Winter 12-2018

THE BIOLOGICAL SIGNIFICANCE AND UTILITY OF FEEDING BY *DERMESTES MACULATUS*

Braymond Adams
University of Nebraska - Lincoln, adams.braymond@gmail.com

THE BIOLOGICAL SIGNIFICANCE AND UTILITY OF FEEDING BY *DERMESTES MACULATUS*

By

Braymond V. Adams

Presented to the Faculty of

The Graduate College at the University of Nebraska

In Partial Fulfillment of Requirements

For the Degree Master of Science

Major: Natural Resource Sciences

Under the Supervision of Professor Leon G. Higley

Lincoln, Nebraska

December 2018

THE BIOLOGICAL SIGNIFICANCE AND UTILITY

OF FEEDING BY *DERMESTES MACULATUS*

Braymond V. Adams, M.S.

University of Nebraska, 2018

Advisor: Leon G. Higley

With their efficient feeding habits and tolerance to very low moisture and humidity, beetles in the Family Dermestidae are especially adapted to variable environments and habitats. Dermestid cultures have been in use since 1922 in cleaning tissue and flesh from bones, and proven benefit in multiple fields, including zoology, ornithology, and forensics. Dermestid feeding behaviors when coupled with known life stage and insect succession information aids in providing significant entomological evidence. However, the feeding activities of insects, like those of vertebrate scavengers and predators, change remains and may leave artifacts that can be sometimes be difficult to assign to a cause. Given their eating habits, dermestids play a distinctive role in many habitats by feeding on dry animal tissue. Rather than representing a physiological adaptation to occupy a specific biome, the physiological preference *D. maculatus* has evolved seeming to show strong evidence of how they've niche specialized for a feeding guild, the carrion insects. The secondary objective stated as a separate study takes an in-depth look at trace marks. Our results from exposing fleshed bones to *D. maculatus* adults and immatures for almost two months after complete tissue removal, indicates that under natural conditions *D. maculatus* do not feed on bones.

Table of Contents

CHAPTER 1. INTRODUCTION AND LITERATURE REVIEW

Introduction

One fifth of all described species on Earth are members of insect Order Coleoptera, the beetles. Beetles have an astonishing range in form, body size, feeding preferences, and ecological activity. They exist at nearly every trophic level above the base in the food chain. Beetles remove millions of tons of waste and organic matter that would otherwise accumulate and foul the environment. They aid as both pollinators and defense for plants. As both nest dwellers and parasites they serve as natural biocontrol agents proving useful in checking mites, lice, scale insects, and other ectoparasites. Possibly one of the most famous quotes regarding beetles comes from the population geneticist J.B.S. Haldane, who was asked what might be learned about a Creator by examining the world. He responded saying, "an inordinate fondness for beetles" (Hutchinson 1959).

In a more significant sense, the most common aspect of beetles is not some aspect of their structure or natural history, but in their sheer numbers - which has prompted some biologists to declare that we live in the age of beetles (Fisher 1988). Currently, 350,000 beetle species are described, and, not surprisingly, they include adaptations for almost all habitats and habits in which insects can occur. Adaptations like flight, exoskeletons, successive metamorphosis, glycerol transport, and intrinsic adaptability to environments are shared with nearly all insects. However, many beetles have exploited the ability to colonize highly diverse areas. This can be magnified when there is a close

association with people. One such group of beetles which capitalized this association are animal decomposers, in the Family Dermestidae.

Insects and Animal Decomposition

The decomposition of both plant and animal matter is a crucial ecological process. However, animal decomposition doesn't get a lot of consideration due to the vast amount of carbon released in plant material; natural organic matter is significant in animals for reasons such as nutrient cycling, the creation of top-soils, resource availability within trophic levels, and associations with lipids, micro nutrients, and various environmental services.

Decomposition also has practical applications and utility. One such application is the art and aptitude of taxidermy, which often calls for the use of dermestid beetles in colony and caters professionally, to museums and the general public. Another and, possibly more significant, association can be found within the broad field where arthropod science and the judicial system interact. This area is forensic entomology. As important mediators in this process, insects play an extremely vital role.

Usually, we rightly think of flies as the most common agents in the decompositional process next to bacteria. In fact, "because of the association with flies, which are of broad importance to human and animal health, and the ecology of decomposition, the field of medico-criminal entomology is rightly recognized as a specialty within medical entomology itself" (Byrd 2001). Similarly, dipterans are found in virtually every conceivable aquatic environment and, in fact, may be the only insects in freshwater habitats with extreme environmental conditions. (Byrd 2001).

What separates beetles from flies and other insects associated with decomposition? Beetles can take multiple ecological roles (predator, detrivore, fungivore, saphrovore, and even herbivore) in association with dead animals. Typically, beetles are not as rapid as exploiting dead tissue as are flies, but beetles are the only group of insects that can occur from immediately after death to the very latest stages of decomposition. One specific role played only by dermestid beetles (and some mites) is the consumption of very dry tissue. With the ability to rapidly colonize under xeric conditions, the dermestids have the capacity to deflesh a carcass at a remarkable pace. The near total removal of all soft tissue from a carcass or cadaver is perhaps the most unique aspect of their fundamental behaviors.

Two key groups of insects are predictably drawn to cadavers and provide the majority of information in forensic investigations: the beetles and the flies. Both groups are diverse, although the beetles have far more known species than any animal group, insect or otherwise (Morse 1988). Flies are much less diverse with nearly 86,000 described species worldwide, of which 16,300 occur in North America (Byrd 2001). Many other types of arthropods are found in association with bodies, many of which are opportunistic feeders who take advantage of the environments and resources. These types are often made up of both scavengers feeding on decaying material and predators feeding on the species that have colonized the carrion.

Both beetles and flies have distinguishing external structures and develop in significantly different ways. In many areas, dermestid beetles (Coleoptera: Dermestidae) are considered to be very late colonizers, frequently arriving when only skin and bone

remains, sometimes months after death (Easton and Smith, 1970 as cited in Byrd 2001; Fuller, 1934; Payne and King, 1970; Reed, 1958; Rodriguez and Bass, 1983; Smith, 1986). In Hawaii, however, some adult dermestid beetles were collected as early as 3 to 10 days after death (Early and Goff, 1986; Hewadikaram and Goff, 1991). In coastal British Columbia, dermestid larvae were first collected from pig carrion in exposed pasture 21 days after death, during the early stages of advanced decay. The majority was collected more than 43 days after death (Anderson and VanLaerhoven, 1996). In the northern region (sub-boreal spruce biogeoclimatic zone) and the interior region of British Columbia, dermestid adults were first found on pig carcasses as early as the bloat stage, and larvae were found by the decay stage (Dillon, 1997; Dillon and Anderson, 1996a). During the summer in the interior region, dermestid larvae were found as early as the bloat stage, but this was rare (Dillon, 1997; Dillon and Anderson, 1996a).

Taphonomy, Decompositional Ecology, and their Application

There are many diverse parameters that can affect the timing and species composition of the carrion fauna. For these reasons it is vitally important to be aware of all the factors that can impact insect colonization of remains and to take them into account. In general, one might expect a predictable sequence of insect colonization as carcasses and carrion go through the stages of decomposition. These stages, signifying increasing destruction commonly have stage-specific agents insects species which arrive throughout the decompositional process. As a comprehensive example, burial influences the time required for insects to reach the remains, the sequence of colonization, the species involved, and the rate of decomposition (Payne 1968). Likewise, different areas

where carcasses are found has influence: as the insect colonization of the remains (Goff and Lord, 1994) noted that suspended carcasses altered the insect colonization by excluding soil-dwelling taxa, thus changing the drying pattern of the body and, consequently, limited the activities of (insect) species. When collecting insects in my free time as a hobbyist I have personally made this observation when hanging carrion and bait. This reduced the numbers of insects collected and influenced which species colonized the remains as well as their times of colonization (Goff and Lord, 1994). Added factors including time, season, soil type and region have a direct effect on arrival times of insect species. These variables have additional influence on colonization and therefore feeding efficiencies.

Taphonomy. Higley and Roe (2018) offer a summary of key processes in decomposition and their use in forensic science, that I repeat here:

> The area of forensic science which addresses decomposition (typically in homicides) is called forensic taphonomy, and forensic entomology (the study of insects as they apply to the law) has applications within forensic taphonomy. Consequently, some forensic entomologists focus exclusively on insect biology, while others work as forensic taphonomists and entomologists.
>
> The process of human decomposition involves internal and external factors, but all processes before skeletonization depend on temperature. Internal processes include autolysis (the breakdown of cells and tissues from enzymes present in the body) and putrefaction (the breakdown of cells and tissues by bacteria and fungi). These processes are manifested by specific visual changes in the body. External processes include feeding on a body by insects or by various vertebrate scavengers. Seasonally when flies, beetles, and other associated arthropods are present and temperatures are adequate for insect flight, decompositional insects…especially blow flies (insects in the family Calliphoridae) can rapidly find and lay eggs on dead animals, including dead humans. Flies can occur only minutes of death, more commonly oviposition occurs within the first couple hours when flies have access to a body. Delays in oviposition may happen when bodies are indoors,

> wrapped, or under various other specific conditions. Also, blow flies do not lay eggs at night.
>
> Estimating the postmortem interval (PMI) when insects are found on a body, involves five key considerations: (1) the species of insect found on the body, and (2) the stage or age of the insect on the body, (3) temperatures at the body from the time of death to the time of discovery, (4) factors associated with delays in insect oviposition, and (5) other (non-entomological) taphonomic indicators of PMI. Insect development depends on temperature, and the relationship between temperature and development differs based on insect species and stage. To determine how long it took insects to reach the stage found at the time of discovery, calculations are made based on scientific data from different insect species and stages, and these calculations also must rely on estimates of death scene temperatures. Because of uncertainties in these factors, as well as possible variation in when eggs were first laid, calculation of a PMI always represents an estimate. Because other taphonomic indicators are independent of insect activity, comparing entomological findings to other time of death indicators is useful in supporting or refining the range of a PMI estimate.

Because they have proven so useful in estimating PMI, most research in forensic entomology focuses on blowflies. In contrast, many issues of forensic and ecological interest remain unknown regarding dermestids, such as feeding rates, feeding differences based on diet characteristics, and distinguishing dermestid feeding from traces left by other agents.

Dermestid Biology

The Family Dermestidae is a group of insects within the order Coleoptera comprising of over 800 species with worldwide distribution. Commonly referred to as carpet, hide, larder or even bacon beetles, dermestids are generalist scavengers who show up on carcasses and can be found various habitats. These insects feed on anything ranging from dried plant and animal tissues to stored grain and fibers. Within this niche however, there is an array of ecological variation ranging from free-living *Dermestes*, which feed on animal carcasses, to generalist scavenger inquilines of spider, insect, bird, and rodent nests, and specialized myrmecophiles in the Thorictini, a monovoltine tribe associated with differential diagnosis in related dermestid species (Kiselyova and Mchugh 2006). These insects are even known to play the role of parasites

in certain instances when infesting the egg cases of mantids (Dresner 1970). Dermestids are known most for their reputation of consuming stored materials and in unfortunate occurrences museum specimens. Along with destruction of property and the characteristic occurrence of a ruined insect collection, these beetles present the threat of food spoilage contamination which can total millions of dollars per year worldwide (Mroczkowski 1968). Even so, their fiber and protein-eating habits constitute both nuisance and value; lending nutrient to cycling and other benefits in ecosystems, thus highlighting their role in decomposition.

Based on their food preferences members of this family also have been given the common names of hide beetles, carpet beetles, and larder beetles. In taking a deeper look into their biology we see that both adults and larvae can feed on nearly all organic matter including dried and stored grain. Dermestid distributions are worldwide are worldwide with over 500 species, approximately 123 of which are found throughout North America (Byrd 2001). Yet, despite the fact they are ubiquitous and quite common, there is an astonishing lack of detailed information known about their feeding habits and behavior.

The body structures and developmental cycles specific to *D. maculatus* shall be discussed in the remainder of this section.

Lifecycle, Diet, and Reproduction. The varied species in this family live in multiple habitats, provided there is a reliable resource available. They are able to digest wool and silk, as well as plant products like cotton. However, being opportunistic feeders, food sources can include anything from keratin, the structural proteins in skin, hair, nails, and claws, to muscle and connective tissues. Although many adult species feed on pollen and other plant tissues, they have well defined mouth structures and mandibles which are specialized for both chewing and boring into meat, animal hides and surfaces. The hind femora fit into recesses of the coxa. After emerging from the pupal stage, adults begin to eat within as little as 36-72 hours. An aggregation

pheromone in the feces of adult hide beetles facilitates the congregation of adults and larvae on food sources (Jaskulska et al 1987; Rakoski 1997). Mediated by this pheromone, the clustering and recruitment of the adult beetles leads to sexual reproduction being initiated. This attraction is defined as the net displacement of one individual toward the chemical pheromone (Jaskulska).

In ideal conditions (i.e., appropriate photoperiod), males stimulate egg production in receptive females. Eggs are laid within the third day, and hatch within 3- 4 days on or near a food source. The ability to deposit varies among induvial females ranging anywhere between 1- 72 eggs in a 24-hour period with averages of about 450 viable eggs in a period of 100 days of life (Russell 1947). After three days (on average), the eggs hatch, and larvae emerge. Larval sizes depend on both instar and species with *D. maculatus*, generally ranging less than 1 millimeter. In the following 15 to 20 days larvae spend most of their time going through rapid growth and feeding. Under ideal circumstances this developmental stage spans between 18 and 20 days. Larvae molt through 8 to as many as 16 instars feeding throughout their growth. The morphology of dermestid larvae is similar to that of other beetles within the superfamily Bostrichodia with the exception of distinctive setae which span the length of the body (Beal 2003). Larvae are scarabaeiform and have three pairs of well-defined thoracic legs which allow easy climbing and mobility. Like adults, they have a sclerotized head, chewing mouthparts, and boast a set of well-defined mandibles which serve them well in their role in decomposition. After their final molt, larvae tend to migrate in search of an innocuous place to pupate. If habitat permits, time is spent excavating a chamber within wood, bone, meat or any available substrate which can provide some degree of protection. Once chambered, pupal stages last between seven to eight days with the larval skin serving as defense during metamorphosis.

Dermestids reproduce quickly which lets large populations form in a hurry as the need for such arises. Being relatively long-lived they are able to survive long periods without food and

periods of near desiccation. Because the beetles are typically found on dry carrion it is assumed that the feeding preferences are exclusively dry tissues. In truth, they are associated with dry carrion through succession and in natural settings prevented from feeding on moist meat by competition from the masses of fly maggots largely (Russell 1947). Blowfly maggots quickly consume soft tissues on most fresh carcasses. But, when the carcass dries to a point where maggots cannot survive, the dermestids typically become the most prominent insect decomposer. Also, because dermestids don't readily feed on skin, egg laying is often delayed until other carrion feeders have opened the hide making the resource more suitable to their needs.

Dermestids vary greatly in the length of their life cycles, with some species going from egg to adult in 6 weeks, and others taking as long as a year or more to complete development. In many insects including dermestids, cuticle secretion and the molt cycle are controlled by ecdysone, a steroid hormone. This hormone is secreted by a gland in the thorax, which is in turn controlled by a hormone from the brain (Jaskulska et al 1987; Rakoski 1997). Whenever the brain receives the appropriate stimulus, new cuticle forms beneath the existing cuticle. This splits the exoskeleton allowing the larva to stretch and emerge. Through time (usually within an hour) the cuticle expands to a new volume as the new exoskeleton hardens.

Cannibalism. Cannibalism or intraspecific predation is defined as the act or behavior of an animal that feeds on others of its own kind. The role of cannibalism in the dynamics of natural populations has been largely neglected; the most elegant and detailed analyses of the population consequences of cannibalism are still provided by laboratory studies of flour beetles (Tribolium) that describe the process, examine its interactions with other population processes, and attempt to derive generalities about its effects (Fox 2003). Although not the main food source for a large majority of insects, cannibalism may occur in any or all of the life stages though it is generally found to be more common in the larval stage. This specific behavior has evolved and become

common place due to pressures akin to lack of food, increased fertility, and inter- and intra-species competition. Also used as a means to control population density cannibalism suppresses populations in certain species (Richardson et al. 2010). In speculating why we commonly saw this behavior several categories of cannibalism are addressed in regard to challenges we faced during this study:

Filial cannibalism occurs in many animal species ranging from mammals to insects, and is especially prevalent in numerous species of fish. Not much is known regarding the exact purposes of this type. However, it is largely believed to have important evolutionary and ecological implications for some species, and is an important source of mortality for various other species.

Total or entire clutch cannibalism occurs when a parent or dominant sibling consumes the entire brood. This scenario usually occurs when a brood or sibling is smaller or of lesser quality. The most obvious purpose of total or whole clutch cannibalism is the termination of care for the parents. The main benefit of this action can only be an investment in the future reproduction of potentially larger or healthier broods (Fox 2003).

Partial clutch cannibalism occurs when a parent consumes a part of its offspring. "Parental manipulation of brood size may allow the parent the maximize lifetime reproductive output by adjusting current reproductive costs in favor of future survival and subsequent opportunities for reproduction (Fox 2003)." Unlike total or whole clutch cannibalism, partial clutch cannibalism invests in both current and future reproduction. Male parents, particularly male fish, may eat some of their offspring to further his current parental cycle, and remain in sufficiently good condition to engage in further breeding cycles (Fox 2003).

Additional benefits of cannibalism may also serve to satisfy ongoing energy or nutrition requirements among broods. This aspect has been shown to place direct evolutionary pressure on offspring promoting quicker development while eliminating individuals which take longer to mature. This method of natural selection removes weaker offspring through purging among siblings in an overproduced brood. It is a form of which is theorized to make the other offspring more likely to be successful. Similarly, among adult dermestids recouping reproductive investment in an unfit reproductive environment may increase the reproductive rate of a parent by making that parent more attractive to potential mates.

Through the use of microscopic imaging and identifying jaw marks made by larvae on their siblings (Kiselyova 2006) we would be able to confirm with certainty what we were beginning to speculate. However, in the trials we ran we had to rely more so from the losses recorded among treatments as individual larvae unaccounted for were speculated to be entirely consumed. This especially held true when there was any size difference among induvial larvae. More than likely, social factors consistent with competition among a species for resources, and dominance are all promoters for this issue. In a study on members from the Order Hymenoptera, tests performed have shown that by consuming their brethren, the lifespan of adult males is increased by 1.5 days, a long time for a short-lived insect (Deyrup et al. 2006). While this may not be exact in the case of dermestid larvae, it is worth some degree of speculation that this finding, along with the others mentioned have a definitive impact on their feeding in natural ecology.

D. maculateus. Dermestid beetles belong to the Class Insecta and fall within the Order Coleoptera. This grand order is split into two separate suborders with dermestids being placed within the group Polyphaga. This is the largest and most diverse suborder of beetles (approximately 90%) comprised of nearly 144 families among 16 superfamilies, and has an

enormous variety of specialization and adaptation (Kiselyova 2006). The Dermestidae is currently placed in the Superfamily Bostrichoidea, the type superfamily of the Infraorder Bostrichiformia which includes the Families Bostrichidae (powder post), Anobiidae (deathwatch), and Ptininae (spider) beetles. The dermestids are a relatively stable group; species are well known and recognized within their global distributions since first described and formally cataloged in 1943 (Beal & Zhantiev, 2001). Generally, the family is regarded as "a well-defined", monophyletic group; this status is accepted by default, as it is untested by cladistic analysis (Beutel 2000). Zhantiev's (2000) phylogenetic analysis includes all recognized and supposed dermestid taxa with characters polarized a priori based on general evolutionary tendencies and morpho-functional analysis (Kiselyova 2006). Most closely related to sister groups Bostrichidae, Anobiidae and Ptinidae, the family's closest relatives include the for mentioned auger and powder post beetles *Amphicerus cornutus*, wood boring beetles *Hedobia imperialis*, and spider beetles *Gibbium scopoli*; each belonging to their respective families.

Several taxonomic schemes have been proposed for the Family Dermestidae. Most notable is the classification proposed by Beal (1959) which is considered the simplest and the least controversial. Ivie (1985) removed *Orphilus*, a genus of beetles native to the Palearctic (including Europe) and the Near East from Dermestidae and proposed it to be a sister taxon to Nosodendron, an important hypothesis later rejected by Beutel in 1996. Despite this work, there remains arguments in revision of this family. The most recently proposed, least controversial and easiest to follow scheme is that proposed in Beals's (2003) Notes on the biology and systematics of the dermestid beetle genus *Apsectus* which places this family firmly between sister groups Marioutini and Egidyellinae as recently as 2001 in the Infraorder Bostrichiforma where biogeographical data suggest traces of a Pangean distribution (Beal & Zhantiev, 2001). This revision dates the origin of this family as far back as the Permian period.

Anatomy. It is generally accepted that the insect predecessor to the insect was elongated, roughly cylindrical, and segmented with paired appendages on each segment. Through time, segments grouped together into functional regions on the insect's body. Three such regions have resulted and are represented on the insect body as the head, thorax, and abdomen. The body wall of an insect called its exoskeleton serves two functions. Internally the exoskeleton provides points of attachment for the muscles. Externally, this *shell* provides a means for protection by way of a hard, outer layer called the cuticle. This aids in the bodies of insects persisting in the environment for lengthy periods of time. Rather than being one continual shell, this skin is composed of many hardened plates which are separated from one another by seams, sutures, and in certain instances by larger membranous areas such as gaps between body segments. With varying degrees of rigidity such as the jaws, or the wing covers in most beetles, tissues are said to be heavily sclerotized. The more lightly sclerotized or membranous areas of the body are those which allow insects range of movement and degrees of flexibility. Both biology and behavior coincide with morphology among insects. Form meets function directly among beetles. Whether or not an induvial is hard or soft bodied is usually determined by many factors primarily that being the habitat in which a species resides. For example, adults such as fireflies, soldier beetles and meloids tend to have soft and flexible elytra and bodies more conducive to sustained flight of distance. Whereas, the robust bodies and exoskeletons of ground beetles, tenebrionids, and scarabs varies from very stiff and rigid articulations meant for protection and sturdiness. In each case the adult beetle form usually consists of hard plates (sclerites) separated by flexible membrane. Occasionally, an insect may spend what might be considered the greater part of their being in the adult life stage and are therefore suited for mating and reproduction. Inversely and in a broader sense, larvae tend to exhibit softer bodies, sclerotized heads, chewing mouthparts and pronounced flexibility. Like adults, the differences in body form of the larvae are closely associated with larval habitats and modes of feeding - ideal for burrowing, forward directed

movement, and generally up taking as much nutrition as readily available. Therefore, a great variation may exist from one life stage of an insect to the next.

Broadly speaking, dermestid species possess oval shaped bodies covered in both fine scales and bristly setae. Most species in this family have clubbed antennae which fit into deep grooves or recesses near the head. This is no different in this species. However, there are key characteristics of *D. maculateus* which separate them from other species within the genus. The adults range from 5 to 10 mm in length and vary from black to reddish brown on the dorsal surface. They also feature characteristic black and white markings on the ventral surface of their elytra. The apex of the elytra are serrated or saw-toothed and merge in a terminal point. The larvae of *D. maculatus* are typically dark brown and have a broad light brown to yellow streak which extends lengthwise along the entire body. They are easily identified by the fact that the spines near the tip of the tail curve forward, towards the head of the larvae (Byrd 2001). This and other species of dermestid larvae are covered with tufts of prominent bristle hairs which differentiate them from other carrion eating taxa in the genus.

Development. All insects pass through a series of stages when developing from egg to adult. The appearance of these stages and the time spent within each varies among species and environmental conditions that are present. The process of undergoing physical changes from one life stage to the next is known as metamorphosis. This is accomplished by means of the insect undergoing ecdysis. This process is analogous to molting or shedding at intervals as it grows and develops. For growth to take place, the exoskeleton must be shed and a new one formed. Initially soft, the body is then expanded before the new one hardens. The larvae can then grow to fill the space created before molting again becomes necessary. Cast skins left behind are called exuviae. The time period spent in any particular life stage is referred to as a stadium or larval instar. Lastly, the insect itself also may be called an instar, especially during larval development. The

most involved and complex growth pattern of development among insects is called holometabolous or complete metamorphosis. Like all beetles, dermestids are equivalent to all holometabolous insects with four distinct life stages: egg, larva, pupa, and adult.

Adults. There are certain characteristics ubiquitous to all beetles; three main body segments, a single pair of antennae, a thorax – which houses two pairs of wings and appendages, compound eyes, etc. Adult dermestids are no exception with these features common to all beetles. More specifically however, they are characterized by having hard wing covers called elytra which serve as general protection while concealing the membranous hind wings used for flight. Generally small beetles, ranging from 2 to 12 mm in length. They are rounded to oval in shape and covered with scales that may form distinctive and patterns. The adult beetles possess chewing mouthparts and most have the ability to fly. Their feeding habits vary greatly among habitats; like larvae they possess powerful enzymes which enable them to easily digest things *uncommon*. However, the expanded preferential (or indifferent) functional role of adult feeding is generally to reproduce on or near a food source.

Larvae. The larvae of D. *maculatus* are dark brown and have a broad light brown to yellow stripe extending lengthwise along the body. They are easily identified by the fact that the spines near the tip of the tail curve forward, towards the head of the larvae (Byrd 2001). Larvae are covered with tufts of long dark hairs and range from size 1 to 15 mm depending on both instar and species. They are further characterized by their two, long, horn-like protrusions are located on the upper surface of the last segment, partially hidden by surrounding hairs (Haines and Rees 1989). The protrusions, called urogomphi, have an ascending curvature away from the tip of the abdomen. This distinguishes the larvae from larvae of *Dermestes lardarius*, the most common in appearance within the genera which having downward curving urogomphi toward the tip of the abdomen. In natural habitats they are typically found on during the dry, skeletal stages of decomposition. In

the following 15 to 20 days larvae spend most of their time going through rapid growth and feeding. They move away from light and will hide in any cavity or recess that is available when disturbed or a significant change in ambient light or shadow is detected. The presence of dermestid beetles or their sawdust-like fecal material termed frass is often an indication that considerable time has elapsed since death (Byrd 2001). The mere presence and amount of frass is evidence that dermestid beetles have fed at a certain length on tissues. In some cases where remains are mummified, living dermestid adults and larvae may still be associated with the remains after a period of years (Byrd 2001).

Both adults and larvae in the Family Dermestidae produce a protective membrane that lines the digestive tract and surrounds their food meal. Considering the varied environments and diets these creatures live in and eat, this lining serves to protect the gut walls from abrasion during the digestive process. As digested food is passed from the beetle's body, the fecal material is enveloped in the protective membrane, which passes in an unbroken tube-like form. This resulting frass material that is passed is light brown, quite dry, and brittle- crumbling very easily when disturbed.

Current and Future Roles of Dermestids in Forensic Science

Over the last decade there has been a resurgence of interest in the estimation of postmortem interval (PMI) by entomological methods. Although, various combinations of data from laboratory rearing of selected species and data from decomposition studies have been used, remarkably, the majority of work which has been reported concerns the insect fauna which infests the human corpses recovered in earlier stages of decomposition (i.e., within first few weeks) (Kulshrestha 2001). Considering the need to speedily resolve cases in forensics involving crime, sensitive evidence, and legality this comes as no surprise. However, limited information is presently available about the insect fauna encountered on corpses in the later stages of

decomposition (i.e. within the first 3-6 months) (Kulshrestha 2001). Undoubtedly, in such cases, particularly where dry human skeletal remains were recovered in the later stages of decomposition, the Coleoptera and associated families, including Dermestidae, compromise the main evidence for determining PMI. Along with the Family Cleridae, the Dermestidae have been found as the most common types of beetles infesting exposed human remains and providing evidence in estimating the minimum postmortem interval (Kulshrestha 2001).

After the bulk of the biomass of a carcass has been consumed by other insects dermestids may be found in large numbers. In these cases where bodies have been decaying for extended periods it might be assumed that they have a distinct preference for drier feeding material. However, dermestids can be introduced artificially in the laboratory to clean soft tissue from bones. This has the advantage of resolving fine damage in situations where a more aggressive method of flesh removal may cause damage to the bone itself. The process is a natural one which, for example, will clean the tissue from human vertebrae within a few days. In many cases that involve extensively decomposed or mummified human remains, skin tissue often remains intact. The remaining tissues often contain many holes and they are sometimes mistaken for gunshot wounds (Byrd 2001). The feeding maggots usually produce symmetrically round holes that are of a uniform size. Dermestid adults and larvae often create the same symmetrical artifacts. However, they also produce holes that are irregular in size, as well as tears that resemble lacerations and abrasions. The edges of these artifacts are often irregular and jagged, not smooth as with those produced by feeding maggots (Byrd 2001). Additionally, the substrate dermestids bed in while feeding is a direct indication of their presence. Being a combination of debris from feeding, fecal matter, shed molts and their peritrophic membrane, this is called frass. In itself, dermestid frass and the associated peritrophic membrane is valuable entomological evidence since it indicates the conditions that were likely present throughout the decomposition process (Byrd 2001).

Although dermestids are often thought to be late colonizers, frequently arriving when only skin and bone remains, sometimes months after death in many areas (Byrd 2001), dermestids have been both observed and collected as early as 3 to 10 days after death (Early and Goff, 1986; Hewadikaram and Goff, 1991). One observation noted that in coastal British Columbia, dermestid larvae were first collected from pig carrion in exposed pasture 21 days after death, during the early stages of advanced decay. In our own observations, beetles have similarly been seen within several to three days feeding on tissues which were not only moist, but consistent with a freshly killed carcass.

"In sufficient numbers, they have been reported as reducing a human body to a skeleton in only 24 days." (Byrd 2001)

Microdamage. There is a great amount of contradictory information in the scientific literature which suggests that dermestids use bone and leave marks on bone tissue. (Parkinson 2012). In large measure, this question has been addressed and answered, but details for other species and specifics remain unresolved. In particular, does a lack of marks extend to all dermestid species and all types of bone? Although it is not likely appreciated whether these marks are species specific, it is important to look at *D. maculatus* because it is a ubiquitous species, commonly used in taxidermic preparation, forensic investigation, and associated studies. The most common species within this Family is Dermestidae and in studying this organism several questions that have emerged are, "do they truly have a preference for dry versus wet tissues and is there some variability on this?" Secondly, in feeding on nearly all available organic matter and as contemporary literatures tend to debate, we ask "are there signs of their feeding on that can occur bone?"

Research Objectives

Given their eating habits, dermestids play a distinctive role in many habitats by feeding on dry animal tissue. This habit lead to various practical implications in areas such as entomotoxicology, agriculture, medicine, and forensic science. Comprehensive evidence on the extent of these roles and functions can be limited or even contradictory for various species. Furthermore, the vagueness in our understanding of areas such as feeding behavior, limit the usefulness of dermestids in fields like forensic science. Further work detailing consumption rates, dietary preferences, and potential bone modifications from dermestid feeding, would help eliminate some of the existing barriers to greater usefulness of dermestids in forensic science and would potentially improve our understanding of ecological contributions by dermestids.

The primary objective in this study was to establish larval and adult consumption rates of both dry and rehydrated pig tissues in seeing if there were differences. The secondary objective stated as a separate study take an in-depth look at trace marks.

References

Beal, R. and Zhantiev, R. 2001. A new California species of EGIDYELLA Reitter (Coleoptera: Dermestidae), a genus previously unknown in the New World. The Coleopterist's Bull. 55: 70-74.

Beal, R. 2003. Annotated checklist of nearctic Dermestidae with revised key to the genera. The Coleopterist's Bull. 57: 391-404.

Beutel, R., and Haas, F., 2000. Phylogenetic relationships of the suborders of Coleoptera (Insecta). Cladistics. 16: 103–141.

Byrd, J. and Castner, J. 2001. Forensic entomology. The utility of arthropods in legal investigations. CRC Press, Boca Raton, FL.

Dresner, E. 1970. A Dermestid (Coleoptera) infesting mantid egg pods. Annals Entomol. Soc. Amer. 63: 1477–1478.

Fisher, R. 1988. An inordinate fondness for beetles. Biological J. Linnean Soc. 35: 313-319.

Fox, L. 2003. Cannibalism in natural populations. Annu. Rev. Ecol. Systematics. 6. 87-106.

Greenberg, B. 1991. Flies as forensic indicators. J. Med. Entomol. 28: 565-577.

Higley, L. G., and Roe, A. . 2018. Forensic analysis report template. Death and Decompositional Sciences, LLC. Lincoln, NE (unpublished document).

Hill, D. 1994. Agricultural Entomology, Timber Press, Portland, Oregon.

Hinshaw, S. II. 2003. Use of dermestid beetles for skeleton preparation. http://deepwater.org/bioteacher/11-Ecology/dermestids/colony-maintenance.htm

Hutchinson G. 1959. Homage to Santa Rosalia or why are there so many kinds of animals? The American Naturalist. 870: 145-159.

Jaskulska, B., Rakowski, G., and Cymborowski, B. 1987. The effect of juvenile hormone on aggregative behaviour of *Dermestes maculatus*. Comp. Biochem. Physiol. 87A: 771-773.

Kiselyova, T., and J. Mchugh. 2006. A phylogenetic study of Dermestidae (Coleoptera) based on larval morphology. Systematic Entomol. 31: 469-507.

Lata, G., Pathak, R., Danlami, H., and Cole, M. 2013. Entomotoxicology: alternative matrices for forensic toxicology. https://www.forensicmag.com/article/2013/08/entomotoxicology-alternative-matrices-forensic-toxicology

Morse, D.R., Stork, N.E., and Lawton, J. H.1988. Species number, species abundance and body length relationships of arboreal beetles in Bornean lowland rain forest trees. Ecological Entomol. 13: 25-37.

Mroczkowski, M. 1968. Distribution of the Dermestidae (Coleoptera) of the world with a catalogue of all known species. Warszawa. 26: 15-191.

Munro, J. (1966). Pests of stored products. Hutchinsons and Co. London, England.

Panagiotakopulu, E. 2000. Archaeology and entomology in the eastern Mediterranean. Research into the history of insect synanthropy in Greece and Egypt, British Archaeological Reports, International Series No. 836.

Panagiotakopulu, E., 2001. New records for ancient pests: archaeoentomology in Egypt. Journal of Archaeological Science. 28: 1235–46.

Parkinson, A. H. 2012. *Dermestes maculatus* and *Periplaneta americana*: bone modification criteria and establishing their potential as climatic indicators. M.S. Dissertation, University of the Witwatersstrand, Johannesburg, South America.

Rakowski G. 1988. Effect of illumination intensity on the response of the hide beetle, D*ermestes maculatus*, to aggregation pheromone. J. Insect Physiol. 34: 1101-1104.

Rowe, A. 2014. Development modeling of *Lucilia sericata* and *Phormia regina* (Diptera: Calliphoridae). Ph.D. dissertation. University of Nebraska-Lincoln, Lincoln, Nebraska.

Russell, W. 1947. Biology of the dermestid beetle with reference to skull cleaning. Journal of Mammalogy. 28: 284.

Schroeder, H., Klotzbach, H., Oesterhelweg, L.,and Puschel, K. . 2002. Larder beetles (Coleoptera, Dermestidae) as an accelerating factor for decomposition of a human corpse. Forensic Sci. International. 127: 231-236.

Sawaby, R., Hamouly H. and Abo-El Ela, R. 2016. Taxonomic study of the main families of Egyptian Coleoptera with forensic importance. Life Sci. Journal. 13: 39-53.

Valdes-Perezgasga M., Sanchez-Ramos F., Garcia-Martinez O., and Anderson G. 2010. Arthropods of forensic importance on pig carrion in the Coahuilan semidesert, Mexico. J. Forensic Sciences. 55: 1098-1101.

Zakka U., Ayertey J., Cobblah M. 2012 Development of *Dermestes maculatus* (DeGeer, 1774) (Coleoptera, Dermestidae) on different fish substrates. Jordan J. Biological Sciences. 6: 5-10.

Zanetti, N., Visciarelli, E., and Centeno, N. D. 2016. The effect of temperature and laboratory rearing conditions on the development of *Dermestes maculatus* (Coleoptera: Dermestidae). J. Forensic Sciences. 61: 375-381.

CHAPTER 2. WATER, FEEDING, EFFICIENCIES AND DEVELOPMENT OF DERMESTES MACULATEUS

Introduction

"Thousands have lived without love, not one without water." − W. H. Auden (1959*)*

Dermestid beetles are unique among decompositional insects because of their consumption of dry tissue. Most insects decomposing animals either feed on soft tissues (e.g., blow flies, flesh flies, filth flies, carrion beetles, cheese skippers, and coffin flies), are predators on decompositional insects (e.g., rove beetles, clown beetles, and wasps), or are drawn to animal carcasses because of an associated change in environmental conditions (often increased humidity) (e.g., springtails) or the need for salts (e.g., butterflies).

The varied species in this family live in multiple habitats, provided there are reliable resources available. Dermestids vary greatly in the length of their life cycles, with some species going from egg to adult in six weeks and others taking as long as a year or more to complete development. Like all holometabolous insects, dermestids have four life stages (egg, larvae, pupa, and adult), with a variable number of larval stages but typically 5-6. While many beetle species have long generation times, after one year or longer, many dermestids can complete a generation in weeks to a couple months, which lets them better use temporary resources. However, like other beetles dermestids are relatively long lived (weeks to months) as compared to many other insects.

For most dermestids larva size ranges between less than 1 millimeter after hatching and up to 12mm pre-pupation. In the 15 to 20 days following hatching, larvae spend most of their time going through rapid growth and feeding. Under ideal circumstances this developmental stage

spans between 18 and 20 days. Dermestids have different numbers of molts depending on species, and within species some may have variable numbers of larval molts (e.g., 5-9 based on rearing temperature in *Dermestes alter*) (Bujang and Kaufman 2010) , facultative diapause (dormancy) (in *Trogoderma granarium*), and even a rare variation in metamorphosis called retrogression (also in *Trogoderma granarium*) arising from limitations in food availability, such as through competition (Beck, 1971). Retrogression is a form of "reverse molting" in which the individual has reduced weight and size after molting and requires additional molts to return to a normal developmental sequence based on limitations in food availability, competition, and completion of other larvae during development.

Although dermestids have varying ecological roles, most in the *Dermestes* are generally associated with decomposition of dry animal tissues (Parkinson 2012), with *Dermestes maculatus* probably the most ubiquitous in North America. Because these beetles are typically found on dried carrion, it is generally assumed that their feeding preference is exclusively for dry tissue. Indeed, dermestids, including *D. maculatus*, are regarded as specialists on dry tissues in the context of the array of insects feeding on dead animals (Smith 1986.) Additionally, *Dermestes* can survive long periods without food and through periods of near desiccation. However, when used for de-fleshing bones, dermestids feed on a combination of wet and dry tissue, and *D. maculatus* and related species are sometimes found on human bodies early in decompositional succession. Consequently, whether or not *D. maculatus* is adapted for feeding solely on dry tissue is unclear.

To address this question, a logical approach is to look for differences in development rates between *D. maculatus* reared on wet and dry food. Development is a dynamic process; many factors beyond food influence the speed and efficiency of development, including temperature, humidity, enzyme regulation, and photoperiod (Nabity et al. 2007). Further

differences may be credited to the fact that oviposition in insects on a specific host is determined by various factors that may determine its suitability as a breeding medium, such as nutritional quality, host abundance (Jansen and Nylin 1997, Barros and Zucoloto 1999, Uzman et al. 2012), morphology, environmental conditions, age and size of individual (Stejskal and Kucerova 1996, Johnson and Kistler 1987) and competition (Siemens et al. 1991). Consequently, these factors can confound experiments to measure development, unless properly considered as part of the experimental design. Measurements in development can become difficult in a laboratory setting when these factors are not taken into consideration.

Besides providing a means for looking at differences in food use, consumption and conversion rates have not been previously examined for *D. maculatus.* Knowing these rates could be of use in forensic science for relating dermestid populations and time to tissue removal on decomposed bodies. So, we examined water content of food and its influence on *D. maculateus* development and survival.

Materials and Methods

Dermestes maculateus for experiments were obtained from colonies and maintained at the University of Nebraska-Lincoln (established in summer 2015) from a combination of purchased and feral beetles. Colonies were maintained at 16:8 L:D, 25°C, and fed dried pork. For experiments, colony adults were placed on a diet consisting of 1 gram of wet liver and moistened sections of paper towel to stimulate oviposition. Eggs were laid on the moist paper towel, were transferred to a separate rearing container and maintained until reaching the third larval stage. For colonies and experiments we used *DigiTherm*® 38-liter Heating/Cooling Incubators which allowed for precision temperature regulation within 0.1°. These incubators have microprocessor-controlled temperature regulation, internal lighting, and a recirculating air system.

We conducted a series of preliminary experiments to establish appropriate larval densities, weight of food, and other experimental conditions before testing the influence of wet versus dry food. In efforts to both reduce error and address variations within stages of insects sampled, we initially examined consumption in first and second larval stages versus later stages and adults. Because the larvae in the first two stages eat so little food (ca. <0.001g dry weight) and this amount represent less than 5% of total consumption, we decided to conduct future experiments with 3rd stage larvae. This decision also reduced problems with survivorship, in that survival rates in the first two stages are highly variable.

We also tested various food sources. As colonies were maintained on pork, we decided early on to use some form of pork in our experiments. Pork liver has long been a substrate of choice in studies of development of carrion insects, however, in looking at wet vs. dry food, liver was unsuitable because of difficulty in establishing water content differences, problems in recovering larvae that had burrowed in the liver, and difficulties in excluding fungal and bacterial contamination in some instances. Other problems were separating frass and shed peritrophic membranes from remaining food. Fortunately, dried pig ears (used as dog treats) proved to be an ideal substrate for testing. We could easily re-hydrate pig ears to provide a workable substrate existing in wet and dry conditions. One potential problem was that in testing small amounts of substrate (specifically, less than 0.5 g) dried pig ears might absorb sufficient moisture from the air so as to confound our treatment and contribute to high variability in measured consumption. We solved this problem by increasing the number of larvae and amount of pig ear per experimental unit, so that the surface area to mass ratio of the pig ear was reduced and therefore absorbed water minimized.

This testing lead to the following observations relative to establishing our pig ear treats: (1) saturated pig ears, which were those soaked water at 25°C for 2 hours to achieve maximum

absorbance (as evidence by constant weight) and a total weight typically double that of a dry pig ear; (2) wet pig ears, which are soaked at 25°C for ca. 30 min to achieve an increase in weight of about 50%; and (3) dry pig, which were ca. <10% moisture content. Preliminary tests showed that when beetles were fed saturated pig ears, the mortality rate in our testing was routinely 100%. To provide measurable consumption on hydrated tissue, we decided to use wet, rather than saturated pig ears for comparisons with dry ears. Additionally, in a natural setting where carrion starts losing fluids immediately after death, the wet pig ear are probably more representative of tissue beetles might natural encounter. One final consideration is the potential influence of intraspecific competition on the larval consumption and associated values. We decided to look at this explicitly by varying starting food amounts to represent different potential resource limitations for larvae.

The wet vs. dry food experiment was a randomized complete block with factorial treatment arrangement. Main effects were food water content (wet pig ears vs. dry pig ears) and food quantity (starting food weights of 6.0, 4.8, 3.6, 2.4, and 1.2 g) with 4 replications. The experimental unit was a 1.7 L plastic box with 10 new molted 3rd stage larvae on pig ear as specified for individual treatments. Experimental units were blocked inside growth chambers by shelves, and maintained at 25.0 ° C with a light: dark cycle of 12:12 and relative humidity (65±5 %), which are reported as optimal conditions for development (Uzman et al. 2013). Individuals were weighed at the beginning of the experiment and every 2 days thereafter. Final larval and consumption rates (used to calculate change in larval weight and food consumption) were based on values from the larval measurement immediately before pupation (results in Table 1). This procedure avoided errors in larval measurements estimated from initial pupal weights, in that pupation can cause a 10-20% reduction in weight (Fraenkel and Blewett 1944).

Because substantial mortality was associated with the wet treatments, and because individual variation among larvae could occur within a treatment given our experimental design, we decided to conduct an additional experiment with individual larvae and adults to provide the best estimate of larval and adult consumption rates. All experimental conditions for this experiment were the same as previously described with the follow exceptions. Single larvae or adults were place on 0.5 g of dried pig ear, and only treatments with dry pig ears were used. N = 23 for larvae and n = 21 for adults (results in Table 2).

Initial testing at all three levels provided enough detail to determine that saturated levels (generally unseen in nature outside of flood events or similar) were deemed excessive. We therefore excluded these treatments from further study and experiments.

Analysis

As previously stated, the goal of this study was to address and determine whether preferences or associations with moist and dry tissue are mitigated through resource availability, then further determine whether this influenced growth and development. For statistical analysis of the wet vs. dry experiment, we used SAS University Edition (https://www.sas.com/en_us/home.html), and mixed models procedures to accommodate missing points in the data set. Response variables were survivorship, change in larval weight, change in food weight, percent conversion, and feeding rates per day (Table 1). We also did linear regressions of proportion survivorship versus food weight for dry food and for wet food in Graph Pad Prism 8.01 (Graph Pad Software https://www.graphpad.com/) (Figure 2).

Results and Discussion

We found a pronounced difference between how much *D. maculatus* (chiefly larvae) feed and their feeding rates on wet versus dry tissues. Our findings illustrate not only a *preference* for dry tissues, but a *dependence* on them. Indeed, hydrated tissue presents a potentially lethal challenge to *D. maculatus.* Adults have approximately 50% the daily consumption rates of larvae (averaged over 3rd to the last larval stage). Finally, conversion rate results indicate that *D. maculatus* must acquire moisture from non-dietary sources.

Survivorship. Table 1 and Figure 1 illustrate the influence of food moisture content on *D. maculatus* consumption rates and survival. As summarized in Table 1, wet and dry food treatments were significantly different for all variables measured. Although some larvae could complete development on moist tissue, high levels (ca. 75%) of mortality occurred on this food. In preliminary experiments with saturated tissues, 100% mortality occurred, and both larvae and adults were observed to “defecate” water droplets, presumably in an effort to maintain osmotic balance.

As compared to limitations in food quantity, we observed no differences in larval survivorship even with food per larvae as low as 0.12 g, however, Figure 2 indicates that there was a linear reduction in survivorship with reduced dry food. In contrast, the linear regression for wet food quantity and survivorship was not significant, which is consistent with the significant interaction noted in our mixed models analysis (Table 1).

One limitation in our experimental design is that wet treatments had less food content than dry treatments (because wet and dry treatments used the same weight, and were not corrected for water content). Consequently, the wet treatment might have been more food limited than dry treatments. However, food limitation does not account for the low survivorship of wet treatments, in that the highest weight wet treatments still had dramatically lower survivorship than the lowest weight dry treatments (Fig. 1). Moreover, our observations of purging water from the anus strongly indicates problems with excess water and osmoregulation.

Because they have large body surface relative to their volume, insects are susceptible to desiccation. While possessing little water in their bodies relative to size causes insects to dry out quickly. Desiccation tolerance in insects is accomplished through numerous physiological and behavioral adaptations including waxy epicuticles, furthered divisions in proto-cuticles and endo-cuticles, glycogen stowage, and eclosion during development. Water conservation is a common issue among all insects and is amplified among those associated with dry habitats. Typically, water conservation in digestion occurs through osmotic regulation in the Malpighian tubules of the hind gut, so insect waste (frass) is usually dry. However, insects processing fluid diets with excess water (such as true bugs and blood feeding insects) may defecate water to allow processing of sufficient food to meet nutritional demands (usually protein for plant feeding insects) and to maintain osmotic balance.

The surprising results and observations that *D. maculatus* regarding defecated water on hydrated food suggest that while *D. maculatus* has evolved to survive on an extremely dry diet, it has lost the ability to survive on normal, hydrated tissue.

Consumption and Conversion Rates. The same trend noted in the wet vs dry experiment is seen from consumption as compared to the amount (weight) of tissues consumed (Table 2). As a rule, only about ten percent of the energy of one trophic level can be transmitted to the next trophic level, the so-called rule of ten. Results of experiment 2 seem to contract this principle, because *D. maculatus* exceeded this ecological limit, averaging a conversion rate of over 44% (Table 2). The explanation for this result is, of course, that the calculated conversion rate is confounded with uptake of water in developing larvae. More specifically, these results indicate larvae are not obtaining the bulk of their water from their food. Consequently, calculating conversion rates only based on food weight over estimates the conversion rate.

Taken in conjunction with the survivorship findings of experiment one, the conversion data confirm that *D. maculatus* does not depend on dietary sources for water. Between wet and dry tissues there were noticeable differences in consumption and survivorship. When we looked at the dry tissues, we found that weight gain is disproportionately higher than would be explained by only the consumption of dry tissues consumed. Like all organisms, insects need water. Looking at the weight gains in our experiments, considering the lack of moisture in the dry tissues, and keeping in mind water requirements, these dermestids are clearly obtaining their water needs from some other source. Indeed, excess dietary water is potentially lethal. So where does the water come from? There are two possibilities: the source may be atmospheric water, or water produced metabolically, as observed in desert-adapted beetles.

Work by Fraenkel and Blewett (1944) showed that *Tribolium confusum* (Coleoptera: Tenebrionidae), *Ephestia kuehniella* (Lepidoptera: Pyralidae), and *Dermestes vulpinus*

(Coleptera: Dermestidae) obtain water from a combination of atmospheric water and metabolic water, with metabolic contributions more important at low relative humidities. The same combination of sources almost certainly applies to *D. maculatus.* What has not been examined, to the best of our knowledge, is the impact of excess dietary water, as we did here in experiment one.

When we look at conversion rate it becomes clear that having to process extra water is unproductive. It is also inefficient when compared to biomass conversion. Moreover, larvae ate more hydrated food, presumably to obtain sufficient nutrients, but in the process exacerbated their problems with excess water. Because larval feeding behaviors did not adapt in response to the danger posed by hydrated food, we assume this is not a commonly experienced phenomenon. Thus, food choice seems likely to prevent mortality as long as sufficient acceptable, i.e. dried, food is available.

Why does excess water kill dermestids? The question itself fits well with one of the observations made during our study. In arid environments (Zachariassen 1996) points out that water conserving physiological adaptations among terrestrial insects, such as the beetles in our study, are accomplished through series of tradeoffs and compromises. Often, there is a substantial lethality due to dehydration hence a strong selection pressure for efficient physiological mechanisms to limit water loss in such species (Zachariassen 1996). In addition to behavioral and circadian responses, there are a series of physiological responses which help insects deal with shortages of free water, extremes of temperature, and desiccation. Reduced cuticular water loss is associated with lowered metabolic rates and variances in oxygen consumption. Oxidative metabolic processes result in the formation of ATP and increases in sodium ion pumping. Most investigators agree that a very substantial part of cellular ATP turnover is spent in trans-

membrane sodium pumping which in turn gives rise to a higher than usual electrochemical energy gradient (Zachariassen 1996).

The high energy costs associated with dehydration are avoided when water can be metabolically produced. Species using metabolic water as a primary water source presumably need access to adequate or even a surfeit of food. Larvae of *T. confusum* and *E. kuehniella* both meet this requirement, and the diet of *Dermestes* like *D. maculatus* and *D. vulpinus* includes calorie-rich proteins. However, the dermestids do not face the same challenges as desert-adapted species because they are not exposed to highly xeric environments. Ironically, although *D. maculatus* can thrive on an extremely dry diet, it is no better adapted to avoid desiccation than most insects.

Rather than representing a physiological adaptation to occupy a specific biome, the physiological preference *D. maculatus* has evolved seem to show strong evidence of how they've niche specialized for a feeding guild, the carrion insects. When contrasted against virtually all other carrion insects, this adaptation for an exclusively dry diet illustrates how *D. maculatus* are able to avoid competition which is usually a challenge for other decomposers species. On decomposing soft tissues we see great competition among insects, whereas dermestids seem to capitalize on a resource inaccessible to other insects and avoid conflict.

Tables and Figures

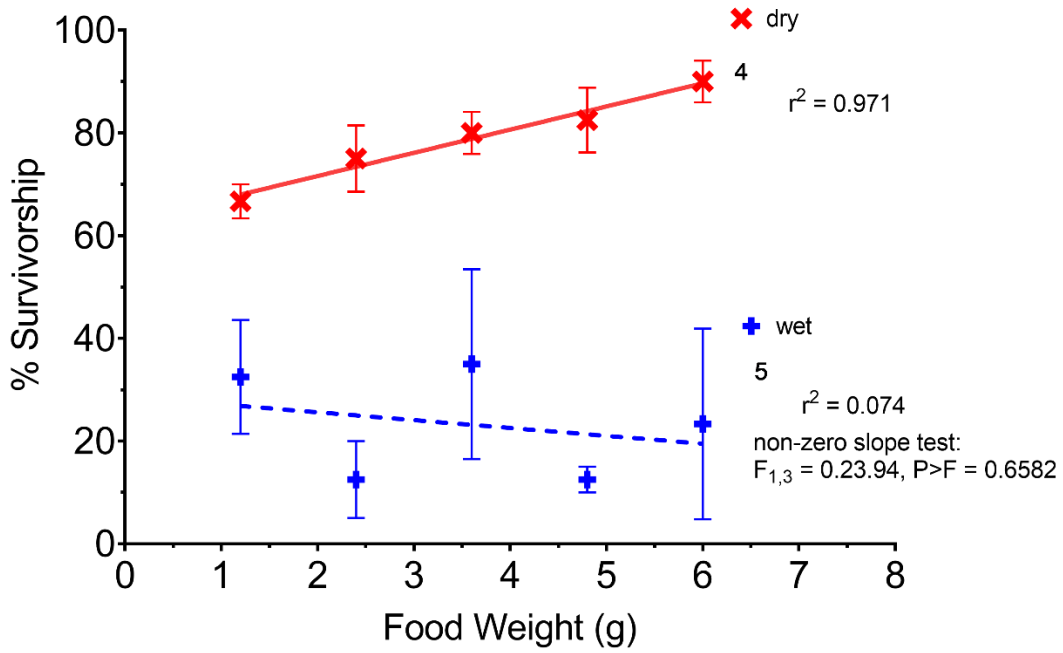
Table 1. Results from a factorial experiment with food water content (wet and dry pig ears) and food quantity (6, 4.8, 3.6, 2.4, and 1.2 g) on growth and food consumption by larval *Dermestes maculatus*. Means are shown for wet and dry, and results from mixed model analysis are shown for main effects and interactions. Proportional variables, % survival and % conversion (final larval weight/total food consumed) were arcsine transformed (arcsine of the square root of variable) and results are shown for the transformed variables. There were 2 missing observations for % survival, and 6 missing observations for all other variables.

	% survival		Δ larval wt (g)		food consumed (g)		% conversion		feeding rate/larva/day (mg)	
Food	**mean**	**SE**	**mean**	**SE**	**mean**	**SE**	**mean**	**SE**	**mean**	**SE**
dry	79.5	2.7	0.051	0.019	-0.218	0.065	93.7	64.8	2.1	0.6
wet	23.2	5.5	0.000	0.003	-0.815	0.196	4.2	2.7	30.0	6.3
food water content: dry vs wet										
df	1, 25		1, 21		1,21		1, 14		1,21	
F	56.27		6.58		12.34		21.35		30.53	
P>F	<0.0001		0.0180		0.0009		0.0004		<0.0001	
food quantity: starting pig ear weight (6, 4.8, 3.6, 2.4, and 1.2g) for 10 larvae										
df	4, 25		4, 21		4, 21		1 ,14		4, 21	
F	0.58		1.60		1.24		1.45		2.43	
P>F	ns		ns		ns		ns		ns	
interaction of food water content vs. food quantity										
df	4, 25		4, 21		4, 21		1, 14		4, 21	
F	1.04		1.74		2.20		0.06		2.84	
P>F	ns		ns		ns		ns		0.0498	

Table 2. Weight gain, food consumption, and conversion percentage for larval (3rd to pupa) and adult food consumption of *Dermestes maculatus* (n = 23 for larvae, n = 21 for adults).

	larvae (3rd to pupa)						adult	
	time in stages (h)	food consumption		larva		% conversion	food consumption	
		Δwt	Δwt/day	Δwt	Δwt/day		Δwt	Δwt/day
mean	548	0.0923	0.0042	0.0345	0.0015	44.5%	0.0386	0.0077
SE	26	0.0105	0.0005	0.0019	0.0001	4.6%	0.0019	0.0004

Figure 1. Survivorship regression showing by stage the contrast between dry vs. wet tissues.



References

Auden, W. H. 1957. First things first. The New Yorker, March 9, 1957 p. 38.

Bujang, N. S., and Kaufman, P. E. 2010. Featured creatures: common name: black larder beetle, incinerator beetle scientific name: *Dermestes ater* DeGeer (Insecta: Coleoptera: Dermestidae). University of Florida Cooperative Extension Service, Pub. No. EENY-480. http://entnemdept.ufl.edu/creatures/misc/beetles/dermestes_ater.htm.

Beck, S. D. 1971. Growth and retrogression in larvae of *Trogoderma glabrum* (Coleoptera: Dermestidae). 1. Characteristics under feeding and starvation conditions. Annals Entomol. Soc Amer. Vol. 64: pp. 149–155.

Byrd, J., and Castner, J. 2001. Forensic Entomology. The Utility of Arthropods in Legal Investigations. CRC Press, Boca Raton, FL.

Fraenkel, G., and Blewett, M. 1944. The utilization of water in insects. Bull. Entomol. Res. 34: pp. 127-139.

Jansen and Nylon, 1997 Development of *Dermestes maculatus* (DeGeer, 1774) (Coleoptera, Dermestidae) on different fish substrate. Jordan J. Biol. Sci. 6, Number 1, March

Johnson, C.D., Kistler, R.A., 1987. Nutritional ecology of Bruchid beetles. Nutritional ecology of insects, mite, spider and related invertebrates. John Wiley & Sons, New York, USA, pp.259-282.

Nabity, P. D., L. G. Higley, and T. M. Heng-Moss. 2007. Light-induced variability in the development of the forensically important blow fly, Phormia regina (Diptera: Calliphoridae). J. Med. Entomol. 44: pp. 351-358.

Parkinson, A. H. 2012. *Dermestes maculatus* and *Periplaneta americana*: bone modification criteria and establishing their potential as climatic indicators. M.S. Dissertation, University of the Witwatersstrand, Johannesburg, South America.

Siemens, D.H., Johnson, C.D., Woodman, R.L. 1991. Determinants of host range in Bruchid beetles. Ecology, 72(5), pp.1560-1566.Smith, K. S. 1986. A Manual of Forensic Entomology. Cornell University Press, Ithaca, NY.

Stejskal, V. and Kucerova, Z., 1996. Reesa vespulae (Col., Dermestidae) a new pest in seed stores in the Czech Republic. Ochrana Rostlin-UZPI (Czech Republic).

Usman, Z., Ayertey, J. N., and Cobblah, M. A. 2013. Development of Dermestes maculatus (DeGeer, 1774) (Coleoptera, Dermestidae) on different fish substrates. Jordan J. Biological Sci. 6: 5-10.

Zachariassen, K. E. 1996. The water conserving physiological compromise of desert insects. European J. Entomol. 93: 359-368.

CHAPTER 3. BONE TRACES AND MODIFICATIONS

Introduction

Insects can use a wide array of biological materials (soft tissues, feces, bones) for feeding, reproduction, and shelter. When they feed on vertebrate tissue, bones may be modified by insect action, and various insect taxa are known to modify bone with their mandibles, including members of the Families Dermestidae, Tenebrionidae, Tineidae, and Termitidae. Despite bone modification being a known behavioral trait of many of these insects, little work has been done to record the frequency of occurrence, species-specific modifications of bone, and concise, definitive criteria distinguishing insect modifications from other agents impacting bones.

Markings on bones can have many causes. In particular, weathering causes many types of bone modifications that can be confused with insect modifications (Roberts, 2007). Weathering itself is potentially valuable as evidence indicating the period of time represented in recent or fossil bone assemblages, including those on archeological sites. It also can be an important tool in censusing animal populations in modern ecosystems (Behrensmeyer, 1978).

Because dermestid beetles are commonly associated with late-stage decomposition of animals, they often feature as entomological evidence in homicides with human decomposition as well as in archeological investigations. Consequently, potential dermestid modification of human bone is of anthropological and forensic importance. Unfortunately, whether or not common dermestid species can alter bone, how they alter bone, or under what circumstances they alter bone are all questions of continuing debate.

The role of dermestid beetles in animal decomposition is well documented; they are known to consume soft sub-dermal tissue and skin, hence their name Dermestes derived from the Greek to “consume skin” (Cornaby 1974, Smith 1986, Byrd and Castner 2009). Their

involvement in animal decomposition and associated consumption of dry and decomposing animal matter typifies members of the *Dermestes*. Because of this they are routinely used for stripping carcasses of meat for skeletal collections and are widely considered as doing little damage to delicate bones.

The older literature largely denies any role of dermestids (principally, *Dermestes maculatus*) in altering bone. Howell (1932) and Borell (1938) state categorically that even skulls of minute sizes are cleaned without the slightest damage to the most delicate of processes. Howell goes on to state that, "…the tympanic bullae of mouse-sized skulls are infrequently eaten by the beetles, possibly in search of blood, processes are not broken off, delicate structures are not destroyed, teeth do not fall out, and sutures do not gape even in the youngest of specimens." This is a personal observation also made in preparing specimens during curation (*Peromyscus leucopus* and *P. maniculatus*). Similarly, Voorhies (1948) states that after being exposed to dermestids for a number of weeks, even delicate bat skulls (e.g., *Myotis sp.*) have tissues removed without any harm to bone which was "cleaned to perfection".

Among more recent workers, Osuji (1975) states that *D. maculatus* larvae may bore into the flesh of dried fish, but do not bore into either their bones or skulls. Similarly, modification of bones has not been regarded as an indicator of dermestid involvement by some forensic entomologists (e.g., Smith 1986, Haskell).

In forums among hobbyists and taxidermists who use dermestids it has been said that "[dermestids] will even eat their way through certain plastics in search of food sources given enough time, but bone remains clean, intact and ready to be bleached if desired" (Taxidermy.net forums 2018). And many museums and institutions use *D. maculatus* colonies for tissue removal from bones prior to scientific analysis of those bones, implying an absence of any bone modification by *D. maculatus.*

In contrast, recent literature reports modifications to bone by terrestrial invertebrates, particularly insects including dermestids (Parkinson, 2012). For some groups, such as the Tenebrionidae and Termitidae, the evidence of bone modification is thorough and convincing. However, for dermestids many reports of modification are from post hoc associations of dermestids with altered bones, rather than from direct experimental evidence. Even in the instances in which the potential for bone alteration by a dermestid has been made, it is unclear the species or conditions associated with the alteration.

Observations on rodents by Hefti et al. (1980), indicated that once available food sources were depleted, *Dermestes maculatus* beetles begin to destroy specific areas on bone, specifically the iliac crest of the pelvis, as well as vertebrae. They went on to state that when bones are modified by dermestids, the modifications are obvious. However, the observations Hefti et al. made did not exclude other possible causes of bone modification, and the reported ability or occurrence *D. maculatus* to modify bones was not confirmed.

Bones on carrion commonly exhibit distinctive weathering characteristics which can be related to the time since death and to the local conditions of temperature, humidity, and soil chemistry. These characteristics can be crucial in archeology and anthropology, as well as in forensic analysis. Indeed, distinguishing weathering from tool marks and other artifacts on bone is a common issue in forensic physical anthropology.

The feeding activities of insects, like those of vertebrate scavengers and predators, change the remains and may leave artifacts that can be sometimes be difficult to assign to a cause. When dry tissues are removed by dermestid beetles, the cause of tissue removal is usually obvious: dermestids are one of the few agents that feed on dried tissue, and sites of dermestid feeding routinely have frass and cast larval skins.

Nevertheless, we find suggestions, and even false confirmations, on both sides of the argument that dermestid beetles can modify bone. One aspect of this debate, contrasts workers in forensic science with workers in paleontology. While forensic scientists seem to be unresolved on this issue, many papers in paleontology treat marks on fossil bones being from dermestids almost as dogma. Hypothesized trace makers most commonly include carrion insects such as dermestid, silphid, and histerid beetles, tineid moths, and a variety of neotropical termite species (Roberts, 2007). Tobien (1965) first recognized Neogene mammal bones from Germany that contained distinctive ovoid chambers (2–7 mm in diameter), which he *interpreted* as dermestid beetle pupal chambers. Similar borings were described in Plio–Pleistocene mammal bones from South Africa by Kitching (1980), who also *associated* them with dermestid beetles. Martin and West (1995) described slightly smaller (2–4 mm) ovoid bone borings from the late Pliocene of Idaho and the middle-late Pleistocene of Kansas. They followed earlier workers and also attributed these traces to dermestid beetles. However, no direct evidence supporting these various claims was provided in these publications.

In the forensic literature, Schroeder et al. (2002) reported that *D. maculatus* larvae damaged the humerus and the acetabulum of a human skeleton recovered from indoor conditions (Schroeder et al 2002), furthering the notion that dermestid use beetles bone for pupation chambers and leave marks on bones, despite having only observational data to support their claims. Roberts and Rogers (2007) conducted a study aimed at establishing modification criteria to bone by dermestids whilst measuring the influences of food availability, food type, and substrata in increasing/decreasing bone modification. Results of this study suggested that a wide variety of modification types were produced by dermestids, including oval-shaped borings into cortical bone and irregular excavations into trabecular (spongy) bone, however, preference was shown for marrow cavities of long bones.

Kenneth Bader, when working with dermestid colonies, observed that dermestids often remove the periosteum from cortical bone. However, the majority of destruction occurred on softer cancellous bone, particularly Aves bones, but also on articular facets of mammal bones (Kirkland and Bader, 2010; Parkinson, 2012). Experiments conducted by Hefti et al. (1980) found that once available food sources have been depleted the beetles, began to destroy specific areas: particularly the iliac crest of the pelvis and vertebrae. Hefti et al. (1980) goes on to state that "the beetles attack bone when they are deprived of other food." we compared dry and ash weights of various bones, cleaned for 1 day with corresponding bones exposed to the beetles for up to 5 days, further stating that However, this is not the case for more vulnerable parts of the skeleton, such as the iliac crest and vertebrae, where macroscopic lesions were observed after leaving skeletons with the beetles for several days. Thus of seven vertebrae analyzed before and after exposure, two were macroscopically damaged.

Microdamage and Taphonomy of Bone Weathering. Weathering is defined as the process by which the original microscopic organic and inorganic components of a bone are separated from each other and destroyed by physical and chemical agents operating on the bone in situ, either on the surface or within the soil zone (Behrensmeyer 1978). This can strongly affect paleoecologic interpretations concerning the faunal composition, relative abundances of taxa and age-structure of the preserved populations because it indicates that taphonomic biases inherent in an attritional bone assemblage must be taken into consideration (Behrensmeyer, 1978). Divided into several stages, bone weathering was referred to in our analysis and is categorized as follows (Behrensmeyer, 1978):

Stage 0. Bone surface shows no sign of cracking or flaking due to weathering. Usually bone is still greasy, marrow cavities contain tissue, skin and muscle/ligament may cover part or all of the bone surface.

Stage 1. Bone shows cracking, normally parallel to the fiber structure (e.g., longitudinal in long bones). Articular surfaces may show mosaic cracking of covering tissue as well as in the bone itself. Fat, skin and other tissue may or may not be present.

Stage 2. Outermost concentric thin layers of bone show flaking, usually associated with cracks, in that the bone edges along the cracks tend to separate and flake first. Long thin flakes, with one or more sides still attached to the bone, are common in the initial part of Stage 2. Deeper and more extensive flaking follows, until most of the outermost bone is gone. Crack edges are usually angular in cross-section. Remnants of ligaments, cartilage, and skin may be present.

Stage 3. Bone surface is characterized by patches of rough, homogeneously weathered compact bone, resulting in a fibrous texture. In these patches, all the external, concentrically layered bone has been removed. Gradually the patches extend to cover the entire bone surface. Weathering does not penetrate deeper than 1.0-1.5 mm at this stage, and bone fibers are still firmly attached to each other. Crack edges usually are rounded in cross-section. Tissue rarely present at this stage.

Stage 4. The bone surface is coarsely fibrous and rough in texture; large and small splinters occur and may be loose enough to fall away from the bone when it is moved. Weathering penetrates into inner cavities. Cracks are open and have splintered or rounded edges.

Stage 5. Bone is falling apart in situ, with large splinters lying around what remains of the whole, which is fragile and easily broken by moving. Original bone shape may be difficult to determine. Cancellous bone usually exposed, when present, and may outlast all traces of the former more compact, outer parts of the bones.

Vertebrate scavenging, in addition to affecting decomposition and insect colonization, may also produce postmortem artifacts that may be initially mistaken for wounds or mutilation. The same can be said for carnivore tooth marks which have been found on bones preserved in formations (Rogers et al., 2003, Anderson, unpublished data; Dillon, 1997; Dillon and Anderson, 1995; 1996a; Patel, 1994). Conversely, wounds originally mistaken as rodent damage may actually have other causes (In: Byrd and Castner (Patel, 1995).

Unlike weathering, ichnites are known as trace fossils or more broadly fossilized footprints, nests, dung, gastroliths, burrow, stomach contents. An ichnotaxon is defined by the International Code of Zoological Nomenclature as "a taxon based on the fossilized work of an organism" that is, the non-human equivalent of an artifact. Ichnites fit into several categories where dermestid beetles are thought to generally be associated. The common indications pertaining to systematic ichnology attribute discrete ovoid borings in bone and hollow, oval chambers with concave flanks bored into inner spongy and outer cortical bone surfaces. *Cubiculum ornatus* (insect-related ichnogenera) borings are interpreted as insect pupal chambers, based on close resemblance to modern arthropod pupae and ancient examples of bone-hosted pupal chambers (Roberts, 2007). Trace fossils referable to *C. ornatus* have been described previously by Tobien (1965), Kitching (1980).

Hypothesized tracemakers most commonly include carrion insects such as dermestid, silphid, and histerid beetles, tineid moths, and a variety of neotropical termite species (Roberts, 2007 Behrensmeyer, 1978; Rogers, 1992; Tappen, 1994; Martin and West, 1995; Kaiser, 2000). Roberts goes on to state that the most commonly cited trace fossil morphologies can be grouped into five general categories: 1) *Cubiculum n. igen*. (i.e., ovoid chambers); 2) shallow circular to elliptical pits; 3) starshaped pit marks; 4) *Osteocallis n. igen.* (i.e., surface trails); and 5) tunnels and subcortical cavities; and furthered that a variety of other bone borings which do not readily fit

into these categories. More recently a forensic entomologist reported damage by *D. maculatus* larvae to both the humerus and the acetabulum of a human skeleton recovered from indoor conditions (Schroeder et al. 2002 as cited by [Parkinson 2012]). Parkinson goes on to state that "unlike the vast body of palaeontological literature which suggests that dermestids modify bones in a number of distinctive ways, particularly the creation of pupation chambers or distinctive borings, the literature that relates to the theory makes absolutely no mention to such features." More recently, it was established while investigating various skeletal preparation techniques that *Dermestes* beetles were capable of destroying bone, making grooves, holes and chew-marks (Fernández-Jalvo and Monfort, 2008). However, providing scanning electron microscope images of the modifications identified, the qualitative descriptions provided by Fernández-Jalvo and Monfort (2008) were shown to be limited in their application for identification and particularly differentiation of dermestid modifications from other reported agents.

While working with dermestid colonies, Kenneth Bader made the common observation that dermestids often remove the periosteum (or outer surface layer) from cortical bone but noted that the majority of destruction occurred on softer cancellous bone, particularly Aves bones, and also on articular facets of mammal bones (Kirkland and Bader, 2010). However, to date no comprehensive descriptions of *Dermestes* modifications have been published that could be used to differentiate such modifications when compared to those created by other potential terrestrial invertebrate agents.

Given the absence of associated body fossils and the paucity of observational and experimental data on the morphology of dermestid borings (Roberts et al., 2003), we also prefer to avoid definitively linking these traces from Cretaceous-age bones to dermestid beetles. However, given the clear and recurrent association with animal remains, we feel confident linking these traces to the activity of necrophagous or osteophagous carrion insect fauna (Roberts 2007).

Other works have briefly described insect borings which may also refer to Cubiculum. Schwanke and Kellner (1999) noted a similarity between certain cylindrical borings observed in Triassic vertebrate bones from Brazil and purported dermestid pupation chambers. Less clear, but possibly referable to Cubiculum, are large (1 cm-2.5 cm), circular (in cross section) borings documented in dinosaur bones from the Upper Cretaceous of Mongolia (Kirkland et al., 1998). Though percussion marks on bone surfaces as a diagnostic of insect, canid, hominid behavior or otherwise, we aim to confirm what is currently seen specific to the feeding of Dermestes maculateus when left to feed en masse i.e. in colony. In particular, this research may prove useful in developing a geographical database of insect succession on carrion in a variety of habitats and scenarios in North America.

In large measure this question has been addressed and answered to a certain degree with *primitive* beetles and insects. However, there are still questions about whether we can see consistent evidence of this with extant dermestids species, although it's not likely appreciated. Many of the aforementioned observations, while reported and accepted, lack experimental data. Across the conflicting nature of existing literatures, termites, ants and beetles are by far the most widely accepted agents of bone modification. Even still, a number of potential limitations of such studies have been identified, such as a lack of standardized descriptive vocabulary, limited comparative case studies, localized applicability, insignificant sample sizes, unrepeatability, and use of single instead of multiple agents to gauge the frequency and intensity of different agents producing similar modification types (Parkinson, 2012). Whether these marks are species specific, it is important to look at *Dermestes maculatus* seeing and their feeding, considering how ubiquitous they are.

For this study the following hypotheses are posited for *Dermestes maculatus*:

1. The bone surface modification distribution and types produced are distinguishable as dermestid modifications.
2. They will modify the surface of bones.
3. They produce a variety of modifications on the surface of bones.
4. They will modify bones in fresh /dry states of preservation, condition and of varying densities (thin cortical, thick cortical, and compact bone).

Material and Methods

Microscope and Imaging. Electron microscopy was used to provide necessary details of dermestid mouthpart morphology in both juvenile and adult stages of development to pair with any observed marks on bone surfaces. The targeted mouthpart areas of interest were enhanced including the pictured clypeus, labrum, mandible, segmented maxilla, and labial palps (Figures 1a-1d) (x-x) in both adult and juvenile specimens (Figures 2a-2e). Bird remains from a food processing plant with dermestid beetles being the confirmed de-fleshing agent were used in conjunction with extensively fed on bones in colony under controlled circumstances. The following sections detail the procedures used in preparing specimens for photography and study.

Procedures. The signals that derive from electron-sample interactions reveal information about the sample including external texture, chemical composition, and crystalline structure, and orientation of materials making up the sample. The Scanning Confocal Electron Microscope (SCEM) is an electron-optical implementation of the Scanning Confocal Optical Microscope (SCOM) which allows observation and characterization of sub-surface structures of thick, optically non-transparent materials. To determine whether or not marks had been made to bone surfaces, we used a Hitachi 3000 variable pressure scanning confocal electron microscope. The microscope featured high resolution thermionic electron scattering, which relayed the specimen chamber vacuum images in real time. This also allowed visual control around the sample from a

range of different angles. The microscope additionally featured a high-density frame memory of 1280 x 960 pixels and an advanced image capture and archiving system for imaging and photography. Four-quadrant solid state backscatter allowed imaging in the compositional, 3D and topographic modes by manipulating samples used from each segment of the detector.

Beetles. *Dermestes maculatus* were obtained from colonies at the University of Nebraska-Lincoln (Lincoln, Nebraska). Three initial colonies were established in June 2015 with the intent of obtaining genetic homogeneity among test subjects. Colonies were kept in modified 25 gallon aquaria modified with mesh and lining to promote essential air circulation, confine insects to their enclosures, and to prevent the entrance of other unwanted organisms such as flies, ants and mites. Sealant was removed from the inner corners of each tank in order to prevent adults and larvae from ascending and escaping colony. To promote environmental conditions where beetles and larvae worked most efficiently, colonies were kept in dark rooms having independent temperature control. An ambient temperature ranging from 27-29 degrees Celsius was maintained using suspended 100 watt Exo Terra® Night heating lamps. Initial substrate consisted of a mixture of shredded paper and cottonwood bedding. A healthy population should be sufficiently large to ensure rapid cleaning of bones and tissues. To achieve a healthy and thriving population of adults, food was introduced as needed to further stimulate egg laying. After several generations, a population large enough to recruit adults for consistent experimentation became attainable.

Bones. Samples were taken from beef, pork, rodent, and chicken bone. However, due to the nature of delicate and intricate bone surfaces, microscopy was primarily focused on chicken bone. In combination, bones were both fresh and aged. Aged bones had been fed on by both adults and larvae. In addition to the bones we used for direct sampling, we gained material from a civil case in which there was a question regarding whether or not dermestid beetles had fed on the remains of a bird carcass. We used these remains as material to make a comparison to our controlled

samples which were fed on by an approximate density of adult beetles and larvae in a 25 gallon aquarium enclosure. At an excess of combination of adult and immature induvials at a ratio of approximately 1:6 per container colony fed on tissues for a period of roughly two and a half months. These conditions in colony closely resemble what might be seen in an infestation in natural settings (Byrd, 2001).

Our aim was to mirror natural conditions that would be found on a carcass with a dermestid infestation– and naturally dermestids, like most insects will remain on a food source until the resource has been exhausted. For this reason, the bones used in the study were exposed to adults and juveniles with tissues intact and without any additional preparations. Tissues were initially eaten down to tendons, ligaments, and cartilage before even these tissues were consumed. Once bare bones were observed (usually within one to two days) they were inspected for thorough cleaning. When it was determined all the tissues had been removed, beetles were placed back into the substrate where they remained until the time of preparation for microscopy.

In general, the *charging effect* is caused by the accumulation of static electric charges on the specimen surface. This can result in many problems which include damage, distortion, or even the eventual destruction of the specimen during observation under the ion beam. In order to avoid focused ion beam charging effects on specimens they were cleaned and heat dried through convection for a period of up to twenty four hours. Once the initial drying time had been attained a graded series of 70, 90, and 100% ethyl alcohol completed the dehydrating process. After dehydration a fixative osmium immersion was used to chemically dry and maintain the structural details of the samples.

An electrically conductive coating must be applied to electrically insulating samples for study in conventional SEM's. To prevent charge build-up on electrically insulating materials, insect samples were sputter coated with a layer of gold as a conducting material. Since it was

determined that the bones provided by the food manufacturing plant had reached desiccation no sample preparation was applied. All samples were mounted on double sided stubs with double sided conductive tape before being placed into the pressure chambers.

Analysis. In evaluating our images, we used fresh bones in comparison to old bones (both available and through images given in other publications) to gain an estimate of what beetles routinely did to the delicate surfaces through extensive feeding. Samples were visually inspected by more than one observer. All images show the pattern as imaged between 5 and 10 kilovolts at up to 12.0kX magnification with backscattered electron detection.

The results shown in Figure (3.1) indicate the linear ridge of the sacrum of the sample used from our civil case. In addition to the linear ridge, the image shows the depression and processes of the left ridge of the sacrum. Damage was determined to be consistent with stage 1 weathering.

Figure 4.1 provides a *dorsal* view of stage 2 weathering on anterior sacral depression (civil case sample) at 10 kV 45X magnification. The image details a pattern of wear showing no marks to the bone surface at increased magnification. Continued imaging along the same sample reveal the same consistencies and pattern of wear ending with fissures along the posterior sacral foramina on a depression at 10 kV 2.0kX magnification

It is noted that on the bone surfaces in Figures (5.1-5.4) which were prepared with both heat and chemical drying processes there is an extensive pattern of wear consistent with stages 3 and 4 weathering. However, the absence of trace marks, mandibular grooves and furrows resulting from feeding parallels with our assumptions and observations.

Figures (6.1-6.4) exhibit what were determined to be micro feathers and fibers derived from processing at a food plant. It is important to note that even on such delicate tissues and

materials we see no damage. This observation illustrates the efficiency and near exactness of bone cleaning intrinsic to dermestid biology. Taken together, these observations indicate that, at magnifications on the order of 120X, the SEM imaging provides a reasonable conclusion that no markings have been made.

Results and Discussion

That *D. maculatus* feeding can leave marks on bones is established in the literature - that *D. maculatus* routinely mark bones is not. Our results here, exposing fleshed bones to *D. maculatus* adults and immatures for almost two months after complete tissue removal, indicates that under natural conditions *D. maculatus* do not feed on bones. The key issue seems to be the ability of *D. maculatus* to seek new food sources versus being confined (and eventually starving) on bones. In a natural setting, once a food supply is exhausted, *D. maculatus* would seek new food sources. If this process is somehow interrupted, such as through placement of an infested body in a sealed container or experimentally over a long (months to a year) period, *D. maculatus* could feed on bone. Based on this conclusion, whether in forensics analysis or paleontological analysis, we think the working assumption should be that *D. maculatus* does not alter bone, with the exception (as we outlined) when beetles and bones are in an enclosure that prevents beetle emigration.

Figures

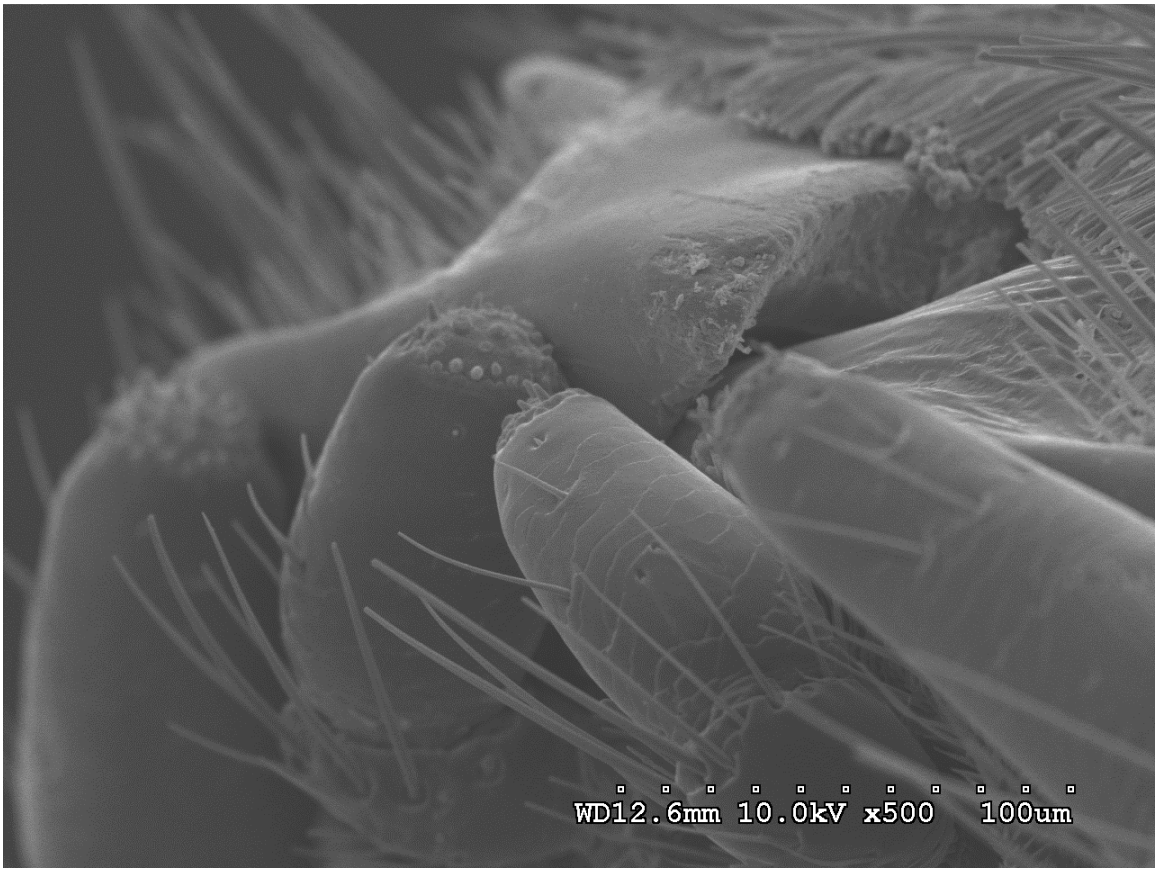


Figure 1.1. *D. maculatus* adult clypheus, labrum, left mandible, and maxillary palps at 10 kV 500X magnification.

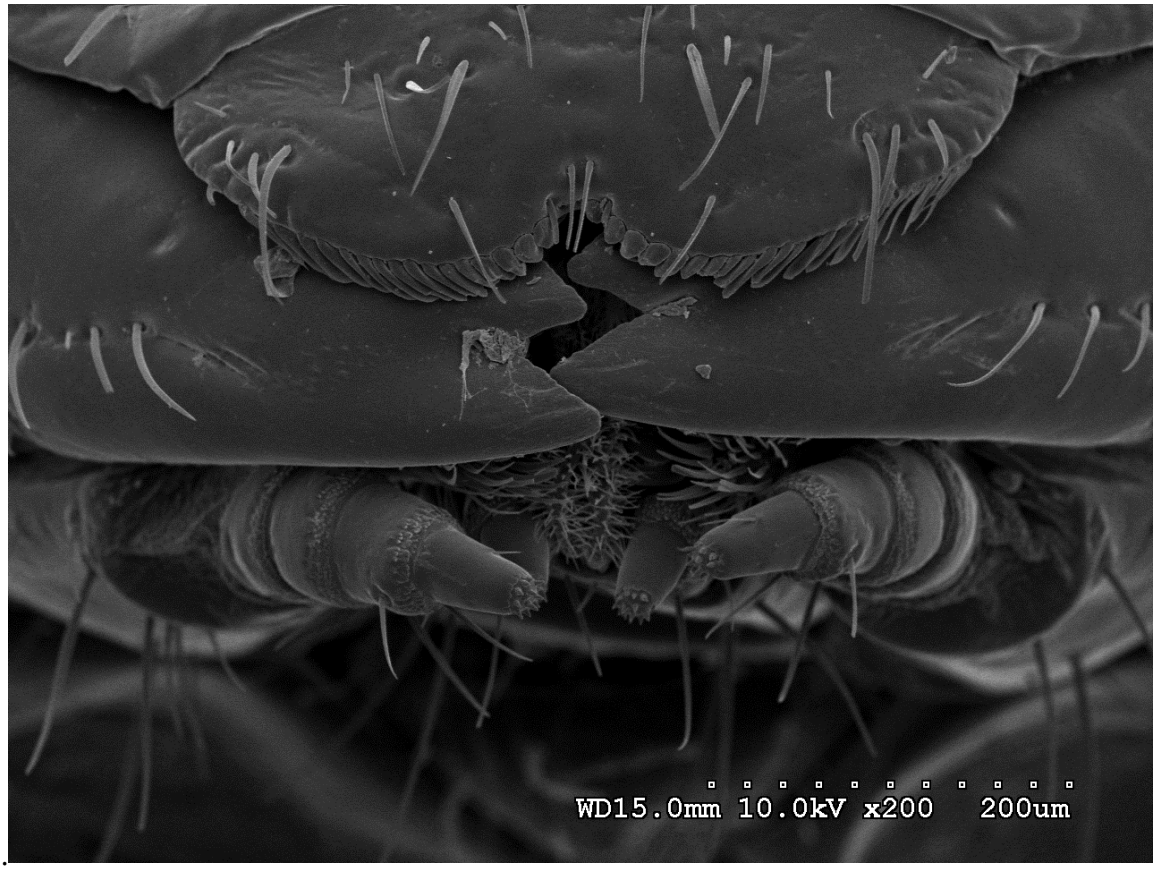


Figure 1.2. *D. maculatus* larvae (3rd instar) clypheus, labrum, and maxillary palps at 10 kV 200X magnification.

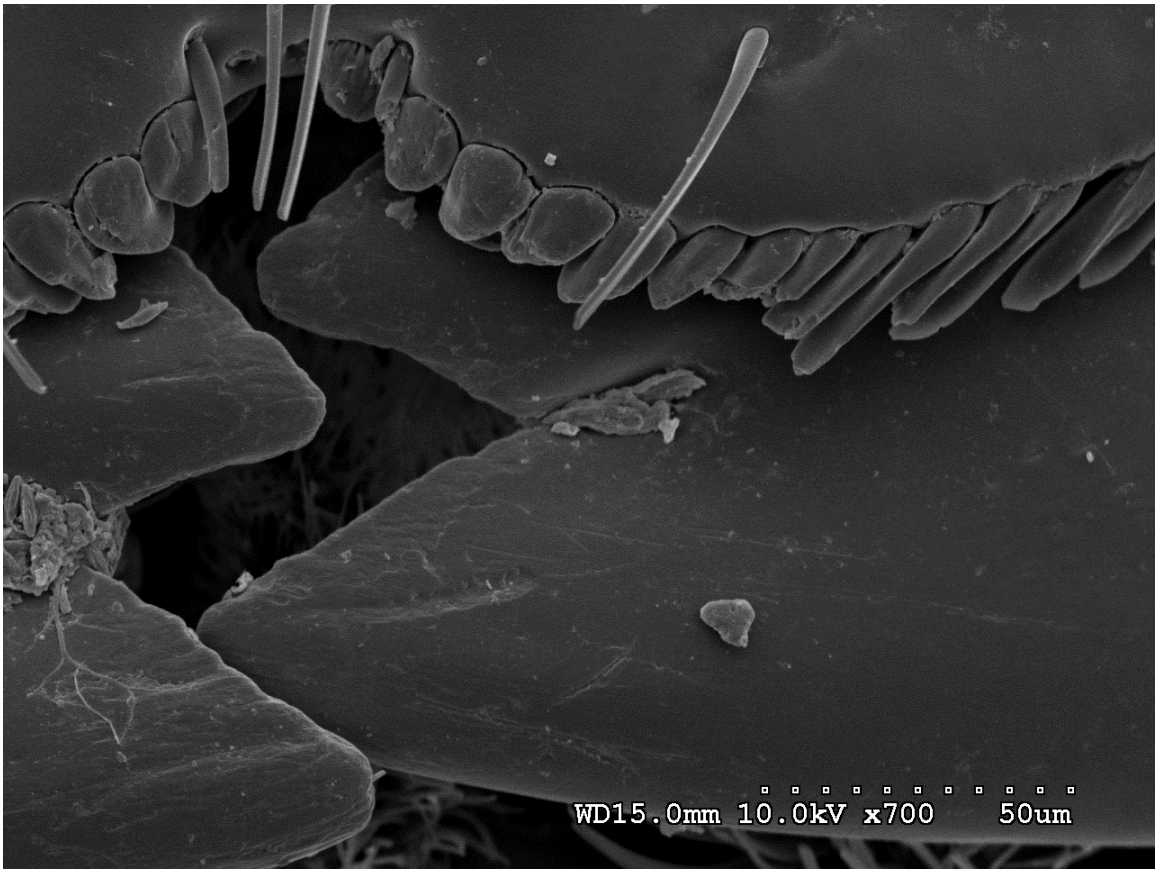


Figure 1.3. *D. maculatus* larvae (3rd instar) labrum and mandibles with feeding debris at 10 kV 700X magnification.

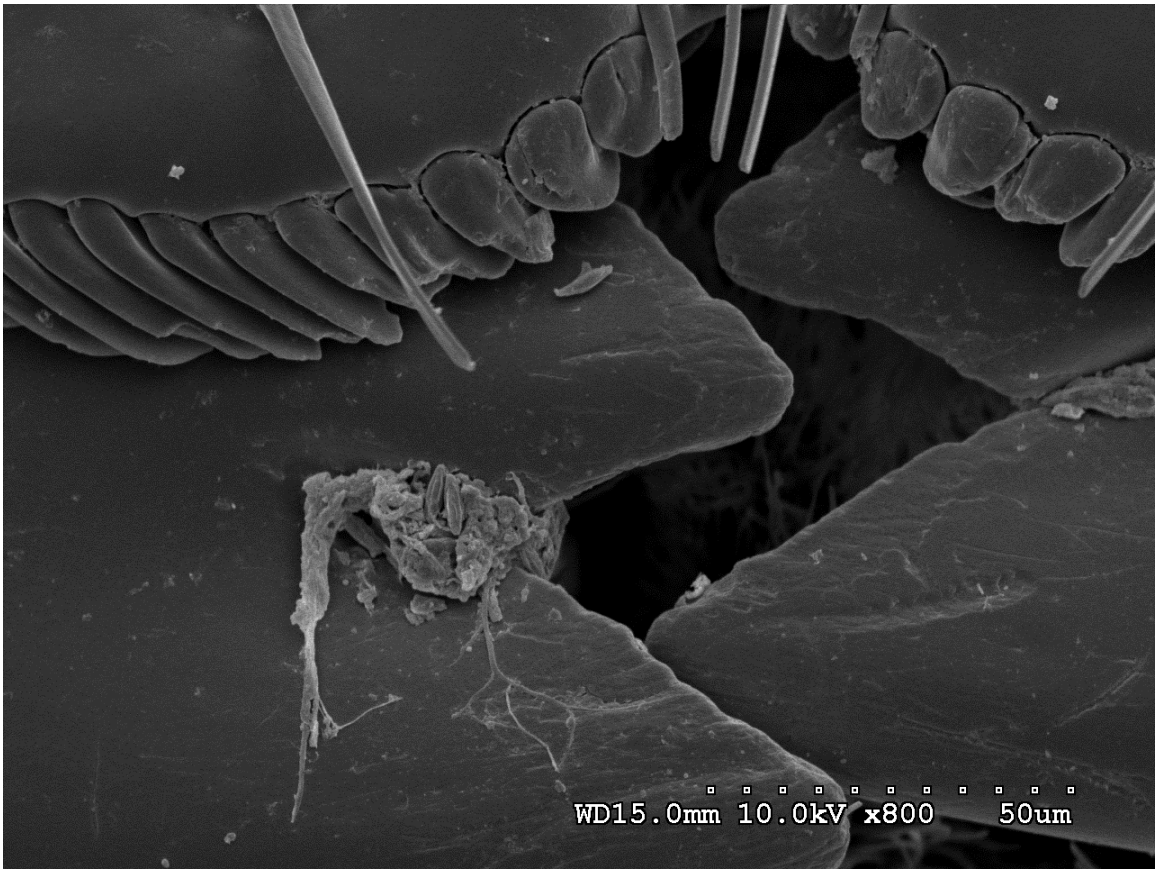


Figure 1.4. *D. maculatus* larvae (3rd instar) labrum and mandibles with feeding debris at 10kV 800X magnification.

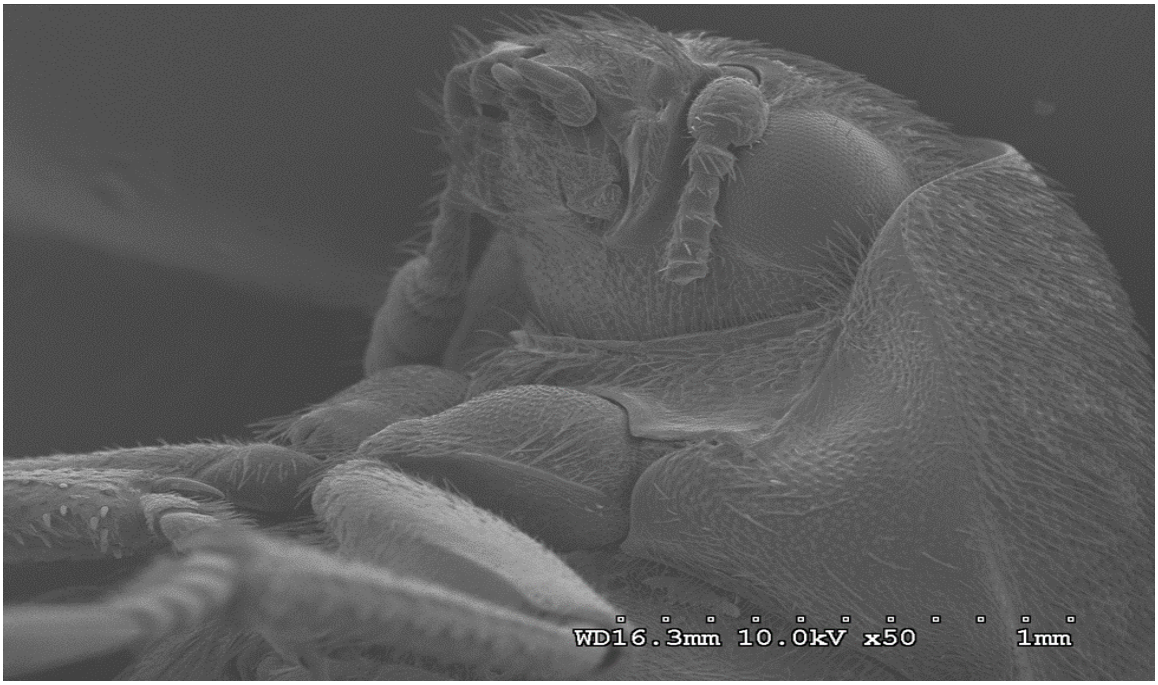


Figure 2.1. *D. maculatus* adult at 10 kV 50X magnification.

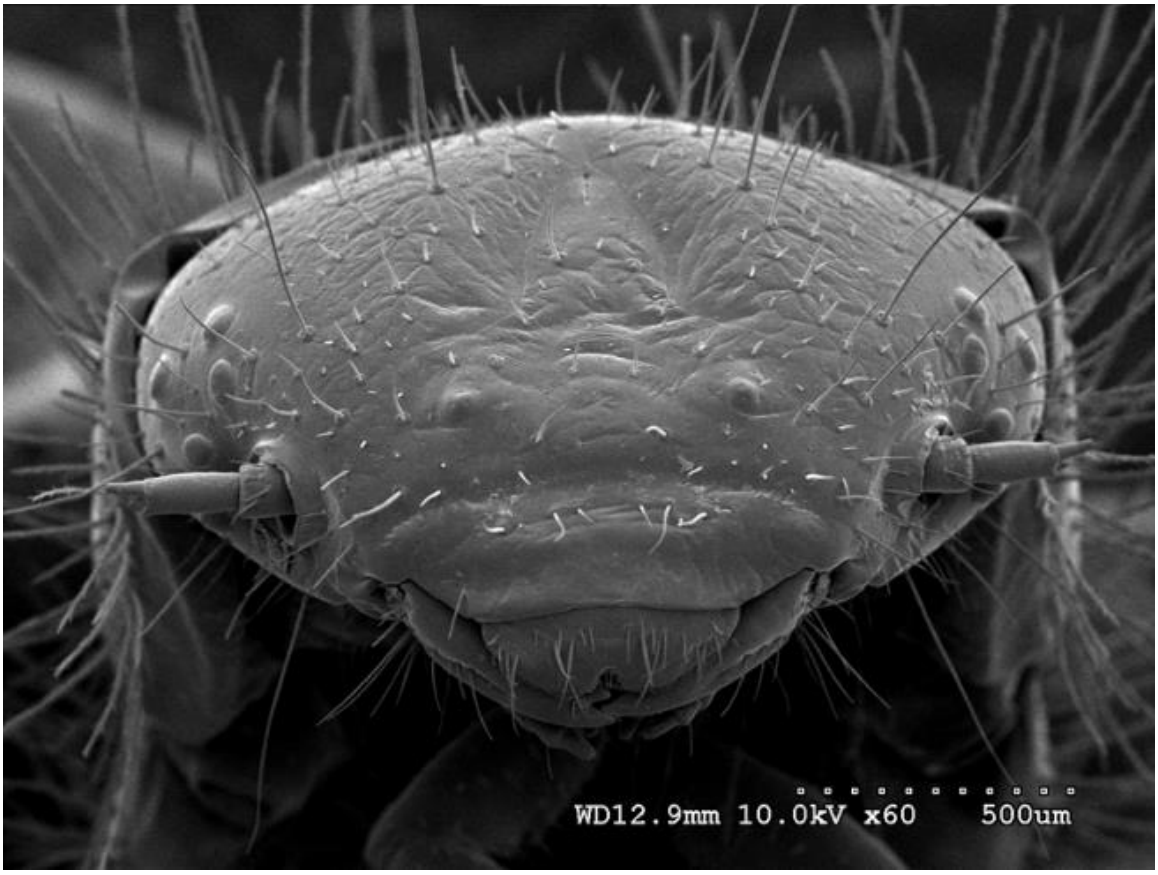


Figure 2.2. Larval (3rd instar) *D. maculatus* at 10 kV 60X magnification.

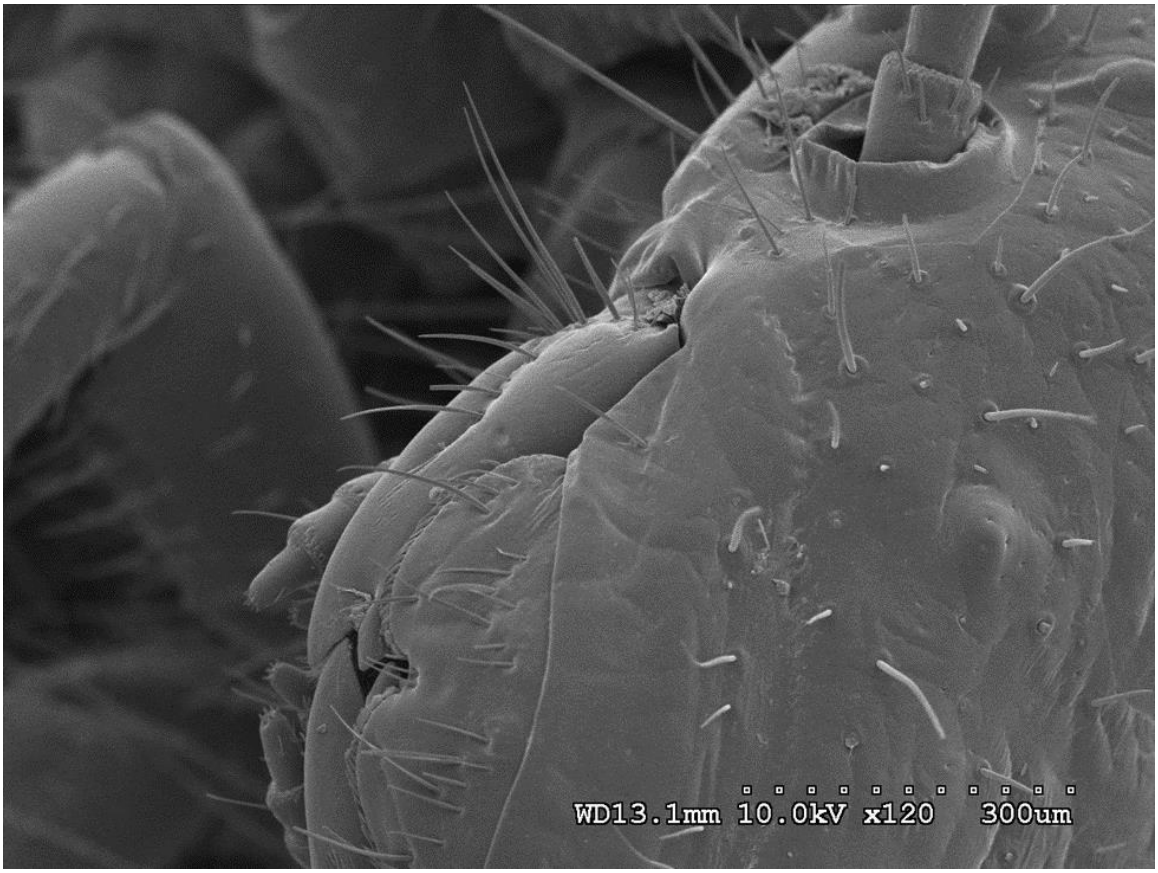


Figure 2.3. Larval (3rd instar) *D. maculatus* 10 kV at 120X magnification.

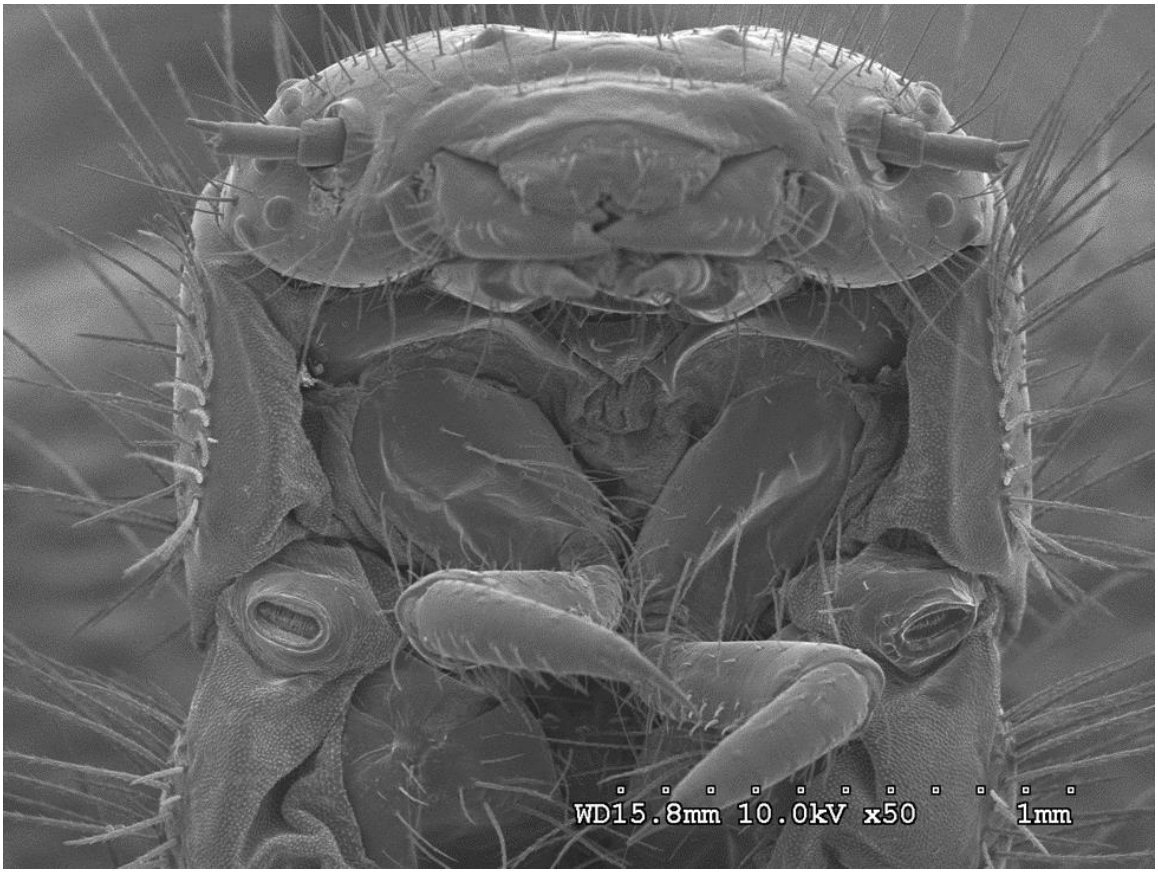


Figure 2.4. Larval *D. maculatus* (3rd instar) at 10 kV 50X magnification.

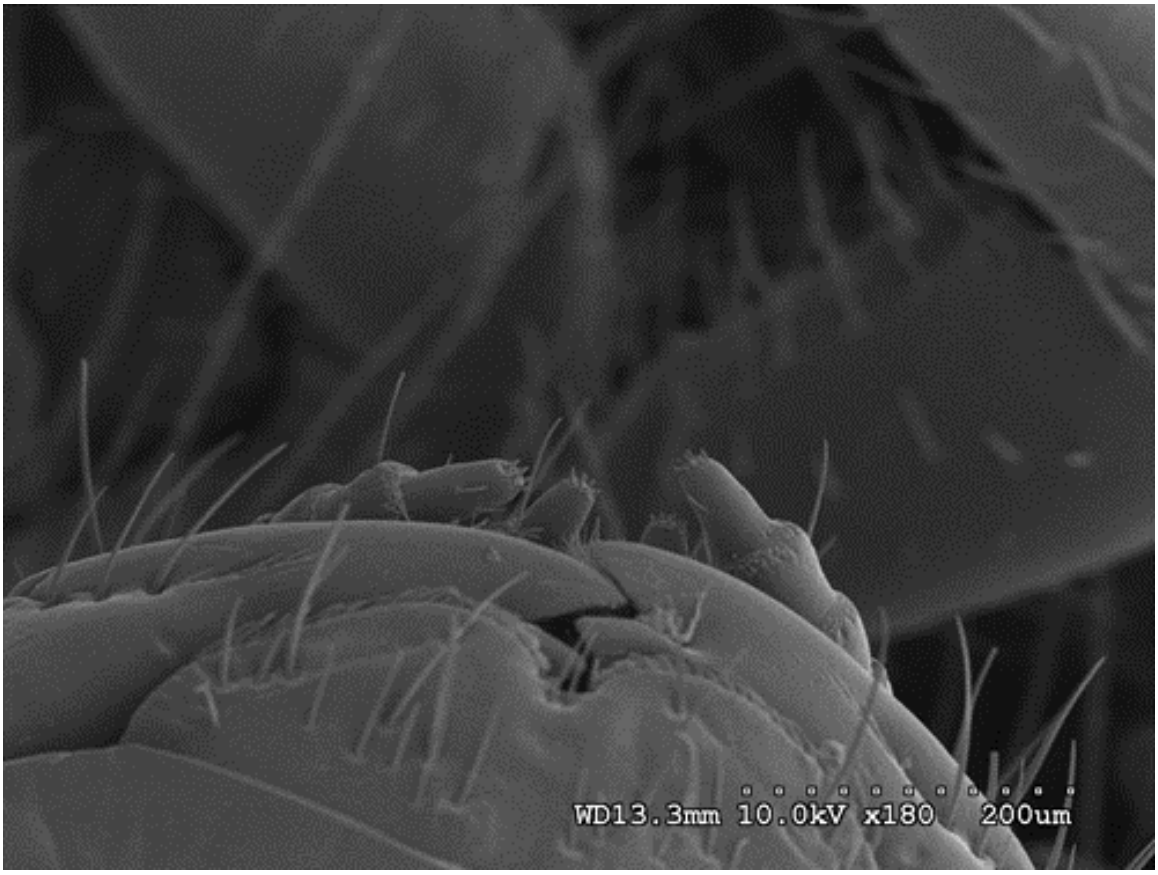


Figure 2.5. Larval *D. maculatus* 10 kV at 180X magnification.

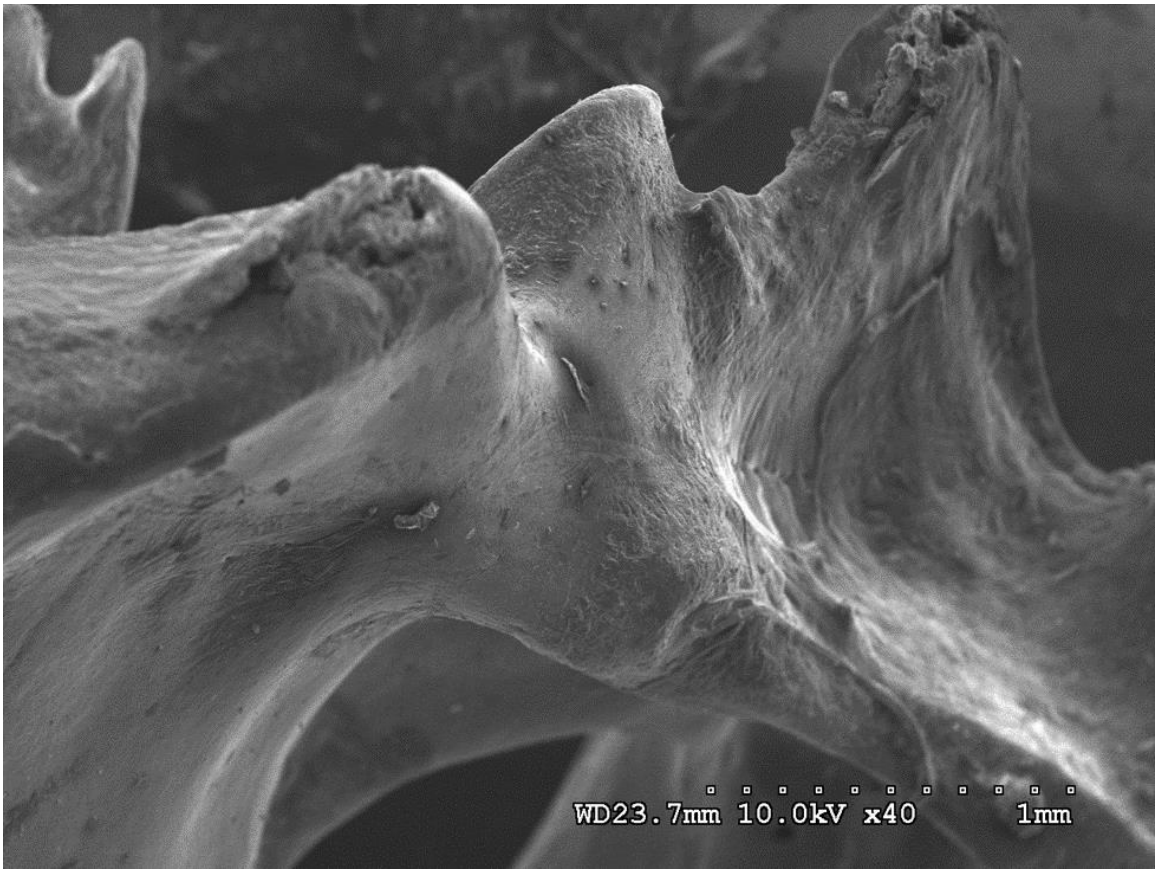


Figure 3.1. Sacrum (dorsal right view) *(civil case sample)* at 10 kV 40X magnification.

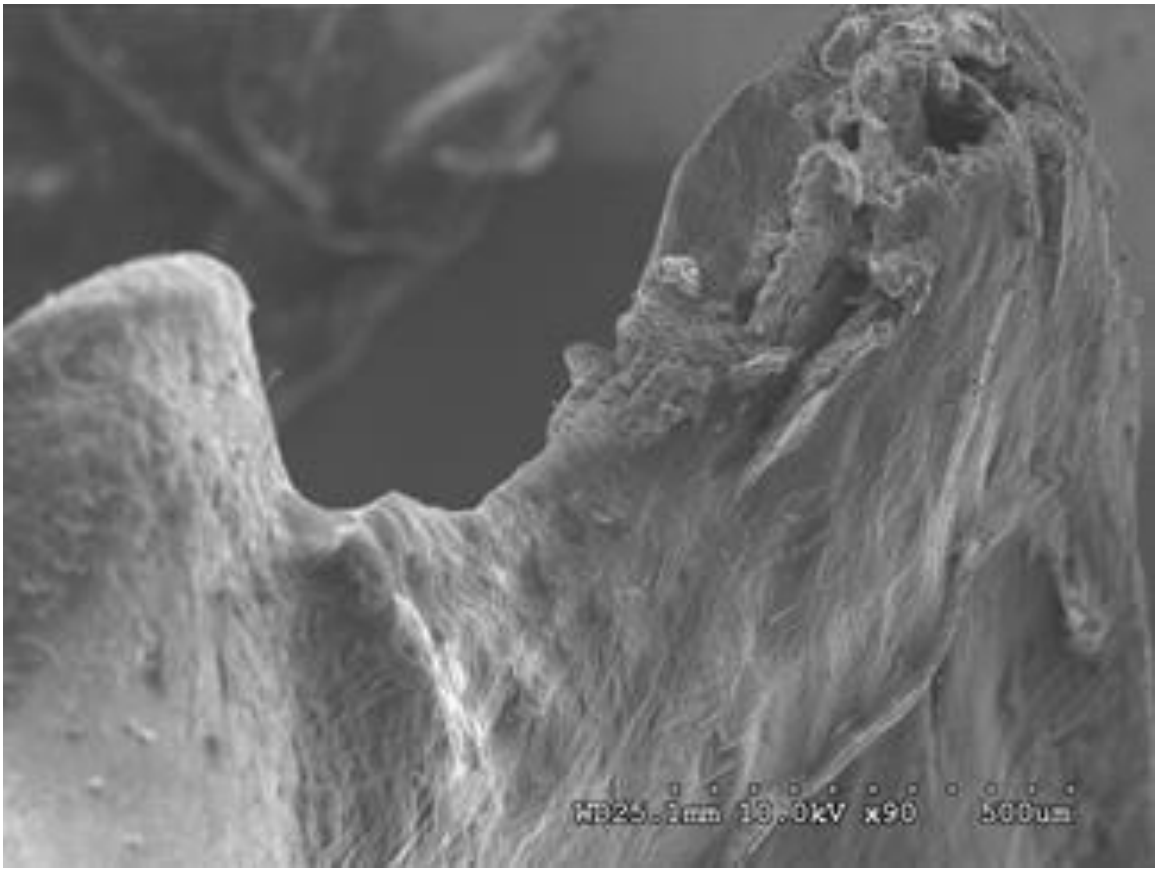


Figure 3.2. Dorsal view of stage 2 weathering on anterior sacral depression *(civil case sample)* at 10 kV 45X magnification.

Fig. 3.3. Sacrum (dorsal right view) *(civil case sample)* at 10 kV 120X magnification.

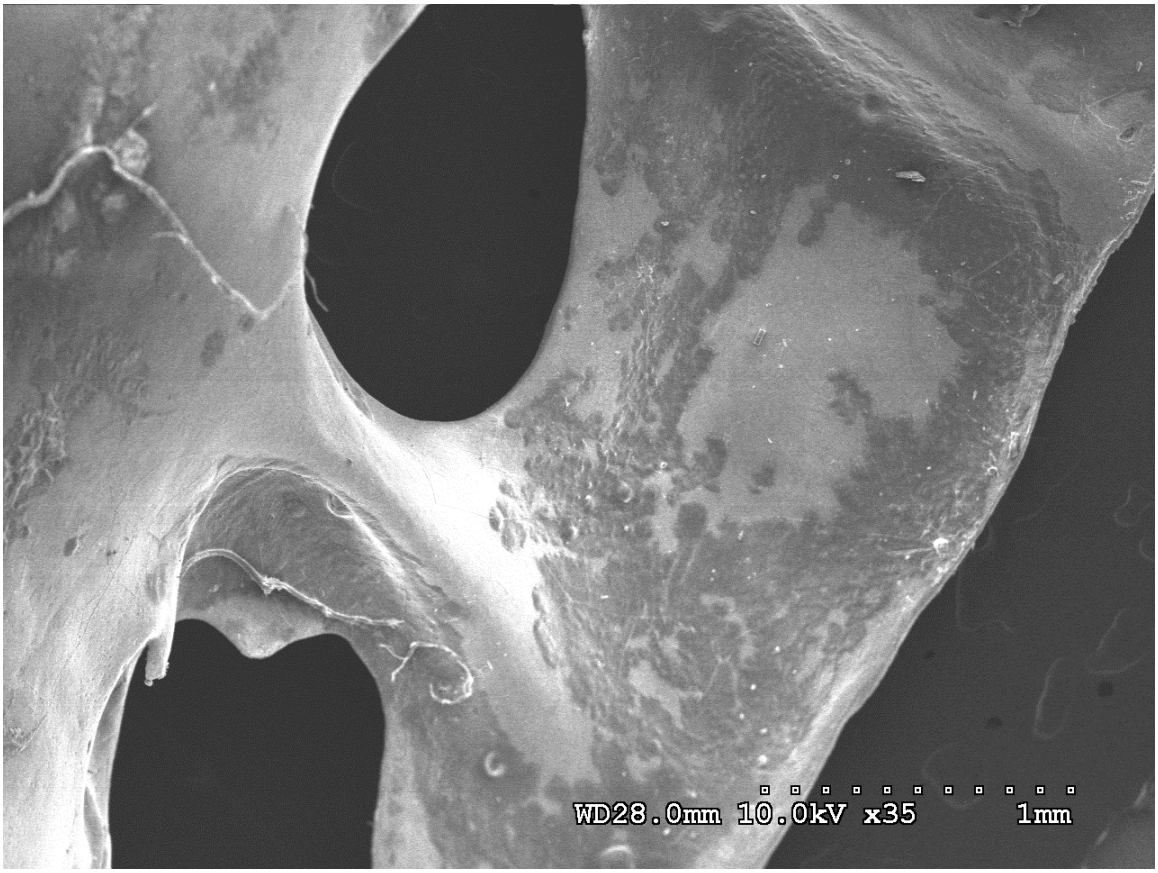


Figure 3.4. Sacrum, tubercles, and sacral groove (sulcus and linear ridge-dorsal right view) *(civil case sample)* at 10 kV 35X magnification.

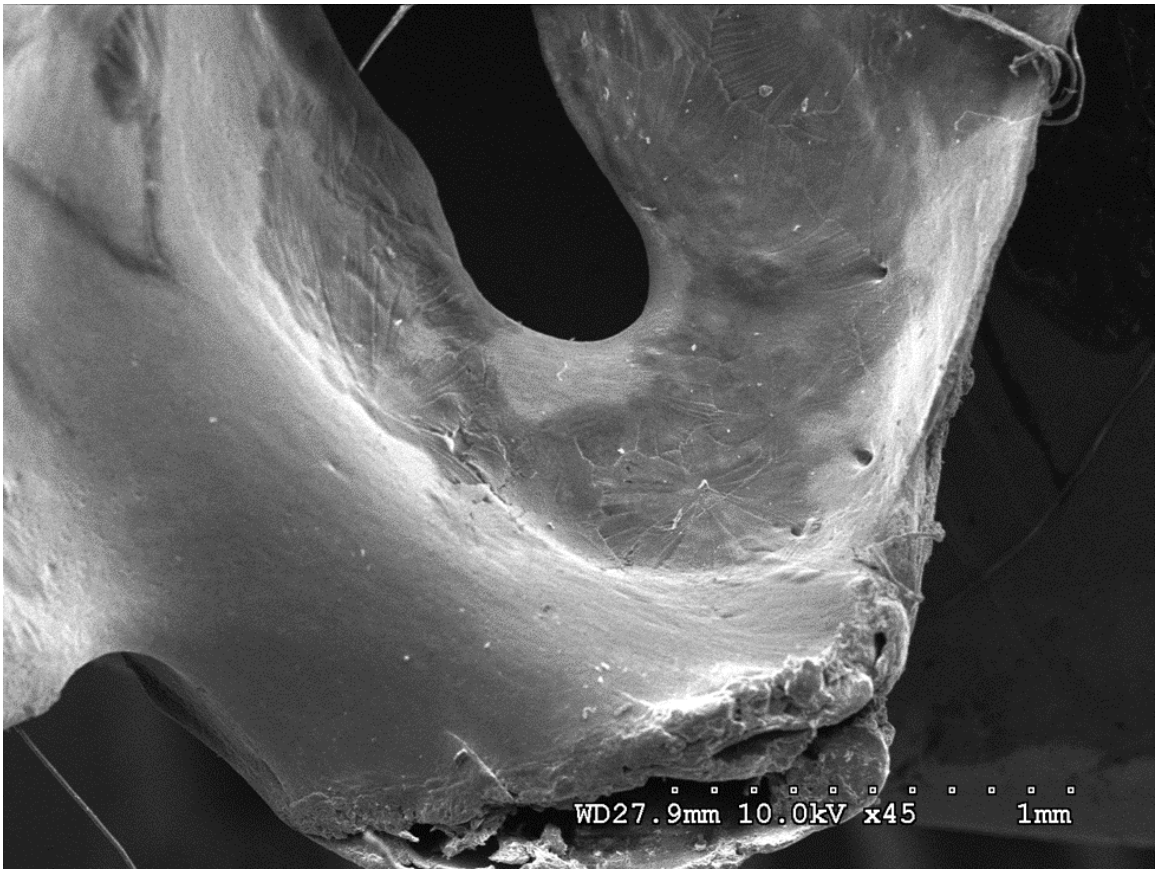


Figure 4.1. Dorsal view of stage 2 weathering (fissures and foramen shown) on anterior sacral depression *(civil case sample)* at 10 kV 45X magnification.

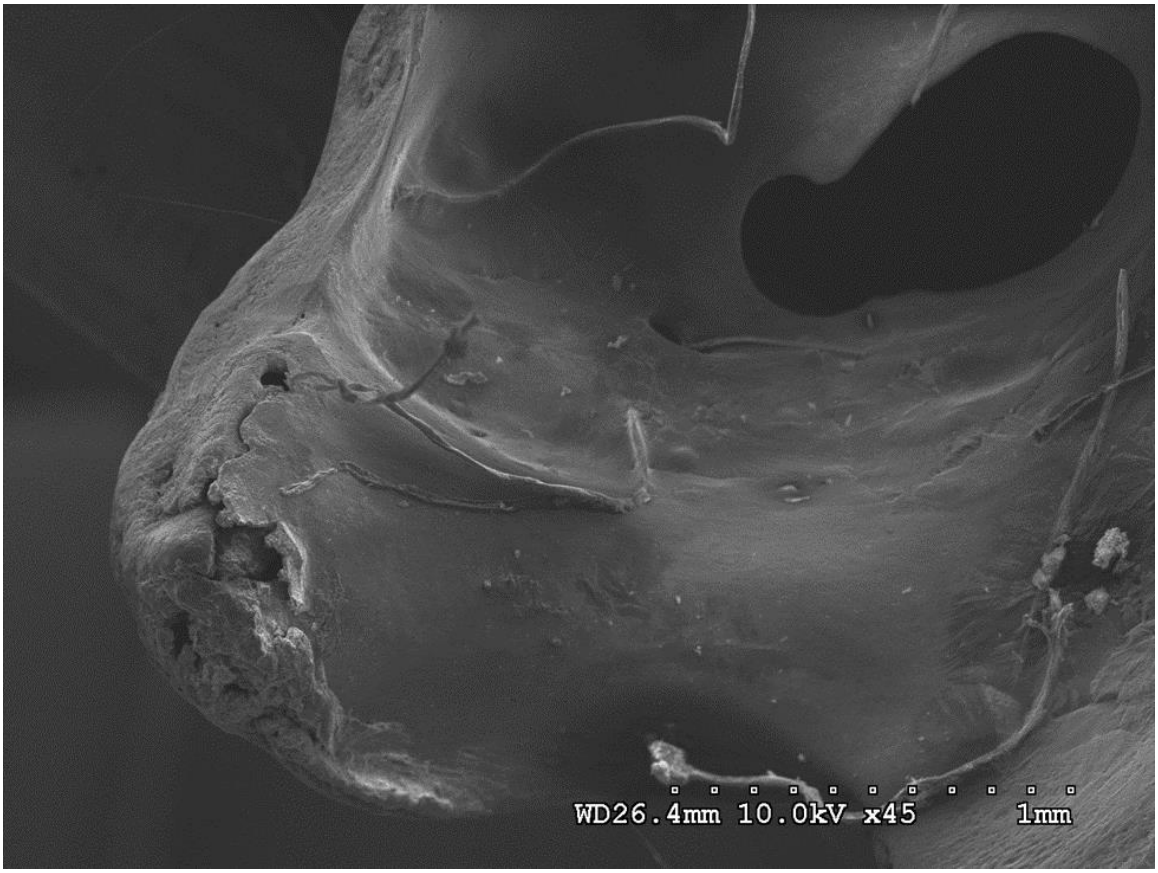


Figure 4.2. Fissures and several posterior sacral foramina on a depression at *(civil case sample)*

10 kV 45X magnification.

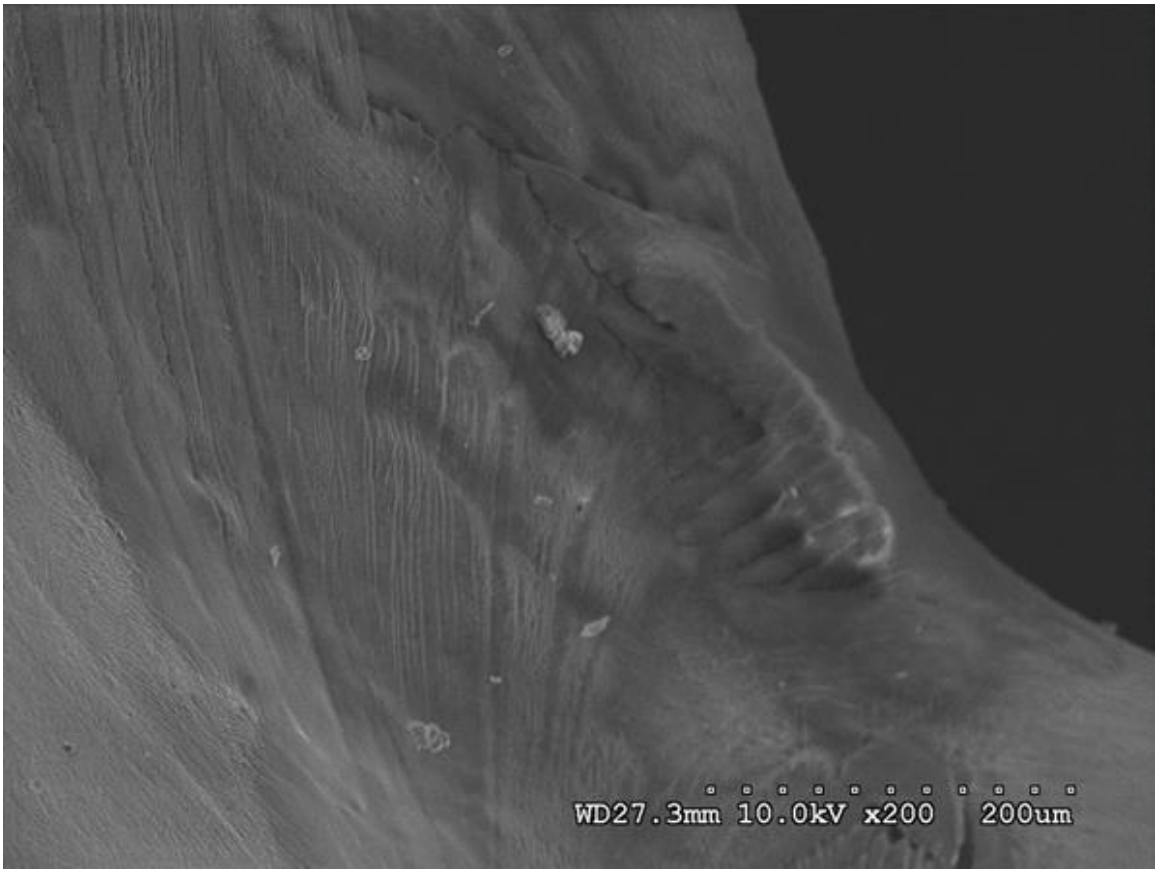


Figure 4.3. Dorsal view of stage 2 weathering on sacral depression *(civil case sample)* at 10 kV 200X magnification.

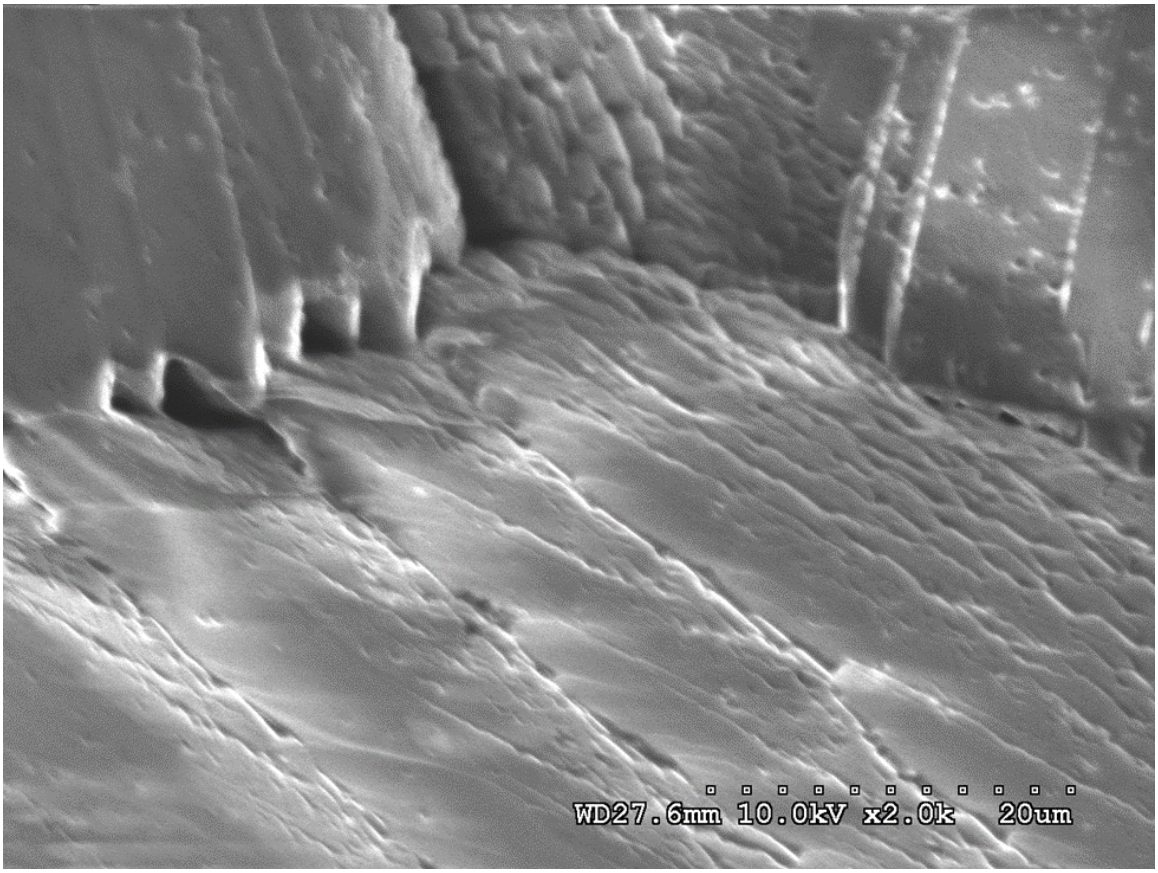


Figure 4.4. Fissures along posterior sacral foramina on a depression *(civil case sample)* at 10 kV 2.0kX magnification.

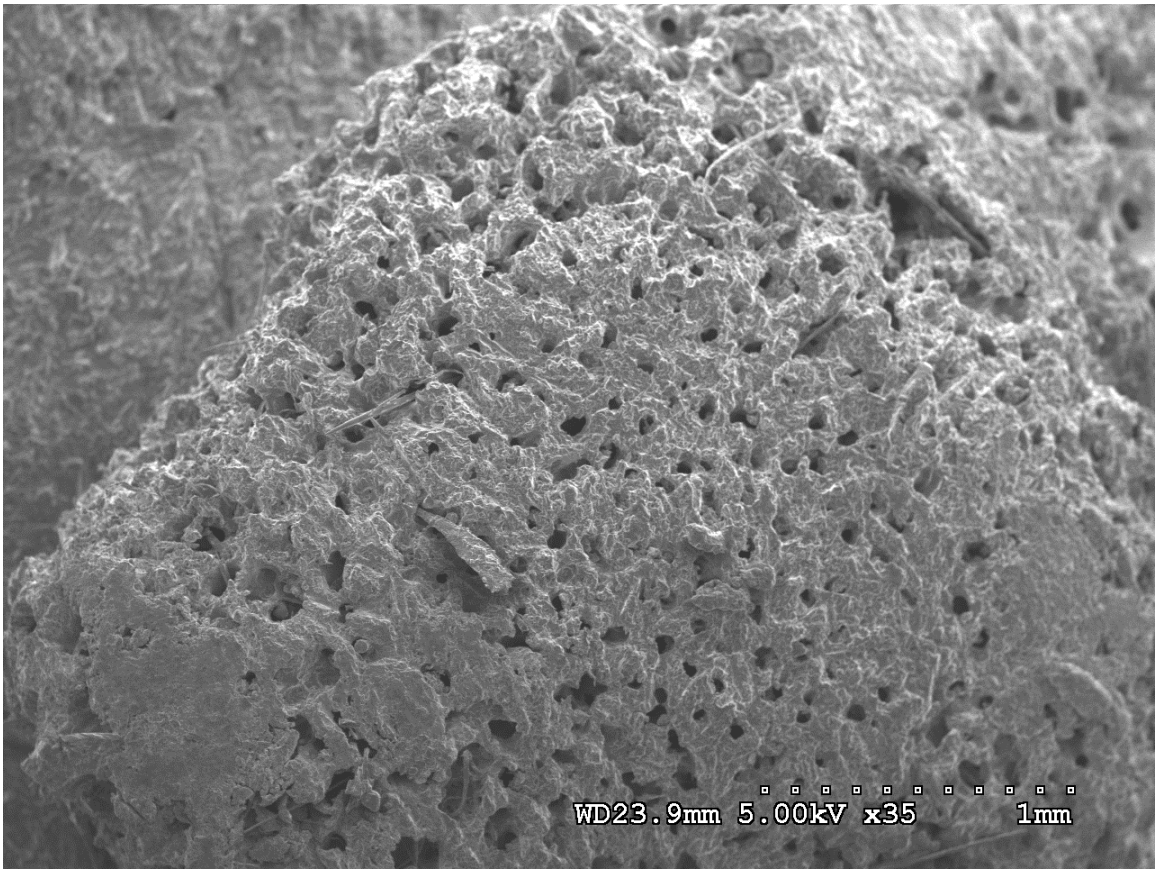


Figure 2.1. Medial sacral crest *(heat and chemical dried sample)* at 5 kV 35X magnification.

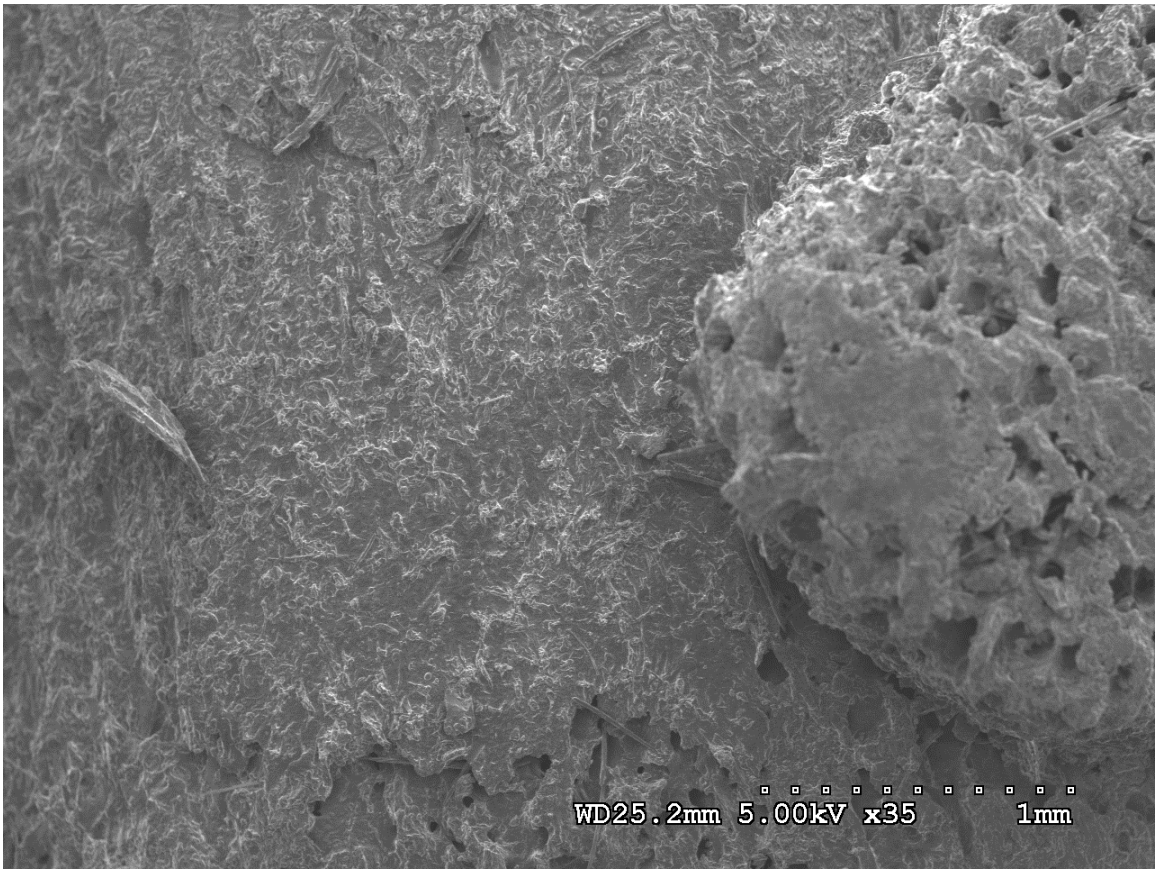


Figure 5.2. Medial sacral crest *(heat and chemical dried sample)* at 5 kV 35X magnification.

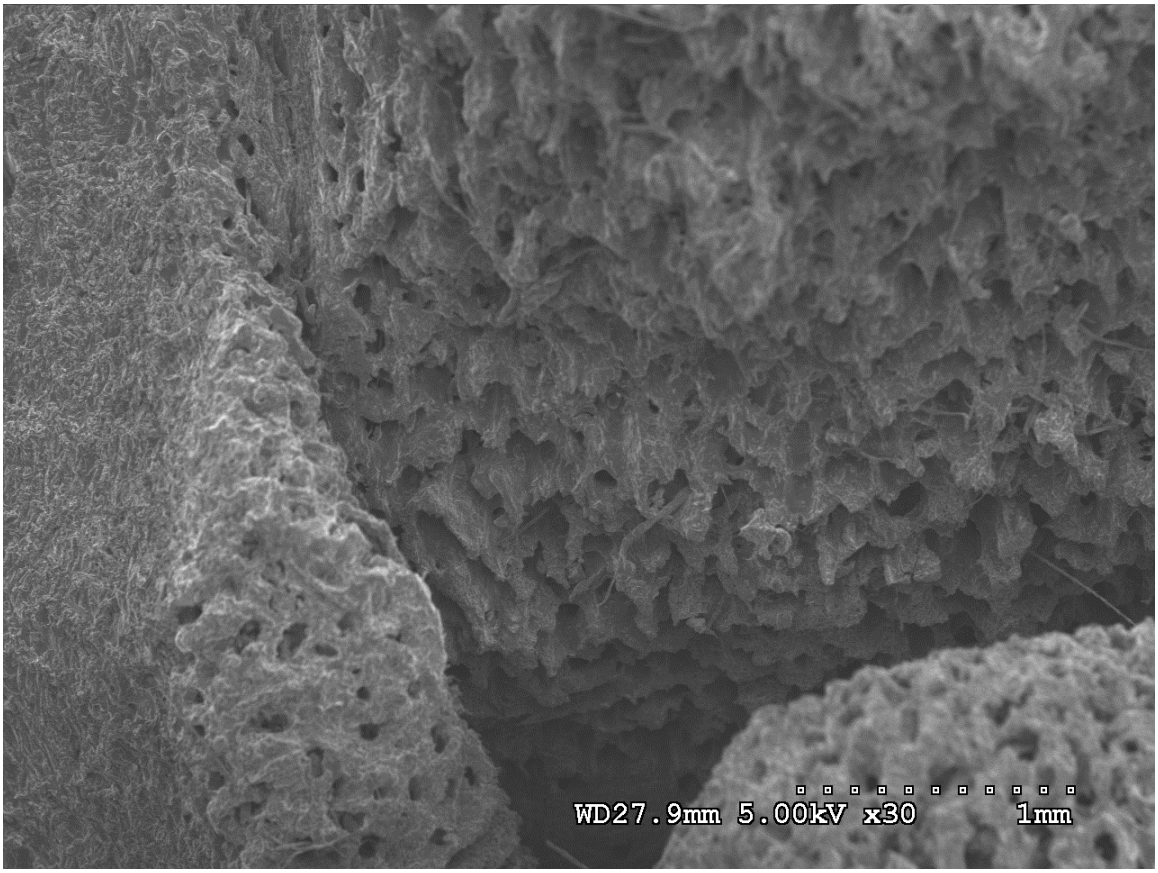


Figure 5.3. Sacrum *(heat and chemical dried sample)* at 10 kV 30X magnification.

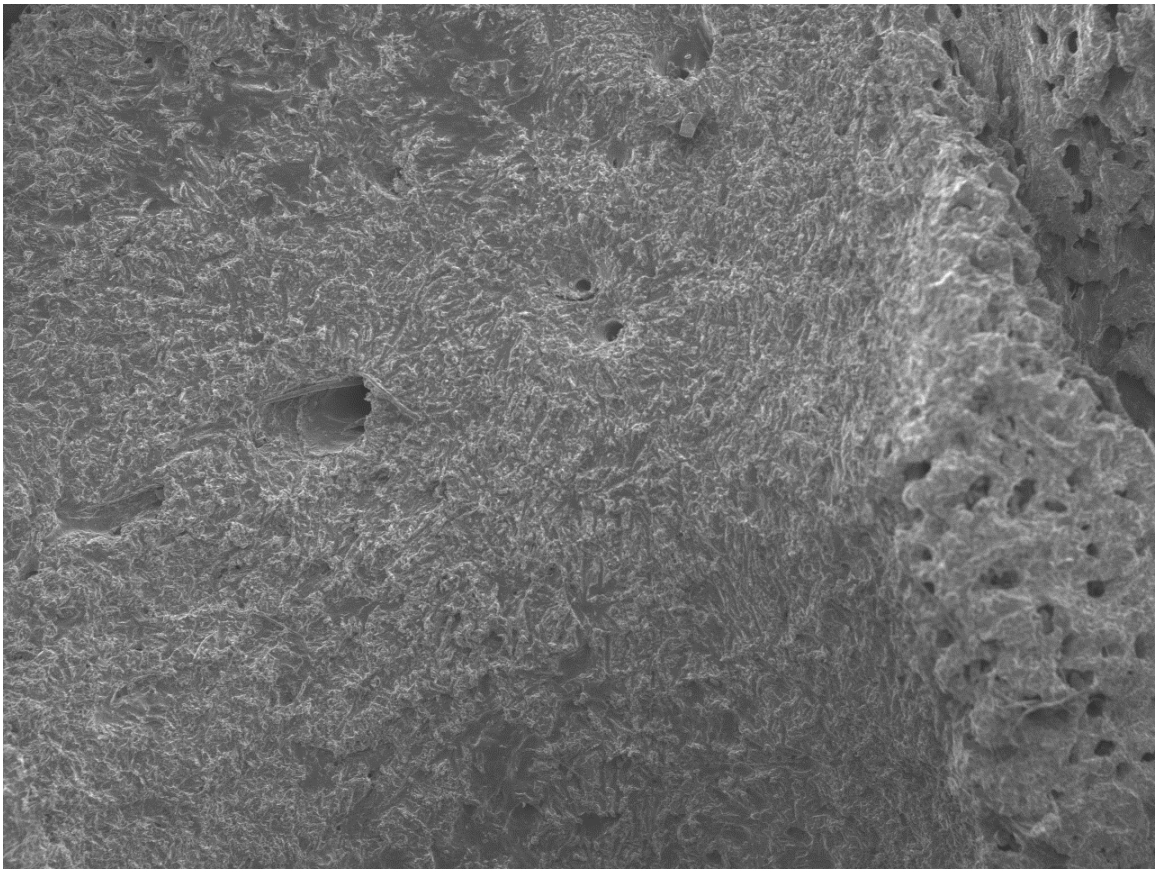


Figure 5.4. Dorsal view of sacrum and foramen *(heat and chemical dried sample)* at 10 kV 120x magnification.

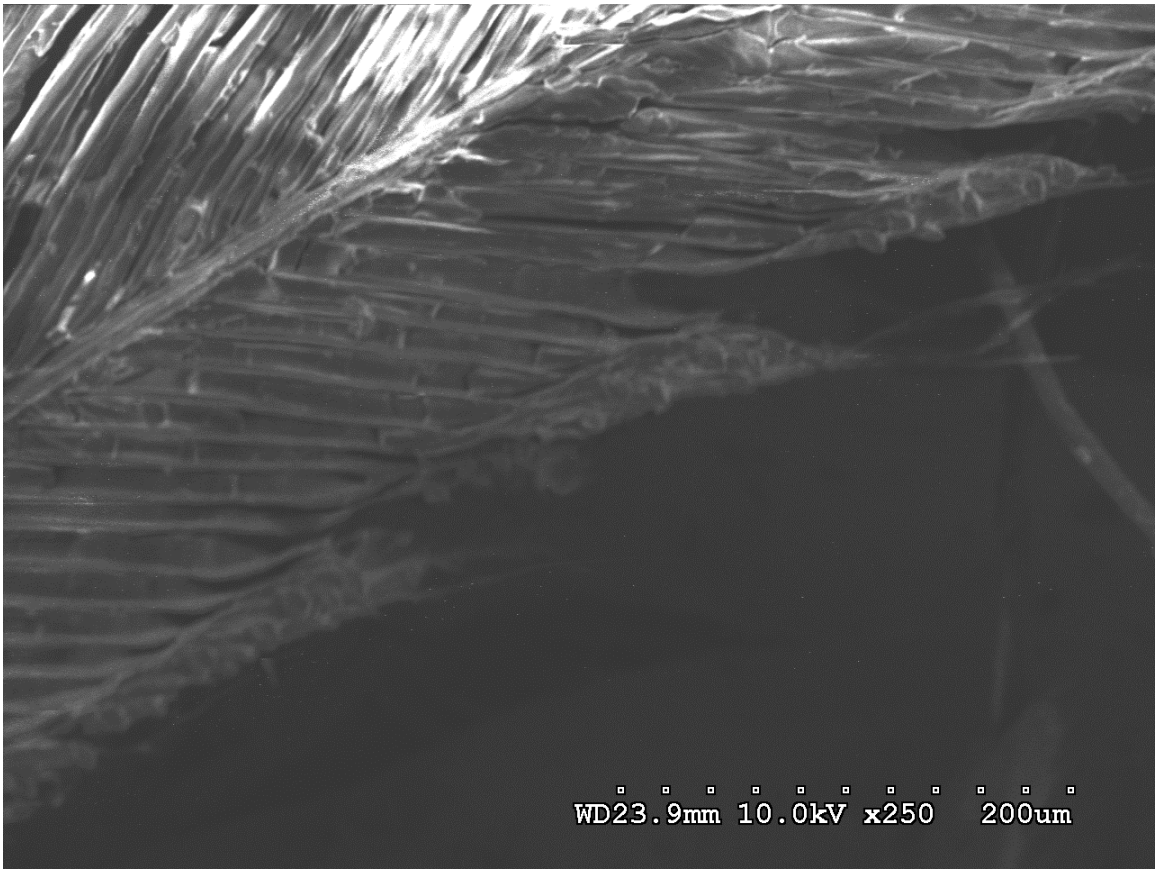


Figure 6.1. Feathers and fibers near nasal process at 10 kV 250X magnification.

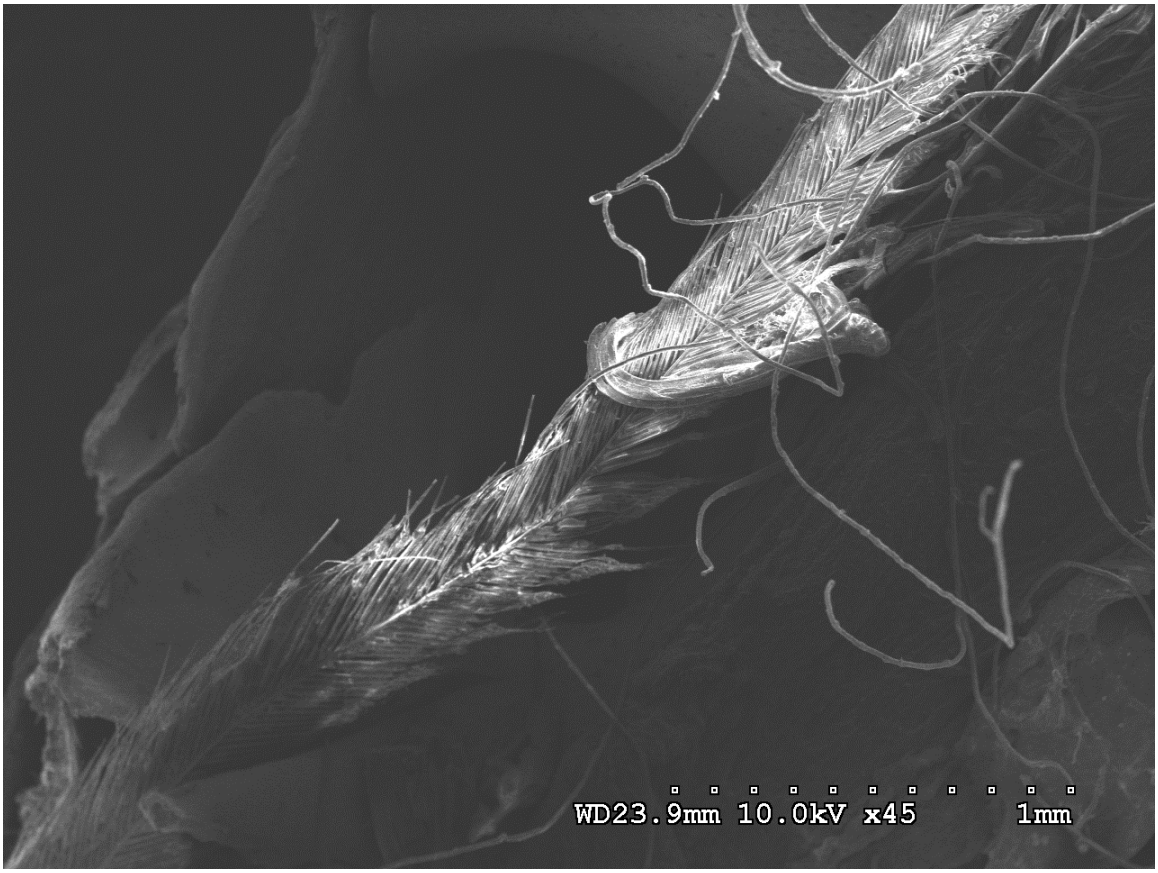


Figure 6.2. Feathers and fibers near nasal process at 10 kV 45X magnification.

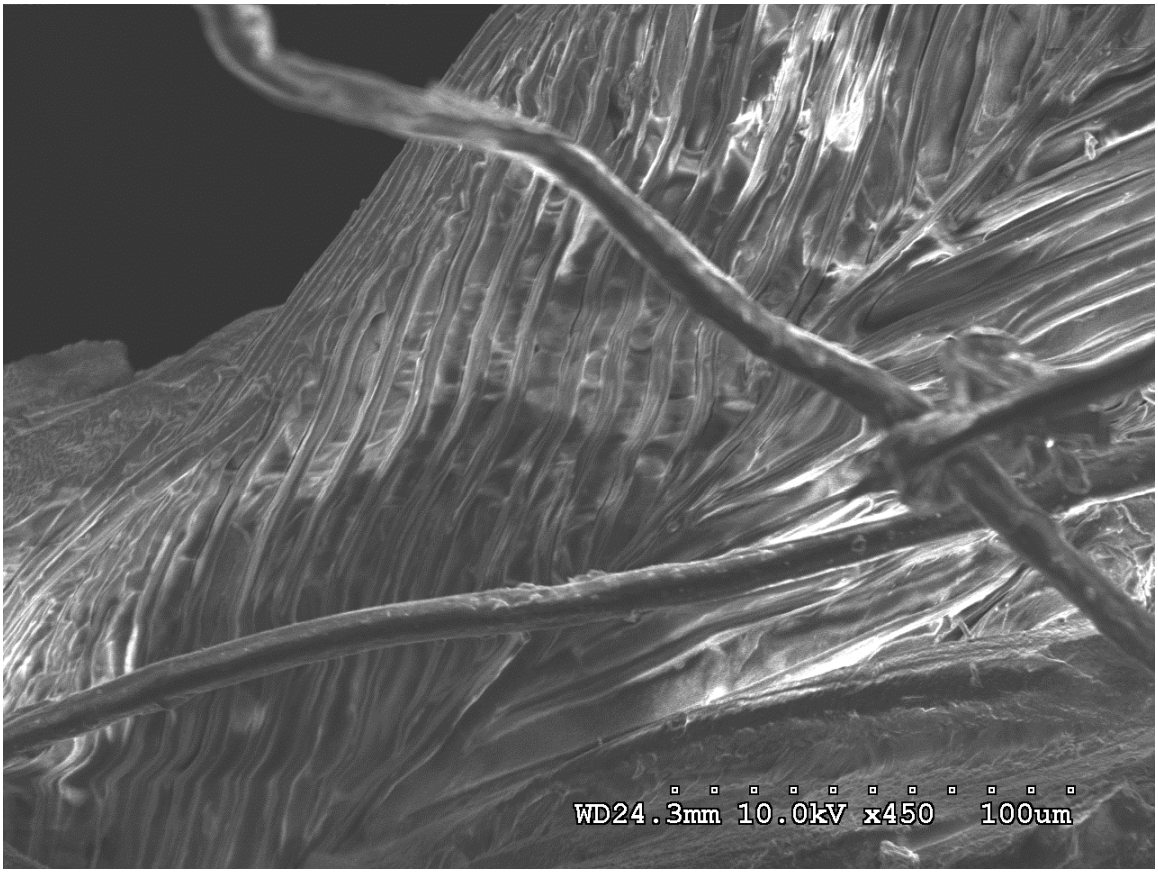


Figure 6.3. Feathers and fibers near nasal process at 10 kV 450X magnification.

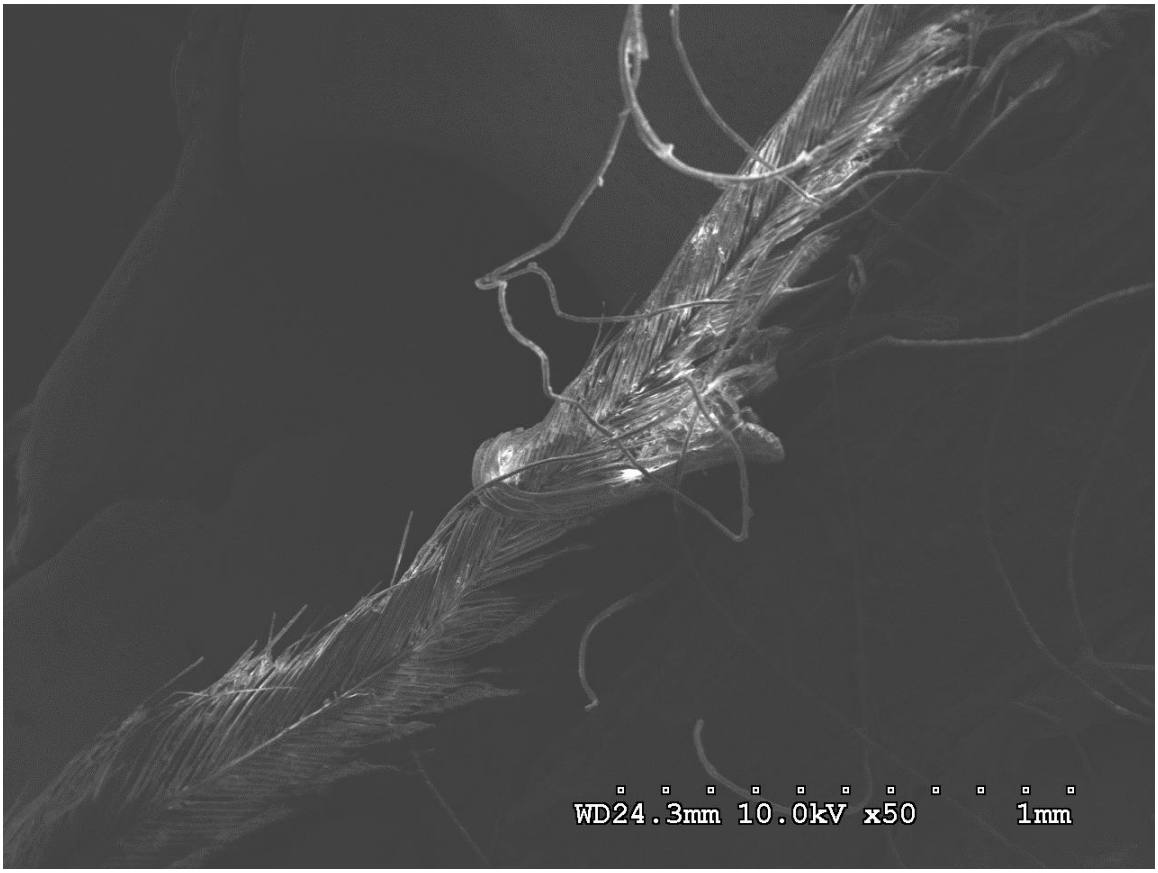


Figure 6.4. Feathers and fibers near nasal process at 10 kV 50X magnification.

References

Behrensmeyer, A. K. 1978. Taphonomic and ecologic information from bone weathering. Paleobiology. 4: 150-162.

Byrd, J. and Castner, J. 2001. Forensic entomology. The utility of arthropods in legal investigations. CRC Press. Washington, D.C.

Borell, A.E. 1938. Cleaning small collections of skulls and skeletons with dermestid beetles. Journal of Mammology 19: 102–10.

Ceciliason, A., Andersson, G., Lindström, A., and Sandler, H. 2018. Quantifying human decomposition in an indoor setting and implications for postmortem interval estimation. Forensic Sci. International. 283: 180-189.

Cornaby, B. W. 1974. Carrion reduction by animals in contrasting tropical habitats. Biotropica. 6: 51.

Fernandez-Jalvo, Y., Monfort, M.D.M. 2008. Experimental taphonomy in museums: Preparation protocols for skeletons and fossil vertebrates under scanning electron microscopy. Geobios 41: 157–18.

Hefti, E., Trechsel, U., Ruffenacht, H., and Fleisch, H. 1980. Use of dermestid beetles in cleaning bones. Calcified Tissue Institute 35: 45-47.

Hinshaw, S. II. 2003. Use of dermestid beetles for skeleton preparation. http://deepwater.org/bioteacher/11-Ecology/dermestids/colony-maintenance.htm.

Howell, A.H. 1932. Dermestid beetles as an aid in cleaning bones. J. Mammalogy 45: 372 – 374.

Kirkland, J.I., Bader, K. 2010. Insect trace fossils associated with Protoceratops carcasses in the Djadokhta formation (upper Cretaceous), Mongolia. Palaeogeography, Palaeoclimatology, Palaeoecology 311: 38-47.

Kitching, J.W. 1980. On some fossil arthropoda from the limeworks, makapansgat, Potgieterus. Palaeontologia Africana 23: 63 – 68.

Martin, L.D., West, D.L. 1995. The recognition and use of dermestid (Insecta, Coleoptera) pupation chambers in palaeoecology. Palaeogeography, Palaeoclimatology, Palaeoecology, 113: 303.Osuji, F.N.C. 1975. Some aspects of the biology of Dermestes maculatus DeGeer (Coleoptera, Dermestidae) in dried fish. Journal of Stored Product Research 11: 25 – 31.

Parkinson, A. H. 2012. *Dermestes maculatus* and *Periplaneta americana*: bone modification criteria and establishing their potential as climatic indicators. M.S. Dissertation, University of the Witwatersstrand, Johannesburg, South America.

Roberts, E., Rogers, R., and Foreman, B. 2007. Continental insect borings in dinosaur bone: examples from the late Cretaceous of Madagascar and Utah. Journal of Paleontology. The Paleontological Society 81: 1.

Ryan, M. J., Chinnery-Allgeier, B. J., and Eberth, D. A. 2010. New perspectives on horned dinosaurs. Indiana University Press, Bloomington, IN.

Schroeder H., Klotzbach, H., Oesterhelweg, L., and Puschel, K. 2002. Larder beetles (Coleoptera, Dermestidae) as an accelerating factor for decomposition of a human corpse. Forensic Sci. International. 127: 231-236.

Smith, K.G.V. 1986. A manual of forensic entomology. Cornell University Press, Ithaca, NY.

Sommer, H. G. and Anderson, S. 1974. Cleaning skeletons with dermestid beetles—two refinements in the method. Curator: The Museum J. 17: 290-298.

Tobien, H. 1965. Insekten-frassspuren an tertiären und Pleistozänen Säugertier knochen. Senckenbergiana Lethaia 46: 441–445.

Voorhies, C.T. 1948. A chest for dermestid cleaning of skulls. J. Mammalogy 29: 188-189.

West, D.L., and Hasiotis, S.T. 2007. Trace fossils in an archaeological context: examples from bison skeletons, Texas, USA. In: Miller, W. (ed). 2011. Trace Fossils: Concepts, Problems, Prospects, Elsevier, Oxford, England.