are opportunistic pathogens isolated from a number of mammalian and environmental sources like soil and water (Berdela et al., 1994). *Vagococcus* spp. were first isolated from chicken feces and river water and were found to be loosely associated with members of the genus *Enterococcus* (Collins et al., 1989). *Lactobacillus* spp. produce antibacterial and antifungal compounds (Olmstead, 2018). The *Lactobacillus* spp. *L. plantarum* alters cuticular hydrocarbons in the fruit fly *Drosophila melanogaster*, altering mating pheromones and causing the fly to select a mate with similar gut microbial communities, ensuring microbial transmission to a similar environment (Olmstead, 2018). *Streptococcus* spp. are typical in animal microbiomes, and while some cause disease, others aid in industrial and dairy processes, or serve as pollution indicators (Patterson, 1996). *Clostridium* spp. are ammonifying bacteria that promote amine accumulation (Vogel et al., 2017). *Wohlfahrtiimonas* spp. catabolize and ferment a number of amino acids and sugars, and reduce nitrate (Hall et al., 2011, Miller et al., 2019).

Morganella spp. and *Proteus* spp. both produce urease which catalyzes the conversion of urea to ammonia and carbon dioxide. *Morganella morganii*, produces a pheromone in the grass grub *Costelytra zealandica* (Scarabidae) that alters mate choice (Olmstead, 2018). *Proteus* spp. produce volatiles that attract carrion-breeding species (Gupta et al., 2014). *Providencia* spp. have been associated with silphids in previous studies and with the blow fly *Cochliomyia macelluria* (Thompson et al., 2013). *Ignatzschineria* spp. may provide accessible nitrogen to silphids due to high urease activity that degrades the cytotoxic compound urea (Kaltenpoth & Steiger, 2014). *Acinetobacter* spp. produce organic acids and acidify their environment (Cray et al., 2013) and produce biofilms and enzymes that degrade vertebrate tissue (Bergogne-Bérézin & Tower, 1996). *Vitreoscilla* spp. metabolize toxic chemicals like nitric oxide, (Stark et al., 2012) and contain genes hypothesized to play a role in gut colonization (Kumar et al., 2014). *Dysgonomonas* spp.

degrade fatty acids (Miller et al., 2019), and finally, *Myroides* spp. produce antibacterial compounds (Dharne et al., 2008). Many of these genera that are abundant and recurring in silphids, particularly *Nicrophorus* spp. are also present in other necrophagous insects and meat (both fresh and decomposing) likely playing a role in the preservation and digestion of carcasses (Shukla et al., 2017).

In this research, I characterized the previously undescribed secretion microbiomes of several species within Silphidae. Silphids contain a microbiome of bacterial taxa from Lactobacillales, Enterobacterales, Bacillales, and Cardiobacteriales that have been repeatedly identified in silphids in a number of studies. Burying beetles within the subfamily Nicrophorinae were found to contain an abundant unique core of Enterobacterales and Peptostreptococcales-Tissierellales. Within the subfamily Nicrophorinae, *N. pustulatus* was positioned as an outlier with fewer genera shared with the other *Nicrophorus* spp. This, in accordance with its reproductive style as a snake egg parasitoid, differentiated it from its congeners. Studying the microbiomes of the four other *Nicrophorous* spp. revealed a core of ubiquitous bacteria shared among nicrophorines excluding *N. pustulatus*. Overall, there is no evidence for a distinct secretion microbiome present exclusively in brood ball preparing *Nicrophorous* spp. Instead, it appears that silphids share a core group of bacteria that differ in abundance across subfamilies and reproductive styles, much as Kaltenpoth and Steiger (2014) found in their initial culture-independent analysis of silphid gut microbiomes. The ability of burying beetles to modify the microbiome of a carcass to more closely match their own secretion microbiomes and suppress the proliferation of carcass borne microbes (Shukla et al., 2017) may be a function of the relative abundances of key bacterial taxa within their guts and secretions. By harboring taxa that are able to successfully colonize carcasses during preparation, *Nicrophorus* spp. are able to interrupt the normal progression of decomposition and maintain their offspring.

I was unable to find strong evidence that secretions of nicrophorines and silphines contained substantially different secretion microbiomes. Instead, the majority of differences between species within the two subfamilies appeared to be from genera present at high abundances in one or two species and low abundances in the rest. However, some of these genera may explain differences in the antimicrobial secretions of certain species. Hoback et al. (2004) found that *N. americanus* and then *N. orbicollis* had the strongest antimicrobial secretions. My analysis of their secretion microbiomes revealed that these two species had the largest numbers of unique accessory genera. Further research into the unique genera of these two species and assays for inhibitory effects may uncover bacteria with significant antimicrobial activity and uncover novel mechanisms.

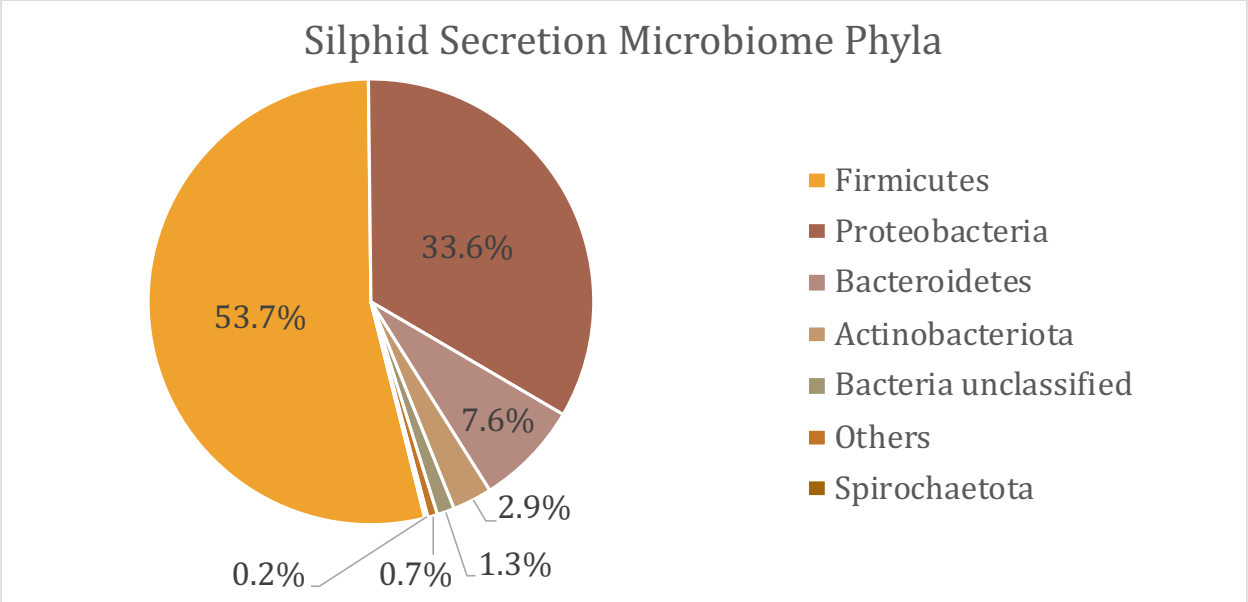


Figure 5. Dominant bacterial phyla in all sampled silphid secretions.

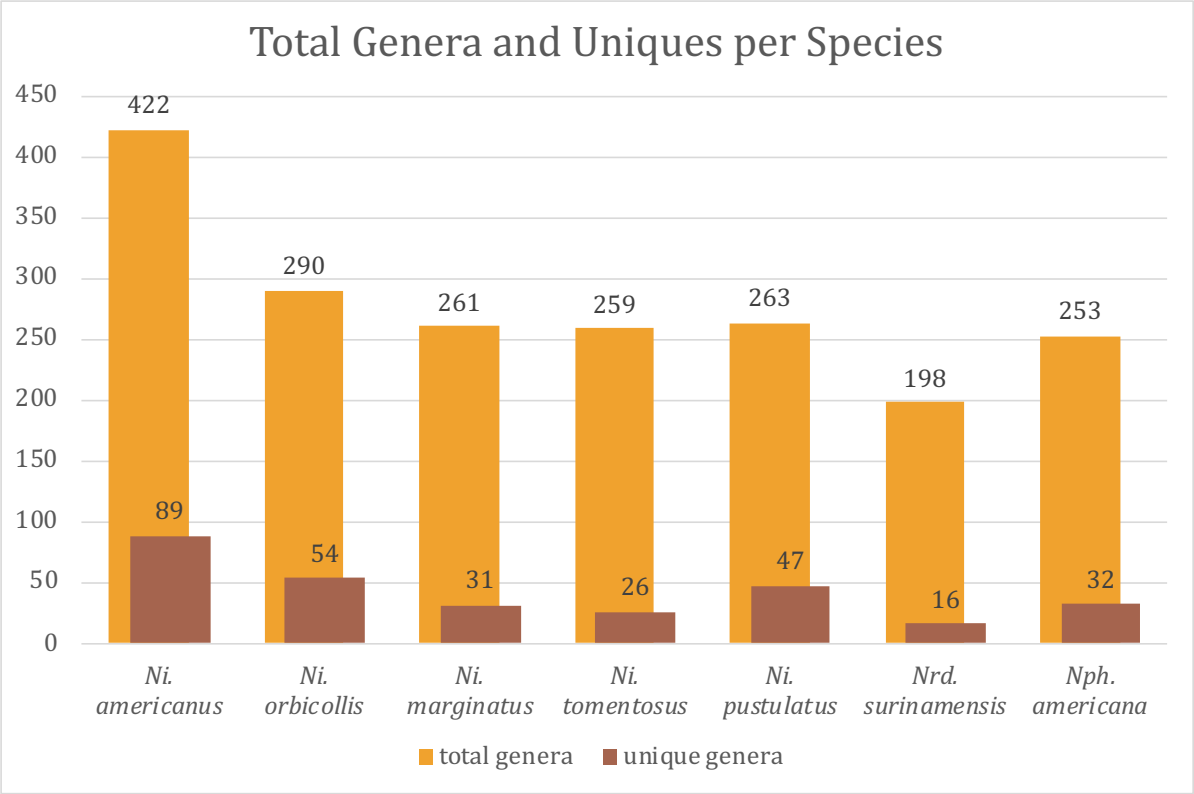


Figure 6. Total number of genera and number of unique genera for each species of sampled silphid. *Nicrophorus* has been abbreviated to *Ni*., *Necrodes* to *Nrd*., and *Necrophila* to *Nph*.

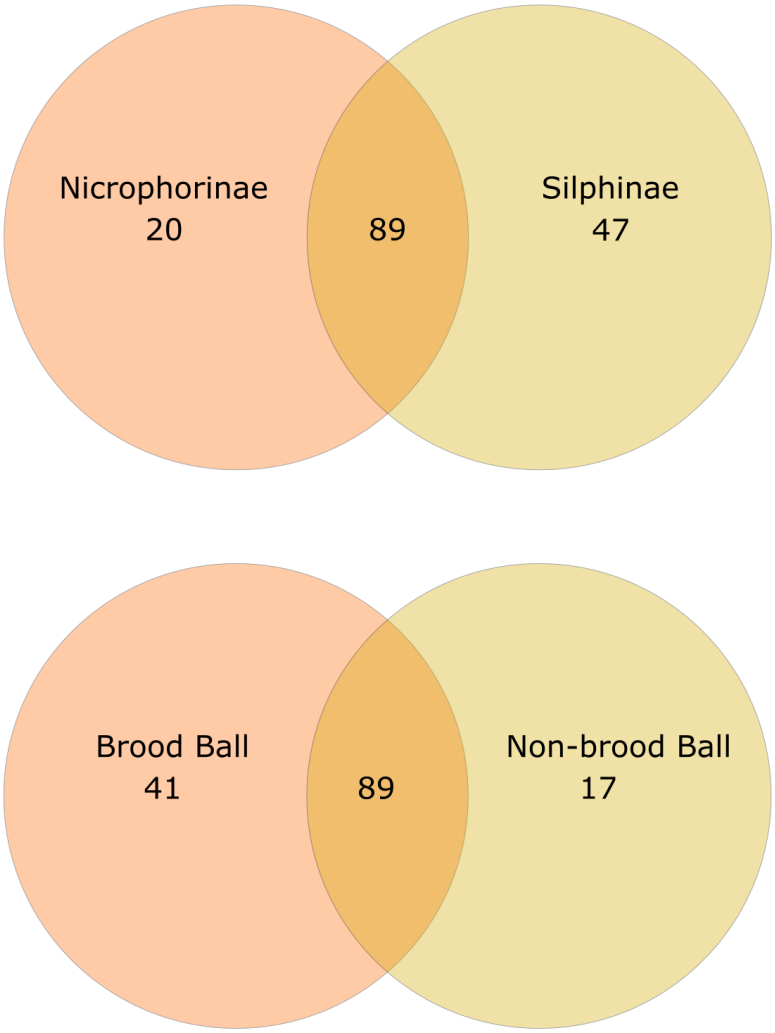


Figure 7. Venn diagrams of bacterial genera, the center shows the number of core bacterial genera shared among sampled silphid secretions. The first Venn diagram divides the genera between the subfamilies Nicrophorinae and Silphinae, and the second Venn diagram divides the genera between the brood ball preparing nicrophorine and non-brood ball preparing silphids.

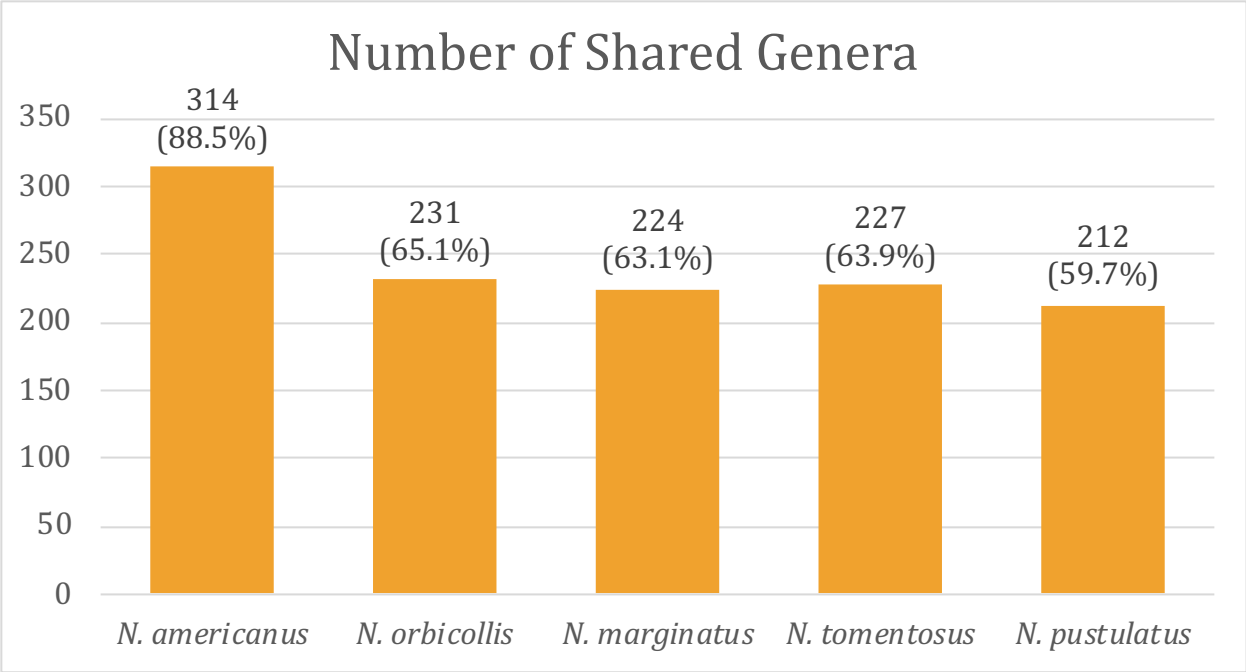


Figure 8. Number of bacterial genera shared with at least one other *Nicrophorus* spp. Percentage of 335 genera shared by two or more *Nicrophorus* spp. is also shown.

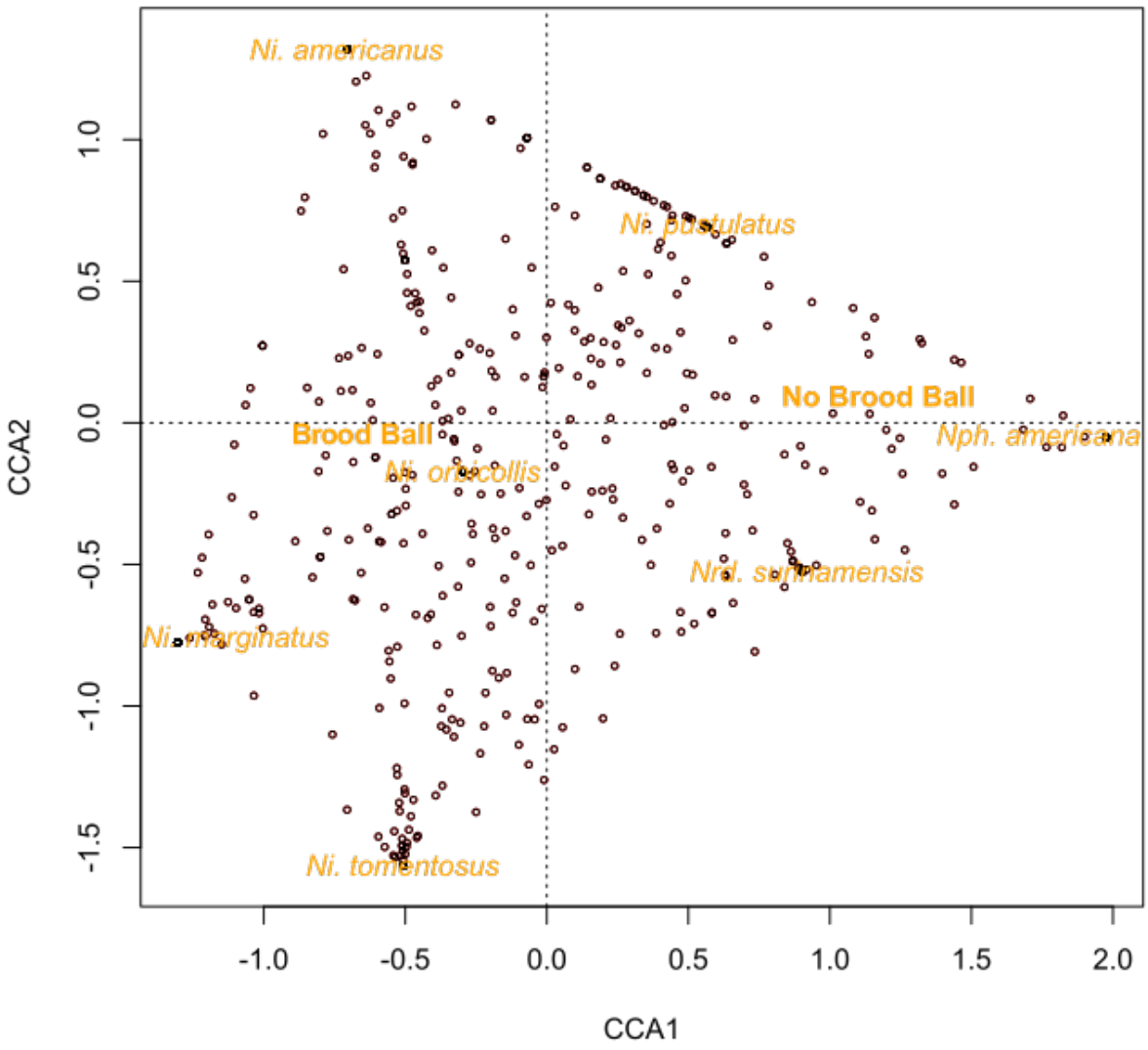
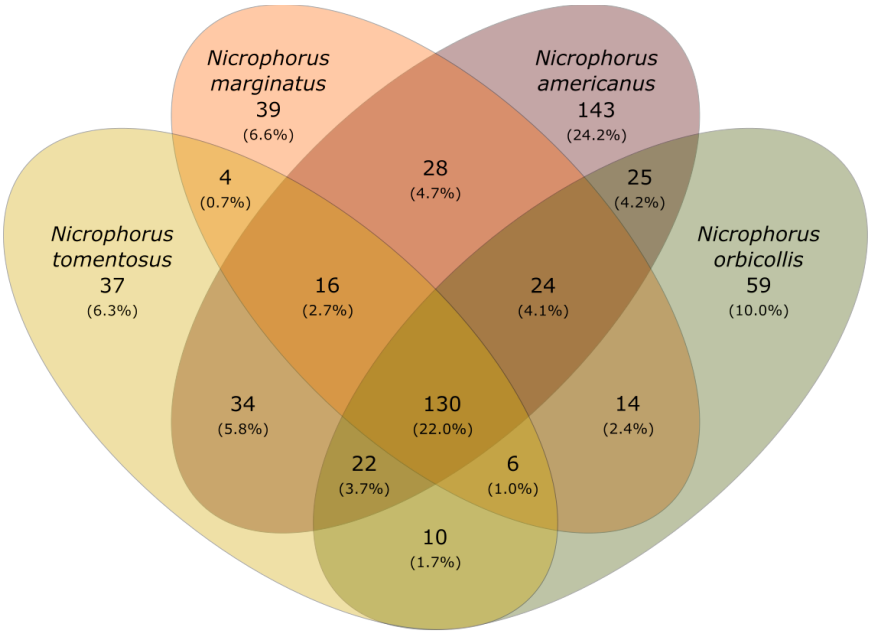
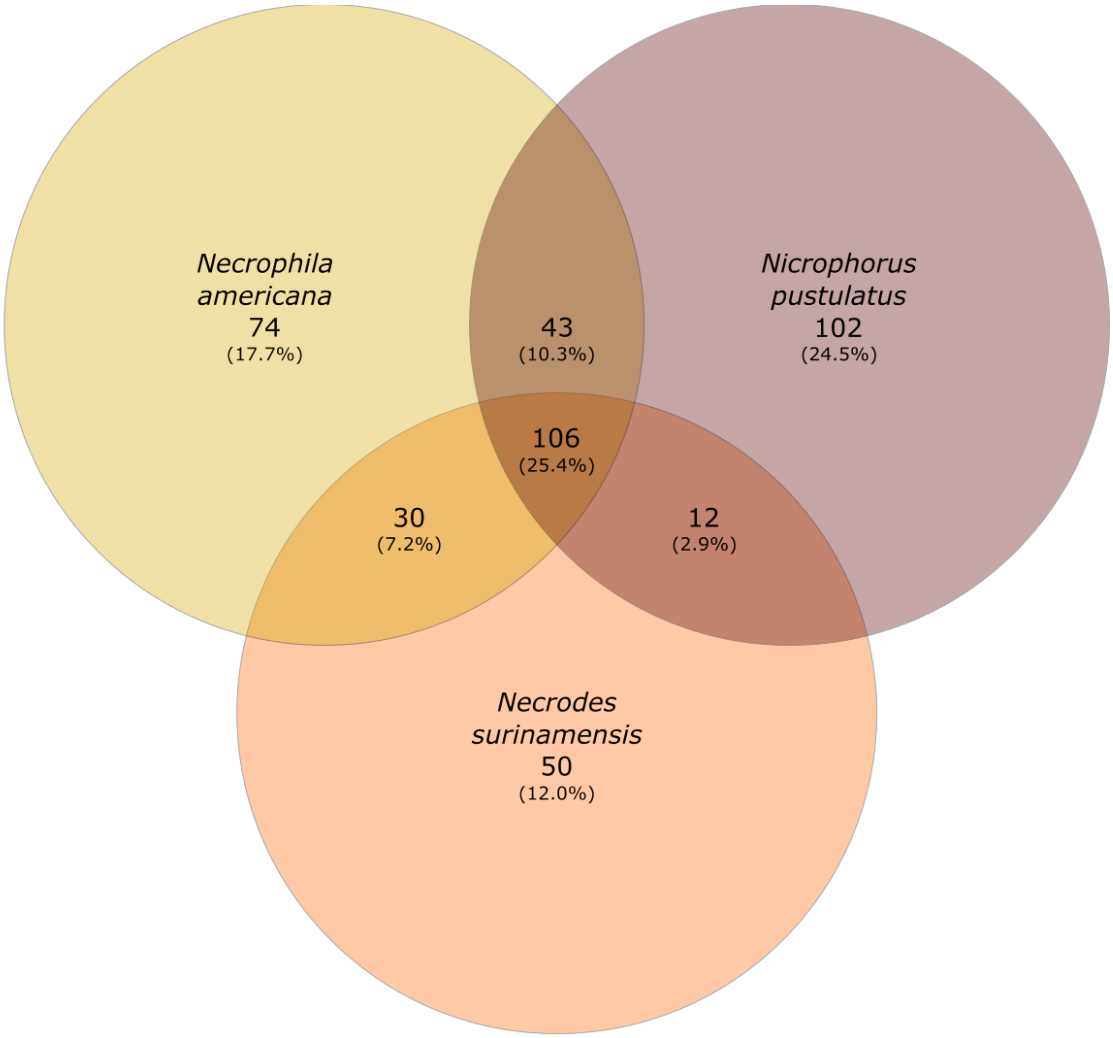


Figure 9. Constrained correspondence analysis of 694 bacterial genera identified in all sampled silphids. *Nicrophorus* has been abbreviated to *Ni*., *Necrodes* to *Nrd*., and *Necrophila* to *Nph*. Dots directly under each of the species' labels represent bacterial genera identified in only that species. Dots nearby species labels represent bacterial genera predominantly identified in that species but also found to some extent in another species.



The number of genera in *Nicrophorus americanus* is 422 (71.4%)
The number of genera in *Nicrophorus marginatus* is 261 (44.2%)
The number of genera in *Nicrophorus orbicollis* is 290 (49.1%)
The number of genera in *Nicrophorus tomentosus* is 259 (43.8%)
The number of genera shared between *Nicrophorus americanus* and *Nicrophorus marginatus* is 198 (33.5%)
The number of genera shared between *Nicrophorus americanus* and *Nicrophorus orbicollis* is 201 (34.0%)
The number of genera shared between *Nicrophorus americanus* and *Nicrophorus tomentosus* is 202 (34.2%)
The number of genera shared between *Nicrophorus marginatus* and *Nicrophorus orbicollis* is 174 (29.4%)
The number of genera shared between *Nicrophorus marginatus* and *Nicrophorus tomentosus* is 156 (26.4%)
The number of genera shared between *Nicrophorus orbicollis* and *Nicrophorus tomentosus* is 168 (28.4%)
The number of genera shared between *Nicrophorus americanus*, *Nicrophorus marginatus*, and *Nicrophorus orbicollis* is 154 (26.1%)
The number of genera shared between *Nicrophorus americanus*, *Nicrophorus marginatus*, and *Nicrophorus tomentosus* is 146 (24.7%)
The number of genera shared between *Nicrophorus americanus*, *Nicrophorus orbicollis*, and *Nicrophorus tomentosus* is 152 (25.7%)
The number of genera shared between *Nicrophorus marginatus*, *Nicrophorus orbicollis,* and *Nicrophorus tomentosus* is 136 (23.0%)
The total richness of all the brood ball preparing nicrophorines is 591

Figure 10. Venn diagram of bacterial genera in secretions of four species of brood ball preparing nicrophorines. The center shows the number of core bacterial genera across brood ball preparing nicrophorines.



The number of genera in *Necrophila americana* is 253 (60.7%)

The number of genera in *Nicrophorus pustulatus* is 263 (63.1%)

The number of genera in *Necrodes surinamensis* is 198 (47.5%)

The number of genera shared between *Necrophila americana* and *Nicrophorus pustulatus* is 149 (35.7%)

The number of genera shared between *Necrophila americana* and *Necrodes surinamensis* is 136 (32.6%)

The number of genera shared between *Nicrophorus pustulatus* and *Necrodes surinamensis* is 118 (28.3%)

The total richness of all the non-brood ball preparing silphids is 417

Figure 11. Venn diagram of bacterial genera in secretions of three species of non-brood ball preparing silphids. The center shows the number of core bacterial genera across non-brood ball preparing silphids.

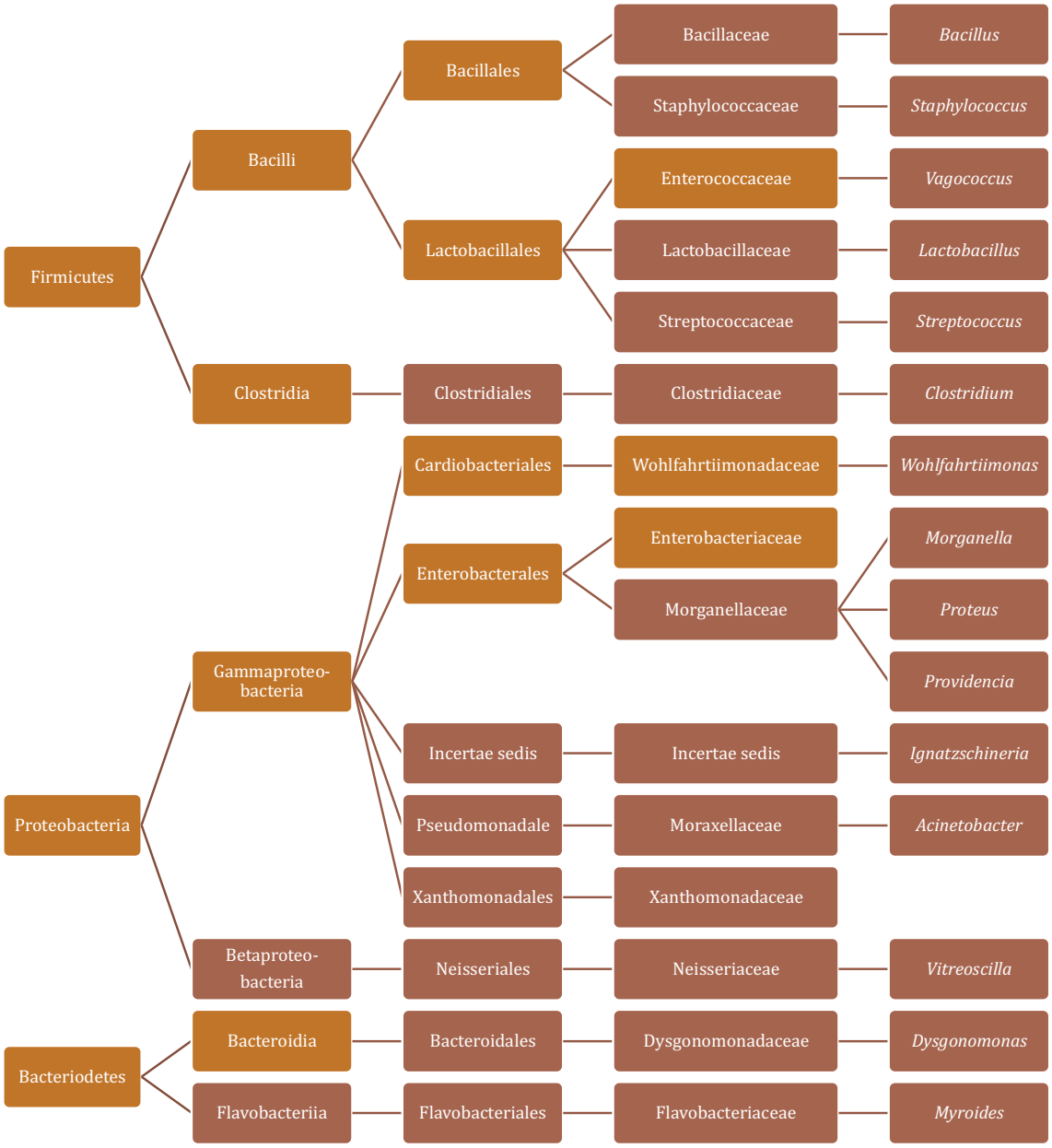


Figure 12. Horizontal hierarchy of common bacterial taxa associated with Silphidae. Orange boxes indicate the most abundant taxa in my silphid samples.

TABLES

Table 4. Most abundant taxa found in iSeq-100 samples. Taxa are listed in taxonomic order and percentages represent percent of total sequences. *Nicrophorus* has been abbreviated to *Ni.*, *Necrodes* to *Nrd.*, and *Necrophila* to *Nph*.

rank	taxon	*Ni. americanus*	*Ni. orbicollis*	*Ni. marginatus*	*Ni. tomentosus*	*Ni. pustulatus*	*Nrd. surinamensis*	*Nph. americana*
order	Lactobacillales	41%	17%	24%	17%	26%	9%	3%
family	Lactobacillales_unclassified	29%	8%	11%	8%	19%	5%	2%
genus	Lactobacillales_unclassified	29%	8%	11%	8%	19%	5%	2%
order	Bacillales	2%	8%	0%	6%	19%	23%	23%
family	Bacillales_unclassified	2%	7%	0%	4%	17%	16%	13%
genus	Bacillales_unclassified	2%	7%	0%	4%	17%	16%	13%
order	Bacilli_unclassified	2%	7%	0%	5%	13%	19%	27%
family	Bacilli_unclassified	2%	7%	0%	5%	13%	19%	27%
genus	Bacilli_unclassified	2%	7%	0%	5%	13%	19%	27%
class	Clostridia	7%	4%	5%	6%	22%	3%	3%
class	Gammaproteobacteria	37%	47%	41%	40%	9%	27%	22%
order	Enterobacterales	25%	33%	27%	36%	5%	13%	3%
family	Enterobacterales_unclassified	13%	31%	26%	18%	4%	7%	2%
genus	Enterobacterales_unclassified	13%	31%	26%	18%	4%	7%	2%
family	Enterobacteriaceae	2%	0%	0%	17%	1%	3%	0%
order	Cardiobacteriales	9%	12%	8%	3%	2%	7%	15%
family	Wohlfahrtiimonadaceae	9%	12%	8%	3%	2%	7%	15%
phylum	Bacteroidetes	4%	4%	12%	7%	5%	7%	18%
class	Bacteroidia	4%	4%	12%	7%	5%	7%	18%

Table 5. Comparison of cultured isolates from Chapter 2 against iSeq-100 samples. *Nicrophorus* has been abbreviated to *Ni*. and *Necrophila* to *Nph*.

Cultured Isolates			iSeq Samples	
Sex	Sample	16S ID	Silva ID	Species
Female	Anal	*Gordonia* sp.		
Female	Anal	*Proteus* sp.	*Proteus*	*Ni. americanus*
Female	Anal	*Acinetobacter* sp.	*Acinetobacter*	*Ni. marginatus*
Female	Anal	*Myroides* sp.		
Female	Oral	*Acinetobacter* sp.	*Acinetobacter*	*Ni. americanus*
Female	Oral	*Pseudochrobactrum* sp.		
Female	Oral	*Corynebacterium* sp.		
Female	Oral	*Myroides* sp.		
Male	Anal	*Vitreoscilla* sp.		
Male	Anal	*Paracoccus* sp.		
Male	Anal	*Glutamicibacter* sp./*Arthrobacter* sp.		
Male	Oral	*Proteus* sp.	*Proteus*	*Nph. americana*
Male	Oral	*Acinetobacter* sp.	*Acinetobacter*	*Ni. americanus*

Table 6. Abundance of cultured isolate genera from Chapter 2 in iSeq-100 sample species. *Nicrophorus* has been abbreviated to *Ni*., *Necrodes* to *Nrd*., and *Necrophila* to *Nph*.

	Ni. americanus	*Ni. marginatus*	*Ni. tomentosus*	*Ni. orbicollis*	*Ni. pustulatus*	*Nrd. surinamensis*	*Nph. americana*
Acinetobacter	91	1395	255	37	14	822	26
Corynebacterium	199	1122	575	2787	41	239	710
Glutamicibacter	89	1122	28	6	0	61	5
Gordonia	25	27	0	3	4	0	3
Myroides	285	383	419	57	3	786	53
Paracoccus	6	0	6	0	0	0	0
Proteus	7518	182	118	250	237	292	64
Pseudochrobactrum	4	10	16	0	1	6	3
Vitreoscilla	7	339	22	28	17	4	7

Table 7. Comparison to other publications. *Nicrophorus* has been abbreviated to *Ni*., *Necrodes* to *Nrd*., and *Necrophila* to *Nph*.

Solter et al., 1989	**Berdela et al., 1994**	**Kaltenpoth & Steiger, 2014**	**Duarte et al., 2017**
guts, hemolymph	guts, hemolymph	guts	guts, anal secretions
Ni. americanus *Ni. tomentosus*	*Ni. americanus* *Ni. orbicollis* *Ni. tomentosus* *Nrd. surinamensis*	*Ni. orbicollis* *Ni. tomentosus* *Ni. pustulatus* *Nph. americana*	*Ni. vespilloides*

Shukla et al., 2017	**Vogel et al., 2017**	**Shukla et al., 2018**	**Olmstead, 2018**
guts	gut, anal secretions	tended carcasses	guts, ovaries, testes, salivary glands
Ni. vespilloides	*Ni. vespilloides*	*Ni. vespilloides*	*Ni. americanus* *Ni. orbicollis* *Ni. marginatus* *Ni. tomentosus*

Miller et al., 2019	**Pratt, 2021 *Nicrophorus* core**	**Pratt, 2021 all samples**
anal secretions	oral and anal secretions	oral and anal secretions
Ni. defodiens	*Nicrophorus* spp. core: *Ni. americanus* *Ni. orbicollis* *Ni. marginatus* *Ni. tomentosus* *Ni. pustulatus*	*Ni. americanus* *Ni. orbicollis* *Ni. marginatus* *Ni. tomentosus* *Ni. pustulatus* *Nrd. surinamensis* *Nph. americana*

Table 8. Comparison to genera present in other publications.

Solter et al., 1989	Berdela et al., 1994	Kaltenpoth & Steiger, 2014	Duarte et al., 2017	Shukla et al., 2017	Vogel et al., 2017	Shukla et al., 2018	Olmstead, 2018	Miller et al., 2019	Pratt, 2021 *Nicrophorus* core	Pratt, 2021, all samples	
x		x	x	x	x	x	x	x	x		*Acinetobacter*
							x				*Actinomyces*
	x		x			x					*Aerococcus*
		x							x		*Akkermansia*
x			x		x	x					*Alcaligenes*
				x							*Alicyclobacillus*
		x									*Alistipes*
							x				*Anaerococcus*
							x				*Aquicella*
				x		x					*Arthrobacter*
x	x		x			x	x				*Bacillus*
	x	x				x					*Bacteroides*
			x								*Brevibacillus*
			x								*Brevibacterium*
							x				*Bryobacter*
							x		x		*Candidatus Soleaferrea*
							x				*Candidatus Udaeobacter*
			x						x		*Carnobacterium*
			x								*Caryophanon*
				x		x			x		*Chryseobacterium*
					x						*Cloacibacterium*
x	x		x	x		x	x	x	x		*Clostridium*
							x		x		*Conexibacter*
			x	x		x			x		*Corynebacterium*
							x				*Cutibacterium*
							x		x		*Desulfovibrio*
			x								*Dietzia*
		x			x	x	x	x	x		*Dysgonomonas*
		x	x		x	x	x		x		*Enterococcus*
		x	x	x	x	x	x		x		*Erysipelothrix*
			x		x				x		*Escherichia-Shigella*
			x								*Finegoldia*
			x	x			x				*Flavobacterium*
							x				*Fluviicola*
		x					x				*Fusobacterium*
			x						x		*Geobacillus*
							x				*Glutamicibacter*
					x		x				*Gordonia*
							x		x		*Halomonas*
			x								*Herminiimonas*
		x									*Hydrogenoanaerobacterium*
		x			x		x		x		*Ignatzschineria*
			x			x					*Jeotgalicoccus*
			x						x		*Kurthia*
			x				x	x	x		*Lactobacillus*
			x			x	x		x		*Lactococcus*

Table 8 cont.

Solter et al., 1989	Berdela et al., 1994	Kaltenpoth & Steiger, 2014	Duarte et al., 2017	Shukla et al., 2017	Vogel et al., 2017	Shukla et al., 2018	Olmstead, 2018	Miller et al., 2019	Pratt, 2021 *Nicrophorus* core	Pratt, 2021, all samples	
		x				x	x				*Leucobacter*
			x								*Lysinibacillus*
							x		x		*Mesoplasma*
							x				*Micrococcus*
x	x	x		x		x	x		x		*Morganella*
			x				x				*Mycobacterium*
		x	x	x		x	x	x	x		*Myroides*
							x				*Neisseria*
			x				x				*Nocardioides*
			x								*Nosocomiicoccus*
						x					*Ochrobactrum*
			x								*Ohtaekwangia*
			x			x	x				*Paenibacillus*
							x				*Paenochrobactrum*
		x									*Parabacteroides*
			x			x					*Paracoccus*
						x					*Pedobacter*
							x	x	x		*Peptoniphilus*
x							x		x		*Peptostreptococcus*
x	x	x		x	x	x	x		x		*Proteus*
x	x	x	x	x	x	x	x		x		*Providencia*
			x	x	x	x	x				*Pseudomonas*
			x			x			x		*Psychrobacter*
		x									*Ralstonia*
							x				*Reyranella*
			x	x		x					*Rhodanobacter*
				x		x	x				*Rhodococcus*
							x				*Rickettsiella*
							x				*Romboutsia*
							x				*Roseiarcus*
			x								*Roseomonas*
			x								*Rothia*
								x			*Ruminococcus*
				x							*Sebaldella*
			x	x		x	x		x		*Sphingobacterium*
						x					*Sphingopyxis*
x	x		x	x	x	x	x		x		*Staphylococcus*
					x	x					*Stenotrophomonas*
x	x								x		*Streptococcus*
				x		x	x				*Streptomyces*
			x			x					*Tepidimicrobium*
		x	x		x		x	x	x		*Tissierella*
		x	x	x	x	x	x		x		*Vagococcus*
				x	x	x		x	x		*Vitreoscilla*
			x	x		x		x	x		*Wohlfahrtiimonas*
											...and 603 other genera

Table 9. Condensed comparison of significant genera present in other publications.

Solter et al., 1989	Berdela et al., 1994	Kaltenpoth & Steiger, 2014	Duarte et al., 2017	Shukla et al., 2017	Vogel et al., 2017	Shukla et al., 2018	Olmstead, 2018	Miller et al., 2019	Pratt, 2021 Nicrophorus core	Pratt, 2021, all samples
x	x		x			x	x			*Bacillus*
x	x		x	x	x	x	x		x	*Staphylococcus*
		x	x	x	x	x	x		x	*Vagococcus*
			x				x	x	x	*Lactobacillus*
x	x								x	*Streptococcus*
x	x		x	x		x	x	x	x	*Clostridium*
			x	x		x		x	x	*Wohlfahrtiimonas*
x	x	x		x		x	x		x	*Morganella*
x	x	x		x	x	x	x		x	*Proteus*
x	x	x	x	x	x	x	x		x	*Providencia*
		x			x		x		x	*Ignatzschineria*
x		x	x	x	x	x	x	x	x	*Acinetobacter*
				x	x	x		x	x	*Vitreoscilla*
		x			x	x	x	x	x	*Dysgonomonas*
		x	x	x		x	x	x	x	*Myroides*

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APPENDICES

APPENDIX A. List of 89 core bacterial genera shared across silphid secretion microbiomes.

Acinetobacter

Akkermansia

Candidatus Soleaferrea

Carnobacterium

Chryseobacterium

Clostridium sensu stricto 15

Corynebacterium

Cosenzaea

Desulfovibrio

Dysgonomonas

Enterococcus

Entomoplasma

Erysipelothrix

Escherichia-Shigella

Geobacillus

Halomonas

Ignatzschineria

Kurthia

Lactobacillus

Lactococcus

Mesoplasma

Morganella

Myroides

Oblitimonas

Peptostreptococcus

Proteus

Psychrobacter

Savagea

Sediminibacterium

Sphingobacterium

Sphingobium

Staphylococcus

Streptococcus

Tepidiphilus

Terrilactibacillus

Vagococcus

Vitreoscilla

Wohlfahrtiimonas

Bacillaceae unclassified

Carnobacteriaceae unclassified

Caulobacteraceae unclassified

Clostridiaceae unclassified

Comamonadaceae unclassified

Corynebacteriaceae unclassified

Dysgonomonadaceae unclassified

Enterobacteriaceae unclassified

Enterococcaceae unclassified

Erysipelotrichaceae unclassified

Lachnospiraceae unclassified

Micrococcaceae unclassified

Morganellaceae unclassified

Neisseriaceae unclassified

Nocardiaceae unclassified

Oxalobacteraceae unclassified

Planococcaceae unclassified

Pseudomonadaceae unclassified

Rhizobiaceae unclassified

Rhodobacteraceae unclassified

Ruminococcaceae unclassified

Sphingobacteriaceae unclassified

Sphingomonadaceae unclassified

Staphylococcaceae unclassified

Streptococcaceae unclassified

Wohlfahrtiimonadaceae unclassified

Peptostreptococcales-Tissierellales fa unclassified

Bacillales unclassified

Bacteroidales unclassified

Burkholderiales unclassified

Corynebacteriales unclassified

Enterobacterales unclassified

Erysipelotrichales unclassified

Flavobacteriales unclassified

Lachnospirales unclassified

Lactobacillales unclassified

Micrococcales unclassified

Oscillospirales unclassified

Peptostreptococcales-Tissierellales unclassified

Rhodospirillales unclassified

Alphaproteobacteria unclassified

Bacilli unclassified

Bacteroidia unclassified

Clostridia unclassified

Gammaproteobacteria unclassified

Actinobacteria unclassified

Firmicutes unclassified

Proteobacteria unclassified

Mitochondria ge

unknown unclassified

Bacteria unclassified

APPENDIX B. Nicrophorine secretion microbiome core bacterial genera, excluding the silphid secretion microbiome core. 20 of 109 genera.

Acidothermus	Budviciaceae unclassified
Conexibacter	Christensenellaceae R-7 group
Massilia	Devosiaceae unclassified
Nissabacter	Pectobacteriaceae unclassified
Orbus	Solirubrobacteraceae unclassified
Paeniclostridium	Burkholderiaceae uncultured
Peptoniphilus	Planococcaceae uncultured
Providencia	Oscillospirales UCG-010 ge
Thermus	Solirubrobacterales 67-14 ge
Tissierella	Clostridia UCG-014 ge

APPENDIX C. List of brood ball preparing *Nicrophorus* spp. secretion microbiome core bacterial genera excluding the silphid secretion microbiome core. 41 of 130 genera.

Acidothermus

Actinomyces

Brevundimonas

Burkholderia-Caballeronia-Paraburkholderia

Conexibacter

Empedobacter

Flavobacterium

Glutamicibacter

Leucobacter

Marmoricola

Massilia

Methylobacterium-Methylorubrum

Nissabacter

Orbus

Paeniclostridium

Peptoniphilus

Providencia

Pseudomonas

Stenotrophomonas

Thermus

Tissierella

Acetobacteraceae unclassified

Bradymonadaceae ge

Budviciaceae unclassified

Burkholderiaceae uncultured

Christensenellaceae R-7 group

Devosiaceae unclassified

Entomoplasmataceae unclassified

Flavobacteriaceae unclassified

Microbacteriaceae unclassified

Moraxellaceae unclassified

Nocardioidaceae unclassified

Pectobacteriaceae unclassified

Planococcaceae uncultured

Rhodocyclaceae unclassified

Solirubrobacteraceae unclassified

Weeksellaceae unclassified

Xanthobacteraceae unclassified

Oscillospirales UCG-010 ge

Solirubrobacterales 67-14 ge

Clostridia UCG-014 ge

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Candidate for the Degree of

Master of Science

Thesis: BACTERIAL COMMUNITIES IN CARRION AND BURYING BEETLE (SILPHIDAE) SECRETIONS

Major Field: Entomology and Plant Pathology

Biographical:

Education:

Completed the requirements for the Master of Science in Entomology and Plant Pathology at Oklahoma State University, Stillwater, Oklahoma in May, 2021.

Completed the requirements for the Bachelor of Science in Biology at the University of North Dakota, Grand Forks, North Dakota in 2018.

Experience:
Collection of arthropod tissues including microdissection
Performance of molecular biology laboratory techniques
Bioinformatic analysis of biological sequences and microbial genomes

Professional Memberships:
Entomological Society of America; Entomology Games Team Captain
EPP Graduate Student Association; Treasurer

Awards:
Fargo/Burton Graduate Student Fund Award, 2020 and 2021

Presentations:
2020 BMBGSA Annual Biological Sciences Research Symposium (Received 1st Place poster, 5th Place oral presentation)
2020 ESA Annual Meeting Virtual Student Competition
2020 ESA Joint North Central & Southwestern Branch Virtual Student Competition (Received 1st Place)