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# IDENTIFICATION AND PHYLOGENETIC CHARACTERIZATION OF SELECT SPECIES OF BUPRESTIDAE (COLEOPTERA) AND SESIIDAE (LEPIDOPTERA) WOOD BORING INSECT FAMILIES OCCURING ACROSS THE SOUTHEASTERN UNITED STATES.

A Dissertation
Presented for the
Doctor of Philosophy
Degree
The University of Tennessee, Knoxville

Jason Andrew Hansen
May 2010

To Meg, Aspen, Joel and Charlotte

## ACKNOWLEDGEMENTS

I would like to thank my committee members Kevin Moulton, Bill Klingeman, Jason Oliver and Mark Windham for their advice and help during my graduate experience at the University of Tennessee. I am especially grateful for my two advisors Bill Klingeman and Kevin Moulton for their patience and help as I grew in the field of entomology and molecular systematics. I would be remiss to forget my undergraduate entomology professor, Richard Baumann, whose "Introduction to Entomology" class in the basement of the Bean Museum at Brigham Young University first captured my interest in insects. I must also thank the many people who taught me the excitement of discovery. Malabi Venkatesan, Jay Vatsan and Ryan Ranallo all contributed to my understanding of basic molecular techniques as I worked under their tutalege at the Walter Reed Army Institute of Research. The knowledge I gained from them formed the foundation that helped me to complete this work.

I am indebted to the many others who helped provide much needed specimens to complete the molecular portion of this work: Josh Basham, Chris Bergh, David Boyd, Daniel Carnagey, Juang-Horng Chong, Ted Cottrell, Michelle Gorman, Linda and Tim Hansen, Ronald Huber, David Kain, Tracy Leskey, Catharine Mannion, Richard Merriment, Mike Reding, Glen Salsbury, Paul Super, Linda Treeful, John Walas, Jim Walgenbach, Stan Wellso, Rick Westcott and Nadeer Youssef. Their cooperation and selfless service were more than I could have asked for.

I would also like to thank Adriean Mayor for access to the Great Smoky Mountains National Park insect collection and his kind encouragement. Stan Wellso, Richard Westcott, Henry Hespenheide and Thomas Echlin all helped unflinchingly with identifications and advice. Corresponding with them was one of the highlights of my time as a graduate student.

I must include in this an acknowledgement of my dear parents for watching over me as a child and supporting my education both financially and with words of encouragement. I appreciate my mother for her sacrifice and caring. As I look back, I understand just how much she gave for her children. I am grateful for my father's example. With three children, he worked through two graduate degrees, first at Rice University and then at Ohio State University. He has continued to inspire me with his love of learning even well into retirement. I would also like to acknowledge my mother-in-law, Jeanne Douglas, who at the age of fifty-five went back to school to earn her undergraduate degree, with honors. She is proof that you are never too old to go back to school and pursue what you love.

Finally, I'd like to thank my wife Meg to whom this work is dedicated. Without her willingness to postpone her own educational aspirations, I would never have been able to accomplish what I have. She is my superior in every way I can imagine and yet she still sticks around. I am truly grateful for her love, encouragement and the hard work and sacrifice she has done to make this degree possible for me. I look forward to many future adventures with her as my companion.

## ABSTRACT

A brief overview of the importance of wood boring insects is provided. Background on the two wood boring insect families Buprestidae (Coleoptera) and Sesiidae (Lepidoptera) is given. Keys and checklists to Tennessee's buprestid fauna as presently known are furnished. Photomicrographs depicting characteristics separating Tennessee buprestid taxa to the level of species are provided for select couplets to aid those unfamiliar with buprestid morphology and terminology. Distribution and flight data of many species within the state are also featured. Results of a phylogenetic analysis of the *Chrysobothris femorata* (Olivier) species complex is presented based on the nuclear gene arginine kinase and the mitochondrial gene cox I. Implications of the resultant phylogenies are discussed. Phylogenetic relationships with the economically important sesiid tribe Synanthedonini are explored using cox I gene sequences. The cox I tree inferred provides interesting new insight into some ambiguous evolutionary relationships. Morphological characters that are used to distinguish genera within Synanthedonini are discussed and compared with the molecular data.

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# CHAPTER 1

# INTRODUCTION

Several orders of insects have evolved the ability to exploit various woody plant tissues to increase their reproductive success (Solomon 1995). Wood boring insects contribute to economic losses in commercially grown crops and can impact international trade (USDA Forest Service 2001, Evans and Oszako 2007). Lax regulation of imported woody material to the United States and other countries has allowed entry of several non-native wood boring species, some potentially devastating to local ecosystems and commerce (Nowak et al. 2001, Haack 2006, Poland and McCullough 2006). Cargo inbound from foreign ports can be sent back to the country of origin if evidence of wood borers is discovered, but with less than 2% of imports being checked some infested material inevitably evades port inspectors (National Research Council 2002, Federal Register 2004). Wood borers are particularly adroit at surviving in untreated ballast material commonly used to brace shipped goods leaving overseas harbors (Haack 2006). Strict regulations on wood packaging material entering the United States are currently in place in an attempt to mitigate the flow of non-native insects but are not always successful in preventing transport of borers across United States borders (Federal Register 2004, Haack and Petrice 2009).

The key to why wood boring insects transport long distances internationally, as well as within the United States, is found in their life history. Borers spend the majority of their life cycle in a protected environment, safely concealed beneath the bark of their host plant. Some survive as adults through the colder months of the year (Wellso 1974, Brewer et al. 1988, Solomon 1995). Many others overwinter as larvae, awaiting warmer temperatures to continue feeding and complete their development. The constant relative

humidity in galleries constructed by adults or their larvae help borers avoid desiccation that would otherwise reduce their numbers. In North America pupation generally takes place in the spring or early summer and can last several weeks before the imago emerges (Solomon 1995).

Though the United States faces a constant threat of exotic wood borers breaching its borders, it is also confronted with losses, both ecological and economical, from its own native borers. Plant damage caused by larvae of native wood borers ranges from merely aesthetic to fatal. Wood borers can cause devastating losses of native plants during droughts and other extreme environmental conditions that can make plants more susceptible to attack (Wygant 1938, Dunbar and Stephens 1975, Wermelinger et al. 2008). Growers of ornamental and shade trees as well as fruit and nut trees, have to continuously protect crops from numerous borers, which are capable of hastening tree decline and reducing yields (Nielson 1981).

The timber industry can especially feel the effect of wood borers with revenue losses as high as $60 per thousand board feet for some oaks (Morris 1977). On occasion feeding activity of larvae can cause degradation of timber to such an extent that harvested logs must be entirely discarded (Akbulut et al. 2008). One study estimated red oak saw-timber alone has a value loss of nearly half a billion dollars in three eastern states due to wood borers; and larval feeding of *Enaphalodes rufulus* (Haldeman) was estimated to cause 40% losses in oak lumber (Donley 1974, Donley and Worley 1976). In pine lumber the long horn beetle *Monochamus scutellatus* (Say) can cause monetary losses of more than one-third the lumber's value (Wilson 1962). Such damage can have a

substantial impact on an industry that has consistently produced more than 40 billion board feet of lumber annually from 1984–2005 (Howard 2007).

Two significant contributors to the economic impact of wood boring insects are clearwing moths (Lepidoptera: Sesiidae) and metallic wood boring beetles (Coleoptera: Buprestidae) (Solomon 1995). Both are easily transported across state and international boarders in larval hosts. Each family possesses native members that routinely attack nursery, landscape and orchard trees. Despite some being recognized pests of forests and commercial enterprises for well over a century, our understanding of their basic biology and life histories is still evolving.

*Metallic wood boring beetles*

Humans for millennia have admired buprestid beetles. Ancient Egyptians fashioned necklaces from their elytra and carved their likeness out of stone (Levinson and Levinson 2001). In Rome "buprestis" was recommended as a cure for facial maladies when applied directly (Cowan 1865). In Europe and most other areas of the world buprestids are known as jewel beetles because of the beautiful elytral colors many display. Recently a renowned artist decorated the palace in Brussells with the elytra of over a million buprestids (Dicke 2004). Not surprisingly, collecting buprestids in some parts of the world has become so popular local legislatures were forced to enact laws protecting them from over harvest and possible extinction (Hawkeswood et al. 1991). Their popularity even extends to the dinner table in some cultures. Ancient Greeks reportedly enjoyed the taste of "buprestis" and even in modern times the largest buprestid

in the world, *Euchroma gigantea* Linné, has been noted as accepted fare of at least one Amazonian tribe (Cowan 1865, Dufour 1987).

Buprestidae contains over 15,000 described species distributed throughout the world on almost every continent (Bellamy 2008). North America alone is home to nearly 800 species of buprestids, which utilize a wide variety of plant hosts, from herbaceous perennial plants to hardwood trees (Nelson et al. 2008). Completion of the buprestid life cycle from egg to adult typically takes about one to two years, but exceptions do exist, giving buprestids the distinction of having one of the longest life cycles of any arthropod in its class (Smith 1962, Bellamy 2007). Adults feed on pollen, bark, leaves and even fungi and can live several weeks (Fenton 1942, Bellamy and Nelson 2002, Hespenheide 2003).

Mating behavior of buprestids is still not thoroughly documented but a few published observations help to enlighten our understanding. Sex pheromones emitted by females are thought to play a role in mate recognition of at least some buprestids (Dunn and Potter 1988, Silk et al. 2009). *Xenorhipis brendeli* LeConte females are believed to release an uncharacterized sex pheromone, attracting male beetles that likely detect the pheromone with their elaborate antennae (Wellso 1966). Males of *Hippomelas planicosta* LeConte have been observed to mount competing males in the act of mating and exhibit courtship behavior before finally loosing interest; presumably attracted by an olfactory response to an unknown sex pheromone emited by the mounted female rather than visual cues (Alcock 1976). Another unusual behavior exhibited by the males of some species is the rapid up and down movement of the abdomen to create an audible thumping when a branch is struck. The sound appears to attract conspecific females even

when imitated by tapping with a fingernail or pencil (Bowditch 1896, Beer 1970). Similarly, male *Euchroma gigantea* also lure mates in with a clicking sound produced by movement of its elytra (Nichols 1910). Males of *Acmaeodera impluviata* Mannerheim use as series of genitalic taps looking for permission from the female to begin copulation (Eberhard 1990). Females do not necessarily mate twice and may refuse advances from subsequent males if their first partner provided sufficient sperm (Eberhard 1990, Akers and Nielsen 1992). Finally, some male buprestids may be attracted by the elytral color of conspecific females as viewed from the air as they fly above (Hawkeswood 2005). Still, the sexual habits of most buprestids are not fully understood and await the study of future coleopterists.

Control of buprestid borers is a continuous problem for growers whose profit margins can be squeezed significantly by tree loss and decline. Managers most often turn to insecticides for reliable and rapid control of flatheaded borers (Potter et al. 1988, Hansen et al. 2009). However, increasing health concerns about chemical pesticide use may limit their employment as a control option in the future (Fenske et al. 2002, Rauh et al. 2006). Several alternatives to broad-spectrum chemical insecticides continue to be investigated.

One alternative are entomogenous fungi. They are attractive as biological controls because of their host specificity. Many insects have been successfully controlled with fungi in the past, but their use against buprestids has been limited (Fan et al. 1990, Liu and Bauer 2008). *Beauveria bassiana* (Balsamo) Vuillemin was effective against buprestid eggs and larvae (Marannino 2006). Given their effectiveness against several buprestid life stages, strains of *Beauveria* could become an invaluable management tool.

Entomopathogenic nematodes have also proven effective against some species of buprestids. The nematode *Steinernema carpocapsae* (Fil.) reduced beetle emergence 75–90% for one economically important buprestid species (Martinez de Altube et al. 2008). A limiting factor seems to be desiccation of the nematodes before they reach their larval host. The highest mortalities are achieved when nematodes are applied on days with relatively high humidity.

Buprestids are food for many other vertebrate and invertebrate organisms. For example woodpeckers take a large toll on buprestid populations (Brooks 1919, Loerch and Cameron 1983). Unfortunately, their method of extracting larvae damages trees, leaving them susceptible to further borer attacks. Predatory insects are also quick to take advantage of the immature stages. Ants, for example, can follow the path of subsurface galleries and extract the immature buprestids from beneath the bark (Brooks 1919). Hymenopteran parasitoids from at least four families (i.e. Ichneumonidae, Chalicidae, Eupelmidae and Braconidae), oviposit on buprestid larvae, which serve as hosts for their eggs (Brooks 1919, Fenton 1942, Bonsignor et al. 2008). Fenton (1942) estimated parasitization rates of 7–58% on *C. femorata* during a three-year drought period. Most specifically, *Cerceris fumipennis* (Say) wasps diligently search during daylight for buprestid prey when beetle adults are most active. Frequently, colonies of this solitary wasp number in the hundreds, each of which rely exclusively on adult buprestid beetles to provision their young. Beetle species from several buprestid genera are actively sought by *C. fumipennis*, some of which are nearly equal in size to their captor (Marshall et al. 2005). Currently, colonies of *C. fumipennis* are being employed as biotic scouts to catch one of the most infamous exotic beetles today, *Agrilus planipennis* Fairmaire

(emerald ash borer). If left unchecked emerald ash borer threatens billions of dollars worth of U.S. ash trees in forests and urban areas, where costs of removing and replacing dead trees are expected to be high (Poland and McCullough 2006, Sydnor et al. 2007).

Though buprestids historically have been recognized as pests, the relatively narrow host range of most species has made them attractive to biologists as biological control agents. Non-native *Agrilus hyperici* (Creutzer) were successfully introduced to North America in 1953 to control St. John's wort (*Hypericum perforatum* L.) (Campbell and McCaffrey 1991). The accidentally introduced *A. subrobustus* Saunders, first discovered north of Atlanta, Georgia in 2006 and then in eastern Tennessee, is suspected to only infest the invasive mimosa tree (*Albizia julibrissin* Durazz), perhaps having a negative impact on its spread (Westcott 2007, Hansen et al. 2010). In another instance, Australian authorities attempted to use *Sphenoptera clarescens* Kerr beetles for biological control of an invasive weed in Australia, but quickly eliminated this buprestid species as an option when it also infested lettuce (Hasan 1978). In North America *Taphrocerus schaefferi* Nicolay and Weiss was considered for control of Yellow nutsedge (*Cyperus esculentus* L.), but damage inflicted by feeding larvae was minimal because larval canabalism kept beetle numbers at low levels (Story and Robinson 1979).

*Clearwing moths*

Clearwing moths (Lepidoptera: Sesiidae) are diurnal wasp-like moths that infest woody and herbaceous plant species (Eichlin and Duckworth 1988). Many adult moths display aposematic color patterns, seen as a clear case of Batesian mimicry (Laštůvka and Laštůvka 2001). By imitating wasps in appearance as well as behavior, adult sesiids

avoid potential daytime predators that might otherwise consume them. The illusion is so complete it is difficult for casual observers to distinguish between the moth and its wasp model. The common name "clearwing" is derived from the lack of scales exhibited by many adult sesiids, lending to a more complete deception. Similar to their hymenopteran models, sesiids are strong fliers that couple their wings when in flight. A hook like projection on the costal side of the hindwing, known as the frenulum, helps to keep the two wings together. In addtion, the moth folds the costal edge of the hindwing around the anal portion of the forewing to keep its wings beating in unison during flight (Engelhardt 1946).

Although life histories differ to some extent, most clearwings follow a general developmental pattern. Responsibility for finding a suitable plant host for larvae falls to the female. Females are capable of laying several hundred eggs in their lifetime (Eichlin and Duckworth 1988). Plant volatiles have been shown to stimulate oviposition in at least two species (Gentry and Wells 1982, Derksen et al. 2007, Cottrell et al. 2008) and likely influences host selection of other clearwing females that routinely seek out wounds on the bark surface of host plants where they will lay their eggs (Hardy 1982, Rocchini et al. 1999). Once hatched from eggs, larvae tunnel beneath the bark and feed in protected gallaries until the following season. After overwintering, larvae either pupate or continue to feed, depending on the species. Most adult moths emerge in spring to late summer, though a few in more southern latitudes emerge year round (Eichlin and Duckworth 1988). Most lepidopteran pheromones targeting conspecific mates originate from females, and sesiids are no exception. Mate finding is initiated by sesiid females that "call" males with the release of sex pheromone from genitalia visibly extruded from the

last abdominal segment (Gentry and Sekul 1972, Bergh et al. 2006). Males may follow the pheromone plume for several hundred meters before finding the receptive female and coupling (Snap and Thomson 1943).

In their natural setting sesiids rarely reach high population densities and therefore present little or no problem. However, commercial and landscape managers can see significant impact from a rise in numbers of the endophagous larvae, which can cause aesthetic damage, reduce crop yields and kill young trees. Mostly for these reasons some clearwings have been the targets of numerous studies to understand their biology and develop methods of control (Solomon and Dix 1979). Nuisance clearwing populations are commonly managed with chemical insecticides, although alternative management options are continually investigated (Miller and Bedding 1982, Shapiro-Ilan and Cottrell 2006).

Tumilson et al. (1974) were the first to identify the molecular composition of a sesiid sex pheromone. They observed the inhibitory nature of the *Synanthedon pictipes* (Grote and Robinson) phermone on *S. exitiosa* (Say) moth communication and vice versa (Tumilson et al. 1974). Since the discovery of the peachtree borer pheromones it has become apparent that each moth has a unique pheromone blend composed of different chemical isomers that help limit responses from non-conspecifics (Greenfield and Karandinos 1979, Cowles et al. 1996, Szöcs et al. 2004). Nevertheless, many pheromone blends attract non-target sesiid species whose pheromones share major chemical components (Bergh et al. 2004). This phenomenon of intergeneric attraction in sesiids was first noticed by Neilsen and Balderston (1973) when caged virgin females attracted males of several different species. Non-conspecific males would often approach the trap

but would not attempt to mate with caged females when they came within visual range. It is possible that other visual cues must be present for species recognition once the male gets close enough to see the calling female and sex-pheromones serves only as a general indicator for long distance signaling (McLaughlin et al. 1976). Many clearwings have yet to have their pheromone characterized, either because they are of little economic importance or due to availability of commercial lures that work sufficiently well to negate the need for a more thorough understanding of the specific moth's exact pheromone composition.

As clearwing pheromones where elucidated, studies investigating their use as a management tool to reduce dependency on potentially problematic pesticides also grew. Not only were pheromones a powerful monitoring tool, they could also be employed to disrupt communication between calling females and seeking mates. By diffusing synthetic sex pheromones though a wide area, male moths become confused between calling females and synthetic lures slowly dispensing sex pheromone. Eventually, the deception leaves males incapable of finding a mate and they die having expended their energy chasing synthetic lures (Gaston et al. 1967).

McLaughlin et al. (1976) showed orchards permeated with peachtree sex pheromones significantly reduced the number of male peachtree borers caught in traps, a fact echoed in later studies (Yonce 1981, Gentry and Snow 1984). In practice pheromone disruption has been successful in just a few cases (Pfeiffer and Killian 1991, Thomas and Burnip 1991). However, the pheromone lure used must be at least as attractive as virgin female moths or any attempt to interrupt communication generally fail (Pfeiffer and Killian 1999).

Despite the obvious advantages of pheromone use for mating disruption, this practice does have limitations. Principal among these drawbacks is immigration of gravid females from areas outside treated plots (Cardé and Minks 1995). Therefore attempts to use this method of control will work best when the area treated is isolated from all immigrating individuals. Furthermore, if population densities are too high some males will inevitably find females using visual cues simply because they come into such close proximity (Carde and Minks 1995). So while pheromone disruption may work in some cases, its use will be limited to instances where population densities are low and isolated.

As with management of buprestid beetles, entomophagous nematodes have been used successfully as a biological control of clearwing larvae of several economically important species (Bedding and Miller 1981, Nachtigall and Dickler 1992, Williams et al. 2002, Cottrell and Shapiro-Ilan 2006). Larval mortality as high as 93% was seen in one clearwing species when nematodes were directly injected into galleries (Kaya and Brown 1986). Two constraints faced with biological control of clearwings using nematodes are method of application and species of nematode selected (Bedding and Miller 1981). Nematodes are sensitive to desiccation and must be applied during periods of high relative humidity or plants must be kept moist until the infective juveniles have had the chance to find and infect larval hosts. Although still not commonly used, nematodes are commercially available to growers at prices that can rival insecticides if applied correctly (Arbico organics 2010).

In some situations clearwing infestations are actually encouraged. Strong plant host specificity has brought these diurnal moths to the attention of biologists around the

world charged with mitigating the damage of invasive plants. Islands are particularly susceptible to the devastation introduced plants can bring. Australia in particular has considered several clearwing moth species as potential biological control agents of select invasive weeds (Scott and Sagliocco 1991, Sagliocco and Coupland 1995, Steinbauer 1998). In one successful case in Hawaii, the African vine borer *Melittia oedipus* Oberthur, was used to reduce the spread of the invasive ivy gourd, *Coccinia grandis* (L.) Voigt, in Hawaii (Chun 2001).

*Need for molecular investigation*

Molecular techniques have proven to be a significant aid where species determination is difficult using only morphological characters (Armstrong et al. 1997, Brown et al. 1999, Wei-Nung Lu et al. 2008). Identification of problem pests is crucial when determining the best management options available. Incorrectly identifying pests can lead to lost effort, revenue and can potentially exacerbate some pest problems by negatively affecting beneficial insects or contributing to pesticide resistance. Pests can be accurately determined and appropiately addressed using molecular tools, saving resources and money.

The two families of boring insects that are the subject of this dissertation include species responsible for enormous economic and yield losses to growers, homeowners and landscape managers (Fisher 1928, Fisher 1942, Eichlin and Duckworth 1988). Though both insect familes have received considerable attention from morphological taxonomists, very few studies have investigated genetic diversity among species (Bernhard et al. 2005, McKern and Szalanski 2008, McKern et al. 2008). Modern molecular approaches allow

development of species-specific diagnostic tools useful not only for identifying adult insects, but also immature stages. Indeed, ability to determine species of larvae molecularly is particularly useful in cases where species can be confused because of their similar outward appearance or when external morphological characters have yet to be described.

Moreover, many buprestids and sesiids species have not had larval stages described, making species identification by morphological means impossible. Molecular diagnostics that prove reliable with adults will also help identify larvae or eggs of species that would otherwise be unidentifiable. This may have an added benefit of helping to speed discovery of plant-species associations and augment our knowledge of species' life histories.

Researchers have advocated the use of standardized DNA sequences or "barcodes" as a means of identifying taxa, rather than relying solely on morphological characters that can be misleading or obscure to non-specialists (Armstrong and Ball 2005). Still this method of species identification is not without controversy (Hurst and Jiggins 2005). Critics argue that treating genes as static barcodes is unwise because direct and indirect selection pressures on the mitochondrial genome can result in inaccurate identifications. These critics instead call for the more thorough and approach of sampling multiple genes from nuclear as well as mitochondrial sources, hoping to retrieve a more accurate picture of evolutionary relationships than could be gleened from a small portion of a single gene. While more independent sequence data will most likely produce more accurate results, the cost and time required to sequence multiple genes for every species would be prohibitive. Though mitochondrial gene sequencing may not

work in all cases, it can be a useful tool to distinguish many species of economic importance, particularly when taxonomic experts are lacking.

Provided in this dissertation are pictorial keys highlighting important morphological characters to all currently known buprestid genera and species in the state of Tennessee. The *Chrysobothris femorata* complex (Coleoptera: Buprestidae) (Wellso and Manley 2007) is investigated using nuclear and mitochondrial markers. Implications of the resulting phylogeny are discussed. A comprehensive molecular analysis of clearwing species is also carried out using mitochondrial cox I sequence data. Evidence suggesting the need to reevaluate taxonomic placement of several species within the tribe is contrasted with morphological characters used to define current species limits within Synanthedonini. Suggestions for future areas of research in these two groups are discussed.

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# CHAPTER 2

# METALLIC WOOD BORING BEETLES (COLEOPTERA: BUPRESTIDAE) OF TENNESSEE

## Abstract

Concerted efforts to assess the buprestid beetle fauna of Tennessee are fragmented when compared to many neighboring states. Although most metallic wood boring beetles persist as unobserved decomposers of wood, a few species are problematic due to their economic and aesthetic impacts on horticultural crops and plants in the urban landscape. When paired with sticky adhesive, purple-panel traps have proven an effective tool to survey the buprestid fauna in Tennessee. A history of color traps used to survey buprestids and other novel trapping methods are discussed as well as their usefulness for monitoring buprestid beetles. An annotated checklist for Tennessee species and photographic keys to genera and species are given (Appendix 2), as well as adult flight phenology of 81 species occurring in Tennessee. In addition to distribution data, 35 new state records for buprestid beetles are reported, as well as three new host records for *Chrysobothris chlorocephala* Gory, *C. azurea* LeConte and *Actenodes acornis* (Say).


## Introduction

While diversity of the metallic wood boring beetle (Coleoptera: Buprestidae) fauna east of the Mississippi has been well documented, the range and occurrence of buprestid beetles, including many economically important species, is still poorly understood in Tennessee (Nelson 2008). In part, this disparity can be explained by the cryptic behavior of buprestids, the time consuming process of rearing adults from host material, and absence of local collectors.

The Great Smoky Mountains National Park (GSMNP) is an internationally recognized biosphere with about half of the park located in Tennessee (Sharkey 2001). The Park's abundant plant life includes over 100 known species of native trees, providing a rich diversity of hosts needed to sustain a variety of wood boring insects. Even so, the GSMNP museum, housed at their Twin Creeks facility in Gatlinburg, TN currently maintains just 34 buprestid species recorded in Tennessee and North Carolina locations and some taxa are represented by a single specimen (Adriean Mayor, personal communication).

Although considered pests of commercial ornamental plant production and urban landscape settings, buprestid beetles play an important ecological role in healthy forest ecosystems. Larval galleries of buprestid beetles provide entry routes for wood decaying fungi and accelerate the beneficial decomposition process of dead and dying hosts (Rayner and Boddy 1988, Hart 1998). Several parasitic insects use buprestids as larval hosts, including the native *Cerceris fumipennis* Say, which stocks its nests with paralyzed adults (Kurczewski and Miller 1984, Marshall et al. 2005). In addition, buprestids often utilize and break down dead or declining host plant tissues, which in turn reduces potential fuel for forest fires and releases nutrients into the environment (Furniss and Carolin 1977).

Some economically important buprestids exploit mechanical injury and abiotic stresses to colonize woody trees and shrubs in landscape settings, commercial nurseries and attack fallen timber (Potter et al. 1988, Evans et al. 2004). The flatheaded apple tree borer, *Chrysobothris femorata* (Olivier), has a broad host range and is a persistent threat to commercial nurseries growing shade trees. Deciduous shade and flowering tree

production in Tennessee accounted for 54% of total annual nursery sales and $78 million in gross sales during 2006 alone (USDA 2007). Nurseries in Tennessee are heavily committed to production of dogwood trees (*Cornus* spp.) (USDA 1998). Though *C. femorata* has a diverse host range, rates of attack have only been quantified in maples (*Acer* spp.). In Tennessee, infestation rates as high as 38% were recorded for one maple cultivar within a non-treated control treatment in an insecticide trial (Oliver et al. 2010). Similar percentages have been observed in separate studies conducted in other southeastern nurseries (Potter et al. 1988, Allen and Alverson 1994, Coyle et al. 2005). As part of a larger species complex, *C. femorata* can be difficult to distinguish from the nine other sympatric species in the complex, making detection and host plant characterization even more complicated (Wellso and Manley 2007).

*Chrysobothris adelpha* Harold has often been confused in the past with adults of *C. femorata*, which shares a similar physical appearance (Fisher 1942). Fortunately, *C. adelpha* can be distinguished from *C. femorata* using various structures of the face and male genitalic morphology (Wellso and Manley 2007). Both pests share similar geographic distribution east of the Mississippi River and infest pecan trees, resulting in frequent misidentifications. Larvae of *C. adelpha* can often be found feeding at the base of pecan twigs, causing twigs to shed by the tree and in turn reducing nut yield (Fisher 1942). Simply implementing cultural controls, like removal and destruction of dead wood, can prevent mature beetles from emerging the following season.

Growers of *Ribes* spp. in Tennessee are likely to experience damage from another economic pest; the red-necked cane borer, *Agrilus ruficollis* (Fabricius), if care is not taken to protect plants from attack. The gall forming larvae can be found in raspberry

and blackberry brambles that grow throughout most of Tennessee. Mundinger (1942) reported over 60% of untreated canes in a single study were infested with red-necked cane borer. In a separate investigation, Hixson (1938) counted red-necked cane borer galls on 18 different varieties of blackberry and dewberry canes. Seventy-two percent of canes in one blackberry variety were infested and a third of all varieties evaluated had infestation rates of 30% or more (Hixson 1938). This is significant because once burdened with galls canes rarely bare fruit (Hixson 1938, Mundinger 1941). Managing populations of these beetles can be accomplished by pruning infested canes, but is more easily achieved by applying pesticides to kill adults (Johnson and Mayes 1989).

Some buprestid species inhabiting Tennessee woodlots also have potential to alter local forest ecosystems. For example, outbreaks of the two-lined chestnut borer, *Agrilus bilineatus* (Weber), a common native buprestid, caused chestnut stand reductions of approximately 75% in northern Virginia before the introduction of chestnut blight (Chittenden 1897). Similarly, *A. bilineatus* may be part of a complex of insects and microorganisms associated with oak decline in several midwestern U.S. states (Scarbrough and Juzwik 2004).

Though not currently known from Tennessee, the emerald ash borer (*Agrilus planipennis* Fairmaire) has been found in bordering states (Bauer et al. 2008, Obrycki 2009) and presents economic and ecological threats to Tennessee populations of ash trees (Sydnor et al. 2007). Ash in the woodlots and landscapes of Tennessee alone are worth an estimated $404 million and are all threatened by the continued southward advance of emerald ash borer (Klingeman et al. 2007). Barring the discovery of an effective control

measure, the future encroachment of emerald ash borer into Tennessee is expected in coming years.

Monitoring economically important buprestid beetles has long been problematic due to their elusive nature and the amount of labor, materials and space needed to rear adult specimens. Semiochemical use in trapping programs has had limited success and only a few are known to attract certain buprestid species (Montgomery and Wargo 1983, McIntosh et al. 2001, Gaylord et al 2006, Miller 2006). While there is evidence of sex pheromone use by some adult buprestids, such pheromones may only be detected by adult males in close proximity to adult females (Wellso 1966, Dunn and Potter 1988, Bartelt 2007, Silk et al. 2009).

Novel approaches to trap buprestids have been employed by numerous enthusiasts, as well as researchers, in an attempt to find more efficient means of buprestid collection (Brooks 1919, Marshall et al. 2005, Wellso and Manley 2007, Lelito et al. 2008). Color appears to be one important factor in buprestid attraction to traps, possibly due to visual acuity within the family. Sakalian et al. (1993) used yellow and white colored traps to collect flower-visiting buprestids belonging to the genera *Anthaxia* and *Acmaeodera*. Other publications appear to support this approach (Wermelinger et al. 2002, Bily et al. 2006). Yellow sticky cards placed in oak tree canopies attracted large numbers of *A. bilineatus* in Kentucky (Johnson and Freytag 2001). Populations of the large Australian buprestid, *Julodimorpha bakewelli* (White), declined significantly when male beetles responded to discarded, orange-colored beer bottles apparently mistaking these for flightless female companions and inhibiting their ability to successfully reproduce (Gwynne and Rentz 1983). Though specific colors may serve as a behavioral

cue within buprestid biology; the function of color cues related to host selection, mate finding, or acquiring food remains unclear in most cases.

One study found red sticky panel traps were more attractive to *C. femorata* and other buprestids among several colors evaluated (Oliver et al. 2004). Upon refinement, purple colors were found to optimize buprestid trap captures, with over 16 different genera of buprestids regularly alighting on traps (Oliver, unpublished). Other studies have also demonstrated affinity of *Agrilus planipennis* (Fairmaire) for the purple traps (Francese et al. 2004, 2008). The purple-colored sticky traps are now an important tool in efforts to monitor invasive buprestids, like *A. planipennis* (Metzger et al. 2007). These same traps are also credited with discovery of another exotic buprestid in northern Georgia and eastern Tennessee, *Agrilus subrobustus* (Saunders), an Asian species that attacks non-native mimosa trees, *Albizia julibrissin* (Durazz) (Westcott 2007, Hansen et al. 2010).

Although traps are useful as a monitoring tool (Hansen et al. 2009), perhaps the greatest disadvantage from trapping studies is the lack of data provided on host plant utilization. Host range is an important factor in buprestid management strategies. Managers must rely on previously published host plant records when using trap studies to direct optimal control actions. Fortunately, the host range of most buprestid beetles is restricted to a single plant family, genus or even specific species (Nelson et al. 2008). Though not always sufficient, the composition of predominant vegetation surrounding traps may be noted, as well as the genus or species of fallen trees or other potential host material in the area. Such records may be indicative of a potential plant host, but should not be relied upon as definitive.

When targeting specific buprestid species for collection using traps, prior understanding of host utilization and habitats will optimize trap placement and increase the likelihood of successful specimen collection. The ecotone border between forest and open fields is an effective trap placement site, where many heliophilic buprestids often fly (Wermelinger et al. 2007, Francese et al. 2008). Traps placed in shaded areas are likely to catch fewer buprestids than those in direct sun due to the affinity of buprestids for light (Francese et al. 2008, Vodka et al. 2009). Vertical placement of traps may also optimize trap collections of some species (Wermelinger et al. 2007, Francese et al. 2008). Nearby weak, stressed, injured or fallen host material may also provide an excellent location for traps.

Colored panel traps are remarkably well suited for faunal survey work. These panels provide a reliable, passive substrate for trapping several buprestid genera, thus eliminating hours of net sweeping vegetation or the time required to rear buprestids from host plant tissues. In cases where the primary objective is to find presence or absence of a pest, purple panel traps are ideal. Commercial availability of the traps also makes them more accessible to growers (AgBio 2009), and their use requires little training.

The seasonal flight phenology of buprestids that are reported represent several years of trapping adults on purple panel traps, rearing specimens from host material, and capture by other methods (e.g. sweep netting, malaise traps, hand collecting). The blue area represents actual collection data whereas the narrower black line only indicates high probability of flight activity in Tennessee, though no actual adults were taken (Figs. 2-1 and 2-2). Because most panel traps were checked on a weekly basis, it is often not possible to report exact collection dates. Regardless, knowledge of seasonal flight

activities of adult buprestids in Tennessee will aid in optimizing commercial grower and landscape management decision-making that in turn will reduce unnecessary pesticide applications.

Several portions of Tennessee, including many promising habitats, have not been surveyed at this time. The GSMNP in eastern Tennessee may yet yield new state records not listed in this publication. Monitoring for the emerald ash borer in the GSMNP is currently taking place in several campgrounds and other public areas (Glen Taylor, personal communication). Traps being used in emerald ash borer survey programs are likely to generate other buprestid records yet to be identified by GSMNP employees or buprestid experts. Continuing taxonomic efforts under the auspices of the GSMNP All Taxa Biodiversity Inventory are expected to yield more new state buprestid records (Sharkey 2001). Although this study did not significantly survey buprestids from western portions of Tennessee, it is likely the western parts of the state share some of the rich diversity of buprestid species (i.e. more than 130 species) that have been well documented in Missouri (MacRae 1991).

A list of buprestid beetle species having a high probability of occurring in Tennessee but are not yet recorded is provided with this manuscript to help direct future trapping studies (Table 2-1). Species listed in Table 2-1 are reported in at least two neighboring states with similar latitudes or have known plant hosts that are also present in Tennessee as summarized from Nelson et al. (2008).

Thirty-five new state records for Tennessee are being reported herein. Most new records represent specimens captured using purple panel traps, which were first developed in middle Tennessee and are now widely used by government agencies to

monitor emerald ash borer (Francese et al. 2008). Insect collections at the Agriculture Campus at University of Tennessee (UT) in Knoxville, Tennessee State University (TSU) Otis L. Floyd Nursery Research Center in McMinnville, the GSMNP collection in Gatlinburg and the Cornell University collection in New York all provided new state records reported here. In addition to new state records, a checklist, distribution data and pictographic keys to known Tennessee buprestid genera and species are provided (Appendix 2). Characters that did not lend themselves to photography or are self-explanatory are not depicted. Records with a date range (e.g. 1–15-V-2009) were captured on purple sticky traps unless otherwise noted. Records with a single date listing were caught by other means (e.g. rearing, sweep netted, hand caught). In the species checklists below, new state records are designated by bold font. Species in checklists without distribution data were not caught in Tennessee during this survey but have been previously published as occurring in Tennessee.

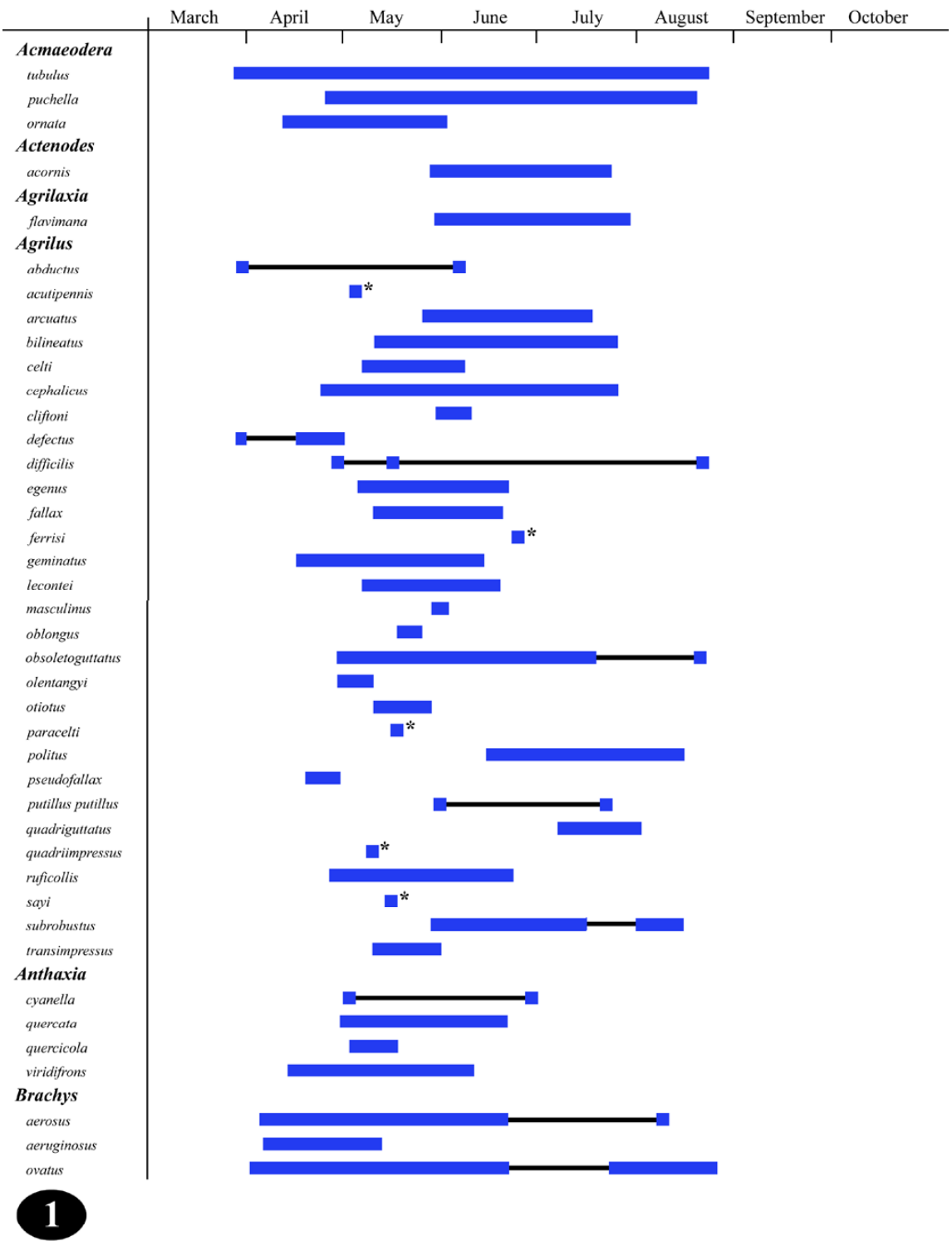


Figure. 2-1. Seasonal flight activity of adult buprestid beetles in Tennessee. Blocks marked with an * are based on one specimen.

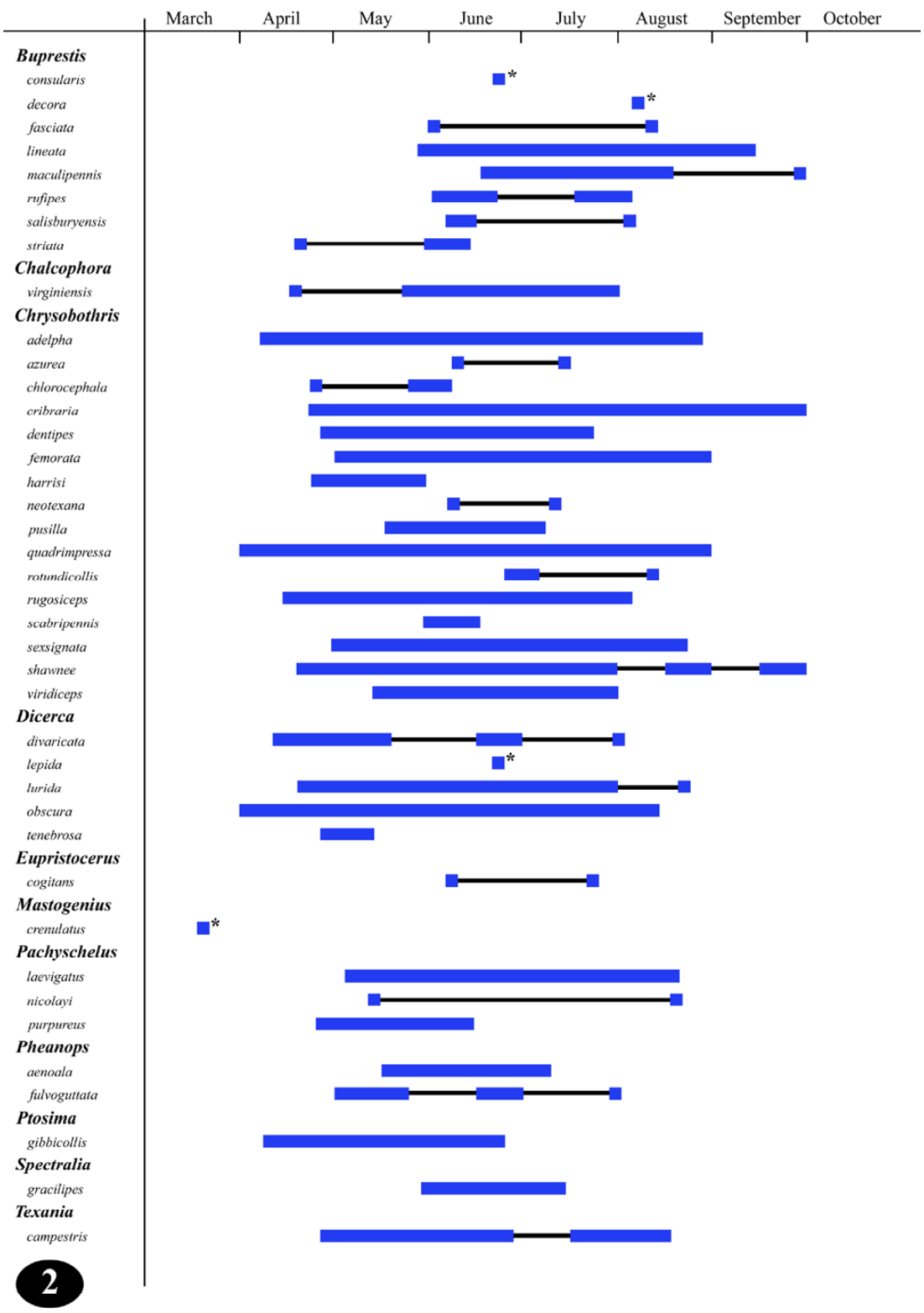


Figure 2-2. Seasonal flight activity of adult buprestid beetles in Tennessee (*continued*), Blocks marked with an * are based on one specimen.

Figure 2-3. Dorsum: *Actenodes acornis*.

Subfamily Buprestinae

**Tribe Actenodini**
Genus *Actenodes* Dejean

*Actenodes acornis* (Say) (Fig. 2-3)

TENNESSEE: Anderson Co., Oak Ridge, UT Arboretum, 7–14-VI-2006, 21–28-VI-2007, J.A. Hansen. Anderson Co., Oak Ridge, UT Arboretum, 12-VI-2004, 3, W.E. Klingeman. Warren Co., McMinnville, NRC, 3–10-VI-2002, J.B. Oliver. Coffee Co., Tullahoma, reared 2008-2009 from *Zelkova serrata* [new host record], N.N. Youssef. Coffee Co., Tullahoma, Tullahoma regional airport, 28-V-2006, J.M. Basham.

Subfamily **Buprestinae**
Tribe **Chrysobothrini**
Genus *Chrysobothris*

*Chrysobothris adelpha*

TENNESSEE: Anderson Co., Oak Ridge, UT Arboretum, 23-VI-2003, W.E. Klingeman. Anderson Co., Oak Ridge, UT Arboretum, 21–28-VI-2007, 28-VI–5-VII-2007, J.A. Hansen. Blount Co., GSMNP, Rich Mt., 26-VIII-1994, collected on *Quercus rubra*, D.

Paulsen. Warren Co., McMinnville, NRC, 13–20-V-2002, 17–23-VI-2002, 22–29-VII-2002, 5–12-VIII-2002, J.B. Oliver.

*Chrysobothris azurea* LeConte

TENNESSEE: Anderson Co., Oak Ridge, UT Arboretum, 9–23-V-2006, 1–14-VI-2006, J.A. Hansen. Anderson Co., Oak Ridge, UT Arboretum, 1-VI-2004, W.E. Klingeman. Warren Co., McMinnville, NRC, 22–29-IV-2002, 6–13-V-2002, 10–17-VI-2002, J.B. Oliver. Coffee Co., Tullahoma, reared 2008-2009 from *Zelkova serrata*, N.N. Youssef.

*Chrysobothris chlorocephala* Gory

This is the first report of this species being reared from *Cercis canadensis* and *Acer* sp., both decidedly different from previously reported plant hosts (Nelson 2008). It emerged from cut wood taken from a redbud tree on the UT Agriculture Campus in Knoxville. In addition, two other specimens were reared from maple in Georgia. Label information of the Georgia specimens is as follows: USA: Georgia, Clayton State College, reared from maple, 21-V-1997, G. Hodges. All three specimens are in the private collection of W.E. Klingeman at the University of Tennessee, Department of Plant Sciences.

TENNESSEE: Knox Co., Knoxville, UT gardens, 15-V-2006, reared from cut *Cercis canadensis*, W.E. Klingeman. Warren Co., McMinnville, NRC, 22-VIII-2001 1♂, J.B. Oliver. Warren Co., McMinnville, NRC 22-IV-2001, J.B. Oliver. Franklin Co., Arnold Engineering Development Center, 7-VI-2003, 5 specimens, collected on *Quercus* sp., J.P. Basham.

***Chrysobothris cribraria* Mannerheim**

TENNESSEE: Blount Co., GSMNP, Cades Cove, 10–24-VI-2008, 5–19-VII-2008, J.A. Hansen. Knox Co., Knoxville, IJAMS Nature Center, 16–30-VI-2006, J.A. Hansen. Anderson Co., Oak Ridge, UT Arboretum, 14–28-VI-07, J.A. Hansen. Warren Co., McMinnville, NRC, 10–17-V-2001 1♀; 29-V–5-VI-2001 1♂; 12–19-VI-2001; 26-VI– 3-VII-2001 2♀; 3–10-VII-2001 1♀; 17–24-VII-2001 1♂; 24–31-VII-2001 1♀, J.B. Oliver. Sequatchie Co., Harrison Ferry Mountain along Highway 8, 30-V-2008, N.N. Youssef.

***Chrysobothris dentipes* (Germar)**

TENNESSEE: Blount Co., GSMNP, Cades Cove, 23-V–5-VI-2008, 30-VI–12-VII-2008, 20-VII–2-VIII-2008, J.A. Hansen. Anderson Co., UT Arboretum, 15–31-V-2007, J.A. Hansen. Warren Co., McMinnville, NRC, 22–27-V-2002, 22–29-V-2002, 17–24-VI-2002, J.B. Oliver. Sequatchie Co., Harrison Ferry Mountain along Highway 8, 30-V-2008, N.N. Youssef.

*Chrysobothris femorata* (Olivier)

TENNESSEE: Blount Co., GSMNP, Cades Cove, 22-V–5-VI-2008 1♂, 16–30-VI-2008, J.A. Hansen. Anderson Co., Oak Ridge, UT Arboretum, 20-V-2007, 2♂, reared from *Cornus kousa*, J.A. Hansen. Warren Co., McMinnville, NRC, 4–12-VII-2005, J.P. Basham. Sequatchie Co., Harrison Ferry Mountain, along Highway 8, 2-VII-2008, N.N. Youssef.

*Chrysobothris harrisi* (Hentz)

TENNESSEE: Blount Co., GSMNP, Cades Cove, 29-IV–5-V-2008, J.A. Hansen. Anderson Co., Oak Ridge, UT Arboretum, 23-V-2006, sweep sample in hardwood forest, W.E. Klingeman. Anderson Co., Oak Ridge, UT Arboretum, 8-V-2007, J.A. Hansen. Warren Co., McMinnville, NRC, 15–22-IV-2002, 15–22-V-2001, J.B. Oliver. Sequatchie Co., Harrison Ferry Mountain along Highway 8, 30-V-2008, N.N. Youssef.

*Chrysobothris neotexana* Dozier

TENNESSEE: Warren Co., McMinnville, 6-VII-2006, 7-VII-2006, 8-VII-2006, collected on *Juniperus virginiana,* J.P. Basham. Davidson Co., Mt. View, Percy Priest Reservoir, 15-VI-2008, collected on Virginia cedar, J.P. Basham.

*Chrysobothris orono* Frost (Nelson et al. 2008)

*Chrysobothris purpureovittata purpureovittata* Horn (Nelson et al. 2008)

*Chrysobothris pusilla* Gory and Laporte

TENNESSEE: Anderson Co., Oak Ridge, UT Arboretum, 21–28-VI-2007, J.A. Hansen. Anderson Co., Oak Ridge, UT Arboretum, 7–14-VII-2003, W.E. Klingeman. Warren Co., McMinnville, NRC, 20–27-V-2002, J.B. Oliver. Sequatchie Co., Harrison Ferry Mountain, along Highway 8, 2-VII-2008, N.N. Youssef.

*Chrysobothris quadriimpressa* Gory and Laporte

TENNESSEE: Blount Co., GSMNP, Cades Cove, 5-VI-2008, 19-VII-2008, J.A. Hansen. Sevier Co. GSMNP, Chimney Tops Trail, 9-VII-2004 1♂, in malaise trap, J.K. Moulton. Anderson Co., Oak Ridge, UT Arboretum, 18–31-V-2007, 11–18-VII-2007, 15–22-VI-2006, 27-VI–3-VII-2006, J.A. Hansen. Warren Co., McMinnville, NRC, 4–12-VII-2005, J.P. Basham. Sequatchie Co., Harrison Ferry Mountain along Highway 8, reared 2008–2009 from *Quercus* sp., N.N. Youssef.

***Chrysobothris rotundicollis* Gory and Laporte**

TENNESSEE: Blount Co., GSMNP, Cades Cove, 10–24-VI-2008 2♀, 29-VI–12-VII-2008, 2–16-VIII-2008 1♂. J.A. Hansen. Blount Co., GSMNP, Foothills Pkwy, 13–27-VI-2008 1♀, J. Love.

*Chrysobothris rugosiceps* Melsheimer

TENNESSEE: Anderson Co., Oak Ridge, UT Arboretum, 23-VI-2003, W.E. Klingeman. Anderson Co., Oak Ridge, UT Arboretum, 18–25-IV-2007, 7–14-VI-2007, 11–18-VII-2007, J.A. Hansen. Blount Co., GSMNP, Cades Cove, 30-V–5-VI-2008, 10–24-VI-2008, J.A. Hansen. Sevier Co., GSMNP, Elkmont, 14-VI-1988, J.K. Watson. Warren Co., McMinnville, NRC, 15–23-IV-2002, 5–12-VI-2002, J.B. Oliver.

***Chrysobothris scabripennis* Gory and Laporte**

TENNESSEE: Blount Co., GSMNP, Cades Cove, 5-VI-2008 2♂ 1♀, 21-VI-2008 2♂, 16-28-VI-2009 ♂ ♀, J.A. Hansen.

***Chrysobothris sexsignata* Say**

TENNESSEE: Blount Co., GSMNP, Cades Cove, Sparks Lane, 23–30-V-2008, 12–19-VI-2008, 3–10-VII-2008, J. Love. Anderson Co., Oak Ridge, UT Arboretum, 10–17-V-2007, 11–18-VII-2007, J.A. Hansen. Coffee Co. AEDC 23-VI-1998, D. Paulsen. Anderson Co. UT Arboretum, 10-V-2001, caught on *Quercus rubra*, D. Paulsen. Warren Co., McMinnville, NRC, 29-V–5-VI-2001 1♀, 19–26-VI-2001 1♂, 19-VI-2001 1♀, 10–17-V-2002 1♀, 5–12-VI-2001 1♀, 12–19-VI-2001 1♀, 26-VI–3-VII-2001 1♀, 10–17-VII-2001 1♀, 24–31-VII-2001 1♀. J.B. Oliver.

*Chrysobothris shawnee* Wellso and Manley

TENNESSEE: Blount Co., GSMNP, Cades Cove, 15–30-VI-2008 1♂, J.A. Hansen. Sevier Co., GSMNP, Park HQ, 1–6-VI-2009 2♂, J.A. Hansen. Anderson Co., Oak Ridge, UT Arboretum, 28-VI–4-VII-2006, 2 ♂, J.A. Hansen. Sequatchie Co., Harrison Ferry Mt. along Highway 8, 30-VII-2008, caught on oak tree, N.N. Youssef. Hamilton Co., Highway 111 and Jones Gap Rd., 22-IX-2005, caught on oak tree, J.P. Basham.

*Chrysobothris viridiceps* Melsheimer

TENNESSEE: Anderson Co., Oak Ridge, UT Arboretum, 22–29-VI-2006, J.A. Hansen. Anderson Co., Oak Ridge, UT Arboretum, 23-VI-2003, W.E. Klingeman. Knox Co.,

Knoxville, IJAMS Nature Center, 30-VI-2006, J.A. Hansen. Warren Co., McMinnville, 14–21-VI-2006, J.A. Hansen. Franklin Co., 3mi NW of Huntland along Bean's Creek, 22-VI-2005, caught on oak tree, N.N. Youssef. Davidson Co. Percy Priest Reservoir, Long Hunter State Park, 15-VI-2008, N.N. Youssef. Sequatchie Co., Harrison Ferry Mountain, along Highway 8, 2-VII-2008, N.N. Youssef.

Subfamily **Buprestinae**
Tribe **Melanophilini**
Genus *Phaenops*

***Phaenops aeneola* (Melscheimer)**

TENNESSEE: Blount Co., GSMNP, Cades Cove, 30-VI-2008 collected on fallen pine, N.N. Youssef. Anderson Co., UT Arboretum, 8-VII-1999, collected fogging tulip poplar, J.M. Laforest. Franklin Co., AEDC, 27-V-1998, collected on Pine, collector unknown. Warren Co., McMinnville, NRC, 17–22-V-2001, 22–29-V-2001, 29-V–5-VI-2001, 12–19-VI-2001, J.B. Oliver.

*Phaenops fulvoguttata* (Harris)

Several specimens of this species were examined by the first author in the GSMNP museum at Twin Creeks. Each was reared from the same piece of hemlock bark. Some had elytral spots very small and faintly indicated. Nevertheless, genitalia of a male confirmed the species as *P. fulvoguttata*.

TENNESSEE: Blount Co., GSMNP, Tremont, 9–23-V-2008, 20-VII–2-VIII-2008, J. Love. Sevier Co., GSMNP, Headquarters, 23–30-IV-2009, 1♂, J.A. Hansen.

***Phaenops drummondi* (Obenberger)**

A single specimen was taken in the GSMNP and is held at Cornell University (Richard L. Westcott, personal communication). This find represents a significant extension of its range, which to this point has reached only as far south as Michigan and New York (Westcott 1991, Wellso et al. 2006). The tamarack tree (*Larix laricina*), its only known larval host, is not known to occur in Tennessee, suggesting it may be using an alternate pine host in the state. Label information for the specimen reads as follows: TENNESSEE, Great Smoky Mtn. N. P., 26-VI-1957, H. G. Liebherr, CUIC. Det: R.L. Westcott.

**Subfamily Buprestinae**
**Tribe Anthaxiini**
Genus *Anthaxia*

***Anthaxia cyanella* Gory**

TENNESSEE: Warren Co., McMinnville, NCR, 29-IV–6-V-2002, 2-VII-2003, 29-IV–6-V-2002, J.B. Oliver.

***Anthaxia quercata* (Fabricius)**

TENNESSEE: Sevier Co., GSMNP, H.Q. area, GRSM 26564, ACC 980, 15-VI-1946, R.R. Dreisbach. Franklin Co., 9-VI-1998, collected on pine, D. Paulsen. Knox Co., Knoxville, UT Plant experiment farm, 10-V-2000, J.M. LaForest. Coffee Co., AEDC, 9-VI-1998, collected from pine, sweeping. Warren Co., McMinnville, NRC, 15–17-V-2001, 3♀, 17–22-V-2001, 7♀, 22–29-V-2001, 3♀, 29-V–5-VI-2001, 1♀, 5–12-VI-2001,

1♂ 1♀, J.B. Oliver. Sequatchie Co., Harrison Ferry Mountain along Highway 8, reared 2008–2009 from *Quercus* sp., N.N. Youssef.

***Anthaxia quercicola* Wellso**

TENNESSEE: Blount Co., Foothills Pkwy, 15-V–28-V-2009, 1♀, J.A. Hansen. Coffee Co., Hillsboro, 27-IV–4-V-2006, 3♀ 5♂, reared from *Quercus falcate*, J.P. Basham. Coffee Co., 27-IV-2006, 1♀, collected on *Quercus falcata*, same tree 5♂ and 2♀ were reared between 28-VI–2-V-2006. J.P. Basham. Bedford Co., Wartrace, VII-2008, caught on oak, J.M. Basham.

*Anthaxia viridicornis* (Say) (Nelson et al. 2008)

*Anthaxia viridifrons* Gory

TENNESSEE: Blount Co., Foothills Pkwy, 15-V–28-V-2009, 1♂ 1♀, J.A. Hansen. Warren Co., McMinnville, NRC, 8–15-V-2001, 15–22-IV-2002, J.B. Oliver.

Genus *Agrilaxia*

*Agrilaxia flavimana* (Gory) 1841 (Fig. 2-3)

TENNESSEE: Warren Co., McMinnville, 5-VI-2007, on Queen Anne's lace (*Daucus carota*), J.P. Basham. Sequatchie Co., 2-VII-2008, on Queen Anne's lace (*Daucus carota*), J.P. Basham.

Figure 2-4. Dorsum: *Agrilaxia flavimana.*

Subfamily **Buprestinae**
Tribe **Buprestini**
Genus *Buprestis*

***Buprestis consularis* Gory**

TENNESSEE: Blount Co., GSMNP, Foothills Pkwy, 27-VI-2008, J. Love.

*Buprestis lineata* Fabricius

TENNESSEE: Anderson Co., Oak Ridge, UT Arboretum, 21–28-VI-2007, 11–18-VII-2007, J.A. Hansen. Blount Co., 16–30-VI-2008, 5–19-VII-2008, 2–16-VIII-2008, J.A. Hansen. Warren Co., McMinnville, NRC, 4–12-VII-2005, J.P. Basham.

***Buprestis decora* Fabricius**

TENNESSEE: McMinn Co., Ocoee R TVA put-in, 4-VIII-2003, landed on blue tarp, W.E. Klingeman.

***Buprestis fasciata* Fabricius**

TENNESSEE: Sevier Co., GSMNP HQ area, GRSM 26555 ACC 980, 1-VI-1943, A. Stupka. Sevier Co., GSMNP, Fighting Creek Gap, GRSM 26558 ACC 980, 9-VIII-1940, A. Stupka.

***Buprestis maculipennis* Gory**

TENNESSEE: Blount Co., GSMNP, Cades Cove, Mill Creek 12–17-VI-2002, caught in malaise trap, Sulton and Steck. Blount Co., GSMNP, Cades Cove, 10–24-VI-2008, 30-VI–12-VII-2008, 2–16-VIII-2008, J.A. Hansen. Coffee Co., 30-VI-1998 hand caught in loblolly pine community, D. Paulsen. Franklin Co., 25-VI-1998 caught fogging pine trees, D. Paulsen. Warren Co., McMinnville, NRC, summer 1999, collected in ETOH baited Lindgren trap, 18–25-VIII-2005, sticky trap, J.B. Oliver.

***Buprestis rufipes* Olivier**

TENNESSEE: Anderson Co., UT Arboretum, 4-VIII-1999, caught fogging tulip poplar, J.M. LaForest. Sevier Co., GSMNP, HQ area, 14-VI-1948 altitude 445 m, GRSM26569, ACC980 Art Stupka. Sevier Co., Gatlinburg, 16-VII-1950, GRS 26570, ACC980, S.G. Baldwin. Warren Co., McMinnville, NRC, 1998, J.B. Oliver. Franklin Co., Woods Reservoir, 9-VI-2007, collected on *Quercus* sp., N.N. Youssef. Warren Co., Rock Island, 2-VI-2008, N. Patton. Davidson Co., Percy Priest Reservoir, Long Hunter State Park, 15-VI-2008, N.N. Youssef.

*Buprestis salisburyensis* Herbst

TENNESSEE: Blount Co., Foothills Pkwy., 1–6-VI-2009, J.A. Hansen. Blount Co., GSMNP, Cades Cove, 7–15-VI-2009, J.A. Hansen. Blount Co., Cades Cove, Ranger Station, 4-VI-2009, Adriean Mayor. McMinn Co., Ocoee R TVA put-in, 4-VIII-2003, landed on blue tarp, W.E. Klingeman.

*Burprestis striata* Fabricius

TENNESSEE: Blount Co., GSMNP, Tremont, 23-V–5-VI-2008, 30-V–12-VI-2008, J. Love. Knox Co., 15-V-2005, came to black light trap, J.K. Moulton. Unicoi Co., Erwin, no date recorded, M. Rice. Warren Co., McMinnville, NRC, 19-IV-2006, N.N. Youssef.

**Subfamily Chrysochroinae**
**Tribe Chrysochroini**
Genus *Chalcophora* Dejean 1833

***Chalcophora virginiensis* (Drury)** (Fig. 2-5)

Many specimens were taken early to mid-afternoon sunning on fallen pines in the GSMNP, Cades Cove. They were fairly slow moving and easily caught by hand. TENNESSEE: Blount Co., GSMNP, Cades Cove, 5-VI-2008, 24-VI-2008, hand caught on fallen pine, J.A. Hansen. Cumberland Co., Fairfield Glade, 29-IV-1996, E.J. Marsland. Warren Co., McMinnville, NRC, 17-IV-2006, 25-IV-2006 N.N. Youssef. Warren Co., McMinnville, NRC, 30-VI-2006, 19-IV-2006, 18-IV-2006, J.P. Basham.

Figure 2-5. Dorsum: *Chalcophora virginiensis.*

**Subfamily Chrysochroinae**
**Tribe Chrysochroini**
Genus *Texania*

*Texania campestris* (Say) (Fig. 2-6)

TENNESSEE: Madison Co., 17-VIII-1993, N. Austin. Warren Co., McMinnville, NRC, 20–27-V-2007, J.P. Basham. Davidson Co. Percy Priest Reservoir, Long Hunter State Park, 15-VI-2008, N.N. Youssef.

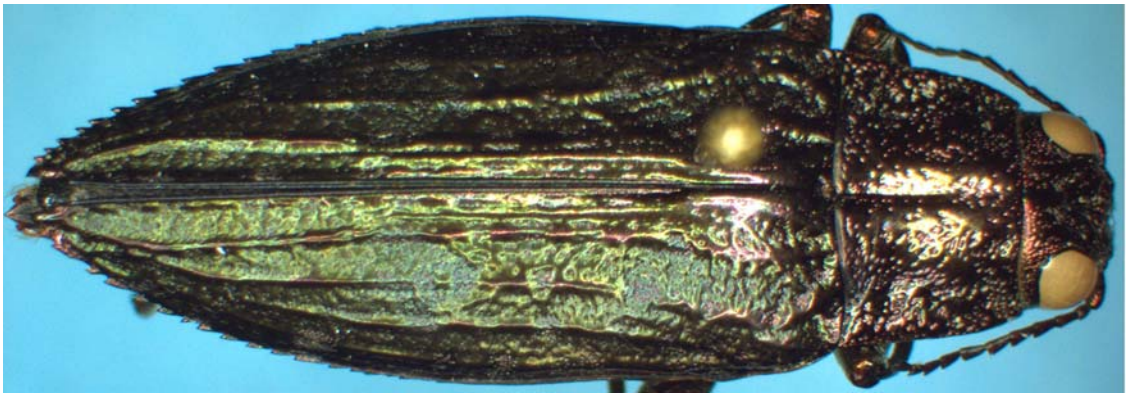

Figure 2-6. Dorsum: *Texania campestris.*

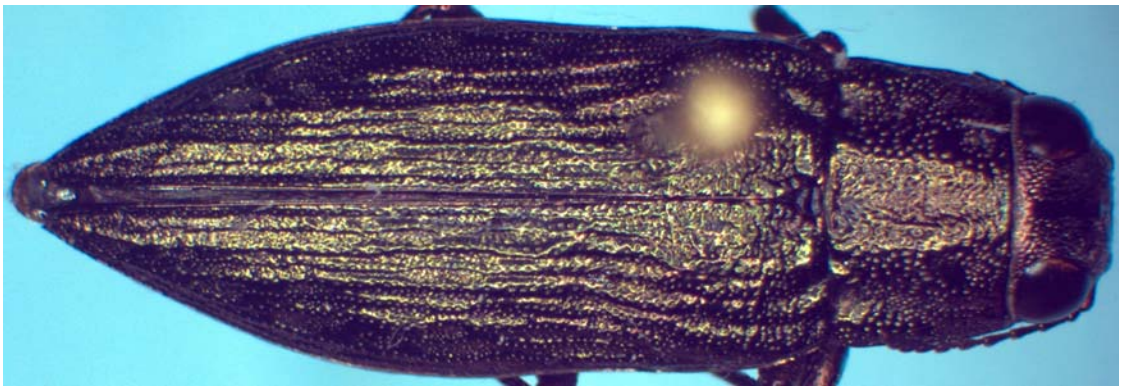

Figure 2-7. Dorsum: *Spectralia gracilipes.*

**Subfamily Buprestinae**
**Tribe Phrixiini**
Genus *Spectralia*

*Spectralia gracilipes* (Melsheimer) (Fig. 2-7)

TENNESSEE: Warren Co., NRC N35°42.47 W85°44.67, 26-VI-2006, J.P. Basham.

Warren Co., near McMinnville, 29-V-2006, hand caught, J.P. Basham.

**Subfamily Chrysochroinae**
**Tribe Poecilonotini**
Genus *Poecilonota*

*Poecilonota cyanipes* (Say) (Fig. 2-8) (Nelson et al. 2008)

Figure 2-8. Dorsum: *Poelilonota cyanipes.*

Subfamily **Chrysochroinae**
Tribe **Dicercini**
Genus *Dicerca*

***Dicerca divaricata* (Say)**

TENNESSEE: Blount Co., GSMNP, Tremont,1–6-V-2009, J.A. Hansen. Sevier Co., GSMNP, Chimney Camp Ground, GRSM 26548 ACC 980, 25-IV-1959, collector unknown. Sevier Co., GSMNP, Headquarters, 23–30-IV-2009, 1♀, J.A. Hansen. Grundy Co., Beersheba Springs, 10-IV-2002, collected on maple (*Acer* sp.), N.N. Youssef. Warren Co., McMinnville, NRC, 17-IV-2004.

***Dicerca lepida* LeConte**

TENNESSEE: Davidson Co., Long Hunter State Park, 20-VI-2006, 2♂ 1♀, All 3 specimens collected on the same slippery elm (*Ulmus rubra*), J.P. Basham.

*Dicerca lurida* (Fabricius)

TENNESSEE: Anderson Co., Oak Ridge, UT Arboretum, 17-V-2000, caught on tulip poplar (*Lirodendron* tulipifera), J.M. LaForest. Blount Co., GSMNP, Rich Mt., 31-V-1994, 19-VIII-1993, caught on *Quercus rubra*, D. Paulsen. Sevier Co., GSMNP, HQ, 23–30-IV-2009, 2♀, J.A. Hansen. Warren Co., McMinnville, NRC, 20–27-VII-2005, J.P. Basham.

*Dicerca obscura* (Fabricius)

TENNESSEE: Henry Co., 12-VIII-1996, N. Austin. Blount Co., GSMNP, Cades Cove, Sparks Ln., canopy trap, 31-V–6-VI-2008, J.P. Love. Warren Co., McMinnville, NRC, 1-IV-2005, J.P. Basham.

*Dicerca tenebrosa knulli* Nelson

TENNESSEE: Blount Co., GSMNP, Cades Cove, east side of loop, 23–30-IV-2009, J.A. Hansen. Warren Co., McMinnville, NRC, 13-V-2005, collected on Virginia pine (*Pinus virginiana*), J.P. Basham. Warren Co. McMinnville, NRC, 24-IV-2006, collected on Virginia pine (*Pinus virginiana*), J.P. Basham.

Subfamily **Polycestinae**
Tribe **Acmaeoderini**
Genus *Acmaeodera*

*Acmaeodera ornata* (Fabricius)

TENNESSEE: Warren Co. McMinnville, NRC, 13-IV-2002, collected on *Hieracium gronovii*, J.P. Basham. Warren Co. McMinnville, NRC, 15-V-2005, collected in ethanol baited trap, J.P. Basham. Grundy Co., Beersheba Springs, 27-V-2005, collected on blackberry flower (*Ribes* sp.), J.P. Basham.

*Acmaeodera pulchella* (Herbst)

TENNESSEE: Blount Co., GSMNP, Cades Cove, 20-VII–2-VIII-2008, J.A. Hansen. Blount Co., GSMNP, Cades Cove, 30-VI-2008, caught on yellow flower, J.P. Basham.

Warren Co., McMinnville, 14–21-VI-2006, J.A. Hansen. Knox Co., Delrose Dr., 3-IV-2005, caught with sweep net, W.E. Klingeman.

*Acmaeodera tubulus* (Fabricius)

On separate occasions two of the authors found adult *A. tubulus* deep inside tree branches. Two adults were extracted in March 2002 from *Cornus* sp. One additional adult was removed from a decaying butternut branch (*Juglans cinerea*) early April 2007. Adults of this species have been observed to overwinter in pupal cells in Texas (Wellso 1974). They may be overwintering as adults in Tennessee as well, which would explain their early appearance in the spring (Fig. 2-1).

TENNESSEE: Morgan Co., Stephen's Switch, N36° 03.587 W084° 25.964, 4-IV-2007, extracted from butternut branch, J.A. Hansen. Anderson Co., Oak Ridge, UT Arboretum, 18–25-IV-2007, 10–17-V-2007, 7–14-VI-2006, 30-VI–5-VII-2006, J.A. Hansen. Blount Co., GSMNP, Cades Cove, east side of loop, 23–30-IV-2009, J.A. Hansen. Sevier Co., GSMNP, Metcalf Bottoms, 17–23-IV-2009, 23–30-IV-2009, J.A. Hansen. Coffee Co. AEDC, 27-V-1998, collector unknown. Warren Co. McMinnville, NRC, 20–26-III-2005, J.P. Basham. Warren Co., McMinnville, III-2002, 2 adults extracted from *Cornus* sp., N.N. Youssef.

Subfamily **Polycestinae**
Tribe Ptosimini
Genus *Ptosima*

*Ptosima gibbicollis* (Say) (Fig. 2-9)

TENNESSEE: Anderson Co., Oak Ridge, UT Arboretum, 18–28-IV-2006, W.E. Klingeman. Anderson Co., Oak Ridge, UT Arboretum, 10–17-V-2007, J.A. Hansen. Blount Co., Foothills Pkwy, 15-V–28-V-2009, J.A. Hansen. Sevier Co., 13-V-1977, P.C. Durr. Knox Co., 4-V-1999, J. Lingenfelter. Warren Co., McMinnville, NRC, 18-IV-2005, 6–7-V-2004, 2–9-VI-2004, J.B. Oliver. Davidson Co. Percy Priest Reservoir, Long Hunter State Park, 15-VI-2008, N.N. Youssef.

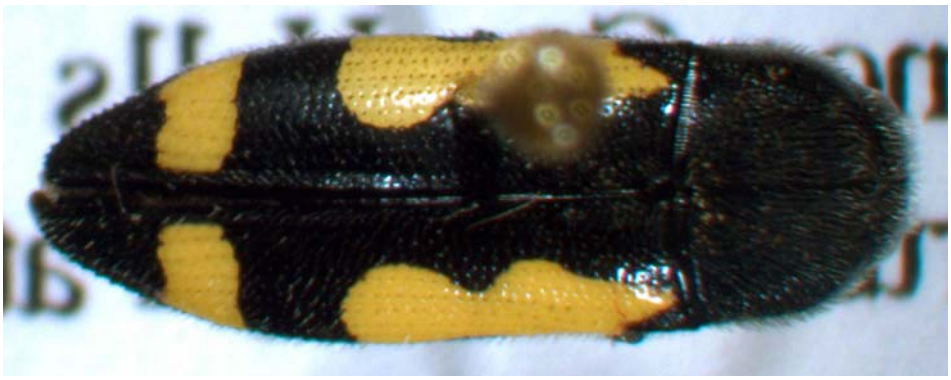

Figure 2-9. Dorsum: *Ptosima gibbicollis.*

Subfamily **Polycestinae**
Tribe **Haplostethini**
Genus *Mastogenius*

*Mastogenius crenulatus* Knull

TENNESSEE: Coffee Co., Hillsboro, 18-IV-2006, reared from *Quercus falcata*, J.P. Basham. Coffee Co., Hillsboro, 15-V-2006, reared from *Quercus falcata*, J.P. Basham. Warren Co., Viola, 5-V-2006, reared from *Quercus falcata*, J.P. Basham.

*Mastogenius subcyaneus* (LeConte) (Nelson et al. 2008)

Subfamily **Agrilinae**
Tribe **Trachyini**
Genus *Pachyschelus*

*Pachyschelus laevigatus* (Say)

TENNESSEE: Anderson Co., Oak Ridge, UT Arboretum, 7-VI-2007, caught sweeping weeds in open field, J.A. Hansen. Blount Co., Cades Cove, 24-VII-2009, J.P. Basham. Warren Co., McMinnville, NRC, 15-V-2005, 27-VI-2005, J.P. Basham. Sequatchie Co., Harrison Ferry Mountain, along Highway 8, 2-VII-2008, N.N. Youssef.

*Pachyschelus nicolayi* Obenberger

TENNESSEE: Sevier Co., GSMNP, Metcalf Bottoms, 1–15-VIII-2009, J.A. Hansen. Warren Co., NRC, 17-VIII-2005, J.P. Basham. Dekalb Co., Center Hill Lake, Four Seasons Resort Marina, 12-V-2007, collected on *Wisteria* sp., N.N. Youssef.

*Pachyschelus purpureus purpureus* (Say)

TENNESSEE: Warren Co., NRC, 24-IV-2006, 8-V-2006, 14-VI-2006, caught on *Geranium maculatum*, J.P. Basham. Warren Co., NRC, 3-V-2006, caught on *Quercus* sp., J.M. Basham.

Genus *Brachys*

At least one species of this genus in Tennessee, *B. ovatus*, appears to have adult populations with numbers abnormally skewed towards the female sex, making male

catches rare. This same phenomenon for *B. ovatus* was observed in Michigan (Wellso et al. 1976). The predominance of the female sex within collections has been hypothesized to result from bacterial infections that kills male embryos, and may have led to parthenogenesis in another southeastern species in the genus (Lawson 2001).

***Brachys aerosus* (Melsheimer)**

TENNESSEE: GSMNP, Tennessee side, 21-VI-1942, D.J. and J.N. Knull. Warren Co., McMinnville, NRC, 21-IV-2003, collected on *Quercus* sp., J.P. Basham. Warren Co., McMinnville, NRC, 5-V-2002, N.N. Youssef. Warren Co., McMinnville, NRC, 23-V-2003, 2-VI-2003, 3-VI-2003, J.P. Basham.

*Brachys aeruginosus* Gory

TENNESSEE: Warren Co., McMinnville, 22-IV-2006, caught on *Quercus* sp., J.P. Basham. Warren Co., 5 miles east of McMinnville, 11-V-2006, caught on *Quercus* sp., J.P. Basham. Coffee Co., Hillsboro, 3-V-2006, caught on *Quercus* sp., J.M. Basham. Warren Co., 10 miles east of McMinnville along Highway 705, 19-V-2006, caught on *Quercus* sp., J.P. Basham.

***Brachys ovatus* (Weber)**

TENNESSEE: Sevier Co., GSMNP, Elkmont, GRSM 26604, ACC 980, 16-VI-1946, R.R. Dreisbach. Warren Co., McMinnville, NRC, 15–22-IV-2002, 6–13-V-2002, 20–27-V-2002, J.B. Oliver. Warren Co., McMinnville, NRC, 25-VIII-2003, J.P. Basham. Warren Co., Morrison, 20-VI-2004, J.P. Basham. Franklin Co., along Highway 127, near

Woods Reservoir, 9-V-2006, collected on hackberry (*Celtis ocidentalis*), J.P. Basham. Grundy Co., Beersheba Springs, 27-V-2005, J.P. Basham. Coffee Co., Hillsboro, 3-V-2006, J.P. Basham. Lincoln Co., Fayetteville, 12-V-2006, collected on chestnut oak (*Quercus prinus*), J.P. Basham.

**Subfamily Agrilinae**
**Tribe Agrilini**
Genus *Agrilus*

*Agrilus abductus* Horn

TENNESSEE: Franklin Co., 3 miles northwest of Huntland along Beans Creek, 6-VI-2003, collected on *Quercus* sp., J.P. Basham. Franklin Co., 3 miles northwest of Huntland along Beans Creek, 31-III-2006, reared from *Quercus* sp., J.P. Basham.

***Agrilus acutipennis* Mannerheim**

TENNESSEE: Blount Co., GSMNP, Cades Cove, GRSM 26571 ACC 980, 5-V-1935, R.J. Fleetwo.

***Agrilus arcuatus* (Say)**

TENNESSEE: Warren Co. Morrison reared Spring 2004 from *Quercus* sp., 1♂, J.P. Basham. Warren Co., NRC, 17–24-V-2004 1♀, 24-V–2-VI-2004 1♀, J.B. Oliver.

***Agrilus bilineatus* (Weber)**

TENNESSEE: Anderson Co., Oak Ridge, UT Arboretum, 30-V–14-VI-2006, J.A. Hansen. Sevier Co., GSMNP, Cosby campground, 16-V–24-VI-2008, A.J. Mayor.

Blount Co., GSMNP, Cades Cove, 30–12-VII-2008, J.A. Hansen. Warren Co., NRC, 3–10-VI-2002 sticky trap, J.B. Oliver.

*Agrilus celti* Knull

TENNESSEE: Warren Co., 2 miles southwest of McMinnville, 20–27-V-2005, J.P. Basham. Franklin Co., along Highway 127 near Woods Reservoir, 9-V-2006, collected on walnut, J.P. Basham. Coffee Co., Hillsboro, 6-V-2006, collected on *Quercus* sp., J.M. Basham. Lincoln Co., near Fayetteville, 15-VI-2006, reared from *Celtis* sp., J.P. Basham.

***Agrilus cephalicus* LeConte**

TENNESSEE: Warren Co., NRC, 5-V-2002, 23-IV-2002, collected on *Cornus* sp., N.N. Youssef. GSMNP, GRSM 26572 ACC 980, 7-VI-1942, D.J. and J.N. Knull.

*Agrilus cliftoni* Knull (Nelson et al. 2008)

***Agrilus defectus* LeConte**

TENNESSEE: Warren Co., NRC, III-2005 1♀, 27-IV-2005 1♀, reared from *Quercus* sp., J.P. Basham. Warren Co., NRC, 4♂ 11♀ collected on *Quercus* sp., 22-IV-2005 1♂, V-2005, 1♂, collected in ETOH, J.P. Basham.

*Agrilus difficilis* Gory

TENNESSEE: Davidson Co., Nashville, Tennessee State University Campus, 22-VIII-2008, collected on *Gleditsia triacanthos* variety 'Skyline', N.N. Youssef. Coffee Co.,

Tullahoma, V-2009, reared from *Gleditsia triacanthos*, N.N. Youssef. Coffee Co., Tullahoma, 28-IV-2009, J.P. Basham.

*Agrilus diospyroides* Knull

First described by Knull 1942 after taking four males on a persimmon tree 11-VI-1942 in the GSMNP. There are no representatives of this species in the Park collection or in any known collection of buprestids in Tennessee. Its distribution includes several eastern and mid-western states. Nelson et al. 1996 describe the female characteristics.

*Agrilus egeniformis* Champlain and Knull (Nelson et al. 2008)

*Agrilus egenus* Gory

TENNESSE: Sevier Co., Chimney Camp, GRSM 26578 ACC 980, 11-VI-1946, G. Steyskal. Warren Co., McMinnville, NRC, 19-VI-2003, collected on hackberry, J.P. Basham. Warren Co., McMinnville, NRC, 19-V-2008, J.P. Basham.

*Agrilus fallax* Say

TENNESSEE: Blount Co., GSMNP, Cades Cove, 30-V–12-VI-2008, J.A. Hansen. Knox Co., Knoxville, IJAMS Nature Center, 23–30-VI-2006, J.A. Hansen. Sevier Co., GSMNP, Park HQ, 28–31-V-2009, 1♀, J.A. Hansen. Warren Co., 2 miles southwest of McMinnville, 20–27-V-2005, 18–27-VI-2005, J.P. Basham. Davidson Co., Nashville, Tennessee State University Campus, J.P. Basham.

*Agrilus ferrisi* Dury

Only one previous specimen of *A. ferrisi* has been reported from Tennessee. MacRae 2006 reported it emerging in "spring 2004", which may point toward a much longer flight period than indicated in Fig. 2-1. However, lab-reared specimens can emerge early depending on environmental conditions. Further collection data will be needed to determine its actual flight time.

TENNESSEE: Coffee Co., AEDC, 25-VI-1998, collector unknown.

*Agrilus fuscipennis* Gory (Nelson et al. 2008)

***Agrilus geminatus* (Say)**

TENNESSEE: Warren Co., NRC, 18–20-IV-2005 1♂, collected in ETOH baited trap, 13-VI-2003 1♂, collected on *Fraxinus* sp., N.N. Youssef. Warren Co., NRC, V-2005 2♂, collected in ETOH trap, 2-V-2005 1♂, collected on *Carya* sp., J.P. Basham.

***Agrilus lecontei celticola* Fisher**

TENNESSEE: Lincoln Co., 1 mile northeast of Fayetteville, 11-V-2005, collected on hackberry (*Celtis occidentalis*), J.P. Basham.

*Agrilus lecontei lecontei* Saunders

TENNESSEE: Blount Co., GSMNP, Foothills Pkwy., 23–30-V-2009, 1♀, J.A. Hansen. Warren Co., NRC, 9–16-VI-2003, J.B. Oliver.

***Agrilus masculinus* Horn**

TENNESSEE: Warren Co., 2 miles Southwest of McMinnville, 2-VI-2003, 1♂, collected on *Sassafrass* sp., 3–10-VI-2005 1♂, 27-V–3-VI-2005 1♂, J.P. Basham. Anderson Co., UT Arboretum, 14-V-2001, collected fogging tree, C.T. Werle. Anderson Co., UT Arboretum, 29-VI-2006, hand caught, 1♂, J.A. Hansen.

*Agrilus oblongus* Fisher

TENNESSEE: Warren Co., near McMinnville, 17–24-V-2004, J.B. Oliver.

***Agrilus obsoletoguttatus* Gory**

TENNESSEE: Warren Co., NRC, 15–17-V-2001 1♀, 15–22-V-2001 1♀, 22–29-V-2001 2♀, 12–19-VI-2001 1♀, 5–12-VI-2001 1♀, J.B. Oliver. Sevier Co., GSMNP, ATBI Twin Creeks canopy trap #1, 18-VII–31-VII-2006, J. Gulbransen. Blount Co., GSMNP, Cades Cove, 23–30-VI-2008, J.A. Hansen. Anderson Co., Oak Ridge, UT Arboretum, 10–17-V-2008, 15–22-VI-2006, J.A. Hansen.

*Agrilus olentangyi* Champlain and Knull 1925

TENNESSEE: Franklin Co., along Highway 127 near Woods Reservoir, 9-V-2006, caught on walnut (*Jugalans* sp.), J.P. Basham.

***Agrilus otiosus* Say**

TENNESSEE: Warren Co., NRC, 20–27-V-2002 1♀, 13–20-V-2002 1♂, 10–17-VI-2002 1♂, J.B. Oliver.

*Agrilus paracelti* Knull

TENNESSEE: Warren Co., McMinnville, NRC, 5-V-2004, collected on elm (*Ulmus* sp.), J.P. Basham.

*Agrilus politus* (Say)

This widely distributed species was common on *Salix* sp. in GSMNP, Cades Cove. Individuals were collected sweeping the upper branches of willows.

TENNESSEE: Knox Co., Knoxville, 3rd Creek Bike Trail near Mann St., collected on *Salix* sp., J.A. Hansen. Blount Co., Cades Cove, Hyatt Ln., 24-VI-2008, 19-VII-2008, 2-VIII-2008, collected on *Salix* sp., J.A. Hansen. GSMNP, GRSM 26582 ACC 980, 14-VI-1942.

***Agrilus pseudofallax* Frost**

TENNESSEE: Warren Co., McMinnville, NRC, 18-IV-2005 1♂, collected in ethanol baited trap, J.P. Basham. Coffee Co., 29-IV-2009, on skyline locust, J.P. Basham.

***Agrilus putillus putillus* Say**

TENNESSEE: Warren Co., 2 miles southwest of McMinnville, reared from (*Acer* sp.), 20–27-V-2005, 27-V–3-VI-2005, 3–10-VI-2005, 10–18-VI-2005, 20–27-VI-2005, J.P. Basham.

***Agrilus quadriguttatus quadriguttatus* Gory**

Two specimens of *A. quadriguttatus quadriguttatus* were caught sweeping upper branches of *Salix* sp. in GSMNP, Cades Cove. It was far less common than *A. politus* in this setting.

TENNESSEE: Blount Co., GSMNP, Cades Cove, Hyatt Ln., 19-VII-2008, 2-VIII-2008, caught on *Salix* sp., J.A. Hansen. Warren Co., McMinnville, Collins River/Highway 127 junction, 22-VII-2008, caught on *Salix* sp., J.P. Basham.

*Agrilus quadriimpressus* Ziegler

TENNESSEE: Warren Co., McMinnville, 8-V-2006, collected on *Prunus* sp., N.N. Youssef.

*Agrilus ruficollis* (Fabricius)

TENNESSEE: Anderson Co., Oak Ridge, UT Arboretum, 10–17-V-2007, J.A. Hansen. Sequatchie Co., near Fredonia, 30-V-2003, J.P. Basham. Warren Co., McMinnville, 9-VI-2006, collected on *Rubus* sp., J.P. Basham. Warren Co., McMinnville, NRC, 4–5-VI-2004, N.N. Youssef. Warren Co., McMinnville, NRC, 25-IV-2004, N.N. Youssef. Franklin Co., 3 miles northwest of Huntland along Beans Creek, 22-VI-2005, J.P. Basham. Grundy Co., Beersheba Springs, 28-V-2005, collected on raspberry *Rubus* sp., N.N. Youssef.

*Agrilus subrobustus* Saunders

Perhaps more common in the southeastern United States than reports indicate, this non-native species has been reported in Georgia and Tennessee (Westcott 2007, Hansen et al. 2010). In both instances this new invasive species was trapped using purple sticky traps. TENNESSEE: Blount Co., GSMNP, Foothills Parkway, 15-28-V-2009, J.A. Hansen. Blount Co., GSMNP, Foothills Parkway, 1-15-VIII-2009, J.A. Hansen.

*Agrilus transimpressus* Fall

TENNESSEE: Franklin Co., along Highway 127 near Woods Reservoir, 9-V-2006, collected on walnut (*Juglans* sp.), J.P. Basham. Giles Co., 2 miles southwest of Pulaski, 15-V-2005, reared from walnut, J.P. Basham.

**Subfamily Agrilinae**
**Tribe Coraebini**
Genus *Eupristocerus*

***Eupristocerus cogitans* (Weber)** (Fig. 2-10)

On 30-VI-2008 a flatheaded borer larva was found in a dissected gall from an alder shrub (*Alnus* sp.) in Cades Cove, but attempts to rear adults from material collected there were unsuccessful. The following year ten galls were taken in April from alder in Metcalf Bottoms and a single adult emerged.

TENNESSEE: Sevier Co., GSMNP, Metcalf Bottoms, reared from *Alnus* sp., 5-VI-2009, J.A. Hansen. Blount Co., GSMNP, Cades Cove, hand collected on *Alnus* sp., 24-VI-2009, J.P. Basham. Franklin Co., Woods Reservoir, reared from *Alnus* sp. 2007–2008, N.N. Youssef.

Figure 2-10. Dorsum: *Eupristoceris cogitans.*

## Glossary to keys*

**Acutely angulated** – forming, or meeting in an acute angle.

**Aedeagus** – the penis.

**Antennameres** – segments of antennae, 11 in most bupresetids.

**Arcuate** – arched or bow-like.

**Bidentate** – having two teeth.

**Carina** – an elevated ridge or keel.

**Cleft** – split or partly divided longitudinally.

**Clypeus** – that part of the head of the insect below the frons.

**Concave** – curved inward.

**Corneous** – leathery.

**Costae** – an elevated ridge rounded at its crest, running longitudinally on elytra.

**Disc (pronotum)** – the central surface of the pronutum.

**Elongate** – drawn out; lengthened; much longer than wide.

**Elytral apices** – tips of elytra furthest from the thorax.

**Emarginated** – notched; with an obtuse, rounded or quadrate section cut from a margin.

**Entire** – with an even unbroken margin.

**Fimbriate** – fringed with hairs.

**Foveae** – a pit with well marked sides.

**Frons** – the unpaired sclerite of the head lying between the arms of the epicranial suture.

**Glabrous** – smooth, hairless and without punctures or structures.

**Intercostals** – between veins or costae.

**Levigate** – smooth, sometimes somewhat shiny, surfaced.

**Maculation** – the ornamentation or pattern of markings.

**Marginal and submarginal carinae (pronotum)** – In *Agrilus* the two elevated ridges running along the lateral edge of the pronotum.

**Median lobe (prosternum)** – an anterior extention of the prosternum.

**Mentum** – the distal sclerite of the insect labrum bearing the moveable parts.

**Mesally** – pertaining to, situated on or in the meson, the median plane of the body.

**Mesosternum** – the underside of the middle thoracic segment.

**Metacoxal plate** – portion of the first ventral segment included above the ventral lines visible on that segment.

**Obtuse** – an angle greater than a right angle.

**Parameres** – two lateral processes or lobes of the male genitalia.

**Piceous** – pitchy black; black with a slight reddish tinge.

**Prolonged** – extended or lengthened beyond ordinary limits.

**Pronotum** – the dorsal surface of the prothorax.

**Protarsal claw**– the claw on the anterior leg.

**Punctate-striate** – with rows of punctures.

**Punctation** – being marked with punctures or very small pits.

**Pygidium** – the terminal tergite.

**Quadrate** – square or nearly so.

**Scutellum** – the triangular sclerite between the elytra.

**Serrate** – saw-like.

**Setae** – slender, hair-like appendages of the cuticle.

**Sinuous** – wavy, curved in and out.

**Sternite** – a subdivision of a sternal plate.

**Striolate** – with finely impressed parallel lines.

**Submarginal ridge** (terminal tergite) – in *Chrysobothris*, jagged ridge running longitudinally sublaterally on the last sternite.

**Sulcate** – deeply furrowed or grooved.

**Suture** – a seam indicating the division of the distinct parts of the body wall.

**Tarsi** – the feet (i.e. jointed appendage connected to the tibia, which often has claws or other structures).

**Tarsomere** – one of the subsegments of the tarsus.

**Tibiae** – the fourth division of the leg.

**Tergite** – a dorsal sclerite.

**Transverse** – running across.

**Triangularly emarginated** – a triangularly shaped notch.

**Truncate** – cut off squarely at tip.

**Vertex** – the top of the head between the eyes.

**Vittae** – broad longitudinal stripes.

*Definitions taken or modified from *A Glossary of Entomology* by Torre-Bueno 1985, published by the New York Entomological Society, New York.

Table 2-1. Buprestid species not yet found in Tennessee, but with host plants known to occur within state borders or found in at least two adjacent states at similar latitude.

| **Species** | **Range** | **Larval Host(s)** |
|---|---|---|
| *Acmaeodera texana* LeConte | AL, AR, GA, MO, MS, NC | Unknown |
| *Polycesta elata* LeConte | AL, AR, MO | *Fraxinus greggi*, *Platanus occidentalis* and *Quercus texana* |
| *Chalcophora georgiana* (LeConte) | AL, GA, MS, NC, VA | *Pinus caribaea, P. echinata, P. palustris* and *P. taeda* |
| *Poecilonota thureura* (Say) | GA, MO, NC | *Salix nigra* and *Salix* sp. |
| *Dicerca asperata* (Laporte and Gory) | AL, GA, MO, MS, NC, VA | *Quercus* sp. |
| *D. pugionata* (Germar) | AR, KY, MO, VA | *Alnus incana, A. serrulata, Hamamelis virginiana* and Physocarpus opulifolius. |
| *D. punctulata* (Schönherr) | AL, GA, MO, MS, NC, VA | *Pinus echinata, P. rigida, P. strobus*, *P. taeda, L. scobina* |
| *D. juncea* Knull | AL, GA, MS, NC, GA | Unknown |
| *D. tenebrica* (Kirby) | GA, MO, VA | *Populus grandidentata* |
| *D. caudata* LeConte | NC, VA | *Alnus* sp. and *Betula nigra* |
| *D. tuberculata* (Leporte and Gory) | NC, VA | *Tsuga canadensis* |
| *Buprestis apricans* Herbst | AL, AR, GA, MS, NC, VA | *Pinus palustris* and *P. taeda* |
| *Xenorhipis brendeli* LeConte | AL, GA, MO, NC | *Betula nigra, Carya illinoinensis, C. laciniosa, C. ovata, Quercus alba* |
| *Actenodes davidi* Nelson | KY, MO, MS | *Gleditsia triacanthos* |
| *Paragrilus tenuis* (LeConte) | GA, MO, MS, NC | *Hibiscus laevis, H. lasiocarpos, H. moscheutos* and *H. moscheutos* spp. *lasiocarpos* |
| *Agrilus anxius* Gory | GA, KY, MO, VA | *Betula alleghaniensis, B. lenta, B. occidentalis, B. papyrifera, B. pendula, B. platyphaylla, B. populifolia, B. pubescens, Populus balsamifera, P. b. trichocarpa, P. deltoides, P. grandidentata and P. tremuloides* |
| *A. cladrastis* Knull | KY, MO, MS | Unknown |
| *A. concinnus* Horn | AR, GA, MO, MS | *Hibiscus laevis* |
| *A. crataegi* Frost | AL, AR, MO, VA | *Amelanchier alnifolia* and *Crataegus* sp. |
| *A. fulgens* Leconte | AL, MO, MS | *Corylus americana* |
| *A. granulatus granulatus* (Say) | AL, MO | *Betula* sp., *Populus deltoides*, *P. deltoides* sp. *monilifera* and *P. nigra* |
| *A. juglandis* Knull | AR, MO, VA | *Juglans cinerea* |
| *A. ohioensis* Knull | GA, MO, NC | *Carpinus caroliniana* |
| *Brachys tesselatus* Kerremans | AL, NC | *Quercus laevis* |
| *B. floricola* Kerremans | AL, GA, NC | Unknown |
| *Taphrocerus agriloides* Crotch | AL, GA, NC, MO | Unknown |
| *T. cylindricollis* Kerremans | AL, GA, MO, NC | Unknown |
| *T. gracilis* (Say) | AL, AR, GA, MO, NC, VA | *Rhynchospora corniculata* and *Schoenoplectus fluviatilis* |
| *T. howardi* Obenberger | AR, KY, MO | Unknown |
| *T. laevicollis* LeConte | AL, MO | Unknown |
| *T. nicolayi* Obenberger | AL, GA, KY, MO, VA | Unknown |
| *T. schaefferi* Nicolay and Weiss | MS, MO, VA | *Cyperus esculentus* |

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# CHAPTER 3

## MOLECULAR PHYLOGENY OF *CHRYSOBOTHRIS FEMORATA* COMPLEX (COLEOPTERA: BUPRESTIDAE) INFERRED FROM A MITOCHONDRIAL AND NUCLEAR GENE.

## Abstract

The number of species that comprise the *Chrysobothris femorata* complex has been slowly increasing for over a century. The most recent revision of the group added seven new *Chrysobothris* species based on recently recognized morphological characters. Many species in the complex are sympatric and even share larval host plant resources. We provide results from a phylogenetic analyisis of the complex using DNA sequences from the mitochondrial gene cox I and the nuclear gene arginine kinase. The primary goal of the analysis was to investigate relationships chiefly between southeastern species in the complex. Neither gene sequence is able to fully resolve the femorata complex as accomplished with morphological species concepts. The concatenated data (i.e. combined nuclear and mitochondrial data) provides the best phyletic resolution, but still falls short. Possible causes of the observed pattern of polyphyly are discussed.


## Introduction

The *Chrysobothris femorata* (Olivier), species complex (*femorata* complex) has been expanding for over a century as additional species within the complex have been described. Nearly fifty years passed after the description of *C. femorata* before the second species in the complex was delineated (Gory and Laporte 1837). Three additional species were described in the 19th century (i.e. *C.* viridiceps Melsheimer, *C. rugosiceps* Melsheimer, *C. adelpha* Harold), bringing the total taxa in the *femorata* complex to five. In Fisher's revision of Chrysobothrini (1942), only four species were included in the *femorata* complex (i.e. *C. femorata*, *C. rugosiceps*, *C. viridiceps* and *C. adelpha*), synonymizing *C. quadriimpressa* with *C. femorata*, while noting the morphological

overlap occurring between members of the complex. Despite his acknowledgement of intermediate forms, Fisher did not attempt to further separate members of the *femorata* complex. Thirty-four years later a rare new species (i.e. *C. sloicola* Manley and Wellso) from American plum (*Prunus americana* Marsh.) was described, adding yet another taxa to the group (Manley and Wellso 1975). Recently, Wellso and Manley (2007) published the first key dedicated entirely to the group, further dividing the *femorata* complex into 12 distinct species, resurrecting *C. quadriimpressa* and describing several new species. The key relies on morphological characters that include color, elytra patterns, male genitalia, structures of the frons and variations in the terminal tergite of adult female beetles. Eight of the 12 species now in the *femorata* complex occur in the southeastern United States with the remaining scattered mostly throughout the western U.S. (Wellso and Manley 2007).

Members of the *femorata* complex are separated from other *Chrysobothris* species by a semicircular clypeus, the row of small teeth on the forelegs of males and the prominent carina of the terminal tergite in females (Fisher 1942). They all share similar life histories, with the first instar larvae boring directly through the bottom of eggs and into the host plant. Healthy plants can mount a defense by exuding sap at the injured area, which slows feeding and can kill larvae, but otherwise larval feeding goes unimpeded. Eventually, larval feeding can influence nutrient flow, further weakening host plants. Damage may continue even during cold winter months when galleries are warmed from direct sunlight and feeding resumes. Pupation takes approximately two weeks at which time the adult chews its way out of the chamber leaving a D-shaped exit

hole characteristic of adult buprestid emergence. The most economically important species of the complex is believed to be *C. femorata*.

Native to the United States, *C. femorata* is unusual among buprestid beetles in that it inhabits all contiguous states in the U.S. as well as northern Mexico and southern Canada (Wellso and Manley 2007). This distribution makes it one of the most cosmopolitan buprestids in North America. *Chrysobothris femorata* has been recognized as a pest of numerous hardwood trees in its native habitat. The ability of the beetle to infest *Malus* spp. hosts earned it the moniker 'flatheaded apple tree borer', but it has also been a noted pest of pecan, dogwood, maple, cherry, chestnut, beech, persimmon, walnut, elm and others (Fenton 1942, Fisher 1942, Wellso and Manley 2007). Its wide host range is unusual among buprestids, which typically utilize a single host plant family or genus. Some are even confined to a single species of plant (Nelson et al. 2008).

Because many of the species in the *femorata* complex are sympatric, have overlapping plant host preferences, and can be difficult to distinguish morphologically, it is important to understand any unique genetic elements that may separate *C. femorata* from other non-economically significant species within the complex. Females are particularly difficult to identify as they lack the unique genitalic characters that separate males. Any unique genetic differences among species of the *femorata* complex will not only yield information that may aid in identification of immature life stages, it could facilitate the timing and placement of insecticides to coincide with activity of economically important buprsetids and reduce unnecessary and costly applications. Optimized control measures could also reduce potential impact on non-target beneficial arthropods in nursery and landscape habitats.

The extensive host plant preferences of *C. femorata* beetles have made it especially difficult for landscape and pest managers to exclude the pest from their managed sites, despite diligent control measures. Several studies have documented first year tree losses of over 30% due to damage caused by *C. femorata* larvae (Potter et al. 1988, Coyle 2005, Oliver et al. 2010). In addition, wild host plants surrounding young trees can harbor infestations and may enhance incidence of attack when crop or managed trees are most vulnerable.

Molecular sequencing techniques provide an additional approach to infer evolutionary relationships of closely related species. Evolutionary hypothesizes are frequently being challenged with genomic data and in some cases has shed new light on species evolution. The objectives of this study were to provide an assessment of the evolutionary relationships of six eastern and one western species in the *femorata* complex based on mitochondrial cox I and nuclear arginine kinase gene sequences and to attempt to validate these results by integrating contemporary knowledge of relavant morphological characters.

## Methods and Materials

*Buprestid beetle sampling, cleaning and preservation*

Purple panel traps were deployed in several locations in Tennessee as well as other eastern states (Table 3-1). Purple panel sticky traps (0.2 m by 1.22 m) were placed vertical at ground level by attaching them to steel or wooden stakes. Traps were placed in transition areas between forest and grassy field exposed to full sun. Pestick™ (Phytotronics Inc, Earth City, MO) was applied liberally to both sides of the panel, which

was stripped and reapplied each time the trap was checked. Traps were serviced on a weekly basis, at which time beetles were removed and placed directly into 5 ml vials containing 95% non-denatured ethanol. Each specimen was kept at 4˚C until it could be cleaned using the clearing agent Histo-clear™ (National diagnostics, Somerville, New Jersey) in the lab by placing the beetles in a 250 ml beaker with 50ml of clearing agent and gentle agitation for 30 minutes or until clean. Once washed clean specimens were rinsed with 95% ethanol, identified to species morphologically using descriptive keys of Wellso and Manley (2007) and sent to Dr. Stan Wellso for confirmation. Each specimen was placed in fresh 95% ethanol and stored at -20˚C until DNA extraction could be performed. A few specimens used in this study were donated and either reared, in which case their hosts were known, or presumably trapped by other unknown means.

*DNA extraction, PCR amplification and sequencing*

Three legs and coxae from one side of each specimen were used to extract total DNA employing a phenol-chloroform based method (Moulton andWiegmann 2004). PCR was carried out using the Ex Taq™ Hot-start PCR Kit (TaKaRa Bio Inc., Shiga, Japan) following manufacturer recommendations for a 50µl reaction. An approximately 600 base pair segment of the cox I gene was targeted using primers C1-J-1718F and K525R (Simon et al. 1994) and about 700 bp of the nuclear arginine kinase (AK) gene was also amplified using primers AK183F and AK939R (Wild and Maddison 2008). Reactions were performed with 1µl of template DNA. The following PCR regime was employed: initial 2 min. denaturing step at 94˚C, then 4 cycles of 30s at 94˚C, 20s at 57˚C

and 90s at 72˚C, followed by 14 cycles of 30s at 94˚C, 15s at 53˚C and 90s at 72˚C, 33 cycles of 30s at 94˚C, 15s at 48˚C and 90s at 72˚C and ended at 72˚C for 7 min.

PCR products were run on 1% agarose gels at 110V for 30 minutes. Bands were excised from the gel, purified using silica spin columns and eluted in 30µl of elution buffer (10 mM Tris, pH 8.5). Purified PCR products served as templates for sequencing reactions using the same primers used to generate bands. Templates were sequenced in both directions with BigDye® v3.1 terminators (Applied Biosystems, Carlsbad, California) in $1/8^{th}$ or $1/16^{th}$ reactions utilizing BetterBuffer (The Gel Company, San Francisco, CA). Sequencing reactions were cleaned using Centri-sep columns (Princeton Separations, Adelphla, New Jersey), electrophoresed through a 6% polyacylamide gel using an MJ Research BaseStation Automated DNA Sequencer (Bio-Rad, Hercules, California), and analyzed using Cartographer 1.2.7 software. Sequencher 4.2.2 (Gene Codes, Ann Arbor, Michigan).

*Sequence analysis*

ClustalX 1.81 (Thompson et al. 1997) was used to conduct a multiple sequence alignment. Modeltest 3.7 (Posada and Crandall 1998) was used to ascertain the optimal evolutionary model for the data. Bayesian analysis was performed with Mr. Bayes 3.1 using the optimal evolutionay model, which was general time reversible with invariant characters and rates following the gamma distribution (Huelsenbeck and Ronquist 2001). Maximum likelihood was performed using RAxML (Stamatakis et al. 2008) *Buprestis* sp. or *B. maniculipennis* Gory served as distal outgroup and *C. dentipes* (Germar) and *C. rotundicollis* Gory and Laporte were used as proximal outgroups for both nuclear and

concantenated sequences (Table 3-1, Fig. 3-1, Fig. 3-2, Fig. 3-3). The distal outgroup for the cox I analysis included *B. lineata* Fabricius, *Chrysochroa fulgidissima* (Tamarmushi) (Hong et al. 2009), *Agrilus planipennis* Fairmaire and *Dicerca lurida* (Fabricius) and the proximal outgroup was represented by six *Chrysobothris* species outside the *femorata* complex (i.e. *C. azurea*, *C. chrysoela*, *C. cribraria*, *C. dentipes*, *C. rotundicollis*, *C. sexsignata*). Uncorrected pairwise distances were calculated using PAUP* (Swofford 1998) to determine the extent long-branch attraction may have had on the resultant inferred phylogeny.

Table 3-1. Regional collection locales of specimens used for the analysis. Numbers not underlined in parentheses indicate specimens used for mitochondrial data and underlined numbers in parentheses represent specimens used for nuclear data.

| **Species** | **Location** |
|---|---|
| *Buprestis maculipennis* | Blount Co., TN (1) |
| *B. lineata* | Anderson Co., TN (2)<br>Peach Co., GA (1) |
| *Dicerca lurida* | Anderson Co., TN (1) |
| *C. azurea* | Anderson Co. TN (4)<br>Knox Co., TN (1)<br>Morgan Co. TN (1) |
| *C. chrysoela* | Peach Co., GA (1)<br>Stone Co., MS (2) |
| *C. cribraria* | Anderson Co., TN (1)<br>Peach Co., GA (1)<br>Stone Co., MS (2) |
| *C. dentipes* | Warren Co., TN (1,1) |
| *C. rotundicollis* | Blount Co., TN (1,1) |
| *C. sexsignata* | Anderson Co., TN (1)<br>Knox Co., TN (1)<br>Sevier Co., TN (4)<br>Stone Co., MS (1) |
| *Chrysobothris adelpha* | Warren Co., TN (2)<br>Morgan Co., TN (1)<br>Stone Co., MS (1) |
| *C. femorata* | Anderson Co., TN (5,1)<br>Morgan Co., TN (1,1)<br>Warren Co., TN (2, 2)<br>Peach Co., GA (3, 2)<br>Stone Co., MS (4, 2)<br>Lake Co., OH (2, 2)<br>Douglas Co., OR (1) |

Table 3-1 continued

| **Species** | **Location** |
|---|---|
| *C. rugosiceps* | Blount Co., TN (2)<br>Warren Co., TN (1)<br>Sevier Co., TN (2)<br>Stone Co., MS (1,1)<br>TX (1)<br>Henry Co., IA (1) |
| *C. shawnee* | Warren Co., TN (1, 2)<br>Peach Co., GA (5, 2)<br>Stone Co., MS (1, 1) |
| *C. viridiceps* | Anderson Co., TN (4, 1)<br>Morgan Co., TN (1, 1)<br>Warren Co., TN (1)<br>Stone Co., MS (1) |
| *C. quadriimpressa* | Morgan Co., TN (1, 1)<br>Anderson Co., TN (1)<br>Peach Co., GA (2, 2)<br>Henry Co., IA (1,1)<br>Wyane Co., OH (1, 1)<br>Lake Co., OH (3, 1)<br>Stone Co., MS (1)<br>Yawapai Co., AZ (1) |
| *C. wintu* | Douglas Co., OR (3, 2) |
| *Chrysobothris* sp. (pupae) | Warren Co., TN (3, 1) |

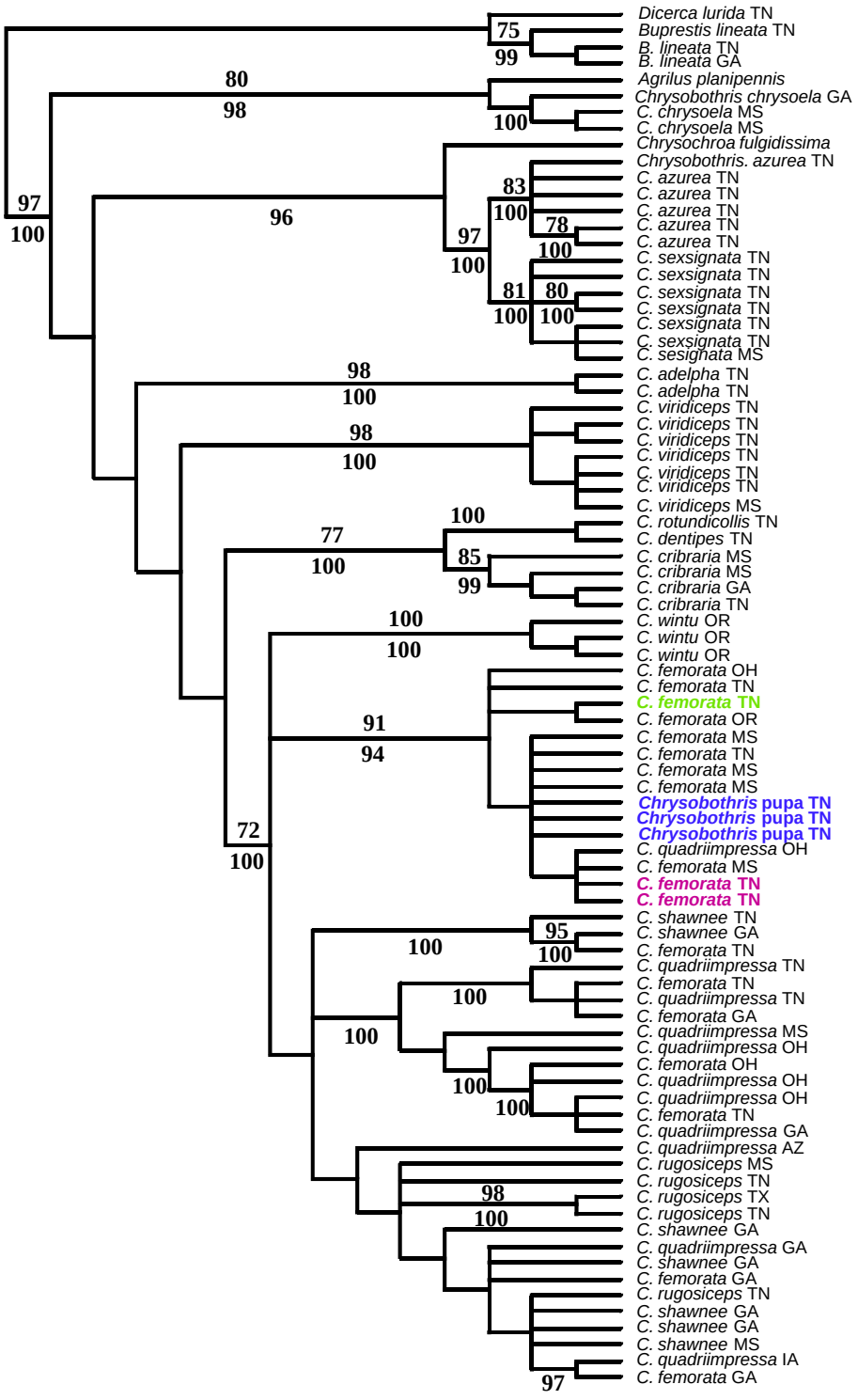


Figure 3-1. Inferred phylogeny based on cox I mitochondrial gene sequence. Specimens indicated with blue text reared from maple (*Acer* sp.); green text reared from dogwood (*Cornus Kousa*), and pink text reared from pear (*Pyrus* sp.).

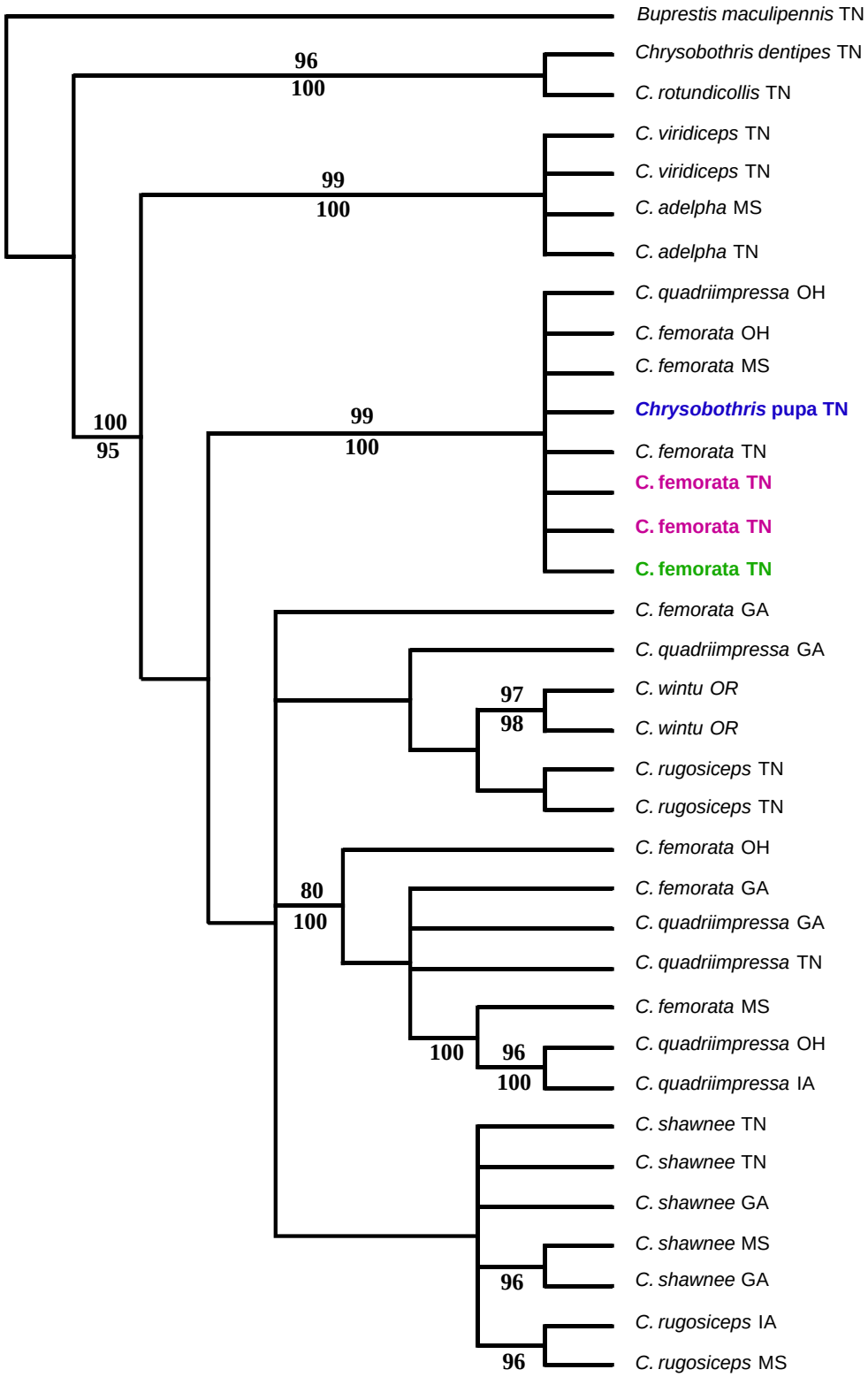


Figure 3-2. Inferred phylogeny based on arginine kinase gene sequence. Specimens indicated with blue text reared from maple (*Acer* sp.); green text reared from dogwood (*Cornus Kousa*), and pink text reared from pear (*Pyrus* sp.).

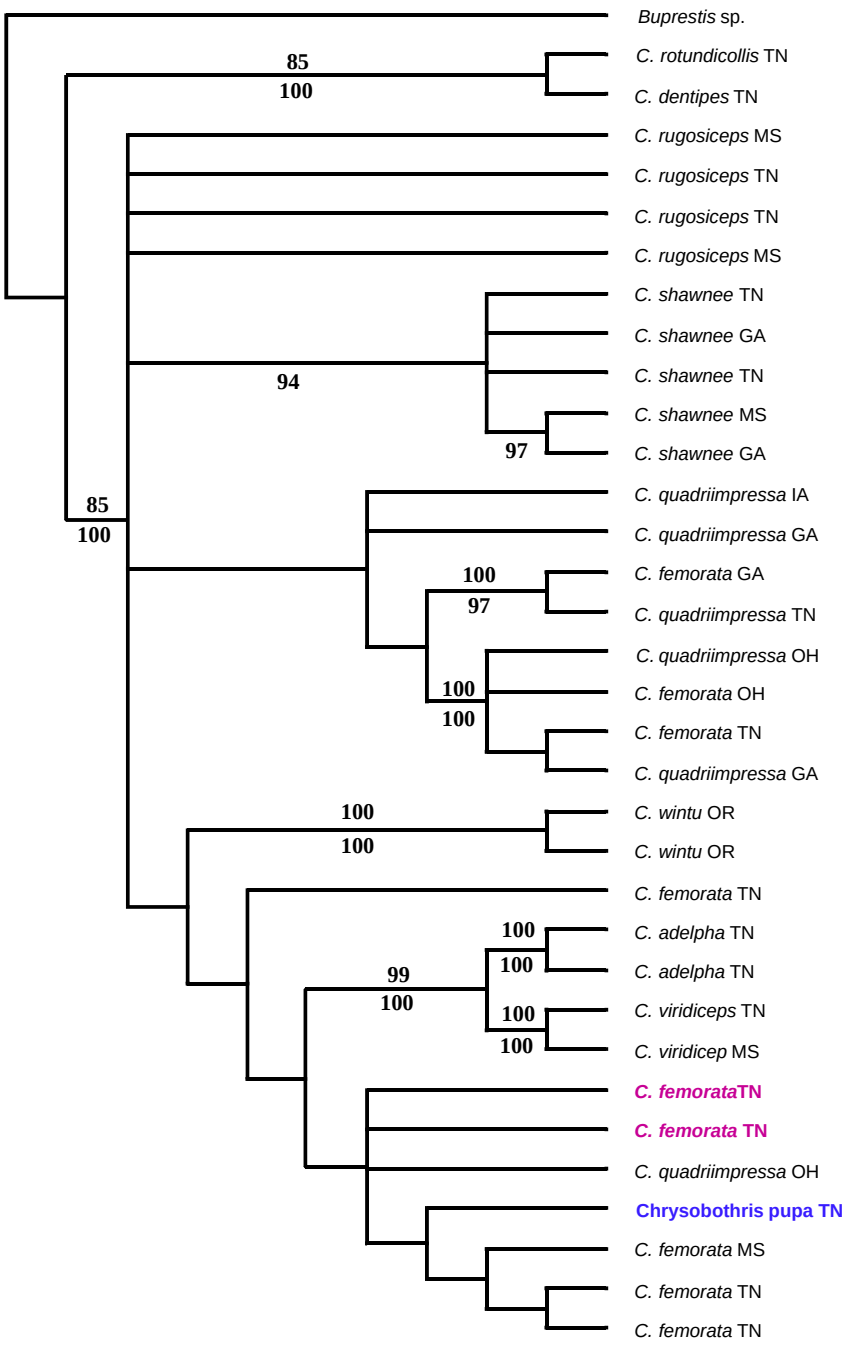


Figure 3-3. Inferred phylogeny based on cox I and arginine kinase gene sequence. Specimens indicated with blue text reared from maple (*Acer* sp.) and pink text reared from pear (*Pyrus* sp.).

## Results

*Cox I phylogeny*

Mitochondrial data support the monophyly of a number of species, which have nodes with posterior probability scores of 90 or more (Fig. 3-1). All *Chrysobothris* species divisions not in the *femorata* complex are well supported (i.e. posterior probability score > 90 and/or bootstrap value > 70) except for *C. dentipes* and *C. rotundicollis*, which both only contributed one sequence to the dataset. Nevertheless the latter two form a cohesive group with *C. cribraria*, which is interesting given their overlapping coniferous host plant preferences (i.e. pines). *Chrysobothris azurea* and *C. sexsignata* appear as sister taxa and they also share overlapping host plant ranges. Finally, *C. chrysoela* sits alone as a valid taxa.

Within the *femorata* complex only 3 taxa are monophyletic (i.e. *C. adelpha*, *C. viridiceps* and *C. wintu*). Other clades with posterior probabilities scores ≥ 90 are composed of at least two polyphyletic species, with the exception of a clade comprised of two specimens, both identified as *C. rugosiceps*. The inferred cladogram reveals two large clades with high posterior probabilities. Though they are polyphyletic, they appear to be rich in one species of the *femorata* complex while other clades contain a more random mix of species. One of the large clades includes 12 species identified as *C. femorata* from four widely disjunct states (i.e. MS, OH, TN and OR) and a single *C. quadriimpressa* taken in Ohio. While most of the specimens in this clade came from unknown host plants, several were reared. Two beetles emerged from dogwood (*Cornus kousa* Hance) and two other specimens came from pear (*Pyrus* sp.) host plants. Also included in the group are three unidentified *Chrysobothris* pupae taken from maple (*Acer*

spp.) near McMinnville, TN. The inclusion of *C. quadriimpressa* appears to be anomalous, but is not too surprising considering the degree of polyphyly exhibited by most individuals in the resultant cox I phylogeny. Similarly, the other major clade is rich in individuals identified as *C. quadriimpressa*, with seven of 11 specimens identified as such. This clade also contains specimens from a wide geographic area. The remaining four individuals in the clade were identified as *C. femorata*.

The two Asian buprestid beetle species included in the analysis (i.e. *Agrilus planipennis* and *Chrysochroa fulgidissima*) fall out within the *Chrysobothris* clade, with both placements well supported. Neither of these exotic species is in the subfamily Buprestistinae, where the genus *Chrysobothris* is currently placed. In fact, *B. lineata*, the distal outgroup species used to root the tree is more closely related to *Chrysobothris* than either of the two Asian species. Distance analysis revealed long-branch attraction is most likely the cause of these inconsistencies.

*Arginine kinase phylogeny*

The phylogeny of the arginine kinase (AK) dataset shows the two pine borer beetles, *C. dentipes* and *C. rotundicollis* as basal taxa in the *Chrysobothris* clade (Fig. 3-2). This is not surprising since conifers are more ancient in origin than deciduous trees. The *femorata* complex is monophyletic but only *C. wintu* is monophyletic within the *femorata* complex. Though not completely cohesive the large *C. femorata* rich and *C. quadriimpressa* rich clades inferred from the cox I data appear to be retrieved by the AK dataset. *Chrysobothris shawnee* forms a weakly supported group with the still

polyphyletic *C. rugosiceps*. Unlike cox I, arginine kinase was not informative enough to distinguish between *C. adelpha* and *C. viridiceps* and the two species form a single clade.

*Concatenated data phylogeny*

When combined, the data set produced a cladogram similar but more resolved than those derived from the two separate genes (Fig. 3-3). *Chrysobothris dentipes* and *C. rotundicollis* remain basal to the *femorata* complex. Three monophyletic clades are apparent within the complex. A monophyletic grouping of *C. shawnee* is also revealed in the combine data set but absent when each gene alone is examined. *Chrysobothris adelpha*, *C. viridiceps*, and *C. wintu* are also monophyletic.

## Discussion

The degree of polyphyly currently seen in the two genes sequenced for this work does not lend itself well to the development of species-specific diagnostic primers. While the sequences produced can be used to positively identify *Chrysobothris* larvae as belonging to the *femorata* complex, long-branch attraction of *A. planipennis* and *Chrysochroa fulgidissima* to other *Chrysobothris* species seen in the cladogram (Fig. 3-1) urges caution when applying cox I sequence comparison to distantly related taxa. Given our lack of knowledge about the extent of interbreeding in the *femorata* complex, identification using molecular markers may not be practical. Neither nuclear or mitochondrial gene sequence in this study were perfectly capable of distinguishing most species in this study as delineated by Wellso and Manley (2007).

According to data from cox I analysis only three of the seven species sequenced have distinct maternal signatures, the remaining four morphospecies are polyphyletic. The lack of resolution given by the cox I data is not too surprising when considering that the overall rate of nucleotide substitutions in mitochondrial genes is much more rapid when compared with nucleotide substitution of the nuclear genome (Moriyama and Powell 1997). In some instances this can be advantageous, allowing for examination of recent speciation events and identifying cryptic species (Murray et al. 2008). However, changes may occur so rapidly that saturation of informative sites occurs, obscuring the underlying gene tree by combining two or more species that are actually more distantly related. Cytoplasmic infections, incomplete lineage sorting and introgressive hybridization also confound evidence of relationships, especially when populations of closely related taxa are sympatric, as is the case with the *femorata* complex. In turn, sympatry may allow interbreeding to occur (Funk and Omland 2003, Ballard and Whitlock 2004).

The region of AK amplified here only recovered one of the species, *C. wintu*, from the *femorata* complex within the resultant gene tree. The failure to recover multiple species may be the product of a slowly evolving nuclear gene, which recovers deeper divergences in the evolutionary timeline of the complex. The nuclear gene tree did recover the *femorata* rich clade present in the cox I tree. However, when the two datasets are analyzed conjointly, support for the *C. femorata* rich clade in the cox I and AK trees falls shy of the predetermined threshold (i.e. posterior probability score ≥90 and/or bootstrap value > 70), causing the clade to collapse.

Nuclear copies of mitochondrial genes can produce the observed results, but sequences evaluated for this study showed no evidence of polymorphic sites that would be expected if nuclear copies of mtDNA evolving at slower rates were present. It is also possible that an undetected bacterial infection within populations sampled is influencing the gene pool. At least one species of *Brachys* from the southeastern United States is known to support such an infection, which influences population sex ratios and encourages parthenogenesis (Lawson et al. 2001). Futher study will be required before the exact cause of the observed polyphyly can be determined.

The recovery of four species within the *femorata* complex by the combine dataset demonstrates the improved accuracy that can occur when utilizing genes from both mitochondrial and nuclear sources. Unfortunately, the *femorata* complex was still left mostly unresolved. The data support previous statements about the *femorata* complex, suggesting that it is rapidly evolving (Fisher 1942, Wellso and Manley 2007). The cause of the observed polyphyly in the resulting phylogenies is uncertain. When taken with evidence from morphology and life histories, however, introgression of genes from neighboring populations of closely related species is likely. Buprestid beetles species from the eastern United States that were examined here not only share similar geographic ranges, but several share larval host plants (Wellso and Manley 2007). Adult beetles are found resting together on many of the same tree species (Wellso and Manley 2007, Nelson 2008). While reproductive couplings between different species within the *femorata* complex has not been reported, it may be occurring.

Mate selection among species in the *femorata* complex is poorly documented, but in some buprestids may involve pheromones emitted from female beetles or male

displays that are aimed at attracting mates (Nichols 1910, Wellso 1966, Dunn and Potter 1988, Eberhard 1990, Silk et al. 2009). Male beetles believed to be *C. femorata* produce a rapid tapping sound that is apparently for attracting conspecifics (Bowditch 1896). Beer (1970) noted observing the tapping behavior among *Chrysobothris* in Oregon, though he does not name the species he observed. Bowditch (1896) and Beer (1970) both describe tapping with a fingernail or pencil to attract buprestids. Although it is not known if the tapping behavior is widespread in the *femorata* complex, it has been observed in at least one other buprestid genus (Beer 1970). If this behavior is shared by species in the *femorata* complex it could encourage interspecies breeding if it acts as a general attractant, but may discourage crossbreeding if each species relies on a unique rhythm of tapping to attract conspecifics.

Differences in genitalic morphology can produce sexual isolation between species by mechanically preventing successful copulation (Sota and Kubota 1998). Three of the four monophyletic species in this study have genitalia that are markedly different from other males in the *femorata* complex (i.e. *C. adelpha*, *C. viridiceps*, *C. wintu*) (Fig 3-4). For example, *C. adelpha* has spines pointing perpendicular to the parameres, *C. viridiceps* has parameres that are more arcuate than any other species in the complex and the parameres of *C. wintu* have a unique "hourglass" shape. The genitalia of *C. shawnee* are similar to other species in the *femorata* complex, but buldge much less in the middle compared to the others (Fig. 3-4).

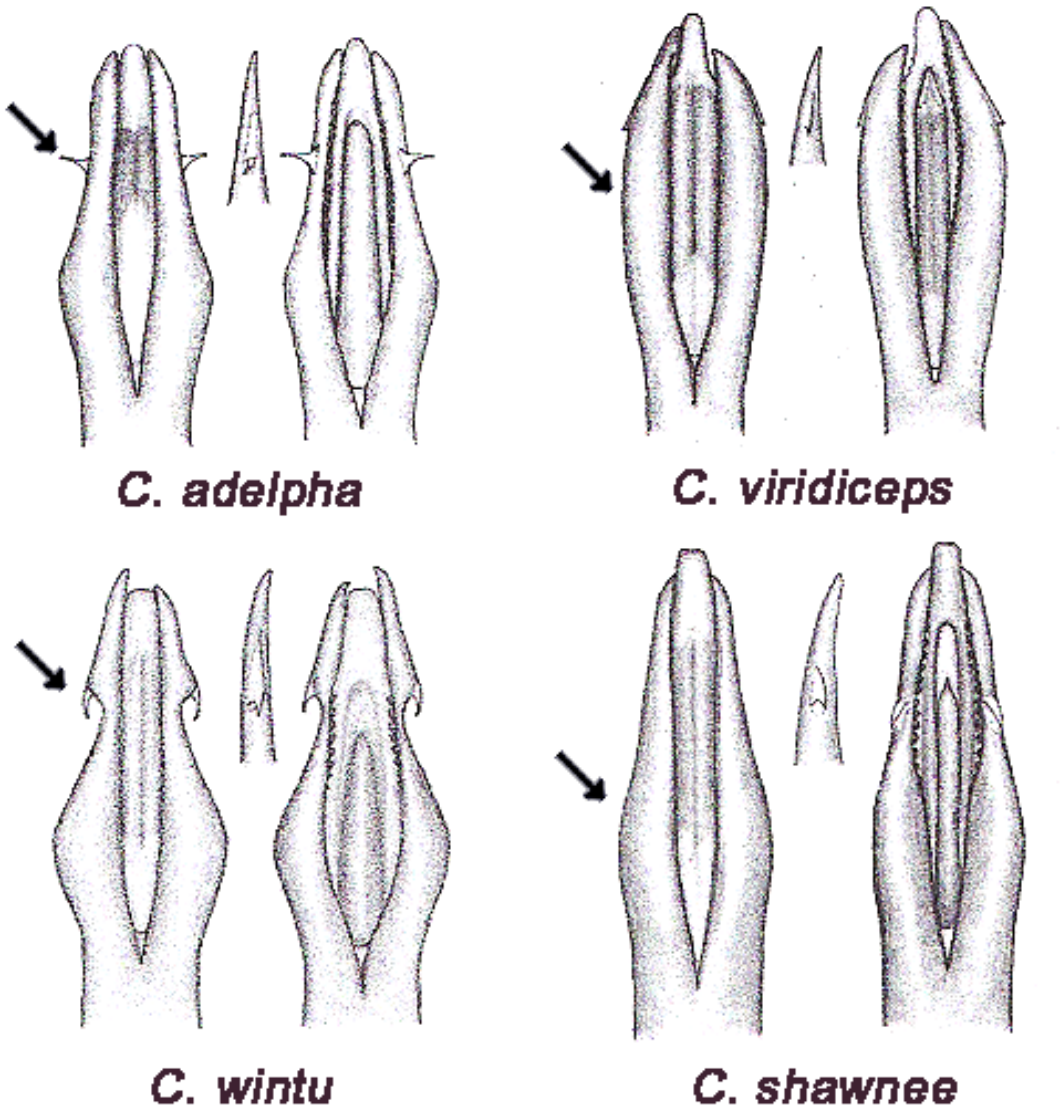


Figure 3-4. Genitalia of monophyletic species showing unique morphology. Figure modified from Wellso and Manley 2007.

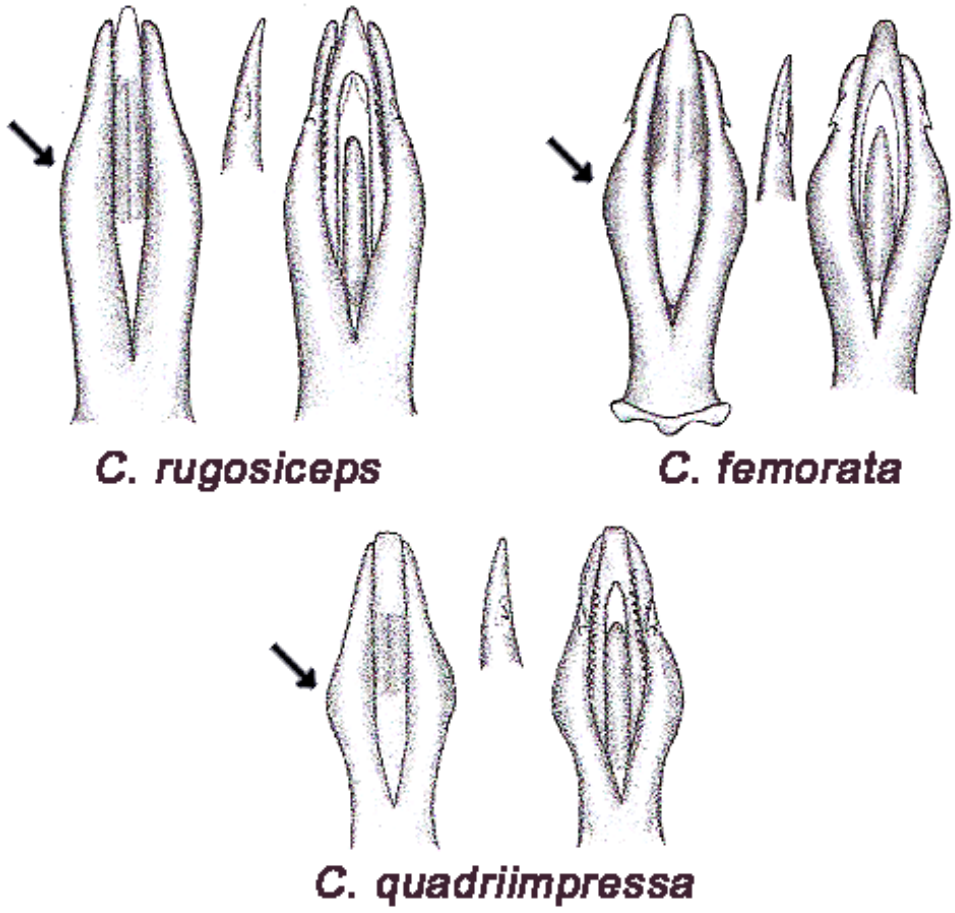


Figure 3-5. Genitalia of polyphyletic species, showing similarities between species. Figure modified from Wellso and Manley 2007.

In contrast, the genitalia of polyphyletic species tend to be similar in shape and structure (Fig. 3-5). Although some taxa in the *femorata* complex have much smaller body sizes than others, there is considerable overlap in the natural size variation of each species. It will be important to know if the relatively minor variation in genitalic morphology of polyphyletic species is sufficient to limit interspecies breeding. If

crossbreeding does occur, frequent gene transfer will not only make attempts to elucidate species boundaries difficult from a molecular perspective, it could also allow unfavorable traits (e.g. pesticide resistant genes) to spread to economically important species in the complex, thus confounding successful pest management.

Further studies of the *femorata* complex are needed to ascertain if barriers to interbreeding exist, whether behavioral, chemical or simply mechanical. Only controlled breeding studies can conclusively provide evidence of hybrid offspring resulting from interspecies coupling. Given the relative ubiquity of most species analyzed here, their collection and subsequent rearing for cross mating is possible. Recently, Gindin et al. (2009) reared buprestid adults in as little as two months on artificial diet containing 20% plant cortical tissue. Ability to manipulate populations in the lab would not only provide valuable information about interbreeding, but would also permit observations on mating behavior, plant host preferences, pesticide resistance as well as make possible a detailed morphological study of immature life stages.

## Acknowledgements

This study was supported by funding from USDA-ARS (58-6404-7-213). Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

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# CHAPTER 4

# MOLECULAR PHYLOGENY OF SYNANTHEDONINI (LEPIDOPTERA: SESIIDAE) USING CYTOCHROME OXIDASE I AND COMPARISON OF MORPHOLOGICAL VARIATION AMONG ITS MEMBERS JUSTIFYING GENERIC PLACEMENT

**Abstract**

Many North American sesiid moths within the tribe Synanthodonini have been studied extensively due to detrimental economic impacts on forest, deciduous shade and fruit trees as well as many other ornamental and native shrub species. Identification of larvae and even adults can be difficult, especially for those unfamiliar with sesiid morphology. Introduction of non-native clearwing moths (e.g., the red-belted clearwing moth, *Synanthedon myopaeformis* (Borkh.), a European pest of apple trees) reinforce a need for reliable and accurate molecular diagnostic tools that can be utilized by non-taxonomic experts. Short DNA sequences can be used to advance knowledge of sesiid species divergence, leading to enhanced understanding of their evolutionary history. The cox I phylogeny produced from sequences of 19 Nearctic Synanthedonini species suggests a close evolutionary relationship of sesiids that rely on similar host plants to complete their life cycle. *Sannina uroceriformis* Walker is positioned firmly with the genus *Synanthedon* despite its genitalic similarities with *Carmenta*. *Podosesia* spp. are also somewhat surprisingly positioned within the *Synanthedon* clade. Our analysis also suggests the current generic placement of *S. rileyana* (Hy. Edwards) may be erroneous. The close evolutionary relationship of the two North American viburnum borers (*S. viburni* Engelhardt and *S. fatifera* Hodges) is briefly discussed. Precise placement of *S. rileyana*, *Sannina*, and *Podosesia* awaits further evaluation of additional taxa, as well as results preferably from the analysis of nuclear genes. Nevertheless, no polyphyletic relationships exist among economically important species making cox I sequences species specific and useful as identifying genetic markers in the tribe.

## Introduction

The tribe Synanthedonini (Lepidopterea: Sesiidae) was established by Niculescu (1964) based on adult morphology and the taxonomic division was supported in a subsequent revision of Sesiidae based upon larval morphological characters (MacKay 1968). Naumann (1971) included *Synanthedon* and other closely related genera in a tribe he called Aegeriini, now considered a synonym of Synanthedonini. Currently, Synanthedonini is considered to be the most species rich sesiid tribe in North America, with over 87 species described (Eichlin and Duckworth 1988). The economic importance of many of its members has made them targets of research to elucidate details of life history and effective methods of control (Solomon and Dix 1979). Equally important is a clear understanding of evolutionary relatedness among members of Synanthedonini, potentially affecting management actions by commercial nursery growers and landscape managers as well as regulatory action by government agencies charged with preventing non-native pest introductions. Immature stages (i.e., eggs, larvae, and pupae), which are often encountered after plant injury is first observed, present a particular problem for professionals charged with pest control because morphological characters required for accurate species identification may not exist in these life-stages or may only be apparent to a taxonomic expert.

Within Synanthedonini, most species having economic importance belong to the genus *Synanthedon* and larvae of several species within this genus feed on commercially grown fruit trees and ornamental plants. For example, larval feeding by greater and lesser peachtree borers (*S. exitiosa* (Say) and *S. pictipes* (Grote and Robinson) respectively) cause root, trunk or branch damage that in turn reduces fruit yield and can

kill plant hosts. Likewise, the dogwood borer (*S. scitula* (Harris)) is recognized as a growing management challenge in apple orchards, particularly where size-controlling rootstocks are used (Leskey and Bergh 2005). In addition to apple and dogwood trees, the dogwood borer has a diverse host plant range and has a geographic distribution extending across the eastern U.S., but has also recently been detected disjunctly in western states (Eichlin and Duckworth 1988, Bergh et al. 2009, LaGassa personal communication). Recent introduction into North American of the red-belted clearwing (*S. myopaeformis* (Borkh.)), as well as several other clearwing moth introductions in the past 150 years, reinforces the need for accurate molecular diagnostic tools to aid with identification of clearwing pests, regardless of life stage (Eichlin and Duckworth 1988, Philip 2006).

Mitochondrial genes can be used to quickly and reliably identify species. Despite criticisms of the possibility of polyphyletic species, leading to incorrect identification and erroneous phylogenies, the mitochondrial genome is capable of reliably identifying species and can provide phylogenetic information congruent with known morphology (Hill et al. 2001, Funk and Omland 2003, Armstrong and Ball 2005, Cameron et al. 2009).

A distinctive cox I genetic signature of economically important species allows identification of all life stages through gene sequencing, eliminating sole reliance on morphological characters that can sometimes be obscured or lacking. Molecular techniques can be particularly useful when specimens are damaged, and therefore difficult to identify morphologically. Specimens caught in pheromone traps are frequently damaged, but can still yield enough genetic material suitable for subsequent

species identification. Exploitation of unique species-specific nucleotide arrangements from the mitochondrial genome have been used in several cases where morphological characters are hard to find or unavailable (Ball and Armstrong 2006, Nwilene 2006, Foottit et al. 2008). Good quality multi-copy mitochondrial DNA is also much easier to obtain than low copy nuclear genes. Over time nuclear genes degrade and become even more difficult to amplify. In contrast, the ease with which mtDNA can be amplified makes it particularly useful even if specimens are decades old (Gilbert et al. 2007).

Despite the relative importance of the tribe little is known about inter- or intraspecific genetic diversity among members of Synanthedonini. Mitochondrial sequences deposited in GenBank to date represent specimens from a limited geographical distribution in the United States and Turkey and are either too broad or narrowly focused within the family to provide reliable analysis of species relationships within Synanthedonini (Kallies 2003, McKern et al. 2008, McKern and Szalanski 2008). Molecular diagnostics based on incomplete genetic data may be inaccurate when individuals from disjunct populations are tested. In addition, some generic relationships within the tribe Synanthedonini have been ambiguous because of overlapping and intermediate morphological characters, leading some in the past to erect genera, further dividing the tribe (Engelhardt 1946, MacKay 1968). Some species of *Synanthedon* and *Carmenta* have been particularly difficult to place at the genus level. Because of their apparent rapid evolutionary rate and common ancestry these two genera sometimes share or exhibit intermediate character traits. Therefore, the objective of this research is to provide a largely phylogenetic study based upon analysis of a portion of cytochrome oxidase I from 25 distinct North American species collected in disparate geographical

areas (insomuch as distribution allows) and the resulting inferred maternal phylogeny of Synanthedonini is contrasted with currently known morphology.

## Materials and Methods

*Taxon sampling*

Starting in March 2007, cooperators across the U.S. and Canada were contacted to determine willingness to trap for clearwing moth taxa of regional and economical relevance, as well as host plant feeding preferences for key ornamental plant species. Several well documented clearwing moth pests were specifically targeted, including greater and lesser peachtree borers, dogwood borer, and viburnum borers. Where possible, modified Multipher-1 traps (Ste-Foy, Quebec, Canada, Bio-Contrôle) were placed in habitats where host plant resources (if known) were present and mounted about 1 meter high on stands in partial shade to retard evaporation of 95% non-denatured ethanol. Non-denatured ethanol was used to quickly kill specimens and to preserve moth DNA between sampling dates. Collection periods were requested to span no longer than seven days between reservoir refills. Dates from deployment to refill were noted on specimen vials collected during each period. All moths were held and shipped to UT-Knoxville in 15 ml vials containing 95% non-denatured ethanol. Species appropriate pheromone lures were used to attract male moths of desired taxa (Table 4.1). In the lab, species were identified using keys in Eichlin and Duckworth (1988) and then stored at -20ºC until DNA was extracted. Specimen collection data and the number of species from each site are given below (Table 4.1).

Table 4-1. Regional collection locales of specimens used in analysis. Number in parenthesis indicate number of specimens of that species from each location.

| Species | Tribe | Collection location (number of specimens) | Lure catalog numbers |
|---|---|---|---|
| *Melittia cucurbitae* (Harris) | Melittiini | MN: Ramsey Co. (1) | PB-SVB (Portland, OR, APTIV) |
| *Paranthrene simulans* (Grote) | Paranthrenini | TN: Knox Co. (1) | GPTB (Adair, OK, Trécé) |
| *Vitacea polistiformis* (Harris) | Paranthrenini | NC: Haywood Co. (1) | Dogwood borer (Zhang et al. 2005) |
| *Osminia ruficornis* (Hy. Edwards) | Osminiini | KS: Cherokee Co. (3) | LPTB (Adair, OK, Trécé) |
| *Alcathoe carolinensis* Engelhardt | Synanthedonini | TN: Knox Co. (2) | L997 (Billings, MT, Sentry Biologicals Inc.) |
| *Carmenta bassiformis* (Walker) | Synanthedonini | KS: Bourbon Co. (1)<br>TN: Knox Co. (1) | Dogwood borer (Zhang et al. 2005) |
| *Synanthedon rileyana* (Hy. Edwards) | Synanthedonini | KS: Bourbon Co. (1)<br>TN: Knox Co. (2)<br>TN: Anderson Co. (1)<br>WV: Jefferson Co. (2)<br>NC: Henderson Co. (1) | PB-SYVE (Portland, OR, APTIV) |
| *S. tipuliformis* (Clerck) | Synanthedonini | MN: Hennepin Co. (3) | PB-SYTI (Portland, OR, APTIV)<br>CCWM (Adair, OK, Trécé) |
| *S. scitula* (Clerck) | Synanthedonini | MN: Ramsey Co. (1)<br>NY: Ontario Co. (4)<br>MS: Pearl River Co. (2)<br>TN: Sevier Co. (1)<br>TN: Knox Co. (5)<br>VA: Fredrick Co. (1)<br>KS: Bourbon Co. (1)<br>IA: Henry Co. (1)<br>GA: Peach Co. (1)<br>TN: Warren Co. (1)<br>WV: Jefferson Co. (1) | Dogwood borer (Zhang et al. 2005) |
| *S. novarensis* (Hy. Edwards) | Synanthedonini | CANADA, Thunder Bay, ONT. (2) | L103 (Billings, MT, Sentry Biologicals Inc.) |
| *S. rhododendri* (Beutenmüller) | Synanthedonini | TN: Knox Co. (1)<br>TN: Sevier Co. (2) | GPTB (Adair, OK, Trécé) |
| *S. kathyae* Duckworth and Echlin | Synanthedonini | TN: Blount Co. (2) | GPTB (Adair, OK, Trécé) |
| *S. sapygaeformis* (Walker) | Synanthedonini | FL: Dade Co. (4) | LPTB (Adair, OK, Trécé) |

Table 4-1. Continued

| Species | Tribe | Collection location (number of specimens) | Lure catalog numbers |
|---|---|---|---|
| *S. fulvipes* (Harris) | Synanthedonini | CANADA, Thunder Bay, ONT. (2) | GPTB (Adair, OK, Trécé) |
| *S. castaneae* (Busck) | Synanthedonini | NC: Haywood Co. (3) | Raspberry crown borer (Delta, British Columbia, PheroTech, Inc.) |
| *Podosesia aureocincta* Purrington and Nielsen | Synanthedonini | MN: Dakota Co. (4) | LILA (Adair, OK, Trécé) |
| *P. syringae syringae* (Harris) | Synanthedonini | TN: Anderson Co. (1)<br>TN: Knox Co. (2) | GPTB (Adair, OK, Trécé)<br>LILA (Adair, OK, Trécé) |
| *P. syringae fraxini* (color form of *P. syringae* syringae) | Synanthedonini | IA: Henry Co. (3)<br>TN: Sevier Co. (1) | LILA (Adair, OK, Trécé) |
| *S. fatifera* Hodges | Synanthedonini | MN: Ramsey Co. (2)<br>TN: Sevier Co. (1)<br>TN: Anderson Co. (2) | GPTB (Adair, OK, Trécé) |
| *S. viburni* Engelhardt | Synanthedonini | MN: Ramsey Co. (3)<br>MN: Hennepin Co. (8)<br>NY: Wayne Co. (4) | L997 (Billings, MT, Sentry Biologicals, Inc.) |
| *S. acerrubri* Engelhardt | Synanthedonini | TN: Knox Co. (5)<br>NC: Haywood Co. (2)<br>OH: Hamilton Co. (1) | Dogwood (Zhang et al. 2005) |
| *S. exitiosa* (Say) | Synanthedonini | CANADA, Thunder Bay, ONT. (2)<br>GA: Peach Co. (2)<br>KS: Cherokee Co. (1)<br>KS: Bourbon Co. (1)<br>TN: Sevier Co. (2)<br>TN: Knox Co. (2)<br>TN: Blount Co. (1)<br>MN: Ramsey Co. (1)<br>MS: Pearl River Co. (2)<br>NY: Ontario Co. (1) | GPTB (Adair, OK, Trécé)<br>L103 (Billings, MT, Sentry Biologicals Inc.) |
| *S. pictipes* (Grote and Robinson) | Synanthedonini | TN: Knox Co. (4)<br>KS: Bourbon Co. (1)<br>CANADA, Thunder Bay, ONT. (1)<br>TN: Sevier Co. (1)<br>IA: Henry Co. (1) | LPTB (Adair, OK, Trécé) |
| *S. pyri* (Harris) | Synanthedonini | NY: Ontario Co. (2)<br>OH: Lake Co. (3)<br>MD: Montgomery Co. (1) | PB-GRB (Portland, OR, APTIV) |
| *S. acerni* (Clemens) | Synanthedonini | GA: Marion Co. (3) | Came to light trap |
| *Sannina uroceriformis* Walker | Synanthedonini | TN: Anderson Co. (2)<br>TN: Knox Co. (2) | Raspberry crown borer (Delta, British Columbia, PheroTech, Inc.) |

*DNA extraction, PCR amplification, sequencing, and analyses*

Legs, head capsule, or thorax tissues were used to extract total DNA from specimens employing a phenol-chloroform based method (Moulton and Wiegmann 2004). Polymerase chain reaction (PCR) was carried out using the Ex Taq™ Hot-start PCR Kit (Shiga, Japan, TaKaRa Bio Inc.) following the manufacturers recommendations for a 50µl reaction. An approximately 700 bp segment of the cox I gene was amplified with the following forward and reverse primers: 5'-ATAATYGGRGGATTTGGWAAYTG and 3'-GTTARTCCNCCYACWGTRAA. Each reaction was performed with 1µl of template DNA. After an initial 2 minute denaturing step at 94˚C, the following touchdown PCR was performed: 4 cycles of 30s at 94˚C, 20s at 57˚C and 90s at 72˚C, followed by 14 cycles of 30s at 94˚C, 15s at 53˚C and 90s at 72˚C, and finished with 33 cycles of 30s at 94˚C, 15s at 47˚C and 90s at 72˚C and at 72˚C for 7 min.

Amplicons were run on a 1% agarose gel at 110v for 30 minutes. Bands were excised from the gel, purified using silica spin columns and eluted in 30µl of elution buffer (10 mM Tris, pH 8.5). Purified PCR products served as templates for sequencing reactions using the same primers used to generate the bands. Templates were sequenced in both directions with BigDye® v3.1 terminators (Carlsbad, California, Applied Biosystems) in $1/8^{th}$ or $1/16^{th}$ reactions utilizing BetterBuffer (The Gel Company, San Francisco, CA).

*Sequence analysis*

A multiple sequence alignment across specimens and species was conducted with ClustalX 1.81 software (Thompson et al. 1997). To ascertain the optimal evolutionary model for the data, we used Modeltest 3.7 (Posada and Crandall 1998). Bayesian analysis was next performed with Mr. Bayes 3.1 (Huelsenbeck and Ronquist 2001). A cox-I GenBank accession for two-year cycle spruce budworm moth, *Choristoneura biennis* (Freeman) (Lepidoptera: Tortricidae), was chosen as a distal outgroup and genetic data from five sesiid moth species belonging to tribes outside of Synanthedonini were used as proximal outgroups (Table 4-1 and *Negotinthia myrmosaeformis* (Herrich-Schaeffer)). Twenty-eight Palearctic species with cox I sequence data were taken from GenBank for the analysis. Their accession numbers are noted in Fig. 4-1. Uncorrected pairwise distances were calculated using PAUP* (Swofford 1998), which provides a measure for determining the extent to which long-branch attraction may have influenced the resulting best-fit, or inferred phylogeny.

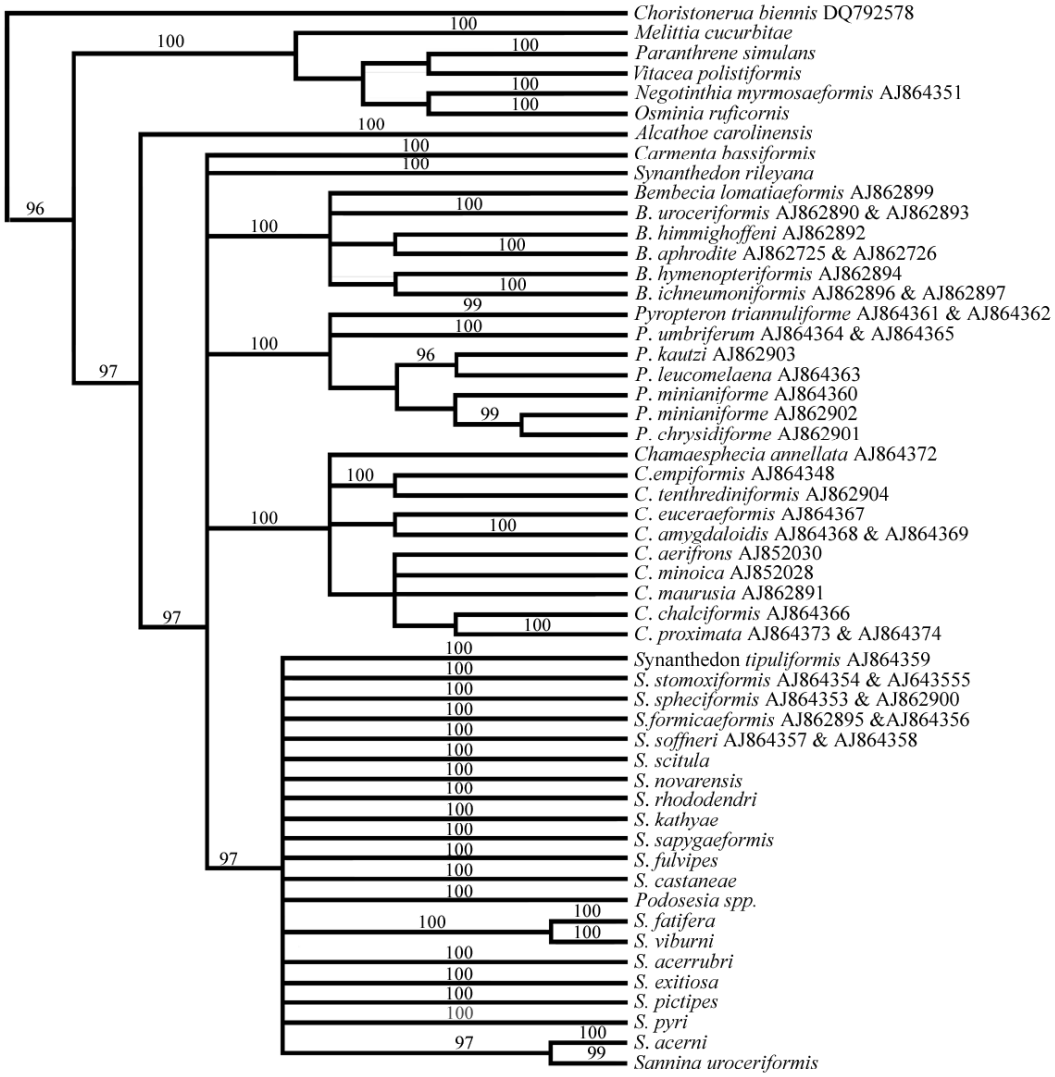


Figure 4-1. Inferred phylogeny of Synanthedonini based on the mitochondrial gene cox I. Accession numbers of species taken from GenBank are shown.

## Results

In total, 21 clearwing species representing Synanthedonini, including 16 *Synanthedon* species were collected from 20 geographic locales. Data from cox I mitochondrial sequences strongly support monophyly of Synanthedonini with a posterior probability score of 97. Among all species sampled, *Alcathoe carolinensis* Engelhardt appears as the most basal species of Synanthedonini in the analysis, with all other taxa

partitioned into one of six clades with posterior probability scores of 97 or higher. *Carmenta bassiformis* (Walker), *Synanthedon rileyana* (Hy. Edwards), and Palearctic genera *Pyropteron* Newman, *Chamaesphecia* Spuler, and *Bembecia* Hübner all use herbaceous larval resources and show a close relationship with the *Synanthedon* species rich clade. This larger clade includes all *Synanthedon* species, regardless of origin in Nearctic and Palearctic regions, except *S. rileyana*. Species comprising this larger clade all exploit woody tissues from host plants for life cycle completion. *Podosesia* and *Sannina* are embedded within the larger clade with other *Synanthedon* species. Their current status as distinct genera is not supported in the cox I phylogeny. The viburnum borers (*S. fatifera* Hodges and *S. viburni* Engelhardt) form a monophyletic group within the *Synanthedon* clade. *Synanthedon tipuliformis* (Clerck), a non-native Palearctic species from Europe, is monophyletic with its invasive Nearctic counterpart.

## Discussion

In recent years criticism of single gene phylogenies has grown, causing many molecular systematists to gravitate toward a more reliable approach using genes from both mitochondrial and nuclear genomes (Springer et al. 2001, Yamamoto and Sota 2007, Ruiz et al. 2008). Polyphyly among species can occur for a number of reasons, making species identification difficult to achieve in some instances with mitochondrial sequences alone. However, nuclear sequences, particularly from variable coding genes, can be difficult to amplify in some taxa as primers may not work for all species due to primer-template mismatch or presence of introns. When nuclear genes are capable of being amplified they are not always phylogenetically informative (Zhang 2004, Regier et al.

2009). In addition, introns can also pose problems when amplifying nuclear genes, either making the amplicon too large to amplify or by rendering primers useless by occurring in the conserved region from which they were designed.

Any practical use of gene sequences for species identification and phylogenetic study first requires targeting an informative gene at the taxonomic level of interest that can be reliably amplified across taxa. To date only a single lepidopteran genome has been characterized, making "discovery" of nuclear gene sequences useful for species level identification or phylogenetic analysis of higher taxa difficult (Mita et al. 2004). Primer availability, ease of amplification and lack of introns all make the mitochondrial genome a much more practical choice, especially for preliminary scrutiny of intergeneric associations.

Cox I is one of several mitochondrial genes for which amplification is relatively straightforward and primers are readily available (Simon 1994). Most Nearctic species included in this study have a unique genetic signature. The few Nearctic species that are not monophyletic are likely the result of undersampling. Sequence from the European *S. tipuliformis* matches its introduced Nearctic counterpart despite their geographic separation. Though cox I seems unable to distinguish some Palearctic taxa included in the analysis, this may also be due to collection of too few specimens of closely related taxa (Fig. 4-1). If species specificity of cox I sequences can be demonstrated for other Synanthedonini species it would be an invaluable tool for the monitoring of pests by authorities at entry points to the United States as well as managers of landscapes, nurseries and orchards who may have little taxonomic expertise, but access to private or public laboratories having the ability to process specimens molecularly.

The generic status of *Podosesia* and *Sannina* has been unchanged for well over a century, yet our inferred phylogeny includes them as congenerics of *Synanthedon* species. Despite resolution of other genera in the tribe these two were not resolved as might be expected. The position of *S. rileyana* outside the *Synanthedon* rich clade is also contrary to its present taxonomic placement. Pairwise distance analysis reveals no signs of long-branch attraction between any of these three species and other nodes or terminal taxa in the tribe.

While the generic designation of *Podosesia* and *Sannina* has never before been questioned, generic placement of *S. rileyana* has been debated several times before its current assignment to *Synanthedon* (Engelhardt 1946, MacKay 1968, Eichlin and Duckworth 1988). Our cox I phylogeny suggests two main groups in Synanthedonini, one relying on herbaceous hosts and the other primarily on woody hosts. Such a division seems unlikely to occur by chance and suggests at the very least the current generic placement of *S. rileyana* may need to be reexamined.

Since its description in 1881, *S. rileyana* has been placed in four different genera by various taxonomists (Duckworth and Eichlin 1977). MacKay (1968) relegated it to an unnamed genus with three other species (*Carmenta giliae* (Hy. Edwards), *C. phoradendri* Engelhardt and *C. anthracipennis* (Boisduval)), based upon larval chaetotaxonomical characters. Ultimately, Eichlin and Duckworth (1988) assigned *S. rileyana* to *Synanthedon* while noting its similarities to both genera. Designation of this species to

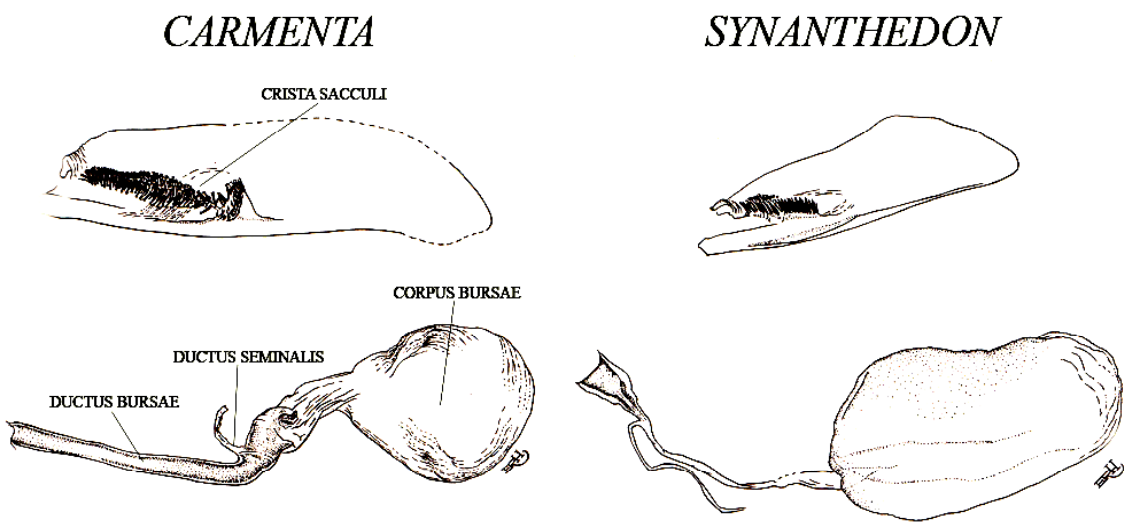


Figure 4-2. Comparison of *Carmenta* and *Synanthedon* genitalia. Illustration modified from Eichlin and Duckworth 1988.

its current genus appears to be based on presence of straight crista sacculi on the valva of male genitalia (Fig 4-2), which is reminiscent of the majority of *Synanthedon* species. Nevertheless, the mostly sclerotized ductus bursae and position of the ductus seminalis in close proximity to the corpus bursae resembles closely the genitalic morphology of *Carmenta* females (Eichlin and Duckworth 1988) (Fig. 4-2). Together with mitochondrial data, the evidence favors inclusion of *S. rileyana* into the genus *Carmenta* or possibly another genus based upon the concept suggested by MacKay (1968). Regrettably, despite two years of trapping in 20 widely separate locales, none of the other clearwing moth species included within MacKay's unnamed genus were collected for analysis in this study and only one *Carmenta* species was successfully sequenced. Until more extensive sampling of clearwing taxa can occur, we are unable to definitively conclude what taxonomic placement for *S. rileyana* would be appropriate at this time.

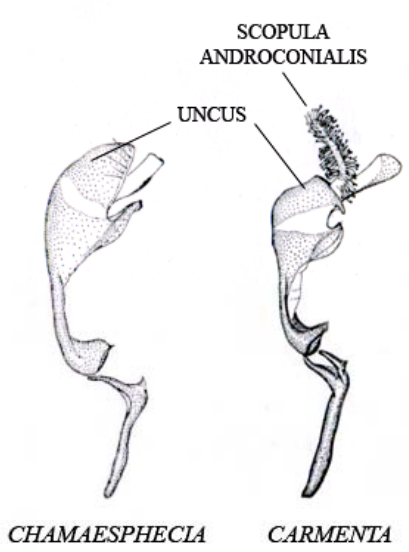


Figure 4-3. Comparison of *Chamaesphecia* and *Carmenta* genitalia. Illustration modified from Naumann 1971.

Our Inferred cox I phylogeny supports a close evolutionary relationship between *Bembecia*, *Carmenta*, *Pyropteron* and *Chamaesphecia* genera, as described by previous work (Naumann 1971, Eichlin and Duckworth 1988), but each is also equal in relatedness to *Synanthedon*. Results from our cox I phylogenetic analysis do not support a more primitive origin for *Chamaesphecia* than other species in the same clade, as has been suggested might be the case (Naumann 1971). Male genitalia of *Chamaesphecia* species entirely lack the scopula andronialis that is typically positioned above the distal end of the uncus, as is apparent in most other Synanthedonini members (Fig. 4-3).
Instead, the uncus in male *Chamaesphecia* is crowned with simple sensory hairs that probably serve the same function as sensory hairs surrounding the scopula andronialis in other species (Naumann 1971).

Regardless, monophyly of *Chamaesphecia* is strongly supported by the mitochondrial data. Its sister clade position with other herbaceous clearwing moths within the tribe implies that the uncal character is more than likely a derived trait and not plesiomorphic. This is further reinforced by the basal position of *Alcathoe carolinensis*, which has a distinct scopula andronialis and not the reduced character state seen in *Chamaesphecia*.

The largest of the clades in this study includes all remaining *Synanthedon* species, each of which have larvae that develop within woody plant hosts. Perhaps the most striking contradiction of current clearwing taxonomy is presented by the inclusion of *Sannina* and *Podosesia* with other species in this clade. Both *Sannina* and *Podosesia* species possess unique morphological characters that have helped justify their rank as separate genera (MacKay1968, Naumann 1971, Eichlin and Duckworth 1988). Interestingly, genitalia of *Sannina uroceriformis* in both sexes conform better to species of *Carmenta* than *Synanthedon* (Eichlin and Duckworth 1988) (Fig. 4-4).

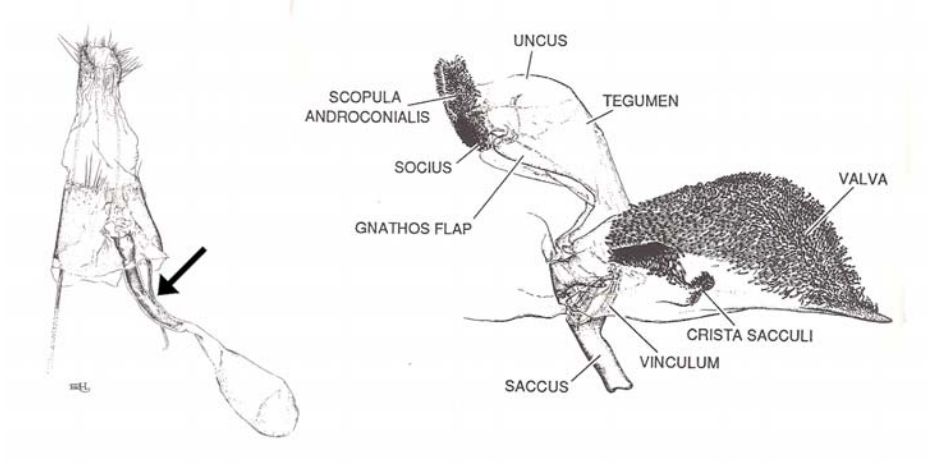


Figure 4-4. Female and male genitalia of *Sannina uroceriformis*. Illustration modified from Eichlin and Duckworth 1988.

Despite morphological similarities, our data place *Sannina* within the larger *Synanthedon* clade, far removed from *Carmenta*. It is probable that genitalic characters relied upon to separate these genera are not always reliable to delineate genera in Synanthedonini. For example, the downward curve of the crista sacculi, common in the genitalia of male *Carmenta*, is also shared by *Synanthedon proxima* (Eichlin and Duckworth 1988). Length of scelerotization on the ductus bursa, which typically extends < 1/3 the length in *Synanthedon* and > 1/2 the length in *Carmenta* (Fig 4-2), is reversed in some species of both genera. At least eight Nearctic *Synanthedon* species, including *S. novarensis* (Hy. Edwards), which was part of our analysis, demonstrate sclerotization of the ductus bursa greater than 1/3 its length. Likewise, *Carmenta querci* (Hy. Edwards) and *C. verecunda* (Hy. Edwards) both have much less than 1/2 the length of the ductus bursa sclerotized, as is generally expected of *Carmenta* species. Location where the ductus seminalis intersects the ductus bursae in females, also a key character, has many exceptions in *Synanthedon*, but is consistent among *Carmenta* species.

Eichlin and Duckworth (1988) describe saccus length in *Sannina* "about 1/3 the length of valva" (Fig. 4-4). Unfortunately, this appears to be an intermediate character that falls somewhere between *Synanthedon* (saccus length < 1/3 the length of the valva) and *Carmenta* (saccus length > 1/3 the length of the valva). The vague description of this character leaves its relationship to other genera, based that character alone, somewhat ambiguous.

Understanding the few exceptions to key characters within the tribe may help to explain the phylogenetic position of *Sannina* in this study. Although Eichlin and Duckworth (1988) suggest that *Sannina* may be most closely related to *Carmenta*,

analysis of cox I does not support that hypothesis. Moreover, *Carmenta* species rely mostly on herbaceous plant hosts and some small shrubs for larval food plants. By contrast, the only known host for *Sannina* larvae is persimmon, *Diospyros virginiana* L., which strengthens the possible close evolutionary connection with *Synanthedon* species whose larvae typically develop in woody plant hosts. Combined with host preference, cox I data suggest that *Sannina uroceriformis* is an autapomorphic *Synanthedon* species.

Three North American *Synanthedon* species found in the western U.S. have been described to use herbaceous host plants during larval development: *S. bibionipennis* (Boisduval), *S. polygoni* (Hy. Edwards) and *S. chrysidipennis* (Boisduval). The first two species are considered more primitive members of *Synanthedon* and a single larval specimen available of *S. chrysidipennis* possesses enough morphological differences to suggest designation of a new unnamed genus (MacKay 1968). Unfortunately, we were not able to acquire suitably preserved material of these western species and therefore cannot deduce where they might fit into our cox I tree.

The genus *Podosesia*, which includes the lilac/ash borer (*P. syringae* (Harris) and banded ash borer (*P. aureocincta* (Purrington and Nielsen)), also appears to have a closer relationship to *Synanthedon* than anticipated. The monophyletic *Podosesia* clade is firmly anchored within *Synanthedon*.

The placement of *Podosesia* within *Synanthedon* was also suggested by the gene tree in another molecular study using a different segment of the mitochondrial genome (McKern et al. 2008). *Podosesia* is a well-known genus that has been recognized in every major revision of Sesiidae. Its unusually long first tarsal segment separates it from other species in Synanthedonini (Fig. 4-5). Males of *Podosesia* have straight crista

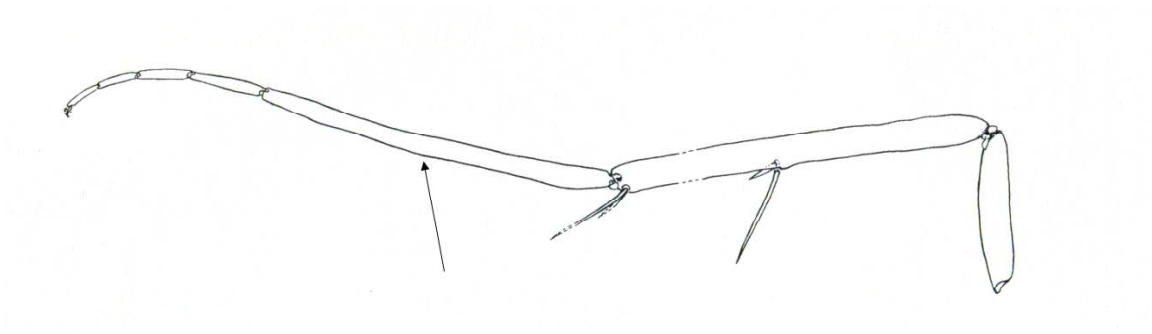

Figure 4-5. Elongation of the first tarsal segment of the hindleg in the genus *Podosesia*. Illustration modified from Eichlin and Duckworth 1988.

sacculi and the ductus bursae of females is not sceleritized for more than 1/3 its length, as is expected among *Synanthedon* species.

Though *P. syringae fraxini* is recognized as merely a subspecies or race, *P. aureocincta* has been separated using slight differences of in saccus morphology, as well as different flight times, and sexual pheromones (Purrington and Nielsen 1979). Regardless, the cox I gene failed to resolve the two recognized species in the genus, perhaps hinting they may not be distinct species, as advocated previously (Purrrington and Nielsen 1979, Eichlin and Duckworth 1988). Mating between the two species does produce viable offspring that exhibit intermediate forms of the genitalic trait used to separate the two (Purrington and Nielsen 1979). It is possible these two or perhaps three species are engaged in incipient speciation.

Unlike *Podosesia* and the vast majority of clearwing species, the host plant range of dogwood borer (*S. scitula*) extends across many plant families. In addition, while the majority of studies argue for a univoltine life cycle, some have suggested the dogwood

borer may be semivoltine or even multivoltine (Underhill 1935, Riedl et al. 1985, Snow et al. 1985, Solomon 1995). Dogwood borer has two peaks during the summer (Rogers and Grant 1991, Bergh et al. 2009). In Tennessee, the first peak was associated with more pupal exuviae on dogwood trees than the second peak, suggesting the second may be emerging mainly from other hosts (Rogers and Grant 1991). Bergh et al. (2009) found that dogwood borers develop more rapidly in burr knot tissue and suggested larval host tissue may influence rate of development. These anomalies raise the question of whether *S. scitula* may represent a species complex within the family. As the dogwood borer becomes an increasing economic threat to apple growers, it is important to understand if it is indeed part of a larger complex, particularly if some siblings are pests while others are not, so populations can be managed effectively (Bergh and Leskey 2003). Evidence from our analysis of individuals of both early and late seasonal flight peaks points to a single monophyletic species within *Synanthedon*, dispelling the notion of a species complex and truly making *S. scitula* unique among sesiid moths for its ability to exploit such a wide range of host plants.

Both viburnum borer species, *S. fatifera* and *S. viburni*, are highly specialized. As the only two North America clearwings whose larvae develop in *Viburnum* spp., these moths share a close evolutionary relationship. As one might predict, cox I reveals a much closer relationship between these two species than to others in the genus. Adult specimens can be readily separated by the green metallic luster of the *S. viburni* abdomen and the duller color scales found on *S. fatifera*.

## Conclusions

The inferred cox I phylogeny obtained in this study shows no evidence of polyphyletic species. Rather it sheds further light on some previous assumptions about relationships among species and higher level taxa within Synanthedonini. Genetic variability of partial cox I sequence analyses provides ample evidence for the monophyletic nature of Nearctic clearwing species included in this analysis. Unique sequence from species can provide rapid and accurate identification of all life stages, offering a proactive alternative to monitoring and control of these pests both in the United States and internationally, where non-native insect introductions are a concern. Species that have overlapping host plant preferences are particularly difficult to identify as immature larvae. For example, *S. viburni* shares the same affinity for *Viburnum* spp. host plants as *S. fatifera*. A chance introduction of the Palearctic viburnum clearwing borer *S. andrenaeformis* Laspeyres could make species identification of the larvae even more difficult. Similarly, *S. scitula* has an extremely large host plant range that overlaps with many other sympatric clearwing species. This overlap can make identification of larvae less certain when samples are collected from an infested host plant.

The dearth of genetic data in regards to sesiid species leaves much room for future molecular exploration of not only Synanthedonini, but also Sesiidae as a whole. Additional nuclear genes will be required to confirm the relationships revealed by cox I in this study. If further nuclear gene evidence validates the phylogeny presented here, synonymizing *Sannina* and *Podosesia* with *Synanthedon*, as our mitochondrial data suggests, will be warranted. Similarly, no clear conclusion can be made about the generic assignment of *S. rileyana* until more *Carmenta* species can be analyzed,

including the three other species suggested by MacKay (1968). Nuclear gene sequences may also help confirm placement of *S. rileyana* outside its current genus and perhaps give a clue to the most appropriate generic placement.

**Acknowledgements**

This study was supported by funding from USDA-ARS (58-6404-7-213). Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

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# CHAPTER 5
# CONCLUSIONS AND RECOMMENDATIONS

Wood boring insects are likely to be an important group of insects for many years to come. As our knowledge of their life histories increases, so will our ability to manage the damage they can periodically inflict. It is hoped that the checklists, distribution, and flight times of buprestid beeltes in Tennessee will help inform future researchers and enthusiasts both in and out of Tennessee and give those unfamiliar with buprestids the tools they need to become familiar with the family in a relatively short period of time. Distribution data may be important to future ecologist as a baseline of state species present in past years.

The *Chrysobothris femorata* complex appears to be molecularly ambiguous, at least among some species. Breeding experiments would show if crosses between polyphyletic species produce viable offspring that are capable of reproducing and would help end speculation about the occurrence of introgressive hybridization among populations of different species within the *femorata* complex. A phylogeny of taxa from western states (i.e. *C. comanche*, *C. mescalero*) as well as inclusion of less common eastern species (i.e. *C. seminole and C. sloicola*) may help to further resolve the *femorata* complex.

Future molecular endeavors in Buprestidae might focus on other species complexes that remain to be unraveled. The *Agrilus otiosus* Say complex is currently comprised of 14 morphologically similar species. Many of the female beetles in this group are indistinguishable even by trained taxonomic experts and all keys available require males in good condition for positive identification. Amplification of gene sequences in complexes like these may reveal new insights into the evolution of buprestids.

Deeper divergences within Buprestidae have yet to be explored molecularly. All currently published material on evolutionary relationships at every level (i.e. subfamily – genus) is based on morphological characters as well as plant host associations. Articles detailing relationships among genera in the family based on molecular tests have yet to be published, although work is being done at Harvard University in this area. There is much work still to be done at all levels of taxonomy that will enable us to understand the relationships within this large beetle family.

Molecular diagnostics for economically important buprestids are lacking at this time. Before this study, the only sequence available for buprestids was a short segment of cox I for emerald ash borer (*A. planipennis*). More effort needs to be placed in developing the molecular diagnostic tools needed for port inspectors to accurately identify any life stage of buprestids (and other wood boring insects) they may encounter. Understanding which species are continually being intercepted will ensure that regulations are adjusted to exclude the most problematic species. Many buprestid larvae remain to be described making their identification impossible or time consuming, having to wait for adult emergence before identification of adult can be accomplished.

Evidence suggesting the overwintering of *Acmaeodera tubulus* adults in Tennessee still needs to be verified. In both cases where adults were found in branches winter was nearing an end and though unlikely adults may have pupated and just not emerged. To firmly establish their overwintering status in Tennessee scouting in mid-winter that recovers live adult specimens in pupal cells is required.

The family Sesiidae also presents many opportunities to apply molecular methods. As seen in our investigation of Synanthedonini, there are still more questions to

be answered. One of the most challenging aspects of molecular work is collecting specimens of the appropriate taxa. Species with geographic ranges spanning thousands of miles require cooperation by many entomologists that must be knowledgeable enough to know where, what and how to collect specimens. Though they require effort to attain, these moth specimens can yield important information for systematists and scientists interested in developing diagnostics. Future molecular work in Synanthedonini should focus on finding informative nuclear genes to test current taxonomic theories in the tribe. This is especially true for the generic division between *Synanthedon* and *Carmenta*. There may be more members of these two genera that have been misplaced because of ambiguous morphological traits.

The *Podosesia* complex needs further investigation determine what if any species delineation would be appropriate. Gravid females of the two *Podosesia* species and "fraxini" color form of *P. syringae* could be used to conduct host preference assays to test survival of larvae on different hosts. Larvae of the two species also have yet to be described, leaving only a single internal genitalic character that requires the distruction of the specimen for positive identification. Breeding experiments producing hybrids would enable tests on hybrid fecundity and host preference. A fast evolving nuclear gene might yet allow separation of these two species and efforts should be made to discover a gene that will be suitable for that purpose.

The clearwing sequences produced by this study are of significant value as diagnostics. Cytochrome oxidase I sequences of additional clearwing species may find equal utility for identification of sesiid taxa. Including additional taxa in future efforts may help to more fully delineate between *Carmenta* and *Synanthedon* as well as other

Syananthedonini genera. However, until nuclear sequence data can confirm the relationships researchers should be cautious about forming any taxonomic reassignments. Even with no nuclear component the mitochondrial evidence has produced strong evidence questioning previous taxanomic hypotheses and allowing for a closer look at some difficult taxa.

Molecular data frequently challenges traditional taxonomy, introducing new hypotheses and sometime generating heated debates. Both approaches have drawbacks and advantages; neither is dispensable. Conventional taxonomy is still and will likely remain a significant part of the framework which molecular taxonomist must build on to answer difficult questions regarding species evolution. The growth of molecular data will continue to increase as more students of taxonomy embrace both morphological and genetic data to address challenging problems of evolutionary importance.

# APPENDIX 1

## RANGE EXPANSION AND ADULT FLIGHT ACTIVITY OF *AGRILUS SUBROBUSTUS* (COLEOPTERA: BUPRESTIDAE) IN TENNESSEE

This chapter is essentially the same as an article accepted by The Florida Entomologist and currently in press. It will appear in September 2010 as a scientific note:

Jason Hansen, John Kevin Moulton and William E. Klingeman. 2010. RANGE EXPANSION AND ADULT FLIGHT ACTIVITY OF *AGRILUS SUBROBUSTUS* (COLEOPTERA: BUPRESTIDAE) IN TENNESSEE. Florida Entomologist 93(3)

My contributions to this publication include collection and identification of specimens, as well as preparation of the figure and manuscript.

**Abstract**

*Agrilus subrobustus* was taken on purple sticky traps in Blount Co., Tennessee, in 2009 extending the southeastern range of this non-native buprestid beetle from northern Georgia where it was first discovered. Based on season-long trap catch data, a preliminary phenology of adult flight activity from May to August is presented.

Several *Agrilus* species have been introduced to the United States, presumably through transport of infested wood packaging material associated with international trade (Haack 2006, Jendek and Grebennikov 2009). The most notable exotic *Agrilus* in the U.S. is the emerald ash borer (*Agrilus planipennis* Fairmaire), now established in 14 eastern U.S. states as of February 2010 (Jendek and Grebennikov 2009, Michigan State University et al. 2010). Lesser known is the arrival of the exotic *A. subrobustus* Saunders, which was first reported in the U.S. after collection of three specimens on purple sticky traps in northern Georgia (Westcott 2007). This exotic species is listed by United States Department of Agriculture Animal and Plant Health Inspection Service (USDA-APHIS) as "quarantine significant", meaning it could require mitigative action if it is determined to have a high risk of reproducing and subsequently spreading (Joseph F. Cavey, Branch Chief, USDA-APHIS, personal communication), but may be limited in host plant range to its only known plant resource in Asia, the silk tree (*Albizia julibrissin* Durazz) (Jendek 1995). However, as of the publication of this note, *A. subrobustus* has yet to be reared from its Asian host in North America, though the plant is commonly found in the southeastern U.S. This note reports further northward extension of the known geographical range of *A. subrobustus* in the United States and gives the first seasonal adult flight records for trap catches of this beetle in North America.

Mimosa, or silk tree was introduced to the United States in the 18th century by André Michaux as part of a nursery established in Charleston, South Carolina (Cothran 2004). Because Michaux cultivated the plant from seeds, it is doubtful *A. subrobustus* could have been introduced at that time. Early establishment of mimosa across eastern North America, originally as a popular ornamental plant, then as a non-native, freely reproducing exotic plant is likely to have enhanced successful establishment by *A. subrobustus*. Though timing of its arrival is not known, it is likely to have arrived from Asian ports as a stowaway in wood packaging material much like the Asian emerald ash borer beetle (*A. planipennis* Fairmaire) (Haack 2006). Alternatively, it may have arrived as one hemipteran pest of the silk tree is thought to have entered the U.S. via prized ornamental mimosa trees shipped from Asia (Wheeler and Hoebeke 2009).

In 2009, four purple panel traps were deployed along the Foothills Parkway in Blount County Tennessee as part of a broader survey of buprestid fauna in the Great Smoky Mountains National Park. Traps were 1.2 m x 0.7 m purple corrugated plastic. Two were positioned 10 m apart at ground level in direct sun under a row of mimosa trees, which are plentiful in the surrounding area. The other two traps were placed in direct sun along a closed road next to a wooded area and about 30 m away from any mimosa trees. One trap was placed at ground level and the other trap suspended 7 m above the first. Each trap was checked biweekly from April to the end of August and terminated four weeks after the last specimen was removed.

Identification of *A. subrobustus* collected on purple traps was confirmed by Richard L. Westcott (Entomologist emeritus, Oregon Department of Agriculture) and Henry A. Hespenheide (Professor emeritus, University of California-Los Angeles).

Voucher specimens were deposited at the Great Smoky Mountains National Park collection in Gatlinburg, TN, as well as the Otis L. Floyd Nursery Crops Research Station in McMinnville, Tennessee, which at present holds the largest single collection of buprestids in Tennessee.

*Agrilus subrobustus* flight activity began late May and continued through early August. All adults were caught on the two sticky traps placed directly below mimosa trees. Though only 9 specimens were collected, peak activity appeared to occur in early to mid-June (Fig. Appendix A-1-1). These Tennessee records of *A. subrobustus* occured approximately 240 km northeast of the first site of discovery in Georgia in 2006. This indicates that *A. subrobustus* has become established in at least two U.S. states. An effort was made to scout for signs of *A. subrobustus* infestation consistent with buprestid larval feeding (i.e. compact frass, winding galleries, D-shaped exit holes) on trunks and stems below 2 m, but none was found. It is possible that adult mimosa borers emerged from upper branches on the 6 trees examined, which reached heights of 8 m and had diameter breast heights of between 12 cm to 25 cm, or from trees other than those examined in the surrounding area. Mimosa is a plant host to other wood boring insects in this area, as several unidentified curculid larvae (Coleoptera: Curculionidae) were removed from a branch about 7.6cm in diameter and tunneling of a small (< 3mm) unidentified beetle was also observed in mid-summer. Further survey work is needed to elucidate the geographic range and assess natural enemies associated with introduced *A. subrobustus*, which may be capable of surviving in eastern states from Florida to Massachusetts where mimosa grows (Elias 1987, Isely 1998). Since very little is known about the life history of *A.*

*subrobustus* in Asia, its establishment in the southeastern U.S. provides an opportunity to further our knowledge of its biology in its new non-native habitat.

Funding for this project was partially provided by a grant from Discover Life in America (DLIA) and by The University of Tennessee Institute of Agriculture in Knoxville, Tennessee. We would like to thank Richard Westcott and Henry Hespenheide for their help in identifying specimens.

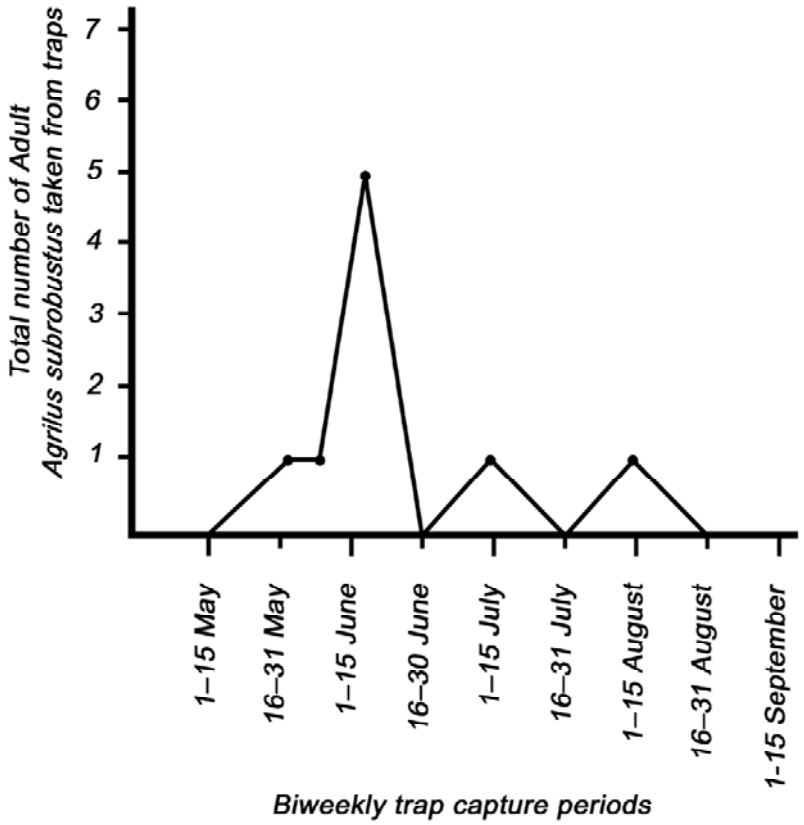


Figure A-1-1. Flight period of adult *Agrilus subrobustus* collected on purple sticky traps along the Foothills Parkway in Blount County, Tennessee.

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# APPENDIX 2

# KEYS TO TENNESSEE BUPRESTIDAE GENERA AND SPECIES

**Key to Tennessee buprestid genera**

1. Metacoxal plates dilated mesally, anterior edge nearly straight (Fig. A-2-1a) … 2

- Metacoxal plates not dilated mesally, anterior edge sinuous (Fig. A-2-1b) … 12

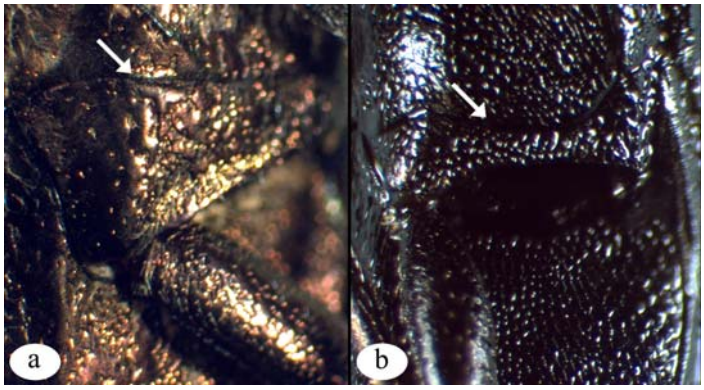


Figure A-2-1. (a) Metacoxal plate: *Chalcophora virginiensis*, (b) Metacoxal plate: *Agrilus ruficollis*

2(1). Prosternum acutely angulated behind coxae (Fig. A-2-2a) … 3

- Prosternum obtusely angulated behind coxae (Fig. A-2-2b) … 4

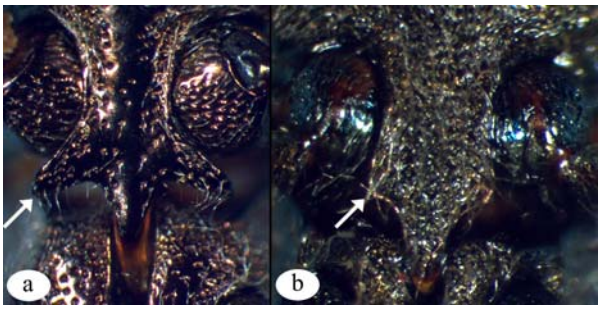


Figure A-2-2. (a) Prosternal process: *Chrysobothris dentipes*, (b) Prosternal process: *Phaenops fulvoguttata*.

3(2). Third tarsal segment elongated laterally (Fig. A-2-3a) … *Actenodes*

- Third tarsal segment not elongated laterally (Fig. A-2-3b) … *Chrysobothris*

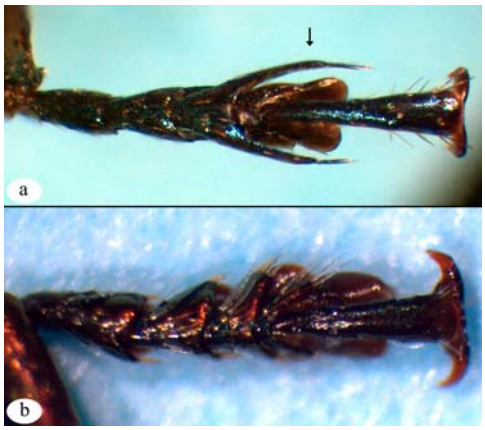


Figure A-2-3. (a) Tarsi: *Actenodes acornis*, (b) Tarsi: *Chrysobothris rugosiceps*.

4(2). Metepimera partly covered by wide abdominal extension (Fig. A-2-4a) … 5

- Metepimera triangular, not covered by narrow abdominal extension (Fig. A-2-4b) … 7

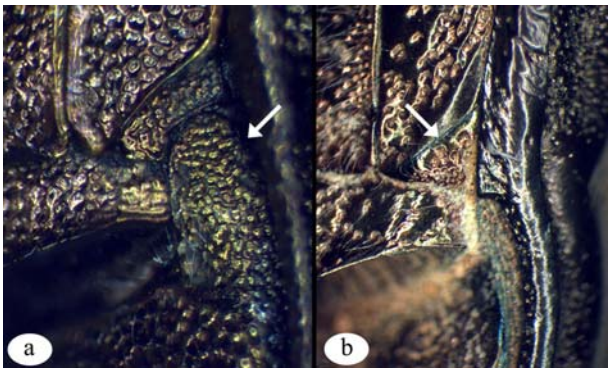


Figure A-2-4. (a) Metepimera: *Phaenops fulvoguttata*, (b) Metepimera: *Chalcophora virginiensis*.

5(4). Pronotal base truncate, mentum hard, opaque (Figs. A-2-5a and A-2-5b) … 6

- Pronotal base sinuate, mentum corneous, translucent (Figs. A-2-5c and A-2-5d) … *Phaenops*

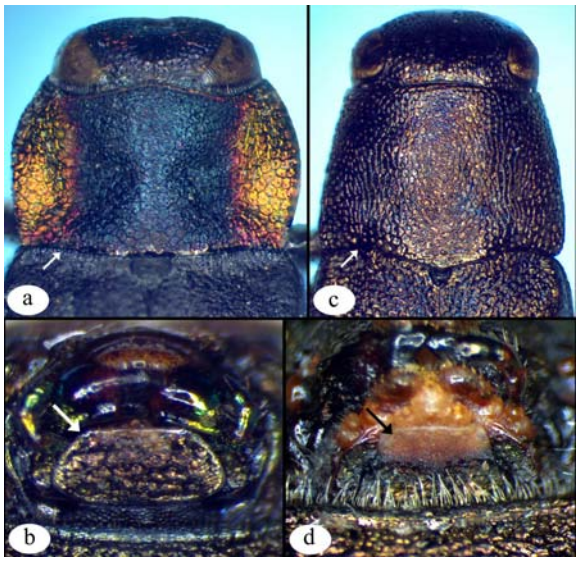


Figure A-2-5. (a) Pronotum: *Anthaxia viridicornis*, (b) Mentum: *Anthaxia viridicornis*, (c) Pronotum: *Phaenops aeneola*, (d) Mentum: *Phaenops fulvoguttata*.

6(5). Body short and broad, clypeus broad and short (Fig. A-2-6a) … *Anthaxia*

- Body long and narrow, clypeus long and narrow (Fig. A-2-6b) … *Agrilaxia*


Figure A-2-6. (a) Dorsum: *Anthaxia viridifrons*, (b) Dorsum: *Agrilaxia flavimana*.

7(4). Antennameres 7–11 subserrate, elongate and narrow (Fig. A-2-7a) … 8

- Antennameres 7–11 strongly serrate, more compact (Fig. A-2-7b) … 10


Figure A-2-7. (a) Antenna: *Buprestis maculipennis*, (b) Antenna: *Dicerca lurida*.

8(7). Meso and metasternum closely united (Fig. A-2-8a) … 9

- Meso and metasternum separated by a suture (Fig. A-2-8b) … *Buprestis*

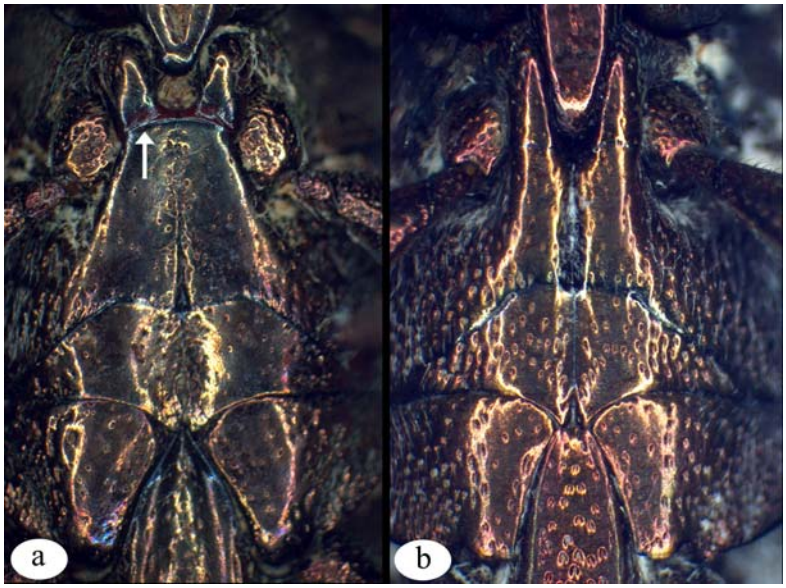


Figure A-2-8. (a) Meso- and Metasternum: *Chalcophora virginiensis*, (b) Meso- and Metasternum: *Buprestis lineata*.

9(8). Pronotum sulcate mesially (Fig. A-2-9a) … *Texania*

- Pronotum elevated mesially (Fig. A-2-9b) … *Chalcophora*

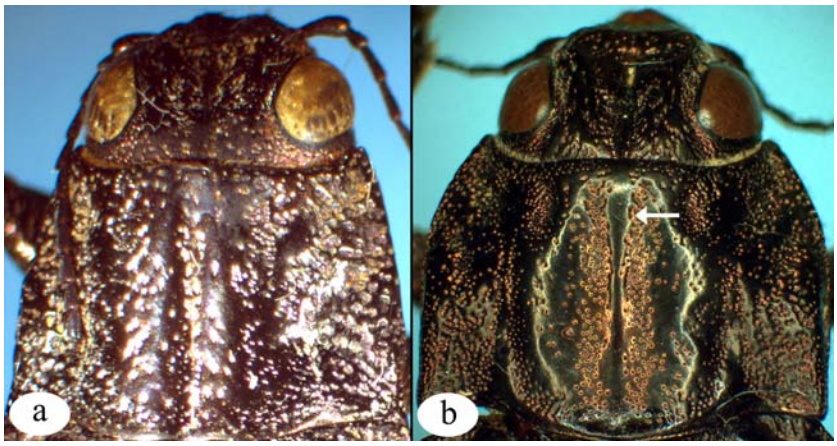


Figure A-2-9. (a) Pronotum: *Texania campestris*, (b) Pronotum: *Chalcophora virginiensis*.

10(7). First metatarsomere longer than second, body narrow (Fig. A-2-10a) … *Spectralia*

- First metatarsomere not longer than second, body broad (Fig. A-2-10b) … 11

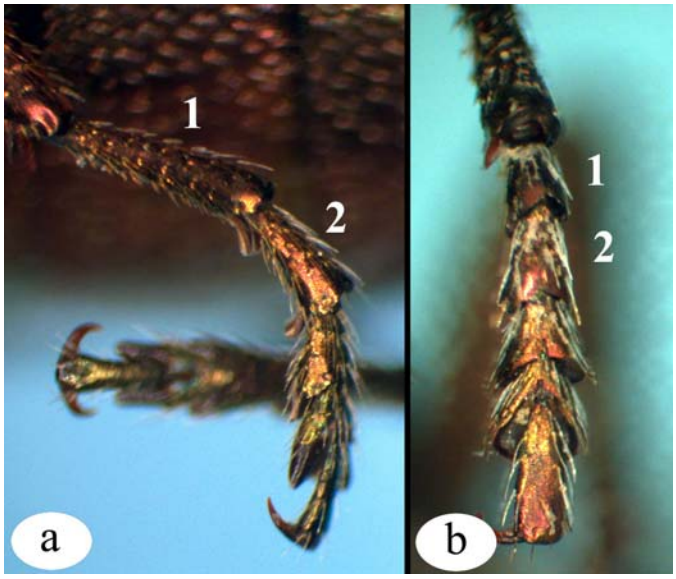


Figure A-2-10. (a) Metatarsi: *Spectralia gracilipes*, (b) Metatarsi: *Dicerca obscura*.

11(10). Scutellum much wider than long (Fig. A-2-11a). Pronotum with a median longitudinal ridge and elytral apices prolonged and more reddish than basal portion of elytra … *Poecilonota*

- Scutellum circular (Fig. A-2-11b). Pronotum lacking median longitudinal ridge, apices usually not prolonged or redder than rest of elytra … *Dicerca*

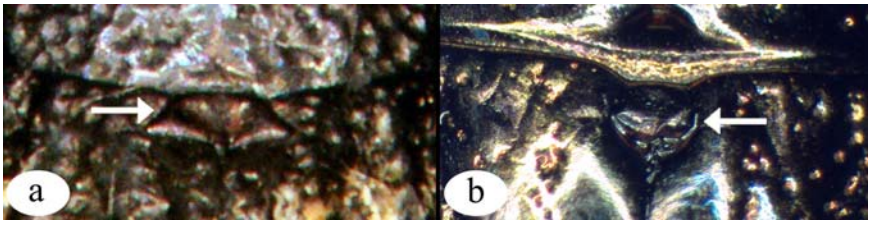


Figure A-2-11. (a) Scutellum: *Poecilonota cyanipes*, (b) Scutellum: *Dicerca obscura*.

12(1). Scutellum absent (Fig. A-2-12a) … *Acmaeodera*

- Scutellum present (Fig. A-2-12b) … 13

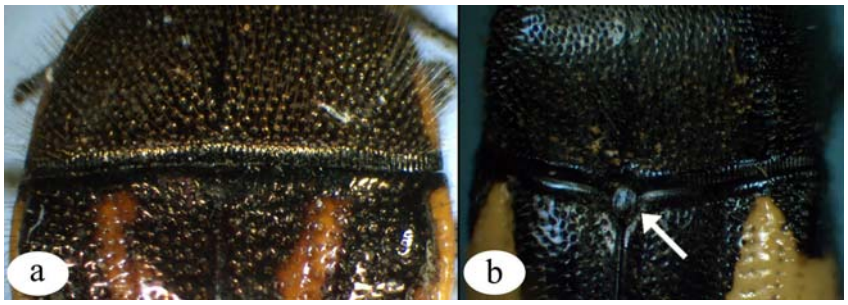


Figure A-2-12. (a) Scutellum: *Acmaeodera pulchella*, (b) Scutellum: *Ptosima gibbicollis*.

13(12). Pronotum base truncate, straight (Fig. A-2-12b) … 14

- Pronotum base sinuate (Fig. A-2-13) … 15

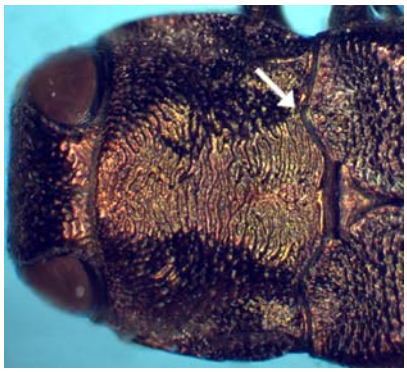

Figure A-2-13. Pronotum: *Agrilus quadriguttatus.*

14(13). Mesosternum scarcely visible (Fig. A-2-14a) … *Mastogenius*

- Mesosternum emarginated (Fig. A-2-14b) … *Ptosima*

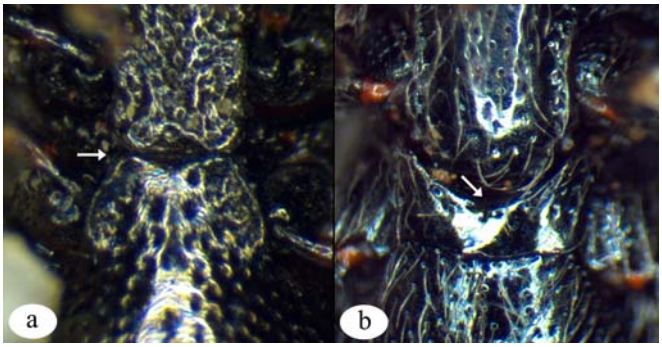


Figure A-2-14. (a) Mesosternum: *Mastogenius crenulatus*, (b) Mesosternum: *Ptosima gibbicollis*.

15(13). Antennae received in groove (Fig. A-2-15a) … 16

- Antennae not received in groove (Fig. A-2-15b) … 17

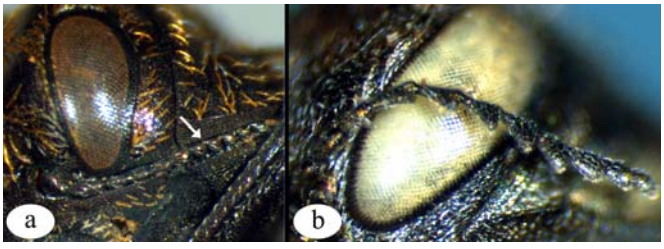


Figure A-2-15. (a) Antenna: *Brachys ovatus*, (b) Antenna: *Agrilus bilineatus*.

16(15). Scutellum large, about 1/3 of body width (Fig. A-2-16a) … *Pachyschelus*

- Scutellum small, much less than 1/3 of body width (Fig. A-2-16b) … *Brachys*

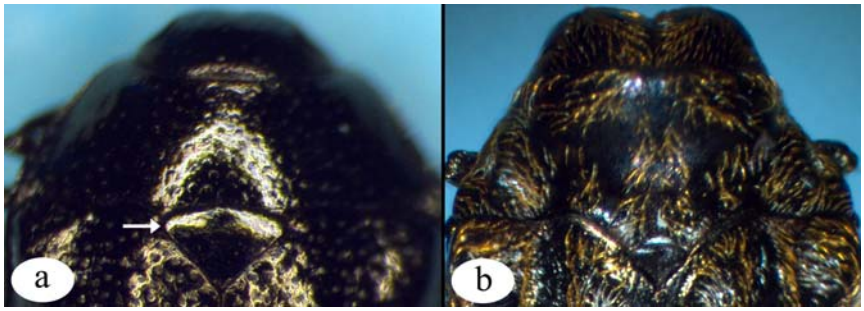


Figure A-2-16. (a) Scutellum: *Pachyschelus laevigatus*, (b) Scutellum: *Brachys ovatus*.

17(15). First metatarsomere subequal to the second (Fig. A-2-17a) … *Eupristocerus*

- First metatarsomere as long as the following three combined (Fig. A-2-17b) … *Agrilus*

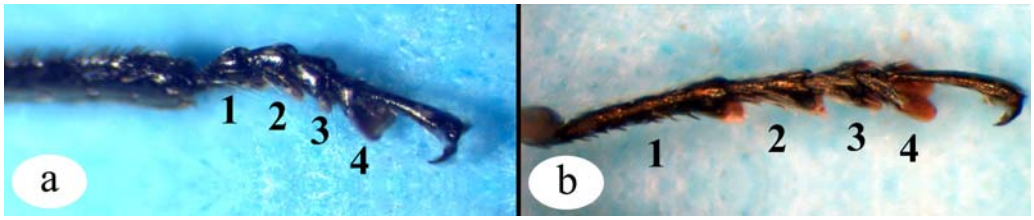


Figure A-2-17. (a) Metatarsi: *Eupristocerus cogitans*, (b) Metatarsi: *Agrilus quadriguttatus*.

**Key to Tennessee *Chrysobothris***

1. Lateral margin of last sternite entire (Fig. A-2-18a) … 2

- Lateral margin of last sternite serrate (Fig. A-2-18b) ... 4

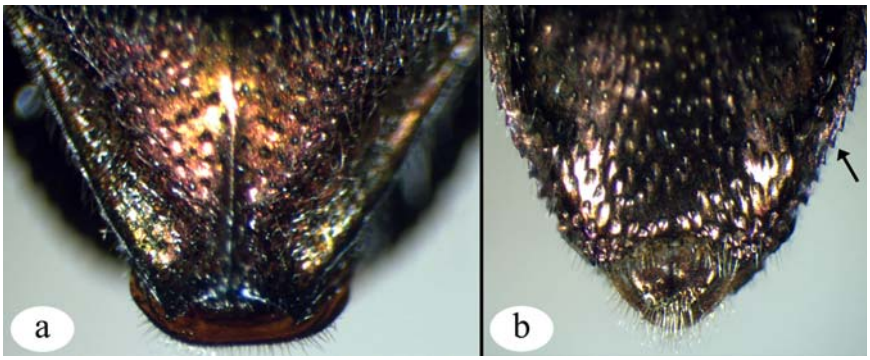


Figure A-2-18. (a) Terminal sternite: *Chrysobothris sexsignata*, (b) Terminal sternite: *Chrysobothris dentipes*.

2(1). Elytra bronze each with gold foveae (Fig. A-2-19a) … *C. sexsignata*

- Elytra blue, violaceous, or sometimes green, foveae without gold spots (Fig. A-2-19b) … 3

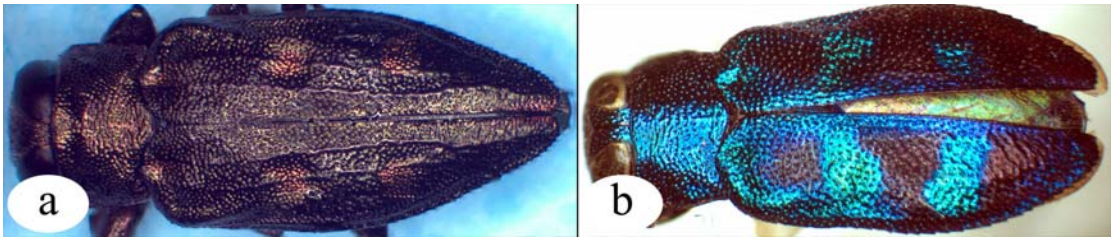


Figure A-2-19. (a) Dorsum: *C. sexsignata*, (b) Dorsum: *C. chlorocephala*.

3(2). Elytral costae absent (Fig. A-2-19b) … *C. chlorocephala*

- Elytral costae present (Fig. A-2-20) … *C. azurea*

Figure A-2-20. Dorsum: *C. azurea.*

4(1). Terminal abdominal sternite with submarginal ridge (short and vague in *C. orono*) … 5

- Terminal abdominal sternite with submarginal ridge absent … 13

5(4). Female with medial longitudinal carina on 8$^{th}$ tergite (Fig. A-2-21a). Male anterior tibia armed with a row of several small acute teeth internally (Fig. A-2-21b) (*Chrysobothris femorata* complex) … 6

- Female lacking medial longitudinal carina (Fig. A-2-21c). Male anterior tibia with a single large tooth or rounded dilation near apex (Fig. A-2-21d) … 11

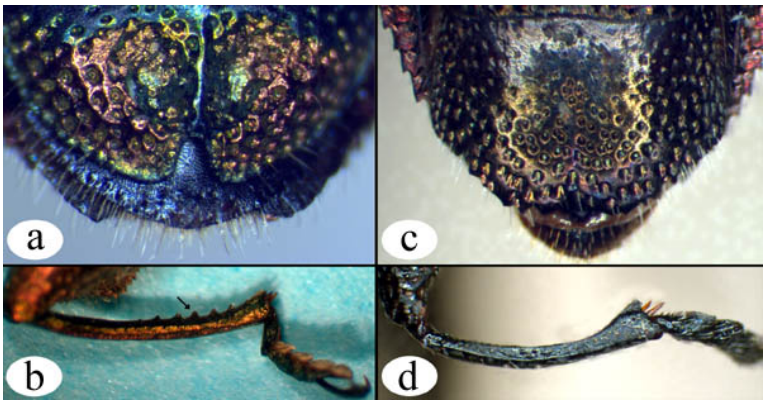


Figure A-2-21. (a) Terminal tergite ♀: *C. quadriimpressa*, (b) Foreleg ♂: *C. quadriimpressa*, (c) Terminal tergite ♀: *C. dentipes*, (d) Foreleg ♂: *C. dentipes*.

6(5). Clypeus acutely emarginated but straight on each side of notch (Fig. A-2-22a). Male genitalia with lateral spines perpendicular to parameres and easily seen from above (Fig. A-2-22b) … *C. adelpha*

\- Clypeus acutely notched in middle semicircular on each side (Fig. A-2-22c). Male genitalia with lateral spines not perpendicular to parameres and sometimes not visible from above (Fig. A-2-22d) … 7

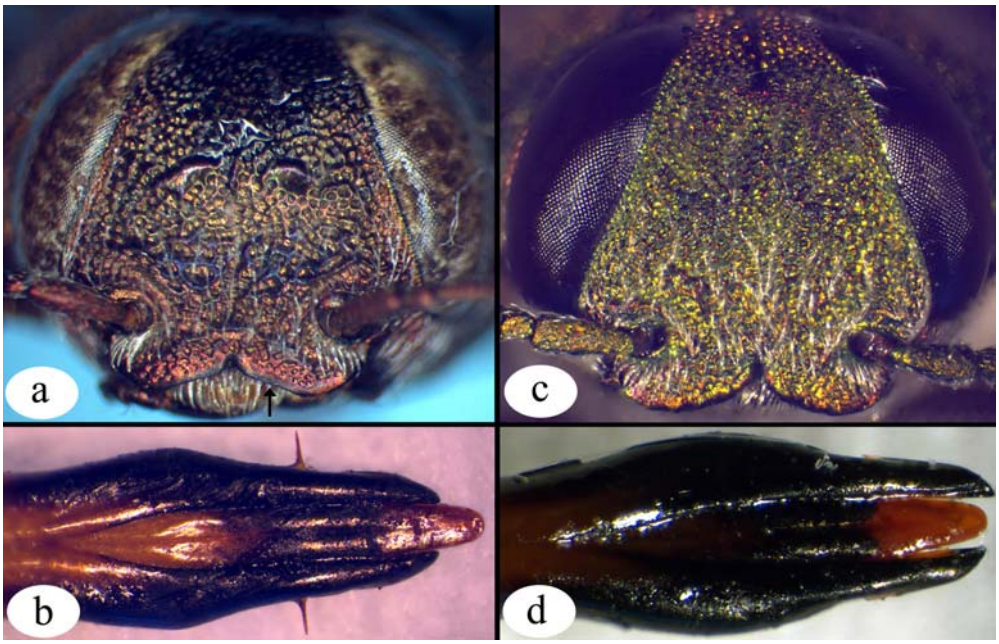


Figure A-2-22. (a) Frons ♀: *C. adelpha*, (b) Genitalia ♂: *C. adelpha*, (c) Frons ♂: *C. femorata*, (d) Genitalia ♂: *C.femorata*.

7(6). Last two foveae near elytral apex nearly circular and separated by costa (Fig. A-2-23a). Male genitalia with longer paramere constricted near apex (Fig. A-2-23b) … *C. viridiceps*

- Last two fovae near elytral apex irregularly shaped (Fig. A-2-23c). Male genitalia without constriction near apex (Fig. A-2-22d)… 8

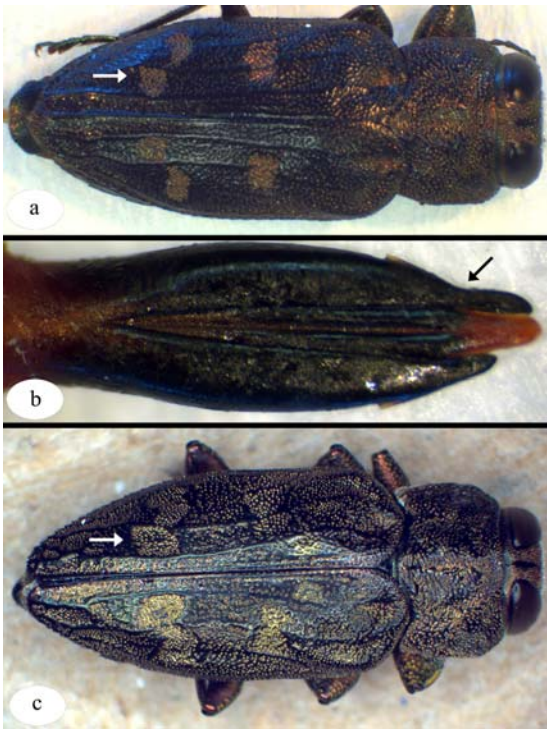


Figure A-2-23. (a) Dorsum: *C. viridiceps*, (b) Genitalia ♂: *C. viridiceps*, (c) Dorsum: *C. shawnee*.

8(7). Eleventh antenomere quadrate (Fig. A-2-24a). Female with extending longitudinal carina of the terminal tergite (Fig. A-2-24b) … *C. rugosiceps*

\- Eleventh antenomere tapering to tip (Fig. A-2-24c). Female without extending longitudinal carina of the terminal tergite (Fig. A-2-21a) … 9

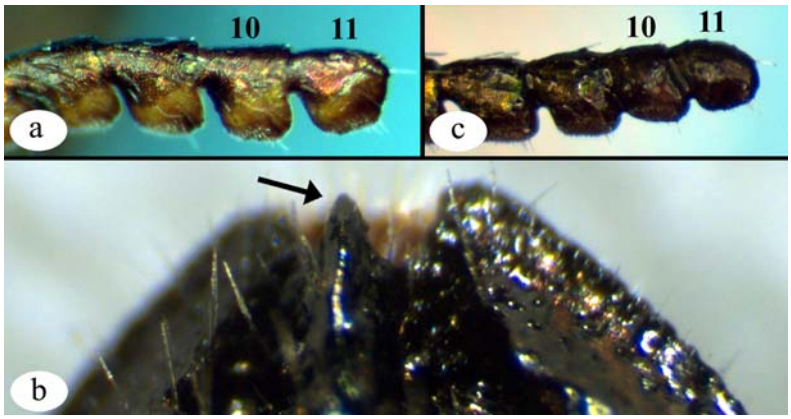


Figure A-2-24. (a) Antennameres 8–11: *C. rugosiceps*, (b) Terminal tergite ♀: *C. rugosiceps*, (c) Antennameres 8–11: *C. femorata*.

9(8). Male with frons dull mottled greenish-bronze, clypeus often greener than frons (Fig. A-2-25). Female pygidium with shallow depressions each side of median carina (Fig. A-2-21a) … *C. quadriimpressa*

\- Male with frons green (Fig. A-2-22c). Female pygidium with deep depressions each side of median carina … 10

Figure A-2-25. Frons ♂: *C. quadriimpressa.*

10(9). Male frons with 2 or 4 transverse maroon-purple irregular chevrons (Fig. A-2-26a). Outer edge of parameres evenly arched (Fig. A-2-26b). Female lacking red on vertex, elytral apices and pygidium … *C. shawnee*

- Male frons with an indistinct purple-copper chevron above middle of frons (Fig. A-2-22c). Outer edge of parameres distinctly bulbous in middle then tapering to distal end (Fig. A-2-22d). Female red on vertex, elytral apices, and usually on lateral margin of pygidium … *C. femorata*

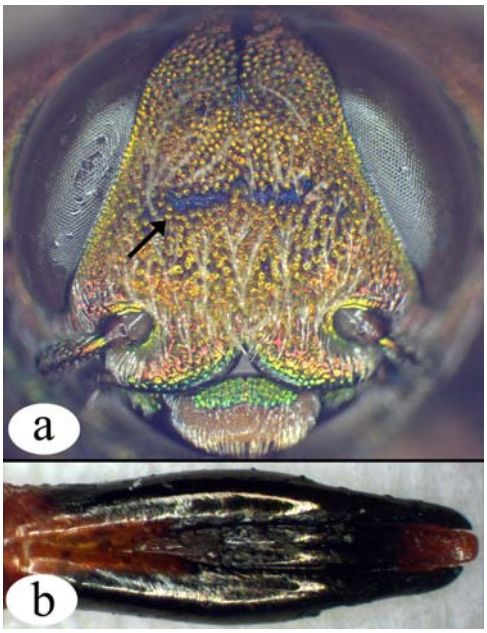


Figure A-2-26. (a) Frons ♂: *C. shawnee*, (b) Genitalia ♂: *C. shawnee*.

11(5). Clypeus triangularly emarginated (Fig. A-2-27a) … *C. rotundicollis*

- Clypeus arcuately emarginated (Fig. A-2-27b) … 12

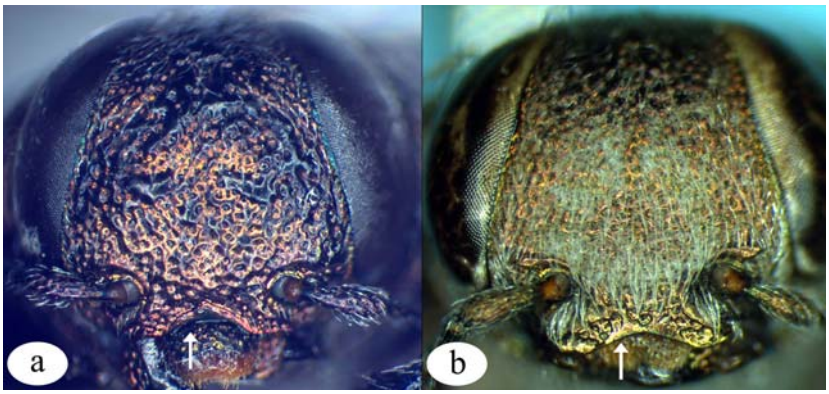


Figure A-2-27. (a) Frons: *C. rotundicollis*, (b) Frons: C. *neotexana*.

12(11). Prosternum with median lobe (Fig. A-2-28) … *C. neotexana*

- Prosternum without median lobe (Fig. A-2-32) … *C. orono*

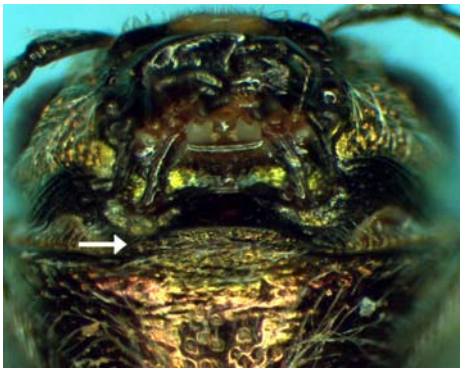

Figure A-2-28. Prosternal lobe: *C. neotexana.*

13(4). Clypeus transversely truncate, somewhat sinuate (Fig. A-2-29a) … *C. cribraria*

- Clypeus not transversely truncate, emarginate at middle (Fig. A-2-29b) … 14

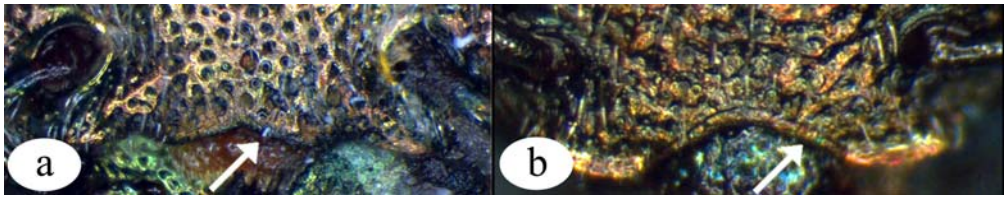


Figure A-2-29. (a) Clypeus: *C. cribraria*, (b) Clypeus: *C. pusilla*.

14(13). Antennomeres 4–11 partially yellow (Fig. A-2-30a) … *C. dentipes*

- Antennomeres 4–11 never with yellow (Fig. A-2-30b) … 15

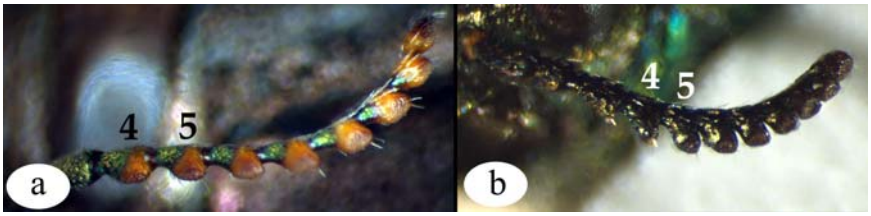


Figure A-2-30. (a) Antenna: *C. dentipes*, (b) Antenna: *C. harrisi*.

15(14). Elytra bronze or brown (Fig. A-2-19a) … 16

- Elytra blue or green (Figs. A-2-19b and A-2-20) … 17

16(15). Clypeus arcuately emarginated (Fig. A-2-29b) … *C. pusilla*

- Clypeus angularly emarginate (Fig. A-2-31) … *C. scabripennis*

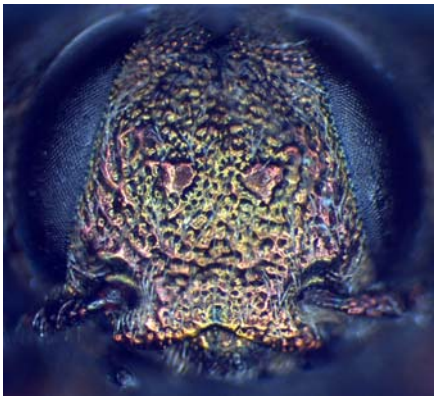

Figure A-2-31. Frons: *C. scabripennis.*

17(15). Prosternum anterior margin with distinct median lobe (Fig. A-2-28). Clypeus arcuately emarginate (Fig. A-2-29b) … *C. purpureovittata purpureovittata*

- Prosternum anterior margin lacking median lobe (Fig. A-2-32). Clypeus triangularly emarginate (Fig. A-2-27a) … *C. harrisi*

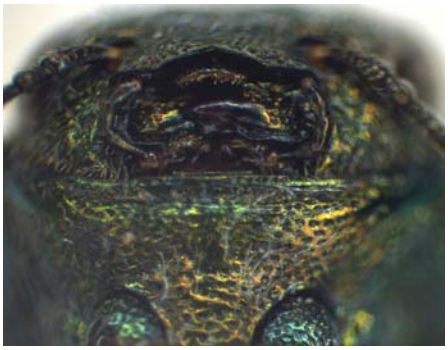

Figure A-2-32. Prosternum: *C. harrisi.*

**Key to Tennessee *Phaenops***

1. Disc of pronotum striolate (Fig. A-2-33a) …. *Phaenops drummondi*

- Disc of pronotum rough with large punctures, not striolate (Fig. A-2-33b) … 2

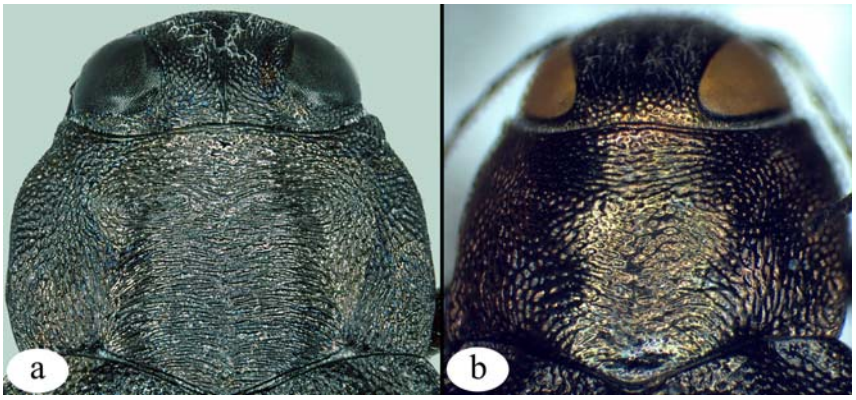


Figure A-2-33. (a) Pronotum: *P. drummondi* (photo courtesy of Steven Valley, Oregon Department of Agriculture.), (b) Pronotum: *P. fulvoguttata*.

2(1). Usually with yellow or white elytral spots, greater than 7mm (Fig. A-2-34a) … *P. fulvoguttata*

- Elytral spots never present, less than 7mm (Fig. A-2-34b) … *P. aeneola*

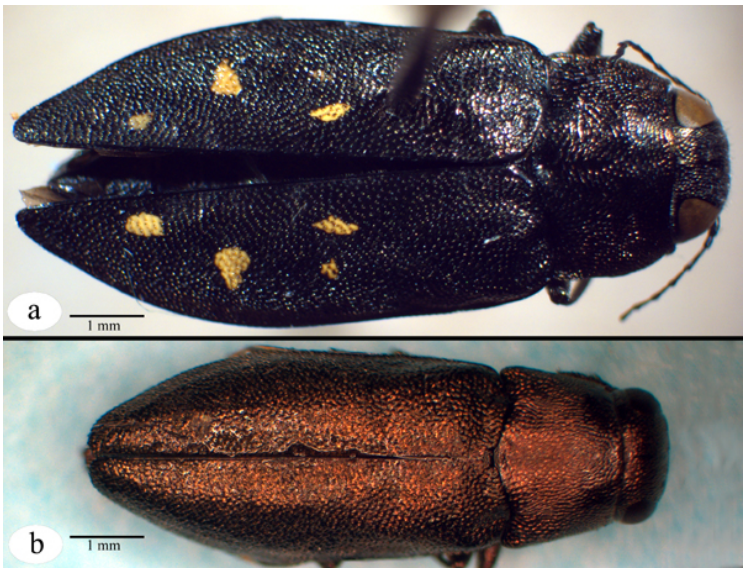


Figure A-2-34. (a) Dorsum: *P. fulvoguttata*, (b) Dorsum: *P. aeneola*.

**Key to Tennessee *Anthaxia***

1. Tarsal claws without tooth at base (Fig. A-2-35a) … 2

- Tarsal claws with tooth at base (Fig. A-2-35b) … 3

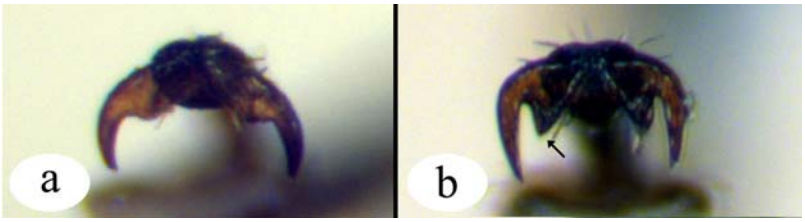


Figure A-2-35. (a) Tarsal claw: *A. viridicornis*, (b) Tarsal claw: *A. quercata*.

2(1). Lateral pronotal margins each for ¼ width of thorax differing from the disc color; elytra dark purplish-black (Fig. A-2-36a) … *A. viridicornis*

- Lateral pronotal margins each more broadly pigmented anteriorly than posteriorly and differing in color from disc; elytra uniformly bronze (Fig. A-2-36b) … *A. viridifrons*

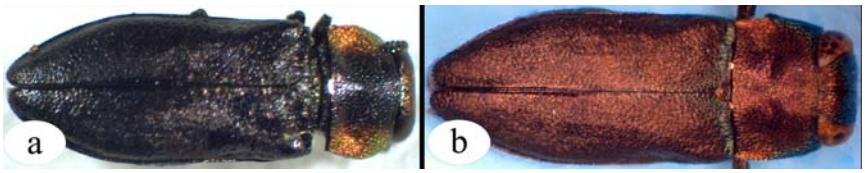


Figure A-2-36. (a) Dorsum: *A. viridicornis*, (b) Dorsum: *A. viridifrons*.

3(1). Prothorax entirely green or purple-blue (Fig. A-2-37a) … *A. quercicola*

- Prothorax bronze or more than one color (Fig. A-2-37b) … 4

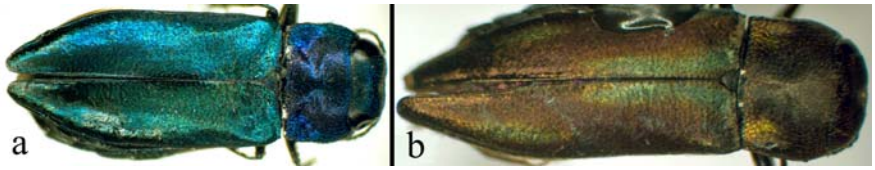


Figure A-2-37. (a) Dorsum: *A. quercicola*, (b) Dorsum: *A. quercata*.

4(3). Male with frons green (Fig. A-2-38a) … 5

- Female with frons not green (Figs. A-2-38b and A-2-38c) … 6


Figure A-2-38. (a) Frons ♂: *A. quercata*, (b) Frons ♀: *A. quercata*, (c) Frons ♀: *A. cyanella*.

5(4). Elytra uniformly colored (Fig. A-2-39a) … *A. cyanella*

- Last third of elytral apices bronze and blue-green (Fig. A-2-39b) … *A. quercata*

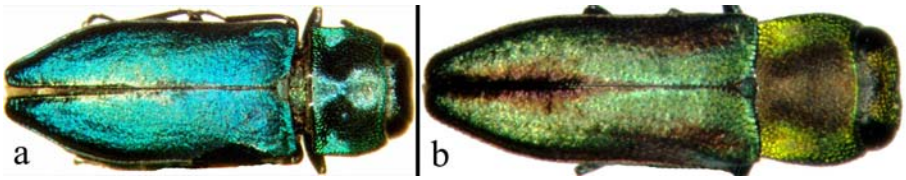


Figure A-2-39. (a) Dorsum ♂: *A. cyanella*, (b) Dorsum ♂: *A. quercata*.

6(4). Elytra and frons blue (Fig. A-2-40a) … *A. cyanella*

- Elytra not blue, pronotum green laterally and along basal margin but bronze on disc (Fig. A-2-40b) … *A. quercata* (Fabricius)

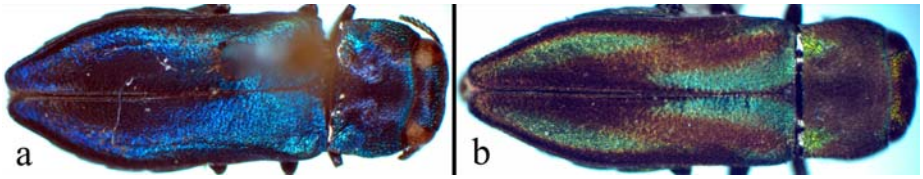


Figure A-2-40. (a) Dorsum ♀: *A. cyanella*, (b) Dorsum ♀: *A. quercata*.

**Key to Tennessee *Buprestis***

1. Pronotum with levigated spaces mesally and laterally (Fig. A-2-41a) … *B. consularis*

- Pronotum without levigated spaces (Fig. A-2-41b) … 2

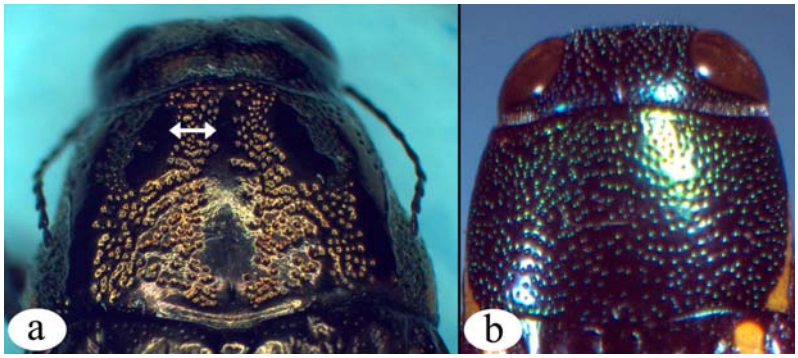


Figure A-2-41. (a) Pronotum: *B. consularis*, (b) Pronotum: *B. rufipes*.

2(1). Elytra intercostal areas broad with dense punctation (Fig. A-2-42a) … *B. striata*

- Elytra striate or longitudinally punctate-striate (Fig. A-2-42b and A-2-44a–A-2-46) … 3

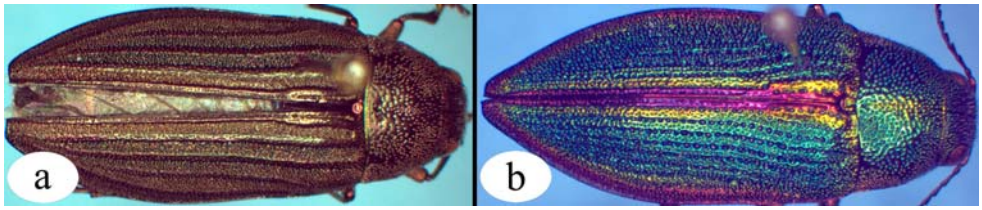


Figure A-2-42. (a) Dorsum: *B. striata*, (b) Dorsum: *B. salisburyensis*.

3(2). First abdominal sternite mesally sulcate (Fig. A-2-44a) … 4

- First abdominal sternite not mesally sulcate (Fig. A-2-44b) … 6

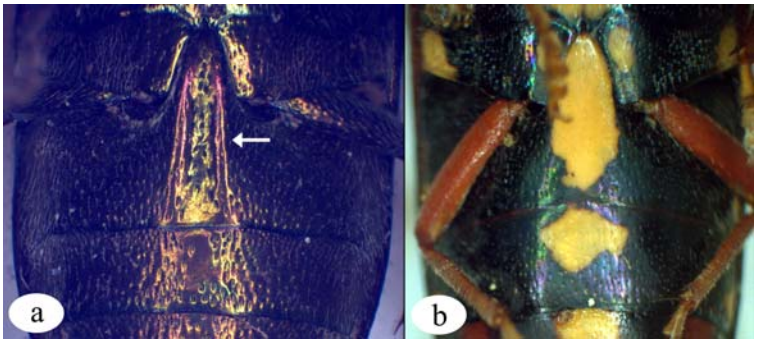


Figure A-2-44. (a) Abdominal sternites 1–2: *B. lineata*, (b) Abdominal sternites 1–2: *B. rufipes*.

4(3). Elytra green with yellow spots (Fig. A-2-45a) … *B. fasciata*

- Elytra black with yellow to orange spots or vittae (Fig. A-2-45b) … 5

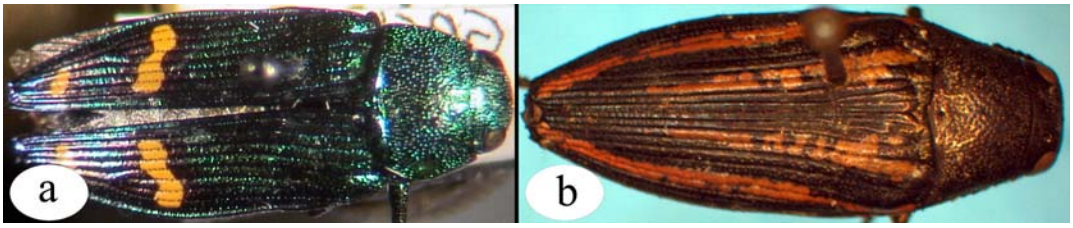


Figure A-2-45. (a) Dorsum: *B. fasciata*, (b) Dorsum: *B. lineata*.

5(4). Elytra with yellow to orange vittae, pattern varies (Fig. A-2-45b); male genitalia with parameres parallel from base to apex … *B. lineata*

- Elytra with yellow to orange spots, which never form vittae, pattern varies (Fig. A-2-46); male genitalia with parameres parallel from base to middle then narrowing to apex … *B. maculipennis*

Figure A-2-46. Dorsum: *B. maculipennis.*

6(3). Elytra with yellow maculation (Fig. A-2-45a) … 7

Elytra without yellow maculation (Fig. A-2-43b) … 8

7(6). Base of elytra with longitudinally elongate yellow spot (Fig. A-2-47) … *B. rufipes*

- Base of elytra without longitudinally elongate yellow spot (Fig. A-2-45a) … *B. fasciata*

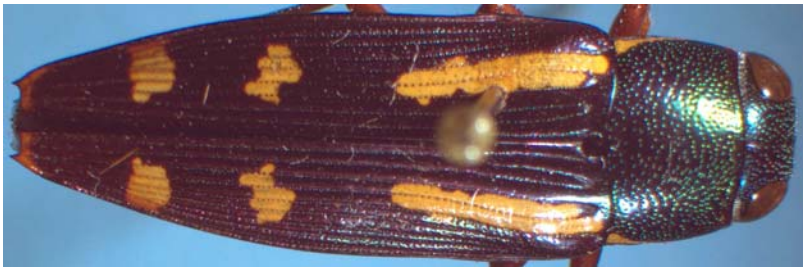

Figure A-2-47. Dorsum: *B. rufipes.*

8(6). Elytral apices bidentate (Fig. A-2-48a) … *B. decora*

- Elytral apices unidentate (Fig. A-2-48b) … *B. salisburyensis*

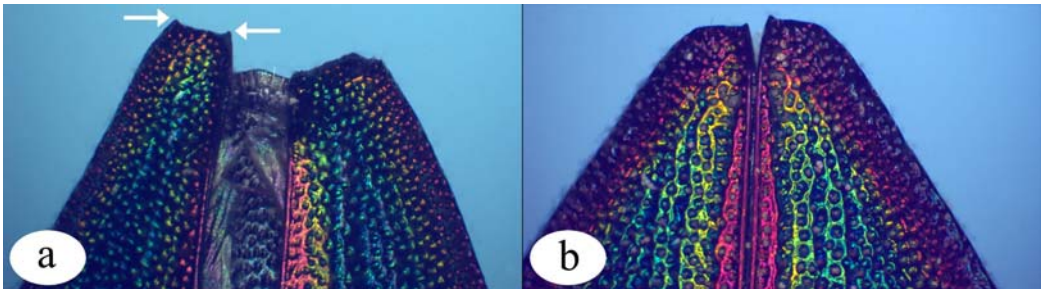


Figure A-2-48. (a) Elytral apices: *B. decora*, (b) Elytral apices: *B. salisburyensis*.

**Key to Tennessee *Dicerca***

1. Elytral apices entire, truncate or at most weakly bidentate (Fig. A-2-49a) … 2

- Elytral apices strongly bidentate (Fig. A-2-49b) … 3

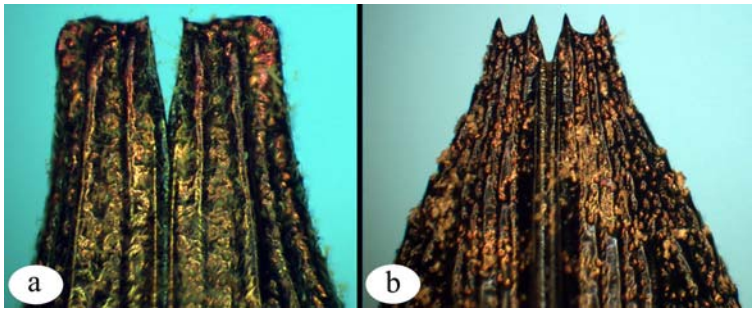


Figure A-2-49. (a) Elytral apices: *D. divarticata*, (b) Elytral apices: *D. obscura.*

2(1). Elytral apices produced (Fig. A-2-50a) … *D. divaricata*

- Elytral apices weakly produced (Fig. A-2-50b) … *D. tenebrosa knulli*

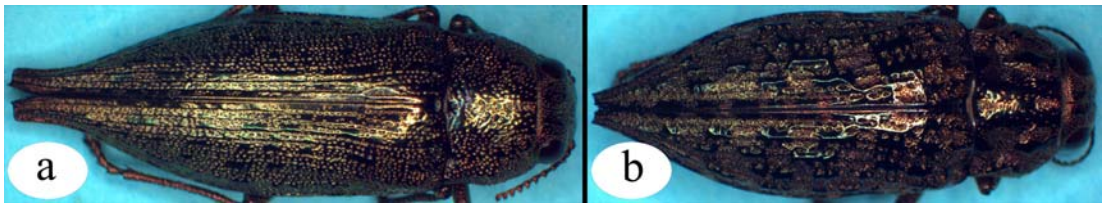


Figure A-2-50. (a) Dorsum: *D. divaricata*, (b) Dorsum: *D. tenebrosa*.

3(2). Pronotum subparallel at base, widening at middle, then narrowing to anterior margin (Fig. A-2-51a) … *D. lepida*

- Pronotum subparallel at base to before middle then converging to anterior margin (Fig. A-2-51b) … 4

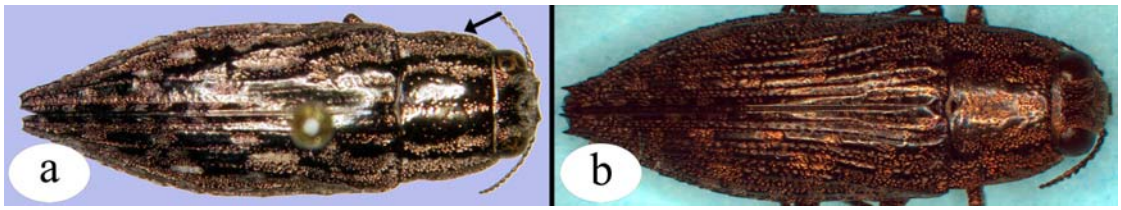


Figure A-2-51. (a) Dorsum: *D. lepida*, (b) Dorsum: *D. obscura*.

4(3). Metacoxal plate distinctly notched (Fig. A-2-52a) … *D. obscura*

- Metacoxal plate indistinctly notched (Fig. A-2-52b) … *D. lurida*

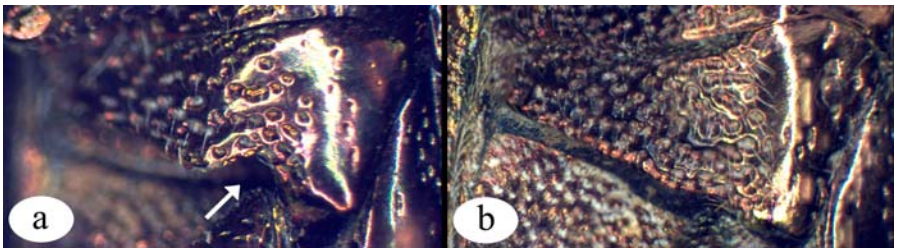


Figure A-2-52. (a) Metacoxal plate: *D. obscura*, (b) Metacoxal plate: *D. lurida*.

**Key to Tennessee *Acmaeodera***

1. Last sternite without subapical plate (Fig. A-2-53a) … *A. tubulus*

- Last sternite with subapical plate (Fig. A-2-53b) … 2

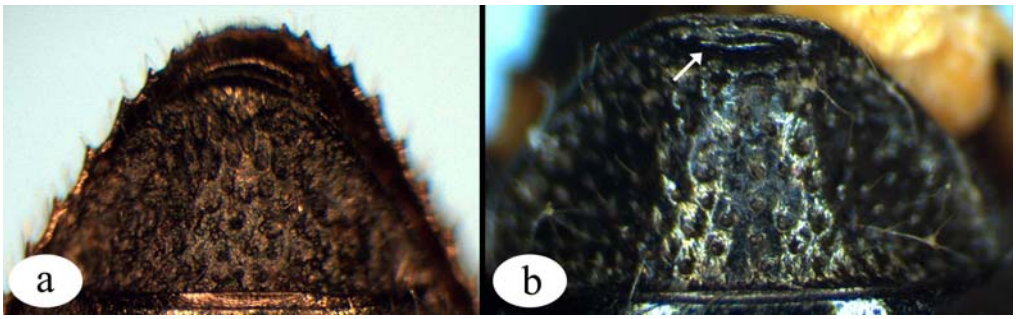


Figure A-2-53. (a) Last sternite: *A. tubulus*, (b) Last sternite: *A. ornata*.

2(1). Basal half of elytra with one confluent yellow macula laterally. Pronotum with yellow macula laterally (Fig. A-2-54a) … *A. pulchella*

- Basal half of elytra with more than one yellow spot laterally. Pronotum without yellow macula laterally (Fig. A-2-54b) … *A. ornata*

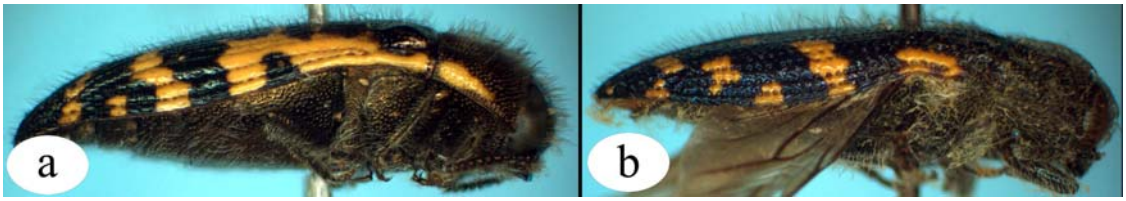


Figure A-2-54. (a) Lateral view: *A. puchella*, (b) Lateral view: *A. ornata*.

**Key to Tennessee *Mastogenius***

1. Elytra shiny black, (Fig. A-2-55a). … *M. crenulatus*

- Elytra with deep blue-violet reflections (Fig. A-2-55b). … *M. subcyaneus*


Figure A-2-55. (a) Dorsum: *M. crenulatus*, (b) Dorsum: *M. subcyaneus*.

**Key to Tennessee *Pachyschelus***

1. Elytra with single transverse white pubescent band near apices (Fig. A-2-56a) ….*P. purpureus purpureus*

- Elytra lacking white band (Fig. A-2-56b) … 2

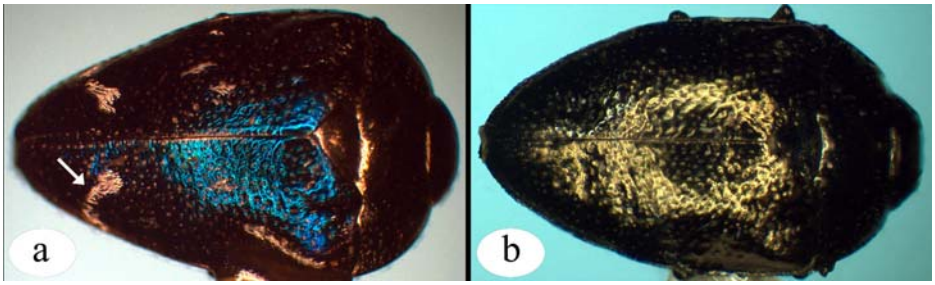


Figure A-2-56. (a) Dorsum: *P. purpureus purpureus*, (b) Dorsum: *P. laevigatus*.

2. Elytra uniformly black (Fig. A-2-56b) … *P. laevigatus*

- Elytra blue (Fig. A-2-57) … *P. nicolayi*

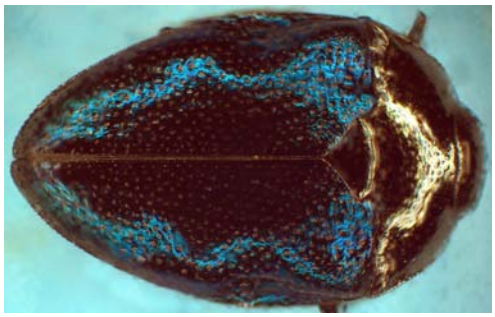

Figure A-2-57. Dorsum: *P. nicolayi.*

**Key to Tennessee *Brachys***

1. Apex of last sternite of female with long white hair (Fig. A-2-58a) (males rarely collected) … *B. ovatus*

- Apex of last sternite sparse short hairs (Fig. A-2-58b) … 2

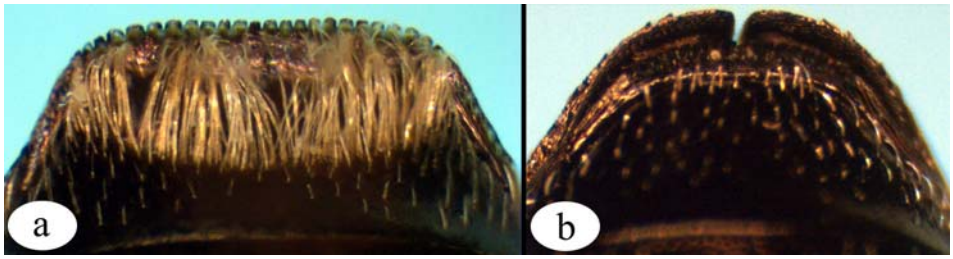


Figure A-2-58. (a) Last sternite ♀: *B. ovatus*, (b) . Last sternite: *B .aerosa.*

2(1). Apical elytral setae mixed light gold to silver (Fig. A-2-59a) … *B. aerosus*

- Apical elytral setae predominately gold (Fig. A-2-59b) … *B. aeruginosus*

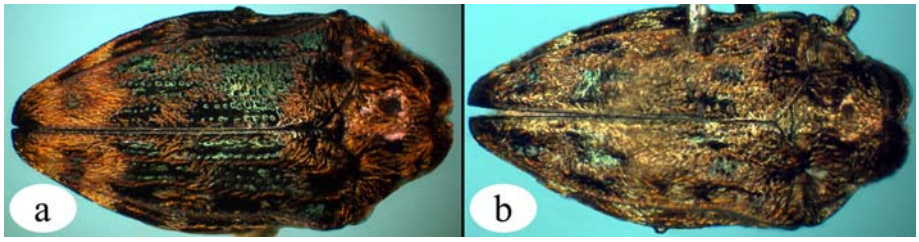


Figure A-2-59. (a) Dorsum: *B. aerosa*, (b) Dorsum: *B. aeruginosus*.

**Key to Tennessee *Agrilus***

1. Prolonged elytral apices (Fig. A-2-60a) … *A. ferrisi*

- Rounded elytral apices (Fig. A-2-60b) … 2

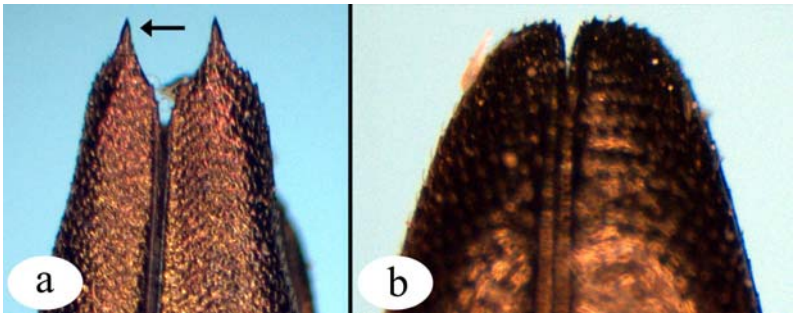


Figure A-2-60. (a) Elytral apices: *A. ferrisi*, (b) Elytral apices: *A. obsoletoguttatus*.

2(1). Antennal segments 4–11 serrate (Fig. A-2-61a) … 3

- Antennal segments 5–11 serrate (Fig. A-2-61b) … 22

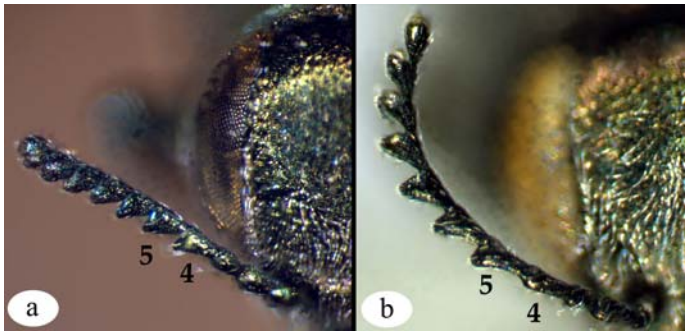


Figure A-2-61. (a) Antenna: *A. cliftoni*, (b) Antenna: *A. pseudofallax*.

3(2). Tarsal claws cleft, inner portion turned inward, nearly or quite touching that of the opposite side (Fig. A-2-62a) … 4

- Tarsal claws cleft, inner portion not or only feebly turned inward (Fig. A-2-62b) … 14

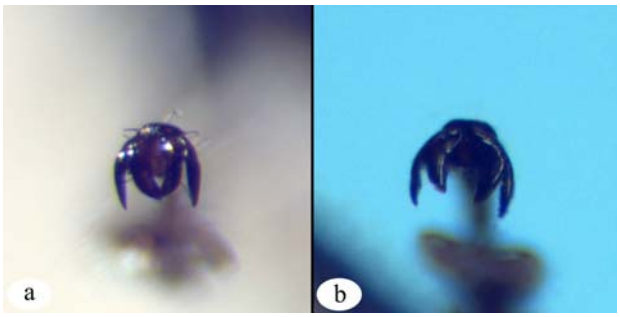


Figure A-2-62. (a) Anterior tarsal claw: *A. masculinus*, (b) Anterior tarsal claw: *A. bilineatus*.

4(3). Pygidium with projecting carina (Fig. A-2-63a), head and pronotum cupreous … *A. ruficollis*

- Pygidium without projecting carina, head and pronotum not cuperous (Fig. A-2-63b)… 5

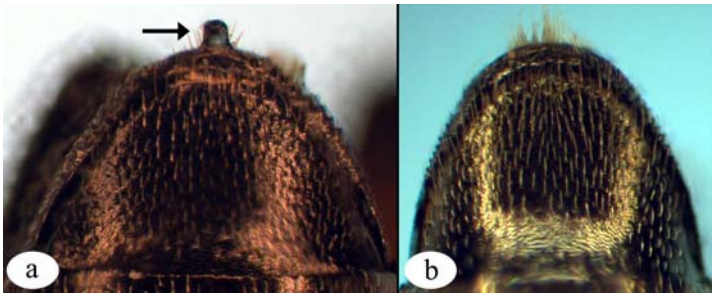


Figure A-2-63. (a) Last sternite: *A. ruficollis*, (b) Last sternite: *A. masculinus*.

5(4). Abdominal segments with distinct pubescent spots laterally (Fig. A-2-64a) … *A. difficilis*

- Abdominal segments lacking spots (Fig. A-2-64b) … 6


Figure A-2-64. (a) Lateral: *A. difficilis*, (b) Lateral: *A. otiosus*.

6(5). Apex of male metatibiae unarmed (Fig. A-2-65a) … 7

- Metatibiae of male with distinct tooth on inner margin at apex (Fig. A-2-65b) … 9

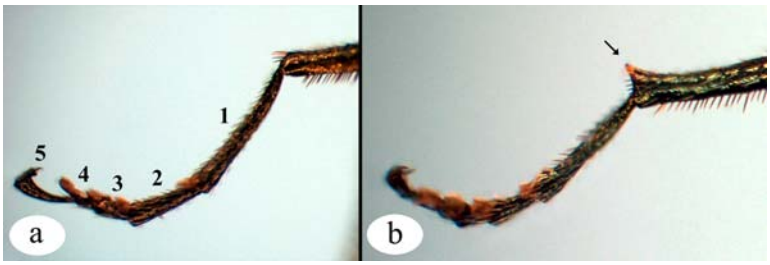


Figure A-2-65. (a) Metatarsi and apex of metatibia ♂: *A. otiosus*, (b) Metatarsi and apex of metatibia ♂: *A. masculinus*.

7(6). Frons deeply concave from epistoma to vertex (Fig. A-2-66a) … *A. fuscipennis*

- Frons not deeply concave (Fig. A-2-66b) … 8

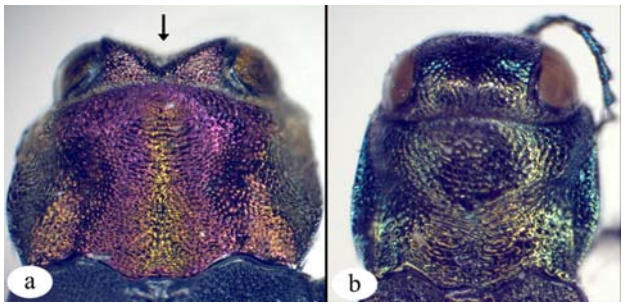


Figure A-2-66. (a) Head and prothorax: *A. fuscipennis*, (b) Head and prothorax: *A. arcuatus*.

8(7). Metatarsi of male as long or longer than tibiae, the first tarsomere as long as the following four united (Fig. A-2-65b) … *A. masculinus*

- Metatarsi of male shorter than tibiae, the first tarsomere shorter than the following united (Fig. A-2-67) … *A. arcuatus*

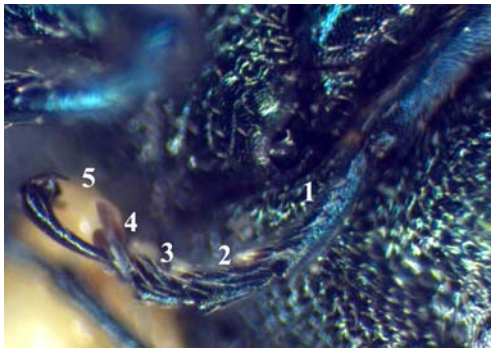


Figure A-2-67. Metatarsi: *A. arcuatus*.

9(6). Male with last ventral abdominal segment fimbriate at apex (Fig. A-2-68a) … *A. defectus*

- Male with the last ventral abdominal segment not fimbriate at apex (Fig. A-2-68b) … 10

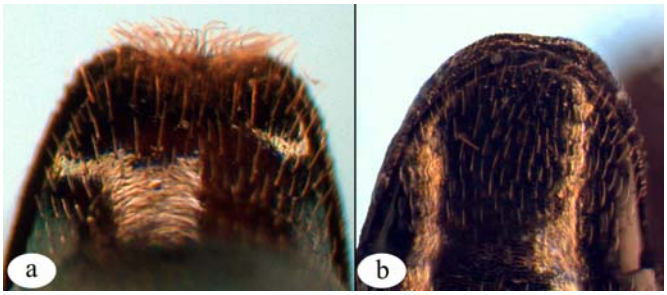


Figure A-2-68. (a) Terminal sternite ♂: *A. defectus*, (b) Terminal sternite ♂: *A. geminatus*.

10(9). Male with prosternum conspicuously pubescent medially (Fig. A-2-69a) … 11

- Male with prosternum not conspicuously pubescent medially (Fig. A-2-69b) … 13

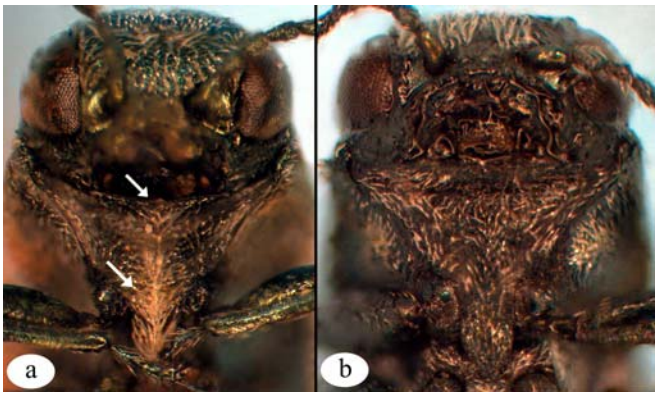


Figure A-2-69. (a) Prosternum ♂: *A. otiosus*, (b) Prosternum ♂: *A. transimpressus*.

11(10). Prosternum deeply emarginate (Fig. A-2-70) … *A. cliftoni*

- Prosternum at most slightly emarginate (Fig. A-2-69a) … 12

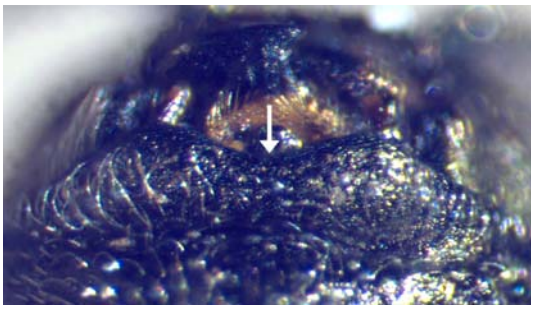

Figure A-2-70. Prosternal lobe: *A. cliftoni.*

12(11). Male genitalia with sides parallel (Fig. A-2-71a) … *A. geminatus*

- Male genitalia with sides arcuately expanded (Fig. A-2-71b) … *A. otiosus*

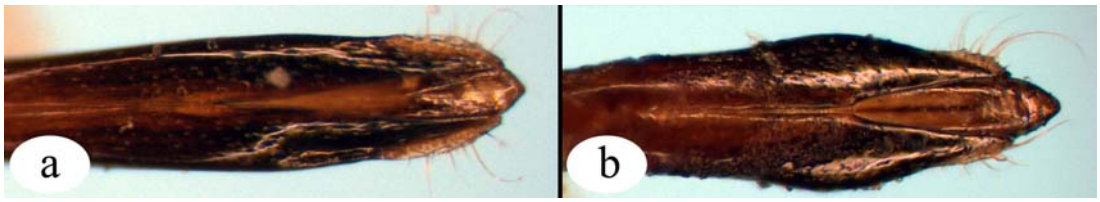


Figure A-2-71. (a) Genitalia ♂: *A. otiosus*, (b) Genitalia ♂: *A. geminatus*.

13(10). Male genitalia with parameres rapidly tapering from middle bulge to apex (Fig. A-2-72a) … *A. transimpressus*

- Male genitalia with parameres gradually tapering to apex (Fig. A-2-72b) … *A. diospyroides*


Figure A-2-72. (a) Genitalia ♂: *A. transimpressus*, (b) Genitalia ♂: *A. diospyroides*.

14(3). Pygidium with projecting carina (Fig. A-2-63a) … 15

- Pygidium without projecting carina (Fig. A-2-63b) … 18

15(14). Elytra with distinct vitta from basal depression to apex (Fig. A-2-73a) … *A. bilineatus*

- Elytra without distinct vitta from basal depression to apex (Fig. A-2-73b) … 16


Figure A-2-73. (a) Dorsum: *A. bilineatus*, (b) Dorsum: *A. quadriguttatus*.

16(15). Vertical portion of second abdominal segment glabrous or not conspicuously pubescent (Fig. A-2-64b) … 17

- Vertical portion of second abdominal segment densely clothed with white pubescence (Fig. A-2-74) … *A. quadriimpressus*

Figure A-2-74. Lateral view: *A. quadriimpressus*.

17(16). Prehumeral carina distinct (Fig. A-2-75a) … *A. quadriguttatus quadriguttatus*

- Prehumeral carina indistinct (Fig. A-2-75b) … *A. acutipennis*

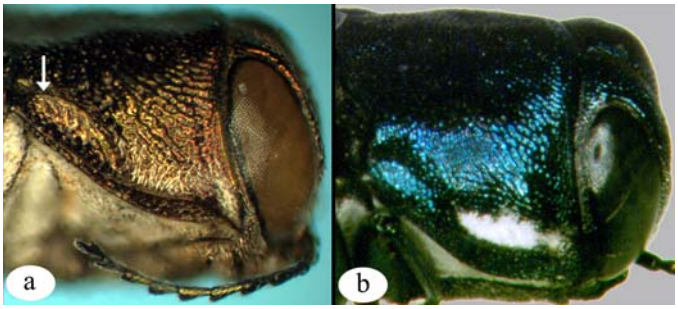


Figure A-2-75. (a) Prehumeral carina: *A. quadriguttatus*, (b) Prehumeral carina: *A. acutipennis*.

18(14). Elytra with distinct pubescent spots (Fig. A-2-76a) … 19

- Elytra without distinct pubescent spots (Fig. A-2-76b) … 20


Figure A-2-76. (a) Dorsal: *A. fallax*, (b) Dorsal: *A. politus*.

19(18). Anterior margin of prosternum deeply emarginated (Fig. A-2-77a), elytra with pubescent spots, middle one elongate … *A. obsoletoguttatus*

- Anterior margin of prosternum not emarginated (Fig. A-2-77b), elytra with 3 pubescent spots, middle one not elongate (Fig. A-2-76a) … *A. fallax*

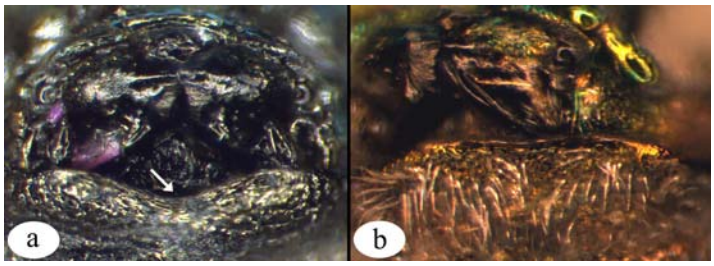


Figure A-2-77. (a) Prosteral lobe: *A. obsoletoguttatus*, (b) Prosteral lobe: *A. fallax*.

20(18). Antenomeres 7–11 distinctly wider than long (Fig. A-2-78a) … *A. politus*

- Antenomeres 7–11 not distinctly wider than long (Fig. A-2-78b) … 21

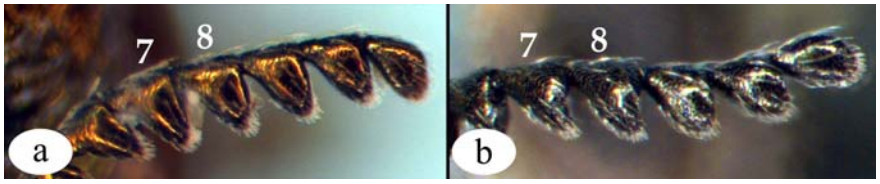


Figure A-2-78. (a) Antennameres 7–11: *A. politus*, (b) Antennameres 7–11: *A. subrobustus*.

21(20). First metatarsomere as long as the following three united (Fig. A-2-79a) … *A. cephalicus*

- First metatarsomere as long as the following two united (Fig. A-2-79b) … *A. subrobustus*

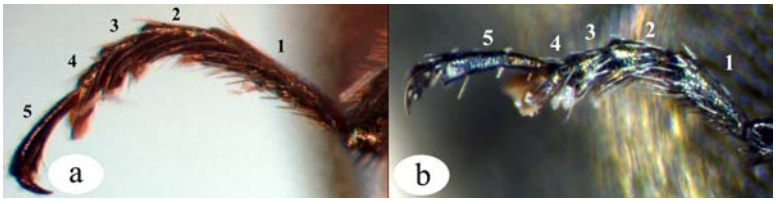


Figure A-2-79. (a) Metatarsi: *A. cephalicus*, (b) Metatarsi: *A. subrobustus*.

22(3). Prehumeral carina absent (Fig. A-2-80a) … 23

- Prehumeral carina present (Fig. A-2-80b) … 24

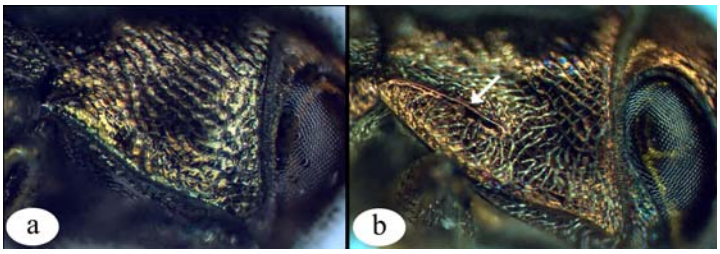


Figure A-2-80. (a) Prehumeral carina: *A. oblongus*, (b) Prehumeral carina: *A. lecontei*.

23(22). Marginal and submarginal carinae of pronotum connected at base (Fig. A-2-81a) … *A. oblongus*

- Marginal and submarginal carinae of pronotum connected behind middle, not at base (Fig. A-2-81b) … *A. putillus putillus*

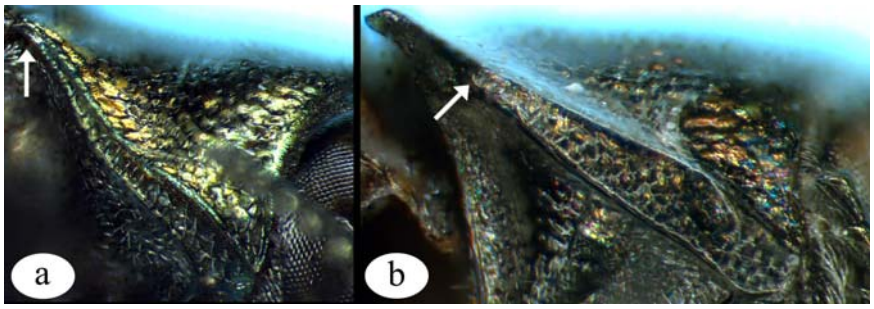


Figure A-2-81. (a) Marginal and submarginal carinae of pronotum: *A. oblongus*, (b) Marginal and submarginal carinae of pronotum: *A. putillus putillus*.

24(22). Elytra ornamented with dense pubescence forming spots or irregular designs (Fig. A-2-82a) … 25

- Elytra pubescence not forming designs but uniformly dispersed (Fig. A-2-82b) … 29


Figure A-2-82. (a) Dorsum: *A. lecontei*, (b) Dorsum: *A. politus*.

25(24). Elytra with three spots one elongated spot in middle (indicated by white arrow) flanked by two rounded spots; one at apical third and one in basal depression (Fig. A-2-83) … *A. abductus*

- Elytral pubescent design without a longitudinally elongated spot in middle (Figs. A-2-82a and A-2-84) … 26

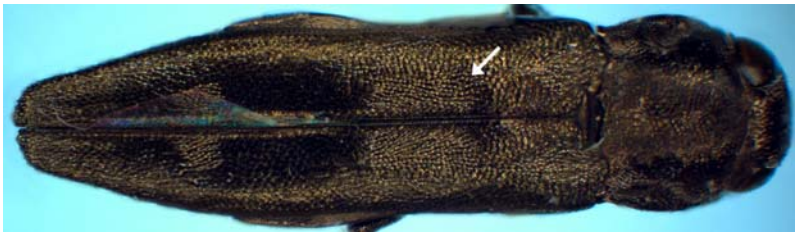

Figure A-2-83. Dorsum: *A. abductus.*

26(25). Elytra with pubescense forming irregular design (Fig. A-2-82a) … 27

- Elytra with three rounded spots (Fig. A-2-84) … 28

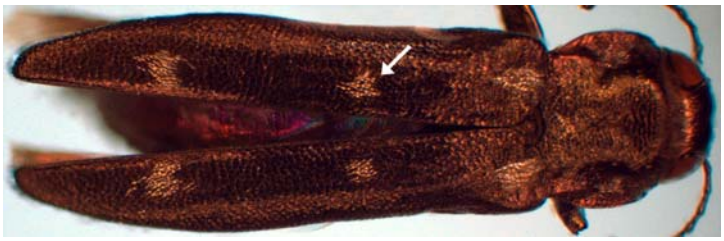

Figure A-2-8411. Dorsum: *A. pseudofallax.*

27(26). Male with protarsal claws dissimilar, one with teeth nearly equal in length the other with inner tooth broader and distinctly shorter than outer (Fig. A-2-85a) … *A. lecontei lecontei*

- Male with protarsal claws similar, both teeth of about equal length (Fig. A-2-85b) … *A. lecontei celticola*

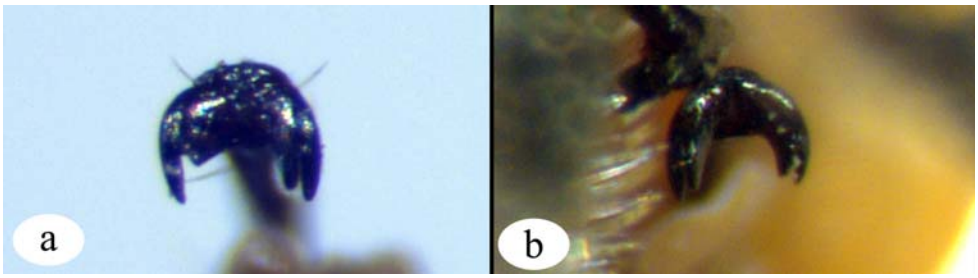


Figure A-2-85. (a) Anterior tarsal claw ♂: *A. lecontei lecontei*, (b) Anterior tarsal claw ♂: *A. lecontei celticola*.

28(26). First metatarsomere as long as the following two united (Fig. A-2-79b), prehumeral carina vague and obtuse … *A. pseudofallax*

- First metatarsomere as long as the following three united (Fig. A-2-79a), prehumeral carina sharply defined (Fig. A-2-80b) … *A. egeniformis*

29(24). Prosternal lobe deeply emarginated (Fig. A-2-77a) … *A. olentangyi*

- Prosternal lobe slightly emarginate or truncate (Fig. A-2-77b) … 30

30(29). Male aedeagus with parameres greatly expanded, middle lobe tip widely rounded (Fig. A-2-86a) … *A. egenus*

- Male aedeagus with parameres parallel (Fig. A-2-86b) … 3

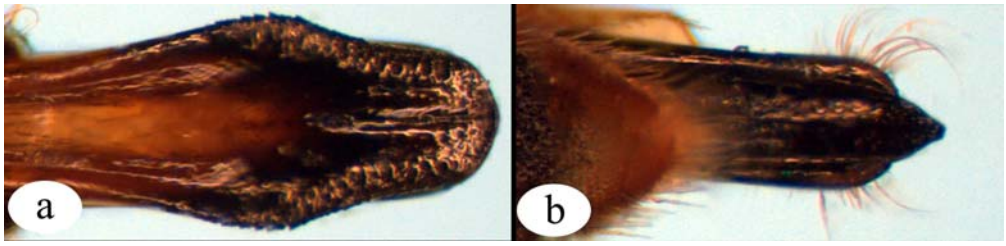


Figure A-2-86. (a) Genitalia ♂: *A. egenus*, (b) Genitalia ♂: *A. celti*.

31(30). Elytra with inconspicuous white hairs of equal length except in basal depression. Male genitalia: tip of middle lobe coming to a sharp point (Fig. A-2-86b) … *A. celti*

- Elytra with uniformly dispersed white hairs, which are slightly longer down middle. Male genitalia: same as Fig. A-2-86b but with tip of middle lobe rounded … *A. paracelti*.

For references see Chapter 2 Literature cited, p. 75.

# APPENDIX 3

## FIRST RECORD OF *ALCATHOE CAROLINENSIS* (LEPIDOPTERA: SESIIDAE) COLLECTED IN TENNESSEE

This chapter is essentially the same as an article accepted by The Florida Entomologist and currently in press. It will appear in the June 2010 edition as a scientific note:

Jason Hansen, William E. Klingeman and John Kevin Moulton. 2010. FIRST RECORD OF *ALCATHOE CAROLINENSIS* (LEPIDOPTERA: SESIIDAE) COLLECTED IN TENNESSEE. Florida Entomologist 93(2)

My contributions to this publication include collection and identification of specimens, as well as preparation of the figure and manuscript.

**Abstract**

The first captures of *Alcathoe carolinensis* Engelhardt in Tennessee are reported from pheromone-baited trap yields taken in 2007 and 2009 from eastern Tennessee locations 100 kilometers apart and at different elevations. Traps were baited with a different pheromone combination than reported in other published accounts. Its capture at 600 meters elevation in the Great Smoky Mountains adjacent to GSM National Park boundaries marks the highest elevation at which *A. carolinensis* has been recorded and is similar to the original type locality described by Beutenmüller for North Carolina.

Known mostly from male specimens, *Alcathoe carolinensis* Engelhardt (Fig. 1) has been reported as rare, but is more likely to be infrequently collected (Thomas D. Eichlin, Senior Insect Biosystematist, *retired*, California Department of Food and Agriculture, personal communication), thus the full extent of its native range is poorly documented. Though most captures have been incidental and typically consist of one to three specimens, only two studies have reported captures of 10 or more males through use of E,Z-3,13–ODDA and Z,Z-3,13–ODDA blends (Reed et al. 1981, Snow et al. 1985). Based on other *Alcathoe* species host plant preferences, larval host plants are assumed to be *Clematis* spp., though *A. carolinensis* remains the only North American member of its genus not reared from any species or cultivar of *Clematis* (Engelhardt 1925, Eichlin and Duckworth 1988). *Alcathoe carolinensis* was once listed as a synonym of *A. autumnalis* Engelhardt, but later the two were recognized as distinct species (Duckworth and Eichlin 1977, Eichlin and Duckworth 1988). When questioned about the lack of label data on the type specimen, Beutenmüller recalled collecting it on *Clematis* flowers somewhere in "the Black Mountains of North Carolina" (Engelhardt 1946).

Engelhardt (1946) reported that subsequent visits to the collecting site showed Beutenmüller's plant identification to be inaccurate, though he did not clarify what the mistakenly identified plant was. Morphological similarities between *A. carolinensis* and two species in the western U.S.: *A. pepsioides* Engelhardt and *A. autumnalis*, also cast some doubt on the capture of *A. carolinensis* in a state so disjunct from other similar *Alcathoe* populations (Engelhardt 1946, Duckworth and Eichlin 1977, Eichlin and Duckworth 1988). Currently, *A. carolinensis* specimens have been collected as far north as Indiana and south to Florida (Sharp et al. 1978, Reed et al. 1981). A lone male captured in Missouri extended the western boundary of its known range and is the most recent reported capture of this species (Brown 1986).

In 2007, as part of an on-going survey of clearwing moth presence in eastern Tennessee, a Multipher-1 moth trap (Les Services BioContrôle, Ste.-Foy, Quebec) was baited with a commercial yellow-legged clearwing moth (*Synanthedon vespiformis* (L.)) lure (Great Lakes IPM, Vestaburg, MI). This modified trap was placed just outside the boundaries of the Great Smoky Mountains National Park in Sevier Co., Tennessee. The trap was retrofitted with an ethanol collection chamber, thus preserving DNA for analyses and preventing damage to important morphological characters. Specimens were captured ~ 70 meters from a Norton Creek in a wooded area on an western-facing slope approximately 600 meters above sea level. Several hemlock, pine, oak trees and rhododendron shrubs had recently been removed from the site. The lure-baited trap was placed on the edge of this canopy opening, where a single male was collected between 29-VI and 5-VII-2007.

In 2009, the same modified trap style was deployed with two viburnum borer (*S. viburni* Engelhardt) lures (Scentry Biologicals, Inc, Billings, Montana) along the wooded edge of a roadside park in Oak Ridge, Tennessee approximately 100 kilometers west of the original Norton Creek site and at about 260 meters in elevation. Canopy mid- and overstory consisted predominantly of oaks (*Quercus* sp.), loblolly pine (*Pinus taeda* L.), bush honeysuckle (*Lonicera maackii* (Rupr.)), privet (*Ligustrum sinense* Lour.), tulip poplar (*Liriodendron tulipfera* L.), sweetgum (*Liquidambar styraciflua* L.) and rusty blackhaw viburnum (*Viburnum rufidulum* Raf.). The trap yielded eight *A. carolinensis* males between 10 and 20-VIII-2009. Identification of the sesiid was verified by Thomas D. Eichlin. Although *Clematis* species were not found within ~160 meters of deployed traps, several other vining forbs were found within 33 meters, including honeysuckle (*Lonicera* sp.), trumpet creeper (*Campsis radicans* (L.)), greenbriar (*Smilax* sp.), grape (*Vitis* sp.), and Carolina moonseed (*Cocculus carolina* (L.)).

In Tennessee, *A. carolinensis* responded to lures which attract *S. viburni* and *S. vespiformis*, both known to be drawn to Z,Z-3,13-octadecadienyl acetate (ODDA)/E,Z-3,13-octadecadienyl acetate at a ratio of 9:1 (Greenfield and Karandinos 1979, Voerman et al. 1983). The commercial lures used were confirmed by vendors as containing the same ratio of isomers reported in the literature. With the exception of a solitary account in which E,Z-3,13–ODDA alone was used, previous *A. carolinensis* captures were accomplished with a 50:50 or 75:25 blend of the two previously mentioned isomers (Sharp et al. 1978, Reed et al. 1981, Snow et al. 1985, Brown 1985, Brown 1986). Regardless of the exact isomer blend used, we expect *A. carolinensis* will continue to be

infrequently collected until larval host plant resources are identified and trapping is focused around habitats containing key plant species.

Although larvae of other *Alcathoe* species rely on *Clematis* plants for development, no specimens of the plant genus were found at the Oak Ridge, Tennessee location. A less common species, *C. glaucophylla* small, is listed on Tennessee's rare plant list as endangered (Crabtree 2008) and is reported from North Carolina, Florida, Georgia and eastern Tennessee. However, older reports of this clematis species may have confused it with closely-related *C. viorna,* thus the range of *C. glaucophylla* may be more restricted than is reported (Estes 2006).

Because many ornamental *Clematis* species are economically important and popular landscape specimens, further efforts to find larvae and rear adults from *Clematis* species are warranted. Engelhardt (1925) noted use of horticultural *Clematis* varieties by *A. caudata* (Harris), but mentioned no specific varieties. We caution that at least one native *Clematis* species, *Clematis glaucophylla* is endangered in Tennessee, thus care should be taken to assess any protected status this plant may have when sampling plants to confirm *A. carolinensis* presence from other localities in the state.


Figure A-3-1. Adult male *Alcathoe carolinenis* Engelhardt, illustrating the hyaline area at base of hind wing (h) and caudal appendage (ca), which are characteristic of *Alcathoe* males.

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# VITA

Jason Hansen was born in Houston, Texas. He spent most of his young adult life in Rockville, Maryland where he attended Rockville High School. After graduating he continued his education at Ricks College in Idaho and received an Associate degree in zoology in 1997. Jason spent two years in Ecuador learning to speak fluent Spanish and serve as a missionary for his childhood faith before returning to continue his education, majoring in zoology. Upon receiving his Bacholor of Science degree in 2000 from Brigham Young University he returned to his home state of Maryland and accepted a job working as a Research Assitant to Jay Vatsan at Walter Reed Army Institute of Research, while pursuing a Masters of Science in Environmental Biology at Hood Colllege. His work on the intercellular location and secretion of $IpaH_{7.8}$ showed translocation of the IpaH protein to the nucleus where it performed a then unknown function. After graduating with his Masters Jason moved to Las Vegas with his wife and two children and worked for a chemical engineering company as a Research Associate. After a year and a half working in Las Vegas he decided to go back to school to pursue a PhD. His search for a program led him to the University of Tennessee.