PHYLOGENETICS, NICHE MODELING, AND BIOGEOGRAPHY OF *MYCOTRUPES* (COLEOPTERA: GEOTRUPIDAE)

By

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To Mom and Dad
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I conducted a study of the molecular phylogenetics, niche modeling, and biogeography of Mycotrupes (Coleoptera: Scarabaeoidea: Geotrupidae). I collected specimens of all five known species of Mycotrupes from across the known distributions. From these specimens, I sequenced a 481 base pair fragment of the mitochondrial gene Cytochrome Oxidase I, providing data for phylogenetic analyses done with both Parsimony as well as Bayesian methods. The resulting phylogenetic hypotheses provided a test of the species boundaries of Mycotrupes, as well as providing the basis for a test of an a priori biogeographic hypothesis of the species of Mycotrupes. I performed a niche modeling study with all Mycotrupes species in order to test the importance of various environmental layers (climate, soil, etc.) in describing the distributions of the five species.

The phylogenetic analyses provided support for four of the five species of Mycotrupes. The fifth species, *M. cartwrighti* Olson and Hubbell, was polyphyletic. Pairwise distances suggested that the haplotype that caused this species to be non-monophyletic may be a cryptic species. Except for the two specimens represented by this haplotype, *M. cartwrighti* is monophyletic as are the other species of Mycotrupes in the analysis. The phylogenetic hypothesis does not support the a priori hypothesis of evolution of the species of Mycotrupes.
As a result, the pre-existing biogeographical hypothesis is also not supported, and I proposed a new hypothesis. In the niche modeling study, I confirmed that well-drained soil is an important factor in the distribution of *Mycotrupes* species.
CHAPTER 1
INTRODUCTION

The beetle genus *Mycotrupes* LeConte is restricted in distribution to the Atlantic Coastal Plain of the Southeastern United States. The five known species are allopatric (non-overlapping) in distribution. Two species, *M. lethroides* (Westwood) and *M. retusus* (LeConte), occur along the Fall Line (a region which separates the Piedmont from the Atlantic Coastal Plain) in Georgia and South Carolina, with their distributions being separated by the Savannah River. *Mycotrupes cartwrighti* Olson and Hubbell is found in several areas in southern Georgia and northern Florida. *Mycotrupes gaigei* Olson and Hubbell is known from an extensive portion of northern Peninsular Florida. *Mycotrupes pedester* Howden occurs only in southwestern Florida.

Species of *Mycotrupes* are flightless and are known to dig deep burrows, up to six feet in depth for *M. gaigei*. Data on feeding is fragmentary although adults can be caught in pitfall traps baited with dung and/or fermenting malt. The larval stage is passed underground in burrows excavated by adults. The larva of only one species, *M. gaigei*, has been described. The larval food for this species was apparently cattle dung (Howden 1954).

*Mycotrupes* species are found most commonly in well-drained areas, such as sandhill, in Florida and the Fall Line of Georgia and South Carolina. This habitat is being developed at a rapid rate for housing and agriculture (Enge et al. 1986; Kautz et al. 2007). This, combined with their restricted geographic distributions, makes *Mycotrupes* quite vulnerable to habitat loss. Some of these sandy environments are associated with ancient seashores, which are the product of a long history of fluctuating climate and sea levels during the Cenozoic Era. In the monograph of the genus *Mycotrupes* (Olson, Hubbell, and Howden 1954), Hubbell (1954) proposed a hypothesis for the evolution of the genus, which he based upon changes in sea level and assumptions of the evolution of certain morphological features. Until now, this hypothesis
has not been tested with modern phylogenetic and biogeographic methods. Multiple characteristics associated with Mycotrupes, such as allopatric distributions, flightlessness, and distribution of the species across a region that has been extensively affected by changes in sea level, suggest that this genus may be well suited for studying patterns of speciation and biogeography.

The first objective of my study was to test the hypothesis, proposed by Hubbell (1954) for the evolution of Mycotrupes species, using modern phylogenetic methods. This component also provided a phylogenetic test of species boundaries and the possible detection of cryptic species. The second objective was to test the biogeographic hypothesis of Hubbell (1954). The third objective was to determine what environmental factors might be associated with, and perhaps limiting the distributions of different Mycotrupes species, using Niche Modeling techniques. This information was later used to further develop a new biogeographic hypothesis and suggest areas that may contain yet undiscovered populations of Mycotrupes.

The development of a character matrix is necessary in order to construct a phylogenetic hypothesis. It is extremely difficult to find morphological features in this genus that consistently vary between species, but which show little or no variation within a species. For this reason, molecular data was used. A 481 base pair fragment of the mitochondrial gene Cytochrome Oxidase I was sequenced from all five Mycotrupes species and analyzed within a phylogenetic analysis. The phylogenetic analysis was conducted by using two methods of phylogenetic inference: 1) Parsimony and 2) Bayesian. Both of these analyses resulted in similar patterns of evolution in Mycotrupes, and both did not support the hypothesis of Hubbell (1954). In addition, both models provided strong evidence for the existence of a cryptic species. The use of Bayesian methods in conjunction with the program BEAST also provided a method for estimating how
long ago each of these beetle species diverged from the next closely related species (e.g., time
since divergence). By combining information from species distributions, phylogenetic
hypotheses and divergence time estimates, new hypotheses for the evolution and biogeography
for the genus *Mycotrupes* have been generated.

I conducted a niche modeling analysis using the program Maximum Entropy (Schapire
2008), which used collection locality, in conjunction with multiple environmental layers to
produce a modeled niche and likelihood of occurrence map for each species of *Mycotrupes*. The
environmental data layers included soil characteristics, elevation, and climate (i.e., temperature
and precipitation). The results showed drainage to be an important factor in the distribution for
all *Mycotrupes* species. This result corroborates data on the known distribution of these species
and supports the hypothesis that the dispersal of *Mycotrupes* species is hindered by poor draining
soils.

This study represents the first molecular phylogenetic study of *Mycotrupes*. These
findings contribute to the study of the historical biogeography of the Southeastern United States
by providing useful comparative data for studying other taxa found in this region. In addition,
data on the environmental variables associated with the distribution of these beetles may help in
future conservation efforts targeted at protecting *Mycotrupes* and their habitat.
CHAPTER 2
LITERATURE REVIEW OF MYCOTRUPES

Taxonomic History of Mycotrupes LeConte

The genus *Mycotrupes* (Coleoptera: Geotrupidae: Geotrupinae) is composed of five species: *Mycotrupes lethroides*, *Mycotrupes retusus*, *Mycotrupes gaigei*, *Mycotrupes cartwrighti*, and *Mycotrupes pedester* (Jameson 2002; Olson and Hubbell 1954). The name *Mycotrupes* was erected by LeConte in 1866 as a subgenus of *Geotrupes* for *Geotrupes retusus*, which he attributed to MacLeay, although no description was published by the later author (Horn 1868). Horn (1868) published an additional description of *G. retusus*, and Blanchard (1888) referred to Horn as the author of the species. LeConte was recognized as the author of *G. retusus* by Olson and Hubbell (1954). The type series of *G. retusus*, consisting of five specimens, was later determined by Olson and Hubbell (1954) to include a specimen each from two other species, *M. lethroides* and *M. cartwrighti*.

Westwood (1837) described *Geotrupes lethroides*, which was later transferred to *Mycotrupes* by Boucomont (1911). Although Westwood recognized the distinctive morphology of his species, stating that it displayed characters that seemed to be “...of higher value than those indicating a species,” he did not propose a new genus. Westwood described the collecting locality of *G. lethroides* as “America Meridionali,” a confusing description that may have been interpreted by later authors (including LeConte) as indicating that the species occurs in South America (Olson and Hubbell 1954). Thus the species went unrecognized as part of the North American fauna until Boucomont (1911).

In 1902, Boucomont placed *Geotrupes retusus* in the genus *Thorectes*, subgenus *Mycotrupes*, and later (1911) moved the subgenus *Mycotrupes* to the genus *Geotrupes*. Felsche (1909) described *Geotrupes aeneus* from what was actually a specimen of *Mycotrupes*.
mistakenly labeled as collected from Senegal. Later, finding much similarity between his specimen and LeConte’s description of *Geotrupes retusus*, Felsche synonymized *G. aeneus* under *M. retusus* (Felsche 1910). In 1911, Boucomont synonymized *M. retusus* under *M. lethroides* after noting the similarity of the descriptions of the two species and recognizing that the locality information given in Westwood’s 1837 description of *Geotrupes lethroides* was incorrect. From this 1911 synonymy until the generic revision of Olson and Hubbell (1954), all authors referred to species of *Mycotrupes* as *M. lethroides*.

Olson and Hubbell (1954) examined the available material of *Mycotrupes* and concluded that multiple species were represented. They recognized the species status of *M. lethroides*, resurrected *M. retusus* as a valid species, and described two additional species: *M. gaigei* and *M. cartwrighti*. Howden (in Olson and Hubbell 1954) described *M. pedester*.

**Distribution and Habitat**

The genus *Mycotrupes* is restricted to the southeastern United States. All five species are known to be allopatric. Although habitat type varies by species and geographic location, well-drained soil and an open understory appear to characterize most occurrences (Woodruff 1973). Many deposits of these well-drained soils, especially in the case of the sand ridges in Florida and the sandhill region of Georgia and South Carolina, are likely associated with ancient shorelines (Hubbell 1954). An account of the distribution and habitat of each species follows. See Figure 2-1 for a map of *Mycotrupes* species distributions; location points represent data from collections made during this study as well as data from borrowed specimens and literature records.

*Mycotrupes lethroides* (see Figure 2-2, a and b) has only been collected from a small number of locations in Richmond and Burke counties, in Georgia (Beucke and Choate 2009; Harpootlian 1995; Olson and Hubbell 1954). Its habitat was described by Olson and Hubbell
(1954) as open forests of pine and oak on sandy soil. These sandy areas include part of the Fall Line sandhills, which extend into South Carolina.

*Mycotrupes retusus* (see Figure 2-2, c and d) is found along much of the sandhills region of South Carolina from Aiken to Kershaw County (Harpootlian 1995, 2001, 2006; Olson and Hubbell 1954). Olson and Hubbell (1954) described the habitat of *M. retusus* as open pine and oak forest on sandy soil. The distributions of *M. retusus* and *M. lethroides* are apparently associated with similar habitat but are separated by the Savannah River. Hubbell (1954) proposed the enlargement of the Savannah River as a vicariant event resulting in the isolation of the ancestors of these two species.

*Mycotrupes cartwrighti* (see Figure 2-2, e and f) is probably the most widely distributed species of the genus and occurs in Georgia and Florida (Hebard 1903 [who referred to it as *M. retusus*]; Olson and Hubbell 1954; Peck and Thomas 1998; Woodruff 1973; Woodruff and Deyrup 1994a, 1994b, 1994c). Most records of *M. cartwrighti* are from a large area extending north from Tallahassee, Florida to Fort Valley, Georgia (Olson and Hubbell 1954). *Mycotrupes cartwrighti* has also been collected from the widely separated areas of Jacksonville and Atlantic Beach, Florida and Hinesville, Georgia (P. Harpootlian, pers. comm.; Olson and Hubbell 1954; K. Beucke, unpublished data). The type locality of *M. cartwrighti* is 6.5 miles East of Tallahassee and was described by Olson and Hubbell (1954) as hardwood forest with little undergrowth, on a slope. The soil was described as Orangeburg sandy loam over clay.

*Mycotrupes gaigei* (see Figure 2-2, g and h) is restricted to Florida and its range extends in a broad swath from Madison County in the North to Sumter County in the South (Olson and Hubbell 1954; Peck and Thomas 1998; Woodruff 1973; Woodruff and Deyrup 1994a, 1994b, 1994c; P. Choate, pers. comm.). This area corresponds to the Peninsular Lime Sink Region, an
extensive area of karst with little surface water and soils predominantly of the Norfolk series (Harper 1921; Olson and Hubbell 1954). *Mycotrupes gaigei* also occurs in Seminole County, Florida (Woodruff 1973). The habitat of *M. gaigei* was described by Olson and Hubbell (1954) as pine and oak forest on well-drained sands.

*Mycotrupes pedester* (see Figure 2-2, i and j), which is also restricted to Florida, has been collected at a limited number of locations in De Soto, Charlotte, and Lee counties (Olson and Hubbell 1954; Peck and Thomas 1998; Woodruff 1973; Woodruff and Deyrup 1994a, 1994b, 1994c). Woodruff (1973) collected *M. pedester* at two sites in Tice and Estero, Florida. One of these sites was located in a cattle pasture at the edge of a drainage canal; the other site had been recently burned and had "Caribbean pine with some scattered large live oaks and a dense mat of saw palmetto in places." The habitat of *M. pedester* may be more fragmented and isolated compared to that of other *Mycotrupes* species. All of the known collecting localities for *M. pedester* are contained within an area labeled as flatwoods vegetation by Harper (1927), indicating that it is generally a poorly drained region.

Several published locality records of *Mycotrupes* require clarification. LeConte (1866) listed *M. retusus* (as *Geotrupes retusus*) from North Carolina to Louisiana. Brimley (1938) and Leng (1920) listed *Geotrupes lethroides* from North Carolina, presumably based on LeConte's distribution information. There are no specific collecting data available that lend credence to *Mycotrupes* occurring this far from its known range. However, Olson and Hubbell (1954) thought that *M. retusus* might occur in North Carolina, because the Fall Line belt of sandhill habitat in which this species is found in South Carolina extends into that state.

In his paper on Florida scarabs, Blatchley (1928) listed *M. lethroides* from St. Augustine and Enterprise. These records probably refer to the currently recognized species *M. cartwrighti*
and *M. gaigei; M. cartwrighti* has been collected from Jacksonville and Atlantic Beach, Florida (both are near St. Augustine) by H. Klages and A.T. Slosson (Olson and Hubbell 1954), and I collected *Mycotrupes gaigei* from Geneva, Florida, which is approximately 12 miles from Enterprise.

Two *M. cartwrighti* collecting localities appear to be doubtful. Three specimens of *M. cartwrighti* are recorded as having been collected in Miami, Dade County, Florida by H.M. Klages (Olson and Hubbell 1954). One of these specimens, on loan from the University of Michigan Museum of Zoology, was examined by the author. A specimen of *M. cartwrighti*, also collected by H.M. Klages, is labeled as having been collected in Comfort, Texas. These two localities (Miami, Florida, and Comfort, Texas) are far from the rest of the known distribution of *Mycotrupes*. In addition, these localities have not produced other specimens of *Mycotrupes*. The H.M. Klages collection is reputed to have numerous mislabeled specimens (Woodruff 1973). For these reasons, I have decided to ignore the Miami, Florida, and Comfort, Texas records in this study.

**Morphology**

*Mycotrupes* are moderately sized beetles and measure between 10 and 20 millimeters in length. The genus may be separated from other geotrupid genera by several distinctive characters. The mesothoracic wings (elytra) are fused, and the metathoracic wings are entirely absent, making *Mycotrupes* flightless. In lateral profile both the pronotum and elytra are strongly convex and are broadly notched at their junction. In males of all species and in females of *M. lethroides*, the pronotum is anteriorly excavated and this excavation is often bordered posteriorly by a pair of polished crests. The body surface is finely sculpted, giving it a dull appearance, and it is covered with rounded granules. The body color is generally black although a metallic blue or coppery sheen is sometimes apparent (Olson and Hubbell 1954).
The species of *Mycotrupes* have a limited number of apparent morphological differences among them. Some characters used by Olson and Hubbell (1954) to distinguish between species include the shape of the phallic theca, presence or absence of elytral striae, pattern of dorsal granulation (whether the granules are separate or confluent), shape of the pronotal depression, shape and position of the cephalomedian pronotal nodule, and shape of the frontal clypeal suture. *Mycotrupes gaigei*, which has evidence of elytral striae as well as confluent (as opposed to distinct) dorsal granules, appears markedly distinct from the remaining four species.

Hubbell (1954) noted some morphological similarities between *Mycotrupes* and the geotrupid genera *Thorectes* Mulsant and *Typhoeus* Linnaeus subgenus *Chelotrupes* Jekel. He believed that these similarities were the result of parallel evolution because of the presence of other seemingly important morphological differences between the genera.

The larva of *Mycotrupes gaigei* was described by Howden (1954). The larvae of the other four species remain undescribed. Gross morphological differences that separate the larva of *Mycotrupes* from those of other Geotrupidae include the “broadly truncate configuration” of the endoskeleton below the anal opening, shape of the tormae of the epipharynx, and presence of a small sclerotized area on the glossa (Howden 1954; Woodruff 1973).

**Biology**

The feeding habits of adult and larval *Mycotrupes* are poorly known. Adult *Mycotrupes* appear to be attracted to a variety of substances, including dung and fermenting malt, and they have been observed feeding on a variety of foods in the field and laboratory (Beucke and Choate 2009; Harpootlian 1995; Olson et al. 1954; Woodruff 1973). Larval food has been observed only once, and in that instance, it appeared to be old cow dung (Howden 1954). See Chapter 3 for additional information on feeding, burrowing, and other *Mycotrupes* behavior.
Associated Life Forms

Geotrupids are known to have a variety of commensal associates and parasites, including mites and nematodes (Théodoridès 1952). Two invertebrate species, a mite and a fly, have a close biological relationship with Mycotrupes. The mite *Macrocheles mycotrupetes* Krantz and Mellott (Acari: Macrochelidae) is phoretic on *Mycotrupes gaigei* and is known from sandhill habitat in Alachua, Levy, and Columbia counties, Florida (Krantz and Mellott 1968). This mite is predaceous, and it presumably feeds on nematodes or other invertebrates present in the food of Mycotrupes. A dihydroxy wax, present on the cuticle of Mycotrupes, is attractive to *Macrocheles* (Krantz et al. 1991). Interestingly, 2,3-dihydroxy alcohol esters found in the uropygial glands of chickens were found to be attractive to *M. mycotrupetes*, suggesting that the attractant in Mycotrupes may be of a similar composition (Krantz et al. 1991). Krantz and Royce (1994) documented, in laboratory experiments, the movement of *M. mycotrupetes* through more than three inches of sand to reach *Mycotrupes gaigei*. As Mycotrupes burrows often appear to be densely aggregated, underground dispersal of mites between beetle burrows may be possible.

The fly, *Ceroptera longicauda* Marshall (Diptera: Sphaeroceridae), is also a phoretic associate of *Mycotrupes gaigei*. Other *Ceroptera* species apparently develop in the underground larval food supplies of dung feeding scarab beetles, and *C. longicauda* presumably has a similar lifestyle (Marshall and Montagnes 1988; Sivinski et al. 1999).

Neither *M. mycotrupetes* nor *C. longicauda* are restricted to *M. gaigei*. *Macrocheles mycotrupetes* is known from the geotrupid *Geotrupes egeriei* Germar (Krantz and Royce 1994), and *C. longicauda* is known from the geotrupid *Peltotrupes profundus* Howden (Sivinski et al. 1999).
Phylogenetics

Hubbell (1954) used several morphological characters that he deemed significant to produce a hypothesis of evolution for members of the genus (Figure 2-3). He assumed $M. \text{gaigei}$ to be the most basal species, as it shares the presence of elytral striae, as well as a punctate pronotum and simple phallic spine, with other Geotrupidae. Hubbell (1954) considered $M. \text{lethroides}$ and $M. \text{retusus}$ to form a group based on the bird head-shaped phallic spine that they share, and $M. \text{cartwrighti}$ and $M. \text{pedester}$ to form another group based on their simple phallic spine and other morphological similarities. He thought that the similarities between $M. \text{cartwrighti}$ and $M. \text{pedester}$ were so great that their differences might not warrant their recognition as distinct species.

Zunino (1984) published diagrams depicting his hypotheses of the relationships of geotrupid genera including Mycotrupes. Zunino’s methods were not clear, and it appears that he simply drew conclusions based on a few morphological characters that he considered important, in a fashion similar to that of Hubbell (1954). Verdu et al. (2004), and Grebennikov and Scholtz (2004) conducted morphological phylogenetic analyses of the Geotrupidae and the Scarabaeoidea, respectively. In both analyses, Mycotrupes gaigei was included as a terminal taxon. Unfortunately, the relationship of Mycotrupes to other genera within Geotrupidae was poorly resolved.

Biogeography

Hubbell (1954) speculated on the biogeographic relationships of Mycotrupes and formulated an explanation that took into account the hypothetical evolutionary relationships depicted in Figure 2-3 and the present-day distribution of the species. A key assumption made by Hubbell was that dispersal in Mycotrupes is limited to walking, restricting them to contiguous habitat and suggesting that their present-day distributions are suggestive of their past
distributions. Hubbell used the Pleistocene terraces studied by Cooke (1945) to formulate a sequence of divergences in *Mycotrupes* in response to repeated submergence and emergence of the land because of changes in sea level. Hubbell (1954) illustrated the sequence of these hypothesized events (see Figure 2-4). An important condition of his hypothesis is the presence of exposed land in Central Florida (“G2” in Figure 2-4) during some of the sea level fluctuations, as *M. gaigei* is assumed to have split from the basal stock early, before the differentiation of the ancestor of the remaining four species of *Mycotrupes*. Complete submergence of this Central Florida area would likely have wiped out any *Mycotrupes* present (Hubbell 1956). Howden (1963) proposed the idea that the seasonality of *Mycotrupes* activity, with most activity being observed during the Fall, Winter and Spring, may be an adaptive shift from summer activity during a cooler (than present) Pleistocene climate. Howden noted that many widespread species of Geotrupidae are active during the cooler months in more southerly, warmer climates, such as Florida, whereas these species are active during the summer farther north. Howden also noted that the deep burrowing habit of *Mycotrupes* could be an additional adaptation to higher temperatures.

**Conservation Status**

The recognition of the unique and threatened nature of the sandy, well-drained habitat of *Mycotrupes*, coupled with the restricted geographic distributions of the species, has promoted some interest in the conservation of species in this genus. However, the poorly known biology of *Mycotrupes*, and an incomplete knowledge of their distributions are impediments in any attempt to assess their vulnerability to human disturbance.

*Mycotrupes gaigei* has been classified as Imperiled (NatureServe 2009a), based on its specific habitat requirements and estimates of its distribution. It has also been classified as Rare by the Florida Natural Areas Inventory (2009) and by the Florida Committee on Rare and
Endangered Plants and Animals (FCREPA) (Woodruff and Deyrup 1994b). *Mycotrupes cartwrighti* has been classified as Rare in Florida by FCREPA, based on its restricted distribution in that state (Woodruff and Deyrup 1994a). *Mycotrupes pedester* appears to have a relatively limited distribution (see Distribution) and has been classified as Imperiled (Florida Natural Areas Inventory 2009), Critically Imperiled (NatureServe 2009b), and Threatened (Woodruff and Deyrup 1994c). While the assignment of a threat level to a taxon as poorly known as *Mycotrupes* may be somewhat subjective, the limited number of known occurrences of *Mycotrupes pedester*, along with the high rate of human development in Florida, should certainly warrant concern (Woodruff and Deyrup 1994c).
Figure 2-1. Distribution map for species of *Mycotrapes*. Locations are approximate and based on location data from Appendix B. Species color code: Red=*M. retusus*, green=*M. lethroides*, yellow=*M. cartwrighti*, maroon=*M. gaigei*, blue=*M. pedester*, pink=possible cryptic *Mycotrapes* species.

Figure 2-3. Hubbell's hypothesis of evolution in *Mycotrupes* (Hubbell 1954, p. 43).
Figure 2-4. *Mycotrupes* speciation events as hypothesized by Hubbell (1954) (Figure 3, p. 49). Shoreline history (Pliocene to recent) is shown with the supposed distributions of the species of *Mycotrupes*, including common ancestors. For example, "C-P₂" represents the common ancestor of *M. cartwrighti* and *M. pedester.*
CHAPTER 3
BEHAVIOR

Introduction

The behavior of *Mycotrupes* is poorly known, partly because they are rarely observed except in pitfall traps, which provides little information besides habitat and relative abundance. In addition, *Mycotrupes* may spend a considerable portion of their lives underground, where their behavior would not be observed except by excavation of their burrows.

Diel patterns of adult activity are usually not indicated by collecting data, because pitfall traps are often left out for 24 hours or more. Limited field observations by Howden (1954) indicate that *M. gaigei* adults are active during the day and early evening. Howden collected (in pitfall traps) adults of *M. gaigei* in High Springs, and near Archer, Florida between the hours of 12:00PM and 8:30PM.

The activity of adult *Mycotrupes*, as indicated by collecting records, appears to be concentrated in the cooler months (fall, winter, and spring) (Howden 1963). Howden hypothesized that this seasonality represents a shift from summer activity during an earlier (last glacial maximum) cooler period, to fall, winter, and spring activity as the climate warmed. Howden reasoned that a taxon adapted to a cooler climate could respond to a warming climate by either 1) shifting its distribution to a cooler latitude, or 2) shift its activity to a cooler season. *Mycotrupes* is flightless, and apparently restricted in distribution to patchy habitat, so the later possibility seems the more plausible of the two.

Little is known of the feeding habits of *Mycotrupes*. In several instances, adult *Mycotrupes* have been observed feeding on fungus. LeConte (1866) mentioned that *M. retusus* was found under decomposing fungi. This prompted him to propose the name *Mycotrupes*. Horn (1868) mentioned that LeConte distributed specimens to some major European
collections...as fungivorous.” Harpootlian (1995) collected *M. lethroides* from under earthball mushrooms (*Scleroderma* sp).

*Mycotrupes*, like many other geotrupid, are known to dig deep burrows. Burrows as deep as 91 cm (*M. retusus*) and 208 cm (*M. gaigei*) have been recorded (Howden 1954). *Mycotrupes* burrows are often indicated at the surface by mounds of soil ("pushups") up to several inches high. Burrows usually open directly underneath these pushups and extend vertically downwards (Howden 1954; pers. obs.). Burrow diameter appears to be approximately as wide as the beetle (pers. obs.). Other geotrupid beetles dig similar burrows, but with experience it is possible to recognize *Mycotrupes* burrows. For example, beetles of the genus *Peltotrupes* occur in many of the localities where *M. gaigei* is found, and this genus also digs very deep burrows (Woodruff 1973). The greater diameter, and larger soil "pushups," of *Peltotrupes* burrows help distinguish them from the burrows of *M. gaigei*. Burrowing activity in *Mycotrupes* often appears to be concentrated in a given area. Howden (1954) excavated an area three feet wide by six feet long that contained nine burrows of *M. gaigei*.

Adult geotrupids typically provision cells (in burrows) with food for larval development (Howden 1955). The larval food of *Mycotrupes* has been observed only once. On March 20, 1953, H.F. Howden and B.K. Dozier excavated a deep (208 cm) burrow of *M. gaigei* in High Springs, Florida. Several larval cells were found at various depths, but most of the larvae had completed development, and little identifiable food matter remained. In one cell, where the resident larva had not yet completed development, there was material that appeared to be old cow dung. The area was being used as cattle pasture at the time, and cattle dung was abundant. The pupal cells constructed by the *M. gaigei* larvae consisted of an extremely thin (1 mm thick) layer of larval feces (Howden 1954).
The following observations of *Mycotrupes* biology were made during my field and laboratory work. The next section combines these observations with information obtained through personal communications.

**Field and Laboratory Observations on *Mycotrupes* Behavior**

**Adult Feeding Behavior**

*Mycotrupes lethroides*

On November 3, 2007, I traveled to the Yu chi Wildlife Management Area (YWMA) near Girard, Burke County, Georgia. The area in which I made observations was generally pine and oak forest with much open sand. It was soon apparent that *M. lethroides* was active at this location in large numbers. At approximately 3:00 PM, beetles were seen crawling on the surface of a sandy road in an area shaded by oak trees. On this road, I observed a male feeding on an acorn in a shallow, cup-shaped depression (not a burrow), which was apparently excavated by the beetle. The acorn was standing upright, and the beetle was straddling it with its legs wrapped around the acorn. The beetle was eating the soft tissue that was exposed through the top of the acorn. In the process of excavating burrows of beetles in the same area, acorns and oak leaves were found in several of the burrows with single adults (Paul Choate, pers. comm.).

At the YWMA, on November 6, 2007, pig dung-baited pitfall traps, which had been set on November 3, 2007, were picked up. In one of the pitfall traps, I observed a beetle feeding on a caterpillar (Lepidoptera) that had apparently fallen into the trap with the beetle.

A live series (5 males; 5 females) was collected at the YWMA on November 6, 2007, and maintained in the laboratory at the Department of Entomology & Nematology, University of Florida, Gainesville, Florida. The beetles were kept in a glass terrarium (approximate dimensions: 40 cm long, 20 cm wide and 20 cm high), which was mostly filled with sand. The sand was periodically moistened with water. I offered a variety of potential foods to these
beetles. The foods were chosen on their similarity to foods *Mycotrupes* have been observed feeding on in nature. Although it is not naturally occurring, dog food was also offered, because other species of *Mycotrupes* have fed on this material in the laboratory (P. Skelley, pers. comm.).

On November 7, 2007, several peanuts and cashews were soaked in water for 30 minutes and placed in the cage. I observed one female feeding on a cashew. Later, on the same day, this female attempted to drag the cashew backwards. She used her fore-tibial spurs to grasp the cashew and used her middle and hind legs to walk backwards. Although this beetle abandoned the cashew after approximately 10 minutes, all peanuts and cashews were buried by the next day (November 8, 2007).

On December 6, 2007, I observed a female feeding on a cashew. She excavated a burrow next to the cashew, and pulled the cashew down into the burrow.

On November 13, 2007, at 2:20PM, three fresh Portobello mushrooms were placed in the cage; two of these were "planted" with their stems in the sand. Ten minutes later, at 2:30PM, a female started to feed on one of the mushrooms; she excavated a small hole in the sand next to the mushroom, and continued to feed from the hole.

On November 27, 2007, I placed four pieces of dog food (moistened with water) on the sand in the cage. I observed a single female attempting to feed on a piece. Minutes later, she pulled the dog food down into a burrow, holding the dog food with her front legs and walking backwards with her middle and hind legs (Figure 3-1).

Other behavior observed included the following. A pair was observed crawling out of a burrow en copula (S. Bybee and B. Smith, pers. comm.). On several occasions, beetles were observed sitting, or "perching," at the top of a burrow, waving their antennae as if "smelling" the air (Figure 3-2). When disturbed, they would often retreat quickly into the burrow.
**Mycotrupes cartwrighti**

On March 7, 2007, in upland pine vegetation at the Tall Timbers Research Station, Leon County, Florida, several burrows were excavated and found to contain *M. cartwrighti*. At the bottom of one burrow that did not contain any beetles, I found a mass of material that appeared to be hair. I identified it as mammal hair, probably opossum (*Didelphis virginiana* Kerr), after studying the mammal collection at the Florida Museum of Natural History (Gainesville, Florida).

In several instances, adults were found associated with dung. In February, 2007, D. Almquist observed deer dung pellets in a beetle burrow in a field at Elinor Klapp-Phipps Park (Tallahassee, Leon County, Florida). Almquist also found a specimen in a pile of deer dung at Tall Timbers Research Station (Leon County, Florida). On December 13, 2007, in a pine-oak-hickory forest at Miccosukee Canopy Greenway (Leon County, Florida), Almquist found a specimen in a 20 cm burrow under what appeared to be old horse dung (D. Almquist, pers. comm.).

**Mycotrupes gaigei**

*Mycotrupes gaigei* adults were observed feeding on, or in association with, several substances, in the vicinity of an alpaca ranch and a residential area in Summerfield, Marion County, Florida. One beetle was collected as it was apparently feeding on an *Opuntia* pad at the alpaca ranch. The beetle was found on the underside of the pad, which had evidence of feeding damage. Beetles were observed feeding on cat food which had been left in a dish on a porch in the front of a house in the residential area. Burrows, apparently of *M. gaigei*, were observed among alpaca dung at the alpaca ranch in June, 2006 (R.E. Woodruff, pers. comm.). This suggests the possibility that the beetles were using the alpaca dung as food for adults and/or larvae.
On two occasions, adults were found associated with dung of an unknown mammal in sandhill vegetation approximately three miles west of Archer, Levy County, Florida. The dung contained hair, and could have been from a dog, coyote, or bobcat. On the first occasion, one beetle was found feeding on the dung. On the second occasion, one beetle was excavated, along with several dung beetles (*Phanaeus igneus floridanus* d'Olsoufieff), from the soil underneath a pile of dung (P. Skelley, pers. comm.).

**Burrowing**

*Mycotrupes lethroides*

At the YWMA, on November 3, 2007, I excavated burrows of *M. lethroides* in a sandy road in an oak forest. These burrows appeared to be concentrated in areas with packed, moist soil, whereas looser, drier sand covered most of the road. Upon excavation, the burrows were found to be plugged with sand for part of their depth. Four burrows were excavated with a single specimen in each (3 males, 1 female). The beetles were found at depths ranging from 8 to 12 cm. At another location in the YWMA, a single specimen was excavated at a depth of 25 cm.

*Mycotrupes cartwrighti*

On March 7, 2007, I excavated, with the help of D. Almquist and P. Skelley, two *M. cartwrighti* burrows in a longleaf pine forest at Eleanor Klapp-Phipps Park in Tallahassee, Leon County, Florida. The area was hilly, and the soil had an orange-red color. Neither burrow had a pronounced "push up" of excavated soil, but instead had a raised rim of clay at the surface. At the bottom of each burrow was a single male, at a depth of 20 cm in one burrow and 24 cm in the other.

On March 15, 2007, I excavated nine burrows at Thomasville, Thomas County, Georgia. The burrows were in a recently burned pine forest with an open understory and scant leaf litter (*Figure 3-3*). These burrows were similar in depth to those at Eleanor Klapp-Phipps Park.
(approximately 20 cm), and the soil here also had an orange color. Some were covered with small (approximately 5 cm in diameter) pushups of excavated soil (Figure 3-4), while others were open at the top (Figure 3-5). A single beetle was found in each burrow; in total, 6 males and 3 females were collected. The beetles were not found in the open space of the burrow itself, but were found a short distance (~3 cm) away in packed clay that appeared to form a distinct layer in the soil.

*Mycotrupes gaigei*

On March 6, 2008, I excavated a single *M. gaigei* burrow at O'Leno State Park, High Springs, Columbia County, Florida. The site was surrounded by typical sandhill vegetation, including Longleaf Pine (*Pinus palustris* Mill.) and wiregrass (possibly *Aristida* sp.). In the burrow, I found one beetle at a depth of approximately 120 cm. The beetle was located in packed sand approximately 5 cm away in a lateral direction from the apparent termination of the burrow.

At the same location in O'Leno State Park, on March 6, 2008 at 12:00PM, P. Choate and I placed seven pitfall traps baited with pig dung and fermenting malt on the ground in a sandy road in the sandhill area. By 1:40PM, five beetles had been trapped. By 4:15PM (when the traps were removed), two additional beetles had been trapped.

On February 17, 2009, a burrow was excavated in O'Leno State Park. A single female was found at a depth of approximately 160 cm. Most of the burrows that appeared to be of *M. gaigei* at this site were covered with a pushup of excavated soil approximately 7 cm in diameter and 7 cm high. These pushups were sometimes obscured, possibly as a result of rain.
Larval Food

_Mycotrupes gaigei_

In the Spring of 2007, P. Skelley placed five _M. gaigei_, collected from a location west of Newberry, Gilchrist County, Florida, in a glass terrarium. This terrarium was filled to a depth of approximately 25 cm with sand. Dry dog food, which was periodically placed in the cage, was buried by the beetles. In August 2007, rotting leaf litter was placed on top of the sand in the terrarium. This material was also buried by the beetles. On October 26, 2007, P. Skelley and I carefully excavated the burrows in the terrarium. Approximately 5 cm below the surface of the sand, a mass of leaf litter measuring approximately two inches in length was found. As the rest of the sand was excavated, several burrows were found to be partially filled with leaf litter at different depths. At the bottom of the terrarium, a large mass of leaf litter was found which measured approximately 7 cm in length. In this mass, a small (probably first instar) _Mycotrupes_ larva was found. On the other side of the terrarium, also at the bottom, another similar larva was found. I placed the two larvae in individual vials with rotting leaf litter and sand for the purpose of rearing them to the adult stage. On November 28, 2007, one larva was found dead. The other larva was still alive, and appeared to be slightly larger than before. It was seen manipulating, with its mouthparts, light brown plant matter, probably leaf litter. It may have been feeding. Unfortunately, this larva died soon after that observation.

Discussion

Field and laboratory observations suggest that adult _Mycotrupes_ are generalist feeders. Among the foods that _M. lethroides_ was observed to feed upon in the field was an acorn, and acorns were found in burrows of this species. Pérez-Ramos et al. (2007) and Verdú et al. (2007) reported acorn feeding by the geotrupid _Thorectes lusitanicus_ Jeckel in Spain, so this habit may be common in the Geotrupidae. The fact that _M. lethroides_ fed on cashews and dog food, foods
which do not occur naturally over the distribution of any *Mycotrupes* species, further suggests that adult *Mycotrupes* will feed on a wide variety of foods. The ability of adult *Mycotrupes* to exploit a wide variety of foods would be advantageous in a heterogeneous and unpredictable environment. This is especially true because *Mycotrupes* are flightless, and cannot cover as much area in search of food. A dependence on one particular food, such as dung, would be a risky strategy for a flightless species. Known foods of adult *Mycotrupes*, including fungus, acorns, and other materials, vary in relative abundance throughout the year (and across years, for acorns [Whitney et al. 2004, p. 99]), and as a result, the diet of *Mycotrupes* probably varies over time as well.

The larval diet of *Mycotrupes* remains unknown except for a single observation by Howden (1954), in which case the larval food appeared to be old cow dung. In this study, adult *M. gaigei* provisioned larval chambers with decomposing leaf litter in the laboratory. The use of a material by adult *M. gaigei* in the provisioning of larval chambers in the laboratory is not evidence that this material is used in this way in nature. The failure of the two larvae to develop could have been caused by a variety of factors, among them, the possibility that the leaf litter was an inadequate food. However, records of other geotrurpids using leaf litter as a larval food suggests the possibility of similar behavior in *Mycotrupes*. Howden et al. (2007) recorded the use of humus as food for larvae in the Australian geotrupid genus *Bolborhachium*. In addition, leaf litter is used as larval food (along with other materials, such as dung) in the geotrupid genera *Geotrupes* and *Peliotrupes* (Howden 1952; Howden 1964; Young et al. 1955). *Peliotrupes profundus* Howden and *M. gaigei* are sometimes found together, and both species dig deep burrows. One possible function of deep burrows in *Mycotrupes* could be to allow, in a more humid underground environment, the decomposition of refractory leaf litter into a microbe and
fungus-rich food of higher nutritional quality. The ability of *Mycotrupes* to utilize leaf litter as a larval diet would be advantageous as this material is widely distributed.

Field observations made over the course of my study indicate that *M. gaigei* and *M. lethroides* are active during the daytime. This supports Howden's observations of daytime activity in *M. gaigei*. It is therefore likely that the entire genus *Mycotrupes* is diurnal, although the possibility of night-time activity cannot be excluded.

The seasonality of adult *Mycotrupes* activity that was noted by Howden (1963) is supported further by Table 3-1, which is based on the specimens available to me in this study *(Appendix B)*. There are several caveats in the interpretation of this data. It is possible that this apparent seasonality is partially due to seasonal bias in collecting effort. Collectors who are attempting to find *Mycotrupes* would likely collect during seasons that are perceived as being the most productive. The analysis of the large number of *M. cartwrighti* collected in unbaited pitfall traps at Tall Timbers Research Station by D.L. Harris, W.H. Whitcomb, and W.W. Baker, which is currently stored at the Florida State Collection of Arthropods (Gainesville, Florida), might provide valuable information of seasonality. Even barring seasonal collecting bias, it should be remembered that most *Mycotrupes* collecting records are the result of pitfall trapping. This means that, at best, these numbers are indicative of adult activity above ground. There could be seasonal differences in the attractiveness of the baits used in pitfall traps; this might be expected if there are seasonal differences in food preference (e.g., feeding during a period of adult maturation versus gathering of food for the provisioning of larval cells).

Laboratory observations suggest the possibility that mating in *Mycotrupes* takes place underground. *Mycotrupes*, being flightless and apparently diurnal, are probably quite vulnerable to predation when above ground. The restriction of as many activities as possible, including
mating, to underground burrows, may reduce the risk of predation. When *Mycotrupes* were excavated from burrows, they were often located some distance away (several centimeters) from the open burrow and were packed in soil. By ensconcing themselves in soil, *Mycotrupes* may protect themselves from predators that enter their burrows.

Adult *Mycotrupes* were excavated from burrows that ranged in depth from 8 to 160 cm. The limited observational data available suggest that burrow depth may depend on the species of *Mycotrupes*, and possibly on the soil type as well. *Mycotrupes gaigei* dig the deepest burrows known for the genus. Howden (1954) found larvae of *M. gaigei* at depths ranging from 140 to 208 cm. In this study, the excavated burrows of *M. cartwrighti* and *M. lethroides* were much shallower (8-25 cm). Moisture may be important in determining the depth of *Mycotrupes* burrows. This possibility can be illustrated with a comparison between the burrows of *M. gaigei* and those of *M. cartwrighti*. The soil at the bottom of excavated *M. gaigei* burrows often appeared to be moister in relation to the soil at the surface. In the case of *M. cartwrighti*, the depth at which adult beetles were found was often marked with a distinct layer of clay. This suggests two possibilities: 1) the clay impeded burrowing, or 2) the clay provided some favorable condition, such as resistance to desiccation. In addition, the soils in which *M. cartwrighti* occurs in may be more resistant to desiccation, and this species may, as a result, not need to burrow as deeply as *M. gaigei*. The soil present at the type locality of *M. cartwrighti* (6.5 mi E. of Tallahassee, Florida) was described as an Orangeburg sandy loam (Olson and Hubbell 1954). Such soils, with greater clay content, have a higher water retention capacity compared to well-drained sands (which are characteristic of the distribution of *M. gaigei*) (Bouma et al. 1982). Howden et al. (2007), who found the brood cells of the Australian bolboceratine geotrupid *Bolborhachium* to be located just above a layer of clay, proposed the
idea that such a position would be ideal for preserving moisture. Desiccation would be a serious problem for *Mycotrupes* larvae or adults, which are found in well-drained soils. Henderson (1939) states that soils of the Norfolk series, which Olson and Hubbell (1954) note as being present in many of the localities where *M. gaigei* occurs, have a layer of friable sandy clay at a depth of 6-8 feet. This further supports the idea that the deep burrows of *M. gaigei* may be a result of a need to access a soil horizon with a higher capacity to resist desiccation. However, *M. lethroides*, which lives in very sandy soil that appears similar to that inhabited by *M. gaigei*, was found in relatively shallow burrows. A careful study of the different soils associated with *Mycotrupes* burrows might yield much useful information. Differences in soil morphology may be responsible for the apparent absence of *Mycotrupes* from scrub habitat in the central ridge of Florida.

It is possible that *Mycotrupes* excavate distinct burrows for different purposes. For example, burrows constructed for adult feeding and resting could differ in depth (and possibly other characteristics) from those excavated for nidification. This is another reason, besides the fact that so few burrows have been excavated, that it may be premature to compare burrows across species until more is known about the biology of each species. It would also be useful to excavate burrows containing larvae for species other than *M. gaigei*, which is the only species for which a burrow containing larvae has been described. Such fortuitous discoveries (in my experience, excavating burrows has only yielded adults, and it is hard work and time consuming) would allow the study of variation (within and across species) of burrow depth, larval food, design of the larval chamber and developmental phenology. This kind of biological information could aid in the understanding of the biogeography of *Mycotrupes* as well as augment conservation measures aimed at protecting these species.
Although knowledge of the biology of *Mycotrupes* is fragmentary, it is possible to speculate on the timing of the life cycle in the genus. As mentioned earlier, H.F. Howden and B.K. Dozier excavated a mature larva of *M. gaigei*, along with the empty cells of larvae which had presumably recently completed development, in March (Howden 1954). Teneral specimens of *M. gaigei* have been collected in March in Florida (P. Choate, pers. comm.). Monthly totals of *Mycotrupes* collections suggest that a larger number of specimens are collected in the spring, compared to the fall (Table 3-1). Taken together, these data suggest that *Mycotrupes* emerge as fresh adults in the spring. There is no data on development time in *Mycotrupes*; this process could take more than a year. Howden (1954) kept an adult female of *M. retusus* alive in captivity for 13 months, suggesting a lengthy life. The possible use by *Mycotrupes* of such abundant material as leaf litter for larval food, combined with a long reproductive life, would allow for the provisioning of a large number of larval cells, possibly over an extended length of time.

Figure 3-1. Female *M. lethroides* pulling a piece of dog food.
Figure 3-2. *M. lethroides* "perching" at the top of a burrow.

Figure 3-3. *M. cartwrighti* habitat at Thomasville, Georgia. Photo by P. Choate.
Figure 3-4. *M. cartwrighti* burrow with "pushup." Photo by P. Choate.

Figure 3-5. *M. cartwrighti* burrow without "pushup." Photo by P. Choate.
Table 3-1. Seasonality of specimen data from Appendix B.

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CHAPTER 4
VARIATION IN THE PARS STRIDENS OF THE STRIDULATORY APPARATUS OF MYCOTRUPES

Introduction

Stridulation, defined as the production of a "shrill creaking noise by rubbing together special bodily structures" (Merriam-Webster 2009), is common in insects. Although it is perhaps most often associated with the Orthoptera, stridulation is known to occur in at least seven insect orders (Ewing 1989). Stridulation is known to occur during mating, and as a response to disturbance, and it has been shown to have a deterrent effect against predators. Stridulations produced in response to disturbance are termed "distress" stridulations (Bauer 1976; Buchler et al. 1981; Hirschberger 2001; Masters 1979; Winking-Nikolay 1975) and can function to repel predators; the effect on the predator may be auditory or tactile (Dumortier 1963). In insects, stridulation appears to repel predators as diverse as wolf spiders, mice, and sandpipers (a bird) (Bauer 1976; Masters 1979).

Stridulation is known in at least 30 families of Coleoptera, and this behavior has evolved numerous times in the order (Wessel 2006). In beetles, stridulation is accomplished through a wide variety of sound producing structures, perhaps due to the highly sclerotized beetle body (Arrow 1942; Wessel 2006). In the beetle family Geotrupidae, stridulation occurs in adults, and probably in larvae as well, as indicated by the presence of stridulatory structures (Howden 1954; Woodruff 1973). In adults, stridulation has been shown to play a role in competition and mating (Winking-Nikolay 1975). At least two different sound-producing mechanisms are known in adult geotrupids: 1) the coxo-abdominal apparatus, and 2) the thorax-elytral apparatus (Carisio et al. 2004). Although they are composed of different structures, both mechanisms produce sound through rhythmic movements of the abdomen.
The coxo-abdominal apparatus is composed of a pars stridens (file) and a plectrum (scraper). The pars stridens is a raised and ribbed area on the posterior surface of the hind coxa, and is mostly hidden within the coxal cavity. The plectrum is located on the inner face of the coxal cavity. The morphology of the plectrum is poorly understood; it has been described as a sclerified ridge on the posterior border of the abdominal sternite (the "hinterrand" of Winking-Nikolay 1975) and as a field of dentiform processes in the area of the coxal cavity (Palestrini et al. 1988; Palestrini & Pavan 1995; Carisio et al. 2004). Sound is produced through the scraping of the pars stridens by the plectrum. The movement of the plectrum is accomplished through shortening and lengthening of the abdomen; this movement may have been derived from the pre-flight "pumping" movement in Coleoptera (Winking-Nikolay 1975). The coxo-abdominal stridulatory apparatus is common in the Geotrupidae and appears to be best developed in (and possibly present in all members of) the subfamily Geotrupinae (Arrow 1904). Zunino and Ferrero (1988) studied the pars stridens in 17 genera of Geotrupidae and found differences in the density of ribs across species and in some cases sexual differences as well.

*Mycotrupes* species are morphologically similar, and a limited number of characters separate them. *Mycotrupes* stridulate when handled; this may be a defensive behavior. My observations indicated variation in the number of ribs of the pars stridens across species of *Mycotrupes*. The pars stridens had not been previously studied in this genus, and the focus of my study was to investigate whether morphological differences in this structure could be useful for delimiting species.

In order to quantify variation in the number of ribs across different species, 38 specimens of *Mycotrupes* were examined (5-10 specimens per species). For each specimen, body size and
number of ribs per pars stridens were recorded. The effects of species, sex, and body size on the rib count of the pars stridens in *Mycotrupes* are described below.

**Materials and Methods**

Dead, pinned specimens of *Mycotrupes* were studied from several collections (American Museum of Natural History, University of Michigan, United States National Museum) as well as self-collected specimens from across the known range of these species (Florida, Georgia, and South Carolina). Sample sizes of specimens used for the morphological study were as follows: *M. cartwrighti* Olson and Hubbell (N=5; 4♂, 1♀), *M. gaigei* Olson and Hubbell (N=9; 5♂, 4♀), *M. lethroides* (Westwood) (N=5; 3♂ males, 2♀), *M. pedester* Olson and Hubbell (N=9; 4♂, 5♀) and *M. retusus* (LeConte) (N=10; 7♂, 3♀).

All morphological observations were made with a Leica MZ16 dissecting microscope fitted with an ocular micrometer. Because of the possible effect of body flexure on resulting measurements of body length, body size was measured as the width of the pronotum, viewed dorsally, at its widest point. All observed ribs on the pars stridens were counted. It was often necessary to place specimens in hot water for several minutes in order to facilitate flexing the coxa and expose the pars stridens.

The following statistics were used to analyze data: The Mann-Whitney test was used to test the effect of sex on rib count (Avery 2008). An Analysis of Covariance (ANCOVA), implemented in R (R Development Core Team 2008), was used to test two null hypotheses: 1) there are no differences between species in the number of ribs in the pars stridens, and 2) there is no effect of body size (width of pronotum) on the number of ribs in the pars stridens. The small sample sizes precluded the use of ANCOVA to test the effect of sex on rib count. Assistance with the ANCOVA analysis was provided by D. Bustamante.
Results

The general form of the pars stridens in Mycotrupes conforms to that present in Trypocopris and other genera of Geotrupinae (Carisio et al. 2004). Ribs along the main portion of the pars stridens are raised and distinctly pronounced (Figure 4-1). The pars stridens has less relief and the ribs become less pronounced (but still are demarcated by striae) at each end of the pars stridens toward the margins of the coxa. There were no apparent morphological differences between the pars stridens in different species of Mycotrupes, other than the number of ribs.

The number of ribs in the pars stridens varies within and across species of Mycotrupes (Figure 4-2). Mean rib counts were as follows: M. lethroides, 224.6; M. retusus, 169.3; M. gaigei, 120.9; M. cartwrighti, 118.2; M. pedester, 104.2. Within all species of Mycotrupes there were no sexual differences in rib number (Mann-Whitney test) at the 0.05 confidence level: (M. lethroides, U=3, p=1; M. retusus, U=14.5, p=0.38; M. gaigei, U=15, p=0.29; M. cartwrighti, U=2, p=1; M. pedester, U=9.5, p=0.91).

After fitting an ANCOVA model to the data including all species of Mycotrupes, a visual assessment of the residual plots (standardized residuals versus predicted values) indicated a strong, non-random pattern that invalidated the assumption of homoscedasticity; in other words, the variance appeared to differ across species. This pattern was due to the M. lethroides data. Data transformation failed to improve model diagnostics. Thus, a second model, excluding M. lethroides, was fitted. This model conformed better with the assumptions of homoscedasticity and normality of residuals, which increased confidence in testing the two proposed null hypotheses. The results of this new model indicated that the value of the slope for the relationship between pronotum size and rib counts was not significantly different among species (F=0.75, p=0.53), however at least one of the intercepts was significantly different from the intercept of other species (F=131.10, p=2.02e-15), indicating that at least one species had a
significantly different number of ribs. There was a significant positive relationship between size (pronotum width) and rib count (slope=4.37, F=2.02, P=0.03). Pairwise comparisons revealed the following significant differences in intercepts: *M. retusus* > *M. cartwrighti*, *M. gaigei*, *M. pedester* and *M. gaigei* > *M. pedester* (Table 4-1). The results of the ANCOVA support rejecting the null hypotheses proposed, indicating that there are differences in the rib counts among species (suggested by significant intercept differences), and that within species, the number of ribs has a moderate positive relationship with body size. Although *M. lethroides* was excluded from this model, visual inspection of the rib counts of five individuals indicates a difference in rib count between this and the other species (Figure 4-2).

**Discussion**

There are differences in the number of ribs on the pars stridens across species of *Mycotrupes*, and there is a positive relationship between body size and number of ribs. No sexual differences in rib count were found in this study.

The behavioral significance of stridulation in *Mycotrupes* remains unknown. Stridulation may be used as a means of defense against predators, as stridulation has been shown to have a deterrent effect against predators in other beetles. In Australia, Howden et al. (2007) occasionally found flightless ground beetles (Coleoptera: Carabidae) feeding on adults of the geotrupid *Blackburnium* "at the bottom of their burrows." As *Mycotrupes* are not able to fly and presumably spend a considerable portion of their time underground, it might be important for them to have an effective means of repelling any predator that might gain access to their burrows. Young et al. (1955) reported stridulation by the geotrupid *Peltotrupes profundus* upon excavation of their burrows.

Though little is known regarding the life history of *Mycotrupes*, it is possible that communication between adults and/or larvae occurs in underground burrows. Stridulation may
be involved in mating. A mating pair of *Mycotrupes lethroides* was observed leaving a burrow in a laboratory setting, suggesting that mating may occur underground (see Chapter 3) (S. Bybee and B. Smith, pers. comm.). Because *Mycotrupes* species are presently understood to be completely allopatric in distribution, there is no reason to expect continued selection for specific differences in acoustic signaling related to mating.

Aggression is another possible function for stridulation in *Mycotrupes*. The apparently aggregated nature of *Mycotrupes* burrows in the field may promote aggressive interactions, and specimens of *Mycotrupes lethroides* have been observed in the laboratory pushing each other out of burrows (S. Bybee, pers. comm.). The geotrupid *Thorectes intermedius* stridulates aggressively when defending a burrow from invading beetles (Palestrini and Pavan 1995). It is possible that similar aggressive stridulation is used by *Mycotrupes*. Behavioral experiments with *Mycotrupes*, similar to those conducted by Winking-Nikolay (1975), might yield important information regarding the significance of stridulation in this genus.

The stridulations of *Mycotrupes* are clearly audible to the human ear. Recordings were made of stridulations of four species of *Mycotrupes* in the hope of finding possible differences in stridulation resulting from the differences in pars stridens rib count. The recorded stridulations were variable within species as well as within individuals. Unfortunately, the poor quality of the recordings made it impossible to study them further.
Figure 4-1. Pars stridens on the posterior face of the right metacoxa of a male Mycotrupes pedester.

Figure 4-2. Relationship between rib counts of the pars stridens and body size in Mycotrupes.
Table 4-1. Results of the analysis of covariance: pairwise comparisons of the differences between intercepts for the four species model (\textit{M. cartwrighti}, \textit{M. gaigei}, \textit{M. pedester}, and \textit{M. retusus}). D is the value of the difference between the intercepts, t (se) denote the t value and standard error for the test of significant differences between the intercepts, and p is the probability of obtaining a value larger than |t|.

<table>
<thead>
<tr>
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<th>\textit{M. cartwrighti}</th>
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<td>\textit{M. retusus}</td>
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<td>D=55.62 (4.62) t=47.37 se=8.72E-14</td>
<td>D=55.62 (4.62) t=65.06 se=18.72 se=2.00E-16</td>
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CHAPTER 5
DELIMITING SPECIES BOUNDARIES AND DIAGNOSING POSSIBLE CRYPTIC SPECIES IN *MYCOTRUPES*

Introduction

Hubbell (1954) hypothesized that *Mycotrupes* speciated allopatrically in response to sea level changes. Modern phylogenetic methods permit a test of this hypothesis with nucleotide sequence data. But before such a study can be done, it is necessary to have some confidence in the known species limits of *Mycotrupes*. Although molecular data is becoming more and more popular within insect phylogenetics, the identification of insect species is still largely accomplished with morphological characters. Distinguishing *Mycotrupes* species is often difficult, as there are few morphological differences between the species. While Olson and Hubbell (1954) presented characters that facilitate their identification, the geographic origin of a specimen is often the primary criterion used for determining its identity.

Given the conservative morphology, patchy habitat, and flightless habits of *Mycotrupes* (leading to little gene flow between populations), I would suspect, and others have suggested, that there is an increased potential for the existence of cryptic (unrecognized) species in *Mycotrupes* (Woodruff 1973). The problem then becomes how to define a species when species concepts abound (Wheeler and Meier 2000). Species concepts vary in their focus on the process of species formation (i.e., theoretical concepts), and in their ability to diagnose species (i.e., operational concepts). Two of the more recent species concepts, both of which are operational concepts, are the Phylogenetic Species Concept (PSC) of Mishler and Theriot (2000), and the PSC of Wheeler and Platnick (2000). The PSC of Mishler and Theriot define species as monophyletic groups that can be supported by a phylogenetic analysis; they leave the decision for the "cut-off point" (the smallest monophyletic group to be recognized) to the systematist. Wheeler and Platnick define a species as "the smallest aggregation of (sexual) populations or
(asesexual) lineages diagnosable by a unique combination of character states.” They do not require that a species be monophyletic or recoverable in a phylogenetic analysis; it must be defined prior to an analysis based on a unique combination of characters. Their (Wheeler and Platnick) species are testable in that the unique characters must be consistent across all members of the species, and unique to that species. For my study, I decided to test the species boundaries of *Mycotrupes* with the PSC of Mishler and Theriot with the criterion that a species will be recovered as monophyletic.

Because of the morphologically conservative nature of *Mycotrupes*, I determined that the acquisition of molecular sequence data would probably be the most efficient means to obtain a phylogenetic data set. Because Hubbell (1954) hypothesized that *Mycotrupes* speciated in response to geologically recent sea level changes, I was inclined to select a more quickly evolving gene with an increased potential to offer differences across closely related species.

Mitochondrial DNA is a relatively rapidly evolving assemblage of genes, and is a popular choice for low-level phylogenetics and population genetics study in insects (Avise et al. 1987; Funk and Omland 2003). In the case of *Mycotrupes*, the use of the mitochondrial Cytochrome Oxidase I gene (COI) would allow extraction of a potentially rich source of phylogenetic character data from a group that is morphologically conservative and possibly recently speciated.

DNA barcoding is a recent development that seeks to use relatively short DNA fragments for the identification of species. Another possible application of such a short “barcoding” fragment is the separation of groups of sequences into probable distinct species based on a cut-off value of sequence divergence (Hebert et al. 2003). This latter application provides a method of discovering possible cryptic species (which are often, at least initially, morphologically indistinct from other species) based on the discovery of a high degree of sequence divergence.
between sequences. This method could be applied to *Mycotrupes* to assess the amount of divergence between species and possibly determine the presence of cryptic species.

The goal of my study was to test "traditional" species limits in *Mycotrupes*, using phylogenetics and pairwise distances, and to obtain a phylogenetic hypothesis of the relationships between the species for use in a subsequent biogeographic study (see Chapter 6).

**Materials and Methods**

**Sampling**

I attempted to use fresh specimens from as many different collecting localities as possible (Table 5-1). All five species of *Mycotrupes* were collected across much of the known distribution of the genus (Figure 5-1). All, except for *M. lethroides*, were represented by material collected from multiple locations. Historic records for *M. cartwrighti* from the vicinities of Jacksonville, Florida and Americus, Georgia could not be substantiated by my recent collecting efforts. Thus, no samples were available for DNA analysis. Most specimens were collected in pitfall traps baited with a combination of pig dung and fermenting malt solution and were brought back alive, if possible, to maximally preserve DNA. Field collected *Mycotrupes* specimens were stored in 95% ethanol at -80°C in the Branham Laboratory at the University of Florida, Gainesville, Florida.

The use of outgroup taxa is currently the most widely accepted method for the polarization of characters in phylogenetic studies (Maddison et al. 1984; Nixon and Carpenter 1993). It was considered that the ideal outgroup for this study would be one that was as closely related to *Mycotrupes* as possible. Unfortunately, the phylogenetics of the Geotrupidae have received little study, and it was not possible to apply such a criterion for outgroup selection. *Peltotrupes youngi* Howden (Coleoptera: Geotrupidae) was chosen as an outgroup taxon. A fresh specimen was collected from the Ocala National Forest in Florida.
DNA extraction, PCR Amplification, Sequencing and Nucleotide Alignments

DNA was extracted, using the DNeasy blood and tissue kit (Qiagen Inc., Valencia, California), from one or two legs of each individual. Amplifications were carried out using 1μL of extracted DNA template and 1μL of each DNA primer in a 25μL polymerase chain reaction (PCR) containing 12.5μL Accuzyme mix (Bioline, Taunton, Massachusetts), 0.4μL Taq polymerase (Bioline) and 9.1μL autoclaved H₂O. To obtain the 481 bp (length after editing) fragment of the mitochondrial gene Cytochrome Oxidase I, the primers Tonya (5'-GAAGTTTATATTTTAATTTTACCGGG -3') and Hobbes (5'-AAATGTTGNNGRAAAAATGTTA -3') were used (Rand et al. 2000). PCR reactions were done in an Eppendorf Mastercycler EP Gradient Thermocycler (Eppendorf International) with the following cycle parameters: 3 minutes at 95º, 29 cycles of 1 minute at 94ºC, 1 minute at 48ºC and 1 minute at 72ºC, followed by 5 minutes at 72ºC and 15ºC for infinity. The PCR product was visualized for fragment length on agarose gels (1.6 grams agarose, 20ml TBE buffer, 180ml H₂O and 200μL ethidium bromide) in TBE buffer solution. Verified PCR product was purified with a QIAquick PCR purification kit (Qiagen Inc.). The purified product was sequenced in both directions with an Applied Biosystems Model 3130 Genetic Analyzer (Applied Biosystems, Foster City, California) at the Interdisciplinary Center for Biotechnology Research (ICBR), University of Florida. Sequences were edited manually in Sequencher (Gene Codes 1998) or by the ICBR, and then aligned by eye in Sequencher. See Appendix A for sequence data.

Phylogenetic Analyses

Two methods were used for phylogenetic reconstructions. A Parsimony analysis was implemented in PAUP (Swofford 2002), and a Bayesian analysis was implemented in BEAST (Drummond and Rambaut 2006). Because these phylogenetic hypotheses would be used later...
for the biogeographic portion of the study, two different Bayesian analyses were conducted with
different "prior" information to give two different estimates of divergence times. Even though
the time since divergence aspect of these Bayesian analyses is irrelevant for the purpose of
Mycotrupes phylogenetics and species delimitation, the methods are described here.

**Parsimony analysis**

The Parsimony analysis was implemented in PAUP 4.0b10. A heuristic search was
performed with the following settings: starting tree(s) obtained via stepwise addition; addition
sequence = random; number of replicates = 10,000; number of trees held at each step during
stepwise addition = 1; branch swapping algorithm = tree-bisection-reconnection (TBR); steepest
descent option = not in effect; initial 'MaxTrees' setting = 100; 'MulTrees' option = in effect.

Bootstrap support values were calculated in PAUP using 10,000 replicates and 10 random-
addition sequence replicates.

**Bayesian analysis**

BEAST ver. 1.4.8 was used to infer phylogenies and obtain divergence time estimates.
The aligned COI data were imported into BEAUTi (Rambaut and Drummond 2008a), and an
xml file was created for each of the two analyses. The monophyly of the ingroup (Mycotrupes)
was constrained.

In order to obtain a temporal scale for dating nodes on the phylogeny, two approaches
were used: a pre-set rate of nucleotide substitution and a biogeographic calibration. This
required two xml files with different settings (Table 5-2).

For the pre-set rate, I used Brower's (1994) estimate of the sequence divergence rate
(2.3% per million years). This pairwise rate was halved to obtain a lineage divergence rate of
1.15% per million years, entered into BEAUTi as 0.0115 (mean rate). This resulted in the node
ages indicated on the Bayesian trees as millions of years before present.
The biogeographical calibration was done as follows: The topology supported by the BEAST analysis, using the pre-set rate, indicated a clade made up of *M. gaigei* and *M. pedester*, the known distributions of which are less than approximately 50 meters above sea level. The sea level history of the past 100 million years was studied by Miller et al. (2005). Approximately 5 mya the sea level was 50 meters above contemporary sea level. After this point, high stands of sea level were successively lower. Assuming that *M. gaigei* and *M. pedester* never occurred at elevations higher than 50 meters above contemporary sea level, we could assume the ancestor of *M. gaigei* and *M. pedester* arose at a time later than the last time the sea level was at this elevation because such an ancestor would have been inundated if it were present before this time. Even with such an assumption, the 5 mya estimate would only be a maximum age limit of the clade made up by *M. gaigei* and *M. pedester*, meaning that all of the node ages of the resulting tree would be maximum limits. A prior was set for the time since the most recent common ancestor (tMRCA) of the clade (*M. gaigei* + *M. pedester*) as normally distributed with a mean of 5 million years with a standard deviation of 0.1 million years. The use of a normally distributed prior age estimate accounts for uncertainty when incorporating biogeographical events in divergence time estimation (Ho 2007).

The model GTR+I+G was found to be the most appropriate according to a likelihood ratio test in Model Test v3.7 (Posada and Crandall 2005) implemented in PAUP. A likelihood ratio test implemented in PAUP failed to reject a molecular clock so a strict molecular clock approach was used for both the pre-set rate as well as the biogeographical calibration runs.

A Monte Carlo Markov Chain with a length of 10,000,000 was used for the pre-set rate runs; a chain length of 20,000,000 was used for the biogeographical calibration, due to the low Effective Sample Size (ESS) values obtained with a shorter chain. Two chains were run for each
analysis. Parameters and trees were logged every 1000 generations. Tracer (Drummond and Rambaut 2007) was used to check on the trace files of the chain. ESS values over 200 were obtained. LogCombiner (Drummond and Rambaut 2008b) was used to combine the trees sampled in the two chains with a burn-in of 1000 generations. TreeAnnotator (Drummond and Rambaut 2008c) was used to produce a maximum clade credibility tree with a posterior probability limit of 0.5, with a burn-in of 1000 generations. The resulting tree viewed in FigTree (Rambaut 2008) ver. 1.1.2 was checked for posterior clade probabilities and divergence time estimates (with 95% highest probability density bars).

**Nucleotide Divergence**

A matrix of uncorrected p-distances was calculated in PAUP 4.10 from the sequence data. I calculated the mean pairwise divergence within each species of *Mycotrupes*, and the mean divergences across all *Mycotrupes*.

**Testing Alternative Phylogenetic Hypotheses**

I used a method from Wuster et al. (2008) to test the support for alternative phylogenetic hypotheses. The following operation was performed when the maximum clade credibility tree did not contain a monophyletic clade for one or more presumed species. I constructed a target tree constraining only the relationship of interest, in this case, a monophyletic clade containing all of the sequences of the presumed species. This target tree was used to filter, in PAUP, the post-burn-in tree files from the molecular clock BEAST run. The percentage of the total trees that had this constrained monophyletic relationship was used as a measure of support. The alternative hypothesis was rejected if it was supported by less than 5% of the total trees.

**Results**

The Parsimony and Bayesian topologies all support *M. lethroides, M. retusus, M. gaigei,* and *M. pedester,* as monophyletic, but not *M. cartwrighti* (Figures 5-2, 5-3, and 5-4). In the
SumTrees analysis, the clade comprised of all of the *M. cartwrighti* sequences (including the Fenholloway sequence) was recovered in 23% of the molecular clock trees and 25% of the biogeographical calibration trees. Pairwise distances show smaller within-species distances compared to across-species distances. The mean pairwise distance between the Fenholloway haplotype of "*M. cartwrighti*" (I-1 and I-2) and *M. cartwrighti* is closer to the mean pairwise distance across *Mycotrupes* species than the mean within species distance (Table 5-3 and Figure 5-5).

**Discussion**

**Phylogenetics**

With the exception of the "*M. cartwrighti*" haplotype from Fenholloway (I-1 and I-2), all known species of *Mycotrupes* were recovered as monophyletic in both the Parsimony as well as the Bayesian analyses (Figures 5-2, 5-3 and 5-4). The "*M. cartwrighti*" haplotype from Fenholloway (I-1 and I-2) fell out basal to the clade (*M. cartwrighti* (*M. gaigei* + *M. pedester*)), and would therefore make the species, presently defined as *M. cartwrighti*, paraphyletic.

If "*M. cartwrighti*" I-1 and I-2 are not considered part of *M. cartwrighti*, the following support values are assigned to the known species of *Mycotrupes*. The Bayesian analyses gave high posterior probabilities to all species (0.9997-1.0). There was more variation in the bootstrap values obtained in the Parsimony analysis. Bootstrap values were high for the species *M. lethroides*, *M. retusus* and *M. pedester* (99-100) and lower for *M. cartwrighti* and *M. gaigei* (84-85). Posterior probabilities are often higher than bootstrap values for a given clade (Erixon et al. 2003). It is possible that the use of additional sequence data would result in higher bootstrap support for *M. cartwrighti* and *M. gaigei*.

The paraphyletic nature of *M. cartwrighti* suggests that "*M. cartwrighti*" I-1 and I-2 may be a cryptic species. The recovery, in both the Parsimony and Bayesian analyses, of all other
sequences as monophyletic clades conforming to the present concept of *Mycotrupes* species boundaries, further supports that the Fenholloway haplotype represents a distinct species. Olson and Hubbell (1954) did not examine specimens from Fenholloway. Since these specimens appear similar to *M. cartwrighti*, and were collected from an area close to known populations of *M. cartwrighti*, they would likely have been considered to be *M. cartwrighti* by most taxonomists.

I examined two male *Mycotrupes* from Fenholloway, and compared them to male *M. cartwrighti* that were collected from Tall Timbers Research Station (Florida) and several localities in Georgia. Specifically, I examined the head, mouthparts (as this required dissection, I compared one specimen of *Mycotrupes* from Fenholloway to two male *M. cartwrighti* from Tall Timbers), thorax, and male genitalia. I was not able to find consistent morphological differences between the Fenholloway *Mycotrupes* and the *M. cartwrighti* from other locations. Further study may result in the discovery of such differences, however.

Recovery of traditionally circumscribed species as non-monophyletic is actually quite common in molecular phylogenetic analyses. Crisp and Chandler (1996) argued that "paraspecies" (a term which they use to refer to poly- or paraphyletic species) are expected to occur with phylogenetic evolution. Crisp and Chandler expect these paraspecies would eventually become monophyletic through the fixation of mutations, though a certain amount of time would elapse before this occurred. Funk and Omland (2003), who reviewed the phenomenon of polyphyletic animal species and discussed the possible causes for such patterns, found 23% of species to be polyphyletic in the studies they sampled. Funk and Omland recognized that sampling protocol often makes it effectively impossible for workers to recognize polyphyletic species because phylogeneticists often use only one sequence from each species
under study, whereas population biologists sample many sequences within only one species. The result in either case is that monophyly is effectively not tested. With a greater degree of sampling within "species," polyphyly may be found to be more common than is currently recognized.

There are a variety of possible explanations for apparent polyphyly of *M. cartwrighti* (if the Fenholloway haplotype is considered to be part of this species). First, it must be remembered that these phylogenies are gene trees rather than species trees, and they represent the evolution of a particular fragment of the Cytochrome Oxidase I gene. Ideally, the gene tree also should reflect the evolution of the species. If this were the case in this study, we are faced with the paraphyly of a traditional species. Strict adherence to a species concept that species are monophyletic requires one of two actions to be undertaken. The first would be to combine the entire clade subtended by the Fenholloway "*M. cartwrighti*" haplotype into one species, resulting in lumping *M. cartwrighti*, *M. gaigei*, and *M. pedester* into one species. The other would be to recognize the Fenholloway "*M. cartwrighti*" specimens as a distinct species. Considering the strong phylogenetic and morphological support for the distinctness of *M. cartwrighti*, *M. gaigei* and *M. pedester*, it would seem preferable to keep these species separate and recognize the Fenholloway specimens as a new and distinct species. This later action would also preserve the greatest amount of taxonomic stability, an important consideration when making nomenclatural changes.

There also remains the possibility that the gene tree does not reflect the species tree. One plausible explanation for this situation could be that there is insufficient signal in the gene sequence used in the analysis (Funk and Omland 2003). For this reason, it is always preferable to use a larger data set, and if possible, multiple genes. Funk and Omland suggested the use of
bootstrap support as a measure of confidence in the polyphyly of a species; they used the largest bootstrap value that grouped any haplotype of the apparently polyphyletic species with one or more haplotypes of another species to the phylogenetic exclusion of one or more haplotypes of the apparently polyphyletic species. The clade that is composed of *M. cartwrighti* (excluding the Fen holloway haplotype), *M. gaigei*, and *M. pedester* is supported by a bootstrap value of 56%. A collapse of this clade, however, would result in a tritomy composed of the Fen holloway haplotype, the *M. cartwrighti* clade, and the clade composed of *M. gaigei* and *M. pedester*. The Fen holloway haplotype would remain distinct from *M. cartwrighti*, and so the low bootstrap support may not indicate a low measure of support for the distinctness of the Fen holloway haplotype. The collecting and sequencing of additional *Mycotrupes* material from Fen holloway may provide additional sequence data and greater haplotype diversity with which to more strongly assess the distinctness of this population with phylogenetic methods. With such additional data, support for the reciprocal monophyly of the possible cryptic species and *M. cartwrighti* could be calculated.

There also remain additional possibilities as to why a gene tree might not represent the true species tree, such as mitochondrial introgression and incomplete lineage sorting. Both of these situations might be recognized through the addition of sequence data from a different gene, which would make possible the recovery of a different phylogenetic pattern as a result. Once again, a larger data set would give greater confidence in the results.

The phylogenetic hypotheses of *Mycotrupes* depicted in Figures 5-2, 5-3 and 5-4 refute the hypothesis of Hubbell (1954), which was based on his assumptions on morphological evolution and biogeography in the genus *Mycotrupes*. The results of the current study are preferred over the hypothesis of Hubbell, because it is based on a modern phylogenetic analysis
that produces a pattern of relationship based on the contribution of all data points (481 base pairs of COI) in the analysis. Consequently, this analysis does not support the hypothesis of Hubbell that *M. gaigei* is a "primitive" *Mycotrupes* and that *M. cartwrighti* and *M. pedester* are sister taxa.

**Pairwise Distances**

The mean pairwise uncorrected p-distances within species of *Mycotrupes* ranged from 0.8 (in *M. lethroides*) to 4.1% (in *M. gaigei*). The mean intraspecific divergence values for *M. cartwrighti*, *M. retusus*, and *M. gaigei* are all higher than the mean divergences between conspecifics of taxa studied by Hebert et al. (2003). High intraspecific divergences are often observed across geographically isolated populations (Hebert et al. 2003). The exclusion of the anomalous Fenholloway haplotype from *M. cartwrighti* reduces the mean intraspecific divergence of that species from 4.6 to 3.5%.

The mean pairwise divergence across *Mycotrupes* species was 9%. This corresponds to a difference of approximately 43 base pairs in the COI fragment used here. This level of interspecific divergence was attained by the majority of the congeneric species pairs studied by Hebert et al. (2003). Nucleotide divergence above a certain threshold is often considered to indicate a species-level difference. However, rather than blindly applying a pre-determined "cutoff" value based on molecular divergence found in other studies, it is important to consider the divergence values calculated here within the context of *Mycotrupes*. Because *Mycotrupes* are flightless, and its populations are probably isolated geographically, we could expect higher molecular divergence values within species, compared to a taxon with stronger dispersal capabilities that occurs in more contiguous habitat. The mean pairwise molecular divergence across *Mycotrupes* species is 9%, which is twice the value for the greatest observed mean intraspecific divergence (4.1%, in *M. gaigei*). The mean pairwise divergence between the *M.*
cartwrighti haplotype from Fenholloway and sequences contained within the monophyletic M. cartwrighti clade is 7.8%, which is closer to a level of nucleotide divergence seen across Mycotrupes species, and would suggest that the Fenholloway haplotype is a distinct species.

Testing Alternative Phylogenetic Hypotheses

Of the 20,001 trees produced by the two molecular clock BEAST runs, 4,642, or 23%, had the monophyletic clade composed of all M. cartwrighti sequences (including the Fenholloway haplotype). This would suggest that it is not possible to reject the hypothesis of M. cartwrighti (including the Fenholloway haplotype) as a monophyletic clade, and thus, a species. It must be remembered, however, that the other 77% of the trees produced in the BEAST run did not have a monophyletic M. cartwrighti. The results may thus be considered somewhat ambiguous.

Species Delimitation

Two lines of evidence, the phylogenetic topology and the high nucleotide divergence, suggest that the specimens from Fenholloway represent a cryptic species distinct from M. cartwrighti. The phylogenetic support appears to be somewhat weak, however. The choice of species concept can dramatically alter the interpretation of a phylogenetic test of species boundaries, including this one. Wheeler and Platnick (2000), who (in their own words) provided a species concept that gives "...the finest level of resolution of kinds of organisms that can be justified on the basis of constantly distributed, observable attributes," state that phylogenetic species "...are the smallest groups of organisms among which historical patterns of common ancestry may potentially be retrieved and which may not be divided into smaller units with similar properties." Although all of the species of Mycotrupes, with the exception of the Fenholloway haplotype, are supported as monophyletic, there appears to be some degree of geographical structure below the species level.
**Recommendation**

Based on molecular evidence, there may be a cryptic species in what has been considered *M. cartwrighti*. To more rigorously test species boundaries, a larger series of specimens from Fenholloway is needed to obtain more sequence data and further assess morphological variation in the population. If analyses using additional data support the Fenholloway population as a separate clade, there would be stronger evidence for a cryptic species. The 481 base pair data set used in this study is small by modern standards of molecular systematics, and the collection of additional nucleotide data would be a valuable contribution to the phylogenetic analysis of *Mycotrupes*. At the very least, sequencing one additional gene across taxa, possibly a nuclear gene, would increase the amount of data and allow a comparison between the phylogenetic "signals." The presently weak support, in terms of the number of Bayesian trees showing the non-monophyly of *M. cartwrighti*, could change in either direction with the addition of more sequence data.

A larger series of specimens from Fenholloway would provide better insight into the morphological variation present in that population, which could provide increased confidence in any discovered morphological differences between Fenholloway and the remaining *M. cartwrighti* populations.
<table>
<thead>
<tr>
<th>Species</th>
<th>Code</th>
<th>Specimens</th>
<th>State</th>
<th>County</th>
<th>Location</th>
<th>Latitude, Longitude</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. lethroides</em></td>
<td>A</td>
<td>2</td>
<td>GA</td>
<td>Burke</td>
<td>Yuchi Wildlife Management Area</td>
<td>N33° 05.017' W81° 46.535'</td>
</tr>
<tr>
<td><em>M. retusus</em></td>
<td>A</td>
<td>2</td>
<td>SC</td>
<td>Lexington</td>
<td>Near Gaston</td>
<td>N33° 49.406' W81° 11.853'</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>1</td>
<td>SC</td>
<td>Richland</td>
<td>Near Sesquicentennial State Park</td>
<td>N34° 06.120' W80° 54.672'</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>1</td>
<td>SC</td>
<td>Aiken</td>
<td>Hwy 78 and Oak Club Road</td>
<td>N33° 30.678' W81° 33.539'</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>2</td>
<td>SC</td>
<td>Aiken</td>
<td>Webb Pond Road</td>
<td>N33° 27.672' W81° 25.987'</td>
</tr>
<tr>
<td><em>M. cartwrighti</em></td>
<td>A</td>
<td>1</td>
<td>FL</td>
<td>Leon</td>
<td>Eleanor Klapp-Phipps Park</td>
<td>N30° 32.294' W84° 17.340'</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>1</td>
<td>FL</td>
<td>Leon</td>
<td>Eleanor Klapp-Phipps Park</td>
<td>N30° 32.310' W84° 17.363'</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>2</td>
<td>FL</td>
<td>Jefferson</td>
<td>Avalon conservation easement</td>
<td>N30° 23.660' W83° 53.763'</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>1</td>
<td>FL</td>
<td>Leon</td>
<td>Tall Timbers Research Station</td>
<td>N30° 40.279' W84° 14.154'</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>1</td>
<td>FL</td>
<td>Leon</td>
<td>Tall Timbers Research Station</td>
<td>N30° 40.317' W84° 14.175'</td>
</tr>
<tr>
<td></td>
<td>H</td>
<td>2</td>
<td>GA</td>
<td>Thomas</td>
<td>Thomasville</td>
<td>N30° 49.693' W84° 00.690'</td>
</tr>
<tr>
<td>(cryptic species?)</td>
<td>I</td>
<td>2</td>
<td>FL</td>
<td>Taylor</td>
<td>Fenholloway</td>
<td>N30° 04.974' W83° 30.476'</td>
</tr>
<tr>
<td></td>
<td>J</td>
<td>2</td>
<td>GA</td>
<td>Liberty</td>
<td>Hinesville</td>
<td>N31° 52.479' W81° 34.452'</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>1</td>
<td>FL</td>
<td>Liberty</td>
<td>Torreya State Park</td>
<td>N30° 33.536' W84° 57.016'</td>
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<tr>
<td><em>M. gaigei</em></td>
<td>A</td>
<td>2</td>
<td>FL</td>
<td>Lafayette</td>
<td>Mayo</td>
<td>N30° 03.706' W83° 11.427'</td>
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<td></td>
<td>C</td>
<td>2</td>
<td>FL</td>
<td>Suwannee</td>
<td>Hildreth</td>
<td>N29° 59.626' W82° 48.633'</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>2</td>
<td>FL</td>
<td>Madison</td>
<td>State Road 53, N of County Line</td>
<td>N30° 16.459' W83° 17.320'</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>1</td>
<td>FL</td>
<td>Marion</td>
<td>Summerfield</td>
<td>N29° 00.618' W82° 08.058'</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>1</td>
<td>FL</td>
<td>Marion</td>
<td>Marion Oaks</td>
<td>N29° 01.382' W82° 14.770'</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>2</td>
<td>FL</td>
<td>Lafayette</td>
<td>W. of Mayo</td>
<td>N30° 08.964' W83° 19.719'</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>2</td>
<td>FL</td>
<td>Seminole</td>
<td>Geneva</td>
<td>N28° 44.754' W081° 07.801'</td>
</tr>
<tr>
<td></td>
<td>O</td>
<td>2</td>
<td>FL</td>
<td>Gilchrist</td>
<td>W. of Newberry</td>
<td>N29° 37.879' W082° 42.313'</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>2</td>
<td>FL</td>
<td>Columbia</td>
<td>O'Leno State Park</td>
<td>N29° 55.001' W82° 35.057'</td>
</tr>
<tr>
<td><em>M. pedester</em></td>
<td>A</td>
<td>2</td>
<td>FL</td>
<td>Lee</td>
<td>Estero</td>
<td>N26° 28.472' W81° 50.165'</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>1</td>
<td>FL</td>
<td>Lee</td>
<td>Babcock Ranch</td>
<td>N26° 45.665' W81° 40.848'</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>1</td>
<td>FL</td>
<td>Lee</td>
<td>Babcock Ranch</td>
<td>N26° 45.578' W81° 40.869'</td>
</tr>
</tbody>
</table>
Figure 5-1. Map of collecting locations (colored circles) of sequenced *Mycotrupes* specimens. Species color code: Red=*M. retusus*, green=*M. lethroides*, yellow=*M. cartwrighti*, maroon=*M. gaigei*, blue=*M. pedester*.

Table 5-2. Settings for BEAUTi .xml files.

<table>
<thead>
<tr>
<th></th>
<th>Pre-set rate</th>
<th>Biogeographical calibration</th>
</tr>
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<tbody>
<tr>
<td>Substitution model</td>
<td>GTR</td>
<td>GTR</td>
</tr>
<tr>
<td>Base frequencies</td>
<td>Estimated</td>
<td>Estimated</td>
</tr>
<tr>
<td>Site heterogeneity model</td>
<td>I+G</td>
<td>I+G</td>
</tr>
<tr>
<td>Gamma categories</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Partition into codon positions?</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Fix mean substitution rate?</td>
<td>Yes (0.0115)</td>
<td>No</td>
</tr>
<tr>
<td>Molecular clock model</td>
<td>Strict clock</td>
<td>Strict clock</td>
</tr>
</tbody>
</table>
Figure 5-2. The single most parsimonious tree (L = 319 steps, CI = 0.56, RI = 0.85) recovered in the Parsimony analysis. Bootstrap values (>70%) are reported below the branches. Note that *M. cartwrighti* specimens and the possible cryptic species from Fenholloway (*"M. cartwrighti"* I-1 and I-2) are highlighted in red.
Figure 5-3. BEAST maximum clade credibility tree, pre-set substitution rate. 95% HPD (in millions of years ago) are in brackets; Posterior probabilities of clades are on branches below clades (red=<0.95). Note that *M. cartwrighti* specimens and the possible cryptic species from Fenholloway ("*M. cartwrighti*" I-1 and I-2) are highlighted in red.
Figure 5-4. BEAST maximum clade credibility tree, biogeographical calibration. 95% HPD (in millions of years ago) are in brackets; Posterior probabilities of clades are on branches below clades (red=<0.95). Note that M. cartwrighti specimens and the possible cryptic species from Fenholloway (“M. cartwrighti” I-1 and I-2) are highlighted in red.

Table 5-3. Mean pairwise distances in Mycotrupes.

<table>
<thead>
<tr>
<th>Within Species:</th>
<th>P-distances (uncorrected)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. lethroides</td>
<td>0.0083</td>
</tr>
<tr>
<td>M. retusus</td>
<td>0.0270</td>
</tr>
<tr>
<td>M. pedester</td>
<td>0.0076</td>
</tr>
<tr>
<td>M. cartwrighti (incl. Fenholloway)</td>
<td>0.0459</td>
</tr>
<tr>
<td>M. cartwrighti (excl. Fenholloway)</td>
<td>0.0345</td>
</tr>
<tr>
<td>M. gaigei</td>
<td>0.0406</td>
</tr>
<tr>
<td>Across species:</td>
<td></td>
</tr>
<tr>
<td>Fenholloway (site I) and all other M. cartwrighti</td>
<td>0.0779</td>
</tr>
</tbody>
</table>
Figure 5-5. Mean pairwise distances in *Mycotrupes*. Mean across *Mycotrupes* species, mean within *Mycotrupes* species and pairwise distance between "*M. cartwrighti*" I-1, I-2 and other *M. cartwrighti*. 
CHAPTER 6
BAYESIAN PHYLOGENETIC INFERENCE AND BIOGEOGRAPHY OF MYCOTRUPES

Introduction

The evolutionary hypothesis of *Mycotrupes* by Hubbell (1954) (Figure 2-1) was based on assumptions of morphological evolution in the genus, and it was inextricably linked with his biogeographical hypothesis as well. Hubbell proposed that speciation in *Mycotrupes* was coincident with, and the result of, the Quaternary fluctuations in sea level that affected the southeastern Atlantic Coastal Plain. Although historic changes in sea level, largely attributed to the growth and melting of continental ice sheets, are taken for granted now, it was not always the case (Miller et al. 2005). Charles Schuchert (1910), the great palaeogeographer, was one of the early proponents of the concept of eustatic changes in sea level. Schuchert developed maps showing various inundations of North America by the sea from the Cambrian to the Pleistocene. His map of the Eocene shows inundation to the point of the Fall Line of the southeastern United States.

Biogeography is, simply stated, the study of how organisms how come to be where they are today. Neill (1957) studied the biogeography of Florida. He focused on patterns of distribution common to multiple taxa and speculated on the mechanisms that may have been responsible for these distributions. None of the patterns he described appear to have any special similarity to distributions of *Mycotrupes* species. In attempting to explain some of these patterns, Neill proposed that the broad embayments along rivers that would have resulted from flooding in Pleistocene interglacials may have been a more important cause of the present-day distributional patterns than the rivers as they are today. Thus, the Savannah River may, at a past time with much higher sea level, have been an even broader (and more significant) barrier between *M. lethroides* and *M. retusus*. In this way, even if the Savannah River has existed since
before the ancestor of *Mycotrupes*, such an ancestor could have been distributed on both sides of the river, and isolation (followed by speciation) may have been a result of a rise in sea level and the consequent formation of a broad embayment along the river's course. Also of possible relevance to this study of *Mycotrupes*, Neill proposed that "a number of distinctive west coast organisms may once have ranged over an extensive territory now largely submerged."

Very little work appears to have been done on insect biogeography in the southeastern United States. Lamb and Justice (2005) studied insect phylogeography across ridges of scrub habitat in Florida. This work was at the population (below species) level. Lamb and Justice found that genetic diversity was partitioned primarily by North-South trending ridges, and they presented evidence that the older ridges (those more inland, such as the Lake Wales Ridge) provided a source of colonists for younger ridges closer to the coast.

Marine terraces are a prominent feature of the Atlantic Coastal Plain of the United States, and they represent prolonged high sea stands. It is generally agreed that the higher terraces are older than the lower ones, and that during fluctuations of sea level, the high stands (interglacials) have been progressively lower in elevation over time until the present (Alt and Brooks 1965; Colquhoun et al. 1991). While marine terraces have been mapped in Florida and Georgia (Healy 1975; MacNeil 1949), such work has not been completed for South Carolina.

Not long after the work of Hubbell (1954), cladistic phylogenetic methods became available that would have enabled inference for the sequence of speciation in the genus, if significant morphological character data had been available. However, to study the biogeography of *Mycotrupes* in relation to particular events such as changes in sea level, it is important to not only resolve the phylogenetic relationships within the genus, but to obtain an
estimate of the divergence times as well. At present, the estimation of divergence times requires the use of molecular sequence data.

To impose a time scale on a phylogeny based on nucleotide change, it is necessary to assume a molecular clock. The molecular clock, proposed by Zuckerkandl and Pauling (1965), has been applied to a wide variety of evolutionary questions. The central assumption is that nucleotide base changes are accumulated at a more-or-less regular rate, and as a result, the amount of divergence between two sequences can be converted to a time scale. Such a tool has obvious applications to biogeography, especially when fossil data are lacking. Two species separated by an obvious barrier may have a divergence estimate congruent with the estimated age of the barrier (for example, a river or mountain range). Such a temporal congruence would provide support for the hypothesis that the formation of the barrier resulted in a vicariant event that separated the two taxa under study. Alternatively, a divergence date later than the age of the barrier could suggest a dispersal event that occurred subsequent to the age of the barrier.

Some concerns, regarding application of the molecular clock to biogeography, include the potentially unrealistic assumption of rate homogeneity across lineages and time, and wide confidence intervals, which make it difficult to correlate a divergence to a particular biogeographic event (furthermore, such events may themselves have wide confidence intervals). These issues and others are discussed in Arbogast et al. (2002) and Ho (2007).

Another, potentially more problematic, issue with the molecular clock is the clock calibration method. Use of dateable fossils may be the least assumption-laden method of calibrating a molecular clock. Unfortunately, there are no known fossils of Mycotrupes. In this study, two other methods were used to calibrate the molecular clock and apply a time scale to the phylogenetic hypothesis: a pre-set nucleotide substitution rate and biogeographical calibration.
When applying a molecular clock with a pre-set nucleotide substitution rate to sequence data, the researcher imports a rate of nucleotide substitution obtained from a taxonomic group that is more or less related to those being studied. Brower's (1994) 2.3% pairwise divergence per million years is probably the most widely used molecular clock rate in insect studies. Brower calculated this rate from a study of exemplar taxa of four insect orders (Coleoptera, Diptera, Hemiptera, and Orthoptera) and one species of decapod crustacean (*Alpheus* sp.); calibration was accomplished through assumed dates of divergence, including geological and paleoclimatological events.

Estimated times of biogeographical events have also been used to calibrate molecular clocks (Shoo et al. 2008; Weir and Schluter 2008; Zhang et al. 2008). Renner (2005) noted that "...constraining nodes in a phylogenetic tree by geological events risks circularity in biogeographic analyses because it already assumes that those events caused the divergence, rather than testing temporal congruence." While this is indisputable, it might be argued that some information can be gained through this method by testing for geological events other than the one(s) used for calibration. By assuming that nucleotide changes are more-or-less clock like, but not wishing to assume a certain value for the rate of change (which has often been calibrated in other taxonomic groups), we can examine the relationship between speciation events and geologic events, calibrating the analysis with the "safest" biogeographical event.

The program Bayesian Inference Sampling Trees (BEAST) (Drummond and Rambaut 2006) implements Bayesian inference in order to sample tree space, allowing the user to impose a great variety of molecular models. Especially significant is the users' ability to apply "priors" to the model. Priors are any constraints that the user wishes to impose on the evolutionary model, and can include dated calibration points such as fossils and biogeographical events.
Because divergence times can be calculated within trees, BEAST has seen much recent application to questions of biogeography and coevolution (Matheny et al. 2009; Light and Reed 2009). The goal of this study was to use the phylogenetic hypotheses and divergence date estimates for *Mycotrupes*, produced with COI data (see Chapter 5), attempting to link divergences within *Mycotrupes* with biogeographic events. The two BEAST analyses used different models of nucleotide substitution rates (pre-set substitution rate and biogeographical calibration) under a strict molecular clock. The biogeographical implications of these new data are discussed.

**Materials and Methods**

I used the maximum clade credibility trees resulting from the BEAST analyses for the biogeographical study. One of these BEAST analyses was done with a pre-set molecular clock rate (Figure 5-3); the other was done with a biogeographical calibration (Figure 5-4). Further details on the BEAST analyses can be found in Chapter 5.

**Results**

**Nucleotide Substitution Rates and Divergence Time Estimates**

The nucleotide substitution rate for the pre-set rate analysis was specified at 1.15% per million years. The mean rate obtained in the biogeographical calibration runs was 0.058 (5.8%), with a standard deviation of 0.00013 (0.013%).

The divergence times (in terms of range of 95% highest posterior density) indicated by the resulting topologies are given in Table 6-1. The two methods produced different confidence intervals for the divergence times.
Discussion

Tree Topologies

There were few differences in BEAST tree topology between the pre-set rate and biogeographical calibration analyses. The mean rate obtained in the biogeographical calibration analysis was 5.8% per million years, which is much higher than the rate used for the pre-set runs (1.15%). This means that the assumption of the maximum age constraint imposed in the biogeographical calibration on the divergence of the *M. gaigei* + *M. pedester* clade at 5 mya is inconsistent with an assumed nucleotide substitution rate of 1.15%. The use of a divergence date younger than 5 mya would have resulted in an even more rapid substitution rate.

Species-Level Biogeography in *Mycotrupes*

Comparison of divergence times of two calibration methods

The biogeographical calibration analysis assumed that the ancestor of *M. gaigei* and *M. pedester* could not have diverged from *M. cartwrighti* earlier than 5 mya, and this assumption was associated with a nucleotide substitution rate of around 5.8%. If this divergence in fact occurred later than 5 mya, this would require an even higher rate of substitution. This rate (5.8%) was much higher than the pre-set rate used in our companion analysis, which then produced an earlier divergence time for *M. gaigei* and *M. pedester* from *M. cartwrighti* (excl. Fenholloway haplotype) between 5.6-11.3 mya.

The 95% highest probability distribution (HPD) values of divergence times were broad (Table 6-1). Such broad confidence intervals make it very difficult to correlate estimated divergence times with events such as a specific shift in sea level, because these changes have occurred frequently over the past 10 million years. In one of the more recent studies, Miller et al. (2005: Figure 4) show hundreds of shifts in sea level over this time. Although a limited number of marine terraces have been mapped in the southeastern Atlantic Coastal Plain, they
represent only the relatively prolonged high stands. The actual history of sea level was more complex, and an inundation of brief duration would be sufficient to extirpate a population of *Mycotrupes*. Alt and Brooks (1965) state that "it is possible to find local topographic evidence somewhere in Florida for an abandoned shore line at almost any elevation." Divergence time confidence intervals of *Mycotrupes* could only be narrowed with the addition of more information, such as dated fossils.

**Dispersal may complicate taxon-area biogeography**

Due to their flightlessness and the conception of their habitat as remnants of old shorelines, it is tempting to consider the distributions of *Mycotrupes* as static, minimizing the role of dispersal relative to that of vicariance. The possible role of dispersal is especially problematic when attempting to relate the evolution of *Mycotrupes* to changes in sea level, long thought to be the primary driving force for speciation within the genus.

The relative importance of dispersal in the resulting distribution of *Mycotrupes* species is not known, but circumstantial evidence reveals that it may have been quite important. For example, the known collecting localities of *M. pedester* (including imprecise label localities of Arcadia and Punta Gorda) are between >1 and 17 meters above sea level (Arcadia is the highest in elevation, at 17 meters asl) (Google 2006). The most recent high-stand in sea level, as indicated in Miller et al. (2005), was almost 30 meters above sea level at approximately 200,000 years ago. Such a high sea level would have completely inundated the known distribution of *M. pedester*. Olson and Hubbell (1954) hypothesized that *M. pedester* might occur farther north, possibly in the central ridge of Florida, but no species of *Mycotrupes* is known from the central ridge.

Another example suggests that dispersal may have been important in *M. gaigei*. Most of the collecting localities for this species range in elevation from 11 to 30.5 meters above sea level
(Google 2006). The Old Town site is at a much lower elevation, 7.6 meters above sea level (Google 2006). The Old Town site is near the Suwannee River, which may have influenced drainage locally through re-working sand deposits. It is possible that *M. gaigei* was able to disperse southwards along the Suwannee River after the ocean last receded from a level that would have inundated this site. A nested clade analysis of *M. gaigei*, with greatly increased sampling, might reveal such a historical pattern.

*Mycotrupes cartwrighti* has been collected from sites that range in elevation from 10 (Hinesville, Georgia) to 80 (Thomasville, Georgia) meters above sea level (Google 2006). This distribution cannot be conveniently related to a given sea level. The lower, more coastal occurrences (such as Hinesville) could be a result of dispersal after a recession of the sea from such areas.

**A vicariance-based biogeographical interpretation**

Figure 6-1 is a depiction of the hypothesized vicariance events supported by the BEAST topologies. Backed up with the phylogeny from the phylogenetic analysis, the following scenario is proposed to explain the biogeography of this genus. Numbers correspond to vicariance events depicted on Figure 6-1.

*Mycotrupes* originated on the Fall Line, or in the vicinity, possibly some time during the Tertiary. The ancestor of (*M. retusus* (cryptic *Mycotrupes* species from Fenholloway (*M. cartwrighti* (*M. gaigei*+*M. pedester*)))) was isolated from *M. lethroides* possibly by the Savannah River (1). At some point after the sea level had dropped below the Fall Line sandhills for the last time, a southward dispersal event from *M. retusus* gave rise to the ancestor of (cryptic *Mycotrupes* species from Fenholloway (*M. cartwrighti* (*M. gaigei*+*M. pedester*))) after an event such as a rise in sea level cut off this area from *M. retusus* (2). An event such as a rise in sea level isolated the ancestor of the cryptic Fenholloway *Mycotrupes* from *M. cartwrighti* (3).
*Mycotrupes cartwrighti* was separated from the ancestor of (*M. gaigei + M. pedester*) possibly in northern Florida (4). Later, *M. pedester* and *M. gaigei* were separated (5). The distributions of *M. gaigei* and *M. pedester* are separated by more than 100 miles. The present land exposed in the state of Florida is only approximately one half of the "Florida Plateau," much of which is shallowly submerged along the west side of the peninsula (Randazzo and Jones 1997; Vaughan 1910). A portion of this now-submerged area was exposed as recently as the last glacial maximum (Cooke 1945). Considering that *M. pedester* is found very close to the coast, it is tempting to consider the possibility that there may have been habitat on the Florida shelf at a time with lower sea level that would have exposed this currently submerged area that allowed dispersal of the ancestor.

The site near Fenholloway, Florida, where the apparent cryptic species of *Mycotrupes* was collected, is approximately 12 miles from Townsend, Florida, where *M. gaigei* has been collected. Land along this 12 mile stretch is predominantly poorly drained pine flatwoods. This is geographically the closest that any two species of *Mycotrupes* are known to occur.

Because all species of *Mycotrupes* are currently understood to be allopatric, vicariance would appear to be the most likely cause of speciation. It might be possible to identify barriers to dispersal, past or present, between species distributions that may have played a role in vicariance. Barriers for *Mycotrupes* could include areas that have been inundated by marine transgressions in the past, bodies of water (such as rivers), and poorly drained habitat.

The general sequence of speciation, as proposed by the results of the phylogenetic study, suggests that sea level played a role in the evolution of *Mycotrupes*. The two most basal species, *M. lethroides* and *M. retusus*, occur on the highest, most inland habitat, which would suggest that an ancestral *Mycotrupes* was restricted to the Fall Line sand hills, when the sea covered much of
the lower elevations of the Atlantic Coastal Plain. The two most derived species (*M. gaigei* and *M. pedester*) occupy lower elevations where more recent high-stands of the sea could have caused vicariance. The species *M. gaigei* appears to occupy a fairly restricted range in elevation. Of the 20 collecting sites used in the niche modeling analysis (Chapter 7), 13 are within the range of elevation listed by Colquhoun et al. (1991) for the Penholloway Terrace (12.9-23.0 m asl). If the elevations for the Wicomico Terrace is included also (12.9-30.3 m asl), 18 sites out of 20 fall within this range. There has been deformation of the bedrock in Florida, which would have changed the terrace elevations (Harper 1921 [in reference to the peninsular lime-sink region]; Opdyke et al. 1984 [in reference to Florida in general]). While the degree to which this has affected terrace elevations is unknown, the use of elevation intervals to indicate marine terraces may be problematic.

The role the Savannah River might have played in the split between *M. lethroides* and *M. retusus* is an interesting problem. The Savannah River is an obvious barrier to dispersal between populations of these two species. The age of the Savannah River is not precisely known, although rivers that drain the Appalachian Mountains, such as the Savannah, could be as old as Early to Middle Miocene (T. Scott, pers. comm.). Such an age would suggest that it was present before much of the lower Atlantic Coastal Plain habitat of *M. cartwrighti, M. gaigei,* and *M. pedester* was most recently exposed. Over time, rivers have changed their course and they presumably change their flow volume as a result of changes in climate. Thus, it may not be realistic to use the present-day Savannah River as a proxy for the Savannah River of the past with regards to its possible biogeographical importance. Under the assumption that the Savannah River was the cause of the split between *M. lethroides* and the ancestor to the rest of the species, there are two possibilities to the mechanism of isolation: 1) a chance dispersal event
across the Savannah River, or 2) a shift in the river's course, dividing a once-continuous population. There is also the possibility that a rise in sea level to the level of the Fall Line could have inundated the area of the river, thus isolating the two populations.

It is possible that climate change over the past 10 million or so years may have had a significant effect on the distribution of *Mycotrupes*. Apart from the obvious impact of inundation with rising sea levels, changes in water table, vegetation, and weather may have resulted in *Mycotrupes* populations moving, making it difficult to study their biogeography. Such changes are undoubtedly occurring at the present time, and the distributions of *Mycotrupes* species are probably changing in response.

**Comparison with studies of other taxa in the same region**

Scrub and sandhill are two major vegetation assemblages that occur on well-drained uplands in the southeastern Atlantic Coastal Plain. Sandhill (which is widespread in the Atlantic Coastal Plain in the southeastern United States) and scrub (restricted to Florida) are often confused, but are characterized by distinctly different plant assemblages (Laessle 1958; Myers 1985). *Mycotrupes* is often collected in habitat that could be characterized as sandhill, but the genus is not known to occur in scrub. *Mycotrupes* is also collected in habitat with vegetation that could not be referred to as sandhill, including grassy fields, although this may have been modified by humans from previous sandhill habitat. The reasons for the apparent preference for sandhill are not known, but they may include factors such as a more open understory in sandhill or differences in soil type.

Sand ridges in Florida, which generally trend North-South, have been named and mapped (Brooks 1981). Although some *Mycotrupes* habitat may be associated with such ridges (for example Brooksville Ridge in Newberry and Archer), sandy soil appears to be more important
than a well-defined ridge, and other localities that host *Mycotrupes* are not part of an obvious ridge system.

A significant amount of work has been done on the population genetics of animals inhabiting Florida scrub. Lamb and Justice (2005) studied insect phylogeography across ridges of scrub habitat in Florida. All of this work, however, was at the population (below species) level, and they did not estimate times of divergence. In general, they found the genetic diversity to be partitioned primarily by North-South trending ridges, and they presented evidence that the older ridges (those more inland, such as the Lake Wales Ridge) provided a source of colonists for younger ridges closer to the coast. These results would somewhat agree with those of this study, as the more derived *Mycotrupes* species occur, in general, at lower elevations.

**Future Research**

Little work has been done using modern phylogenetic tools to study the species-level biogeography of southeastern insects. More attention has been given to the molecular phylogeography (below the species level) of Florida scrub animals, including insects (e.g. Lamb and Justice 2005). This work on *Mycotrupes* will provide an interesting comparison to future work on species-level biogeography in the southeastern United States. One remaining significant barrier to such work that remains is the lack of clear-cut, dateable vicariance events. It should still be possible in a historical biogeographic framework to compare the speciation patterns of different groups to support or reject common speciation mechanisms in this region.

The study of plants that occur in similar situations may offer some information that could aid in understanding the biogeography of *Mycotrupes*. *Ceratiola ericoides* Michaux, or Scrub Rosemary, a common shrub in sandhill and scrub habitat in the Atlantic Coastal Plain of the southeastern United States, is thought to have expanded its distribution during the glacial maxima (Trapnell et al. 2007). There is evidence that much drier conditions in the past, along
with a lower water table, favored xeric vegetation in Florida (Watts 1975). *Mycotrupes* may have expanded in distribution during these dry periods, and what today appear to be isolated populations may have then been more extensive with a higher degree of connection.

It is likely that further *Mycotrupes* character data will be in the form of nucleotide sequences. As I used only a fragment of a single gene (481 base pairs of Cytochrome Oxidase I) in this study, additional sequence data may result in significantly different topologies and a different biogeographic hypothesis for *Mycotrupes*. 
Table 6-1. Selected divergence time estimates with 95% HPD.

<table>
<thead>
<tr>
<th>Divergence</th>
<th>Pre-Set Rate</th>
<th>Biogeographical Calibration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peltotrupes-Mycotrupes</td>
<td>15.5-42.5 mya</td>
<td>7.9-16.8 mya</td>
</tr>
<tr>
<td>M. lethroides-(rest of Mycotrupes)</td>
<td>12.0-28.4 mya</td>
<td>7.0-13.7 mya</td>
</tr>
<tr>
<td>M. retusus-(rest of Mycotrupes)</td>
<td>8.9-20.8 mya</td>
<td>5.8-10.8 mya</td>
</tr>
<tr>
<td>M. gaigei-M. pedester</td>
<td>4.8-9.7 mya</td>
<td>4.8-5.2 mya</td>
</tr>
</tbody>
</table>

Figure 6-1. Collecting locations of Mycotrupes specimens used in this study with numbered (1-5) vicariance events. Green = M. lethroides, red = M. retusus, yellow = M. cartwrighti, pink = Mycotrupes from Fenholloway, maroon = M. gaigei, blue = M. pedester.
CHAPTER 7
EMPLOYING ECOLOGICAL NICHE MODELING TO PREDICT SPECIES DISTRIBUTIONS IN MYCOTRUPES

Introduction

Because of the secretive habitats of the adults, Mycotrupes are rarely collected except when they are specifically targeted with baited pitfall traps. Hence, it is possible that significant portions of their distribution remain unknown. The only way to know with certainty if a species occurs in a given area is to collect a specimen there. However, it isn't practical or efficient to collect across a large geographic area. In addition, it would be beneficial to know why a species occurs where it does, i.e. what its habitat requirements are. Such information can be used to target surveys for the species, guide conservation efforts, and it can help us understand the biogeography of the group in question. Niche modeling can provide possible answers to these questions.

The goal of niche modeling is to develop a model of a species' distribution, based on areas where the species is known to occur (and sometimes also incorporating areas where the species is known not to occur), using environmental data. An inherent assumption is that the biology of the organism is related to its environment, and that environmental data can then be used to predict the geographic distribution of the species. This is, of course, only possible if biologically informative data are used in the analysis.

The Geotrupidae have received little attention with regard to niche modeling. Lobo et al. (2006) studied the distribution of two species of the flightless geotrupid genus Jekelius in the Iberian Peninsula, by using logistic regression. They found that both climate and substrate were important factors in the distribution of these species. Barriers to dispersal (in this case, rivers) appeared to be important in further limiting the distributions of the species.
Maximum Entropy (MaxEnt) has become a popular program for predicting species distributions. MaxEnt exhibits superior performance, compared to other niche modeling programs, in terms of area under receiver operating characteristic curves (AUC) and omission rates (Elith et al. 2006; Phillips et al. 2006). In addition, MaxEnt appears to perform well even at small sample sizes of presence data (Hernandez et al. 2006). MaxEnt is a maximum likelihood algorithm that attempts to find the model with the highest entropy, in other words, the model explaining the distribution of a species with the fewest constraints. Two types of data are necessary for MaxEnt: 1) environmental layers, and 2) presence data (i.e., points of known species occurrence). All environmental layers must be in raster form with identical dimensions and coverage, because MaxEnt uses a grid in which each cell is assigned a certain value for each environmental layer, as well as a presence or absence status for the species under study. MaxEnt does not require that known absence data points be input; a pseudo-absence method is used to approximate absence data from background pixels chosen at random. The MaxEnt output includes a map with a likelihood of occurrence value given for each grid cell. The reader is referred to Phillips et al. (2006) for a more in-depth discussion of MaxEnt.

I used MaxEnt to generate predicted distributions of the five recognized species of *Mycotrupes* by using several types of environmental data. The Fenholloway site was excluded because of the evidence supporting a cryptic *Mycotrupes* species at that site, and because only one locality is known (see Chapter 5). There were four goals of this study: The first was to improve the knowledge of the distributions of *Mycotrupes* species. The second was to observe the effects of using different types of environmental data (climate and soil data). Because of the burrowing habits of *Mycotrupes*, soil was expected to be critical in describing the niche of *Mycotrupes*. Elevation was included as a layer, because changes in sea level are thought to have
been important in speciation in *Mycotrupes*. The third goal was to examine how the niches suggested by MaxEnt relate to the distributions and habitat of each species of *Mycotrupes*, especially the habitats as I observed and as described in the literature. The fourth goal was to consider how biogeography might explain the comparisons of the likelihood of occurrence map with the known distributions.

**Materials and Methods**

**Location Data**

*Mycotrupes* species presence data points were made up mostly by recent (2006-2008) field collections by the author and other individuals. A Garmin GPSMAP 60CSx (Garmin International, Inc., Olathe, Kansas) hand-held GPS unit allowed precise geographic coordinate information to be logged at collecting sites. Literature records guided much of the recent collecting.

The amount of available presence data was distributed unequally across the five species (*Table 7-1*); this is a result of the restricted geographic distributions of the species, and regional differences in collecting effort. The reliability of a model produced from few sites is expected to be poor, thus in the case of *M. lethroides* (which had only three, closely-spaced collecting sites), several literature records were used which were precise enough to obtain coordinates from the program GoogleEarth (Google 2006). Only three collecting sites were available for *M. pedester* and the available literature records were not precise enough to use as presence data. The total numbers of presence sites used, per species, were as follows: *M. pedester*, three sites; *M. retusus*, four sites; *M. lethroides*, six sites; *M. cartwrighti*, nine sites; *M. gaigei*, 20 sites.

**Environmental Layers**

The following databases of environmental layers were obtained for this study: Elevation data, Bioclim data, and U.S. soil maps. A total of 27 layers (*Table 7-2*) were obtained from these
databases. Geographic data coverage included the majority of Florida, Georgia, and South Carolina, as well as a portion of North Carolina. All layers were formatted as a raster grid (with uniform dimensions) with a 0.001 degree cell size, which is approximately 111 meters. Elevation data were downloaded from the National Map Seamless Server (USGS 2008). Bioclim layers, described as "biologically meaningful variables" derived from temperature and precipitation values, were downloaded from the WorldClim web site (WorldClim 2006). Soil data from the U.S. General Soil Maps were downloaded from the USDA Natural Resources Conservation Service web site (USDA 2008).

Maximum Entropy

MaxEnt (Version 3.1.0) (Schapire 2008) runs were done with the following settings: 50% of sites used for testing; random seed selected; 500 iterations (for *M. cartwrighti* 10,000 iterations were used because 500 iterations were not sufficient to converge); jacknife variable analysis selected. A convergence threshold of 0.00001 was used. For each species of *Mycotrupes*, two separate MaxEnt runs were conducted; one with all of the environmental layers included, and one excluding the BioClim data to test the effect of climate data on the model.

Results

The algorithm converged for all species, resulting in the maps of likelihood of occurrence depicted in Figures 7-1, 7-2, 7-3, 7-4 and 7-5. Maps are given for both with and without the Bioclim layers, as there may be reason to expect climate to bias the models (see below). Species-specific model characteristics are given in Table 7-2. The "most important" layers in Table 7-2 are those layers that contributed at least 5% to the model. The area under the curve (AUC) is the area under a Receiver Operating Characteristic curve depicting the relationship between sensitivity (y-axis) and (1-specificity) (x-axis) for all thresholds. Higher values for the AUC, which indicate that the model was sensitive enough to include all or most of the presence
sites, while still being specific enough that a minimum of geographic area is predicted as being of high likelihood of occurrence, are considered to indicate a better model fit. The P-value and threshold value are those for the likelihood of occurrence threshold that maximizes the sum of sensitivity+specificity. The P-values obtained for this threshold were significant for all analyses (<0.05).

**Discussion**

**Areas Predicted by MaxEnt**

*Mycotrupes lethroides*

The area of high likelihood of occurrence for *M. lethroides* ([Figure 7-1](#)) is focused in two major areas. The first area extends South, Southeast and to a lesser extent Southwest of Augusta, Georgia, and includes the presently known distribution of this species. The other area straddles part of the Oconee River south of Dublin, Georgia.

When Bioclim layers are omitted from the analysis, the likelihood map for *M. lethroides* becomes the broadest obtained in any of the analyses. Most of the Atlantic Coastal Plain region (below the Fall Line) is assigned a likelihood of occurrence of at least 0.5. The AUC is relatively low (0.930), which suggests that there was less information in the presence data to build a model.

*Mycotrupes retusus*

The map of likelihood for *M. retusus* ([Figure 7-2](#)) suggests that this species is restricted to the Fall Line sandhill region. There is an area of high likelihood of occurrence northeast of Aiken, S.C. This map also shows a relatively broad area of unsuitable habitat corresponding to the upper Congaree River, which suggests that this area could act as a barrier to gene flow. The Fall Line appears to drop off in likelihood abruptly northeast of Columbia, S.C. This agrees with the known distribution of *M. retusus*, which only extends approximately 10 miles northeast of
Columbia to Blaney, in Kershaw County. Another large area that was assigned somewhat high likelihood is the vicinity of Marlboro County, SC, which lies outside of the known distribution of *M. retusus*, and would be an interesting area to sample in the future.

When the Bioclim layers are excluded, the map of likelihood is somewhat different. A large area in Florida, within part of the distribution for *M. gaigei*, is assigned high likelihood of occurrence for *M. retusus*. Scattered patches of high likelihood extend along the Fall Line across Georgia. Within South Carolina, the pattern does not change much, although there is now a small area of much higher likelihood (0.9) in the vicinity of Chesterfield County, SC.

*Mycotrupes cartwrighti*

Several areas were assigned a high likelihood of occurrence for *M. cartwrighti* (Figure 7-3). One is roughly coincident with the Tallahassee red hills region, and is well represented by collecting records, except for the area west of Torreya State Park (Harper 1914). Another area of high likelihood for *M. cartwrighti* is located in North-Central Florida. The Hinesville, Georgia locality was not assigned high likelihood, although there is an area with moderate likelihood of occurrence located NW of Hinesville. The known localities (not represented by collections in this study) in the vicinity of Americus and Vienna, Georgia are assigned low likelihood.

When Bioclim layers are excluded, the predicted distribution of *M. cartwrighti* becomes much broader. There are still moderate to high likelihood values assigned to the Tallahassee Hills region of the Florida Panhandle and the areas in North Central Florida, but the pattern in Georgia has changed. Much of the Upper Coastal Plain region of Georgia is assigned a moderate level of likelihood, and the known localities of *M. cartwrighti* near Americus and Vienna are now represented. This moderate likelihood region extends Northeast into South Carolina, and includes the Fall Line region (where *M. lethroides* and *M. retusus* occur). The collecting site in Hinesville, Georgia still occupies a relatively isolated position.
*Mycotrupes gaigei*

Most of the area assigned high levels of likelihood of occurrence for *M. gaigei* is in Florida (Figure 7-4). In addition, there are some scattered areas with moderate likelihood in Georgia and South Carolina. Much of the large area assigned high likelihood in Florida is represented by known occurrences. The Brooksville Ridge and the area along the Suwannee River, both of which host *M. gaigei*, have been assigned high likelihoods. Several large areas that have been assigned high likelihood are outside of the presently known range of *M. gaigei*. These areas include the Southern Brooksville Ridge, the Central Ridge, and several areas near Jacksonville.

The Seminole County, Florida sites are isolated in terms of distance to the nearest known occurrence (Marion County). The map of likelihood of occurrence suggests a relatively broad swath of suitable habitat extending west from Seminole County with the likelihood becoming patchier in the vicinity of the Central Ridge.

When Bioclim layers are excluded, the map of likelihood of occurrence is apparently little changed. The area of known distribution of *M. gaigei* is still represented. The few differences include an area of moderate likelihood now present in the Florida Panhandle. *Mycotrupes gaigei* would appear to be the species with the least difference between the model produced with, and that produced without the Bioclim layers. Two possibilities that might explain this difference (both possibilities assume that climate is not an important factor in the distribution of the species) are: 1) *Mycotrupes gaigei* is represented by more collecting localities than the other species, and more data are expected to yield a better-fitting niche model. 2) *M. gaigei* is widely distributed geographically, and its distribution is probably represented by more climatic variation, meaning that climate would be found to be less important by MaxEnt.
*Mycotrupes pedester*

The model for *M. pedester* is remarkable, because the entirety of the area assigned moderate or high likelihood is restricted to southwest Florida (Figure 7-5). This area includes the known localities, and it also suggests the existence of suitable habitat further to the East along the Caloosahatchee River, and South into Hendry and Collier counties. The model for *M. pedester* had the highest AUC value of all the analyses (0.999). These results should be interpreted with caution, because the use of a small number of presence data points with similar environmental characteristics would be expected to give the same results, and it is possible that there are presently unknown populations of *M. pedester* that are not included in the areas of moderate to high likelihood of occurrence. The use of additional presence localities would give more confidence in the results, however, it is possible that *M. pedester* has an extremely limited geographic distribution, and the addition of presence localities may be difficult. In that case, surveying for *Mycotrupes* in the areas suggested above (from which *M. pedester* is not presently known) may be advisable.

When Bioclim layers are excluded, the likelihood of occurrence map for *M. pedester* includes much of coastal Florida and parts of coastal Georgia and South Carolina. This coastal pattern is interesting, as it is a different pattern from that seen in the other *Mycotrupes* species.

**Effect of Bioclim Data on Predicted Distributions**

For all *Mycotrupes* species, the models including Bioclim layers have higher AUC values compared to those excluding Bioclim. This indicates that the Bioclim layers are providing information that allows MaxEnt to build a more precise niche model for each species, at least based on the presence localities available. In addition, the areas of high likelihood of occurrence produced by the models including Bioclim show less overlap between species (for instance, when Bioclim layers are excluded, the likelihood of occurrence areas for *M. retusus* overlaps...
with that of *M. gaigei*). The use of the Bioclim layers produces areas of likelihood of occurrence that agree more with what is known about the distributions of *Mycotrupes* species.

It is highly possible that species of *Mycotrupes* are adapted to the climate characterizing their distributions. For example, the climate present across the distribution of *M. pedester* is different from the climate farther north, where other species of *Mycotrupes* are present (Mitchell and Ensign 1928). Likewise, Lobo et al. (2006) found climate data to be an important factor in the distribution of the geotrupid genus *Jekelius* in the Iberian Peninsula. Minimum winter temperatures can cause significant mortality in insects living underground, and it is possible that different species of *Mycotrupes* could have different tolerances for winter ground-freezes (especially considering the observed species differences in burrow depth) (Mail 1930). It is also possible, however, that the Bioclim layers, while being associated with the distributions of species of *Mycotrupes*, are not important factors in limiting the distribution of *Mycotrupes* species. The geographic distributions of certain climate layers could be coincident with the distributions of *Mycotrupes* species. If this were true, it would limit the ability of the model to predict unknown populations and skew the analysis of the determinants of habitat suitability.

Of all of the species of *Mycotrupes* studied, *M. gaigei* appears to have the least difference between the likelihood maps produced with, versus without, Bioclim data. *Mycotrupes gaigei* is also the only species in which the most important layer in the model including Bioclim data was found to be a non-Bioclim layer (drainage). This result may be due to the wide geographic spread and large number of presence locations available for *M. gaigei* relative to the other species of *Mycotrupes*, which may have lessened the potential influence of coincidental climate layers.
Mycotrpes are burrowing beetles, and the larvae live and feed in underground chambers (Howden 1954). These facts, combined with the apparent association of Mycotrpes with well-drained habitat (sandhills, etc.), suggest that soil characteristics would have an important influence on the distribution of these beetles. This suggests to me that a dependence on deep well-drained soil and barriers to dispersal are the primary reasons for the restricted distributions of Mycotrpes. Because the non-Bioclim layers are more readily interpretable, further discussion will be limited to them.

Relative Importance of Environmental Layers Across Species of Mycotrpes

The modeled niche of each species of Mycotrpes was different in terms of which environmental layers were found to be important, and the preferences within each of these layers. As discussed above, the association of different species with distinct variables across their geographic distributions does not mean that there are differences in habitat preferences between such species. The restricted geographic area of distribution for many Mycotrpes species may increase the likelihood that the distribution will correlate, even if by chance, with some variable. This could apply to soil as well as climate. For example, if a species is restricted to a very small area with one soil type, this species may be capable of living in other soil types, but it may not only because of an inability to disperse to those other areas. Depending on the size and location of the distribution of each species, there might be different layers that could correlate with the distribution. A possible example of such an effect from the present study is as follows. The niche model for M. pedester had the highest contribution from the elevation layer (22.3%). It is possible that this is because all of the presence locations are at low elevations relative to the wide range of values in the elevation layer (which includes mountains in northern Georgia).

Perhaps more significantly, some environmental layers appeared to be of general importance across all or most species of Mycotrpes. Drainage was important for all, with most
species preferring more well-drained soil. Low silt content was important for *M. gaigei*, *M. pedester*, and *M. retusus*. Percent clay, elevation, and percent sand were important for only one or two species. An interesting difference was observed in the response to the soil bulk density layer between two species. *Mycotrupes cartwrighti* occurs in low bulk density soils, while *M. retusus* occurs in high bulk density soils. Bulk density is often negatively correlated with organic matter, which suggests that *M. retusus* prefers soil with less organic matter relative to *M. cartwrighti*.

**Soil Types Associated with *Mycotrupes* Species**

In an attempt to further characterize the soils associated with the distribution of *Mycotrupes* species, I obtained the taxonomic classification of the soil at each site from the USDA General Soils Map (Table 7-4). The following generalizations are based on these data. Proportions indicate the number of sites, of the total sites used in the niche modeling analysis, with a particular type of soil. It must be remembered that these associations between site and soil type are based, not on sampling at the site and identification, but on digital soil maps derived from soil surveys, and there is the potential for inaccuracy.

Not surprisingly, most of the soils at the sites are well drained. *Mycotrupes gaigei* soils are mainly Quartzipsamments (10/20) and Paleudults (8/20), both of which are well-drained. The Quartzipsamments are in the Entisol order, and have little or no diagnostic horizons. They are sandy throughout their depth (USDA 2009). Quartzipsamments are also present at many of the *M. lethroides* and *M. retusus* sites.

*Mycotrupes cartwrighti* inhabits a different group of soils. While these soils are also well-drained, most (8/9) sites are in Kandiudult soils, meaning they have a clay-rich horizon. The soils at six of these sites are fine-loamy, kaolinitic, thermic typic Kandiudults, which is the classification of the Orangeburg series (USDA 2009). The soil present at the type locality of *M.
cartwrighti was described as in the Orangeburg series (Olson and Hubbell 1954). This soil is widely distributed in the Tallahassee Red Hills region.

The soils at the four Mycotrupes pedester localities are classified under the Aquod suborder, which is characterized as having a shallow water table (USDA 2009). It is possible that the sites in which M. pedester occurs may represent small areas of relatively well-drained soil amongst larger areas of poorly drained soil. Such "islands" of better-drained soil could have gone unnoticed at the scale of soil mapping. In Florida, slight differences in elevation are known to be associated with dramatic differences in drainage and vegetation (P. Skelley, pers. comm.).

**Detailed Discussion of the Distribution of Mycotrupes gaigei**

There are more presence records available for M. gaigei than any of the other species of Mycotrupes, hence there is reason to place more confidence in the modeled niche of this species. In addition, the distribution of this species appears to be associated with certain geological features. Factors that may have affected the distribution of M. gaigei, are discussed in detail here.

The apparent association of M. gaigei with well-drained soils is not surprising, as adults are burrowing (to a depth of six feet [Howden 1954]) and larvae live underground, and even a single inundation event would pose an obvious problem for their survival. In this study, it was found that all Mycotrupes species are associated with somewhat well-drained soils. **Figure 7-6** is a map of drainage in the study area. Dark areas are those that have been categorized under the three highest drainage classes (from USDA soil map). Dark points represent the M. gaigei localities. Note that all of the points are contained within the dark areas or near them.

Many of the areas in which M. gaigei has been collected could be characterized as sandhill, a habitat that is defined by well-drained sandy soil and a characteristic mix of vegetation including turkey oak (*Quercus laevis* Walt.), longleaf pine (*Pinus palustris* Miller),
and wiregrass (*Aristida* spp.) (Laessle 1958; Myers 1985). The apparently strong association of *M. gaigei* with both a particular vegetation type as well as a soil type supports the observations of Harper (1914), who noted the influence of soil on vegetation in Florida. Another widely distributed (in Florida) type of vegetation that occurs in well-drained sandy soil in Florida is scrub. Scrub and sandhill are sometimes confused as they both are dominated by pines and oaks and have sandy soil. Scrub is a distinct habitat type characterized by a dense canopy of "scrubby" vegetation, including sand pine (*Pinus clausa* (Chapm. ex Engelm.) Vasey ex Sarg.) and various oaks (*Quercus* spp.) (Laessle 1958; Myers 1985).

There are no records of *Mycotrupes* from scrub habitat. *Peltotrupes* (Coleoptera: Geotrupidae), which is also a deep burrowing beetle, does occur in scrub, and it is also found in sandhills with *M. gaigei* (Woodruff 1973; Young 1950). Scrub and sandhill habitat are often in close proximity, with "islands" of one habitat often being found within areas of the other habitat (Myers 1985). Kurz (1942) hypothesized that there were differences in subsoil water retention between sandhill and scrub, and that this was the cause of the different vegetative associations. Kurz thought that scrub soils tended to retain water at a shallower depth than sandhill soils.

Laessle (1958) also suggested soil differences as a factor in the distribution of the two habitats. Laessle hypothesized that scrub developed preferentially on the higher-energy, better washed dune sands, whereas sandhill represented the offshore deposits of less washed sands. Such a difference in the origin of soils between the two habitats would suggest soil differences as a reason for the absence of *Mycotrupes* from scrub. Myers (1985), however, found evidence that frequency of fire played a role in the distributions of sandhill and scrub. In his study sites at the Archbold Biological Station (Florida), Myers found evidence that sandhill had been replaced by scrub in the absence of fire. In some cases, sandhill burns on an almost annual basis, whereas
scrub is thought to burn less frequently (Laessle 1958). Deep burrowing by Mycotrupes may be an adaptation not only to warmer post-Pleistocene temperatures, but to frequent burning as well. As sandhill and scrub often are close together, and even grade into each other, a pitfall trap transect across a sandhill-scrub ecotone could yield interesting data on the habitat preferences of Mycotrupes.

A striking characteristic of the likelihood of occurrence map for M. gaigei is that most of the presence localities (except those in Seminole County) are located in a relatively large, contiguous area of high likelihood of probability. Because M. gaigei has been collected in so many localities, and there are no records from sites outside of this apparently contiguous area (except for the Seminole County records), it would seem unlikely that there would be large populations in the other large areas of high likelihood of occurrence in Florida, such as the area within Citrus and Hernando Counties or the Lake Wales Ridge in Central Florida. This is because of the amount of collecting that has occurred across Florida, not because of an inability of MaxEnt to properly model the niche of this species.

The predicted distribution of M. gaigei appears to be coincident with two geological features. Florida is composed of a sequence of marine terraces, remnants of past sea levels caused by climate cycles. It is assumed that the higher (in elevation) terraces are the oldest, and that there was a progressive lowering of maximum interglacial sea level. The Wicomico and Penholloway terraces, which are adjacent and considered by Colquhoun et al. (1991) to include 12.9-30.3 meters above sea level (based on stratigraphic units in South Carolina), contain 18 of the 20 localities for M. gaigei (Figure 7-7). There has been some deformation of the Florida bedrock, resulting in changes in elevation, so elevation is only a rough estimate of the terraces (Opdyke et al. 1984). In addition, all attempts to map marine terraces in Florida have relied on
elevation, rather than mapping terrace features (T. Scott, pers. comm.). Nevertheless, the apparent relationship between the distribution of *M. gaigei* and elevation suggests that sea level changes may have been an important factor in the distribution of the species. The distributions of the subspecies of the snake *Stilosoma extenuatum* Brown (a Florida endemic) are separated by areas of less than 100 feet in elevation. It is possible that the subspecies inhabited separate islands at a time when the sea level was at the Wicomico shoreline. *Stilosoma* inhabits similar habitat to that of *M. gaigei* (sandhill and upland hammock) (Highton 1956).

The predicted distribution of *M. gaigei* is also mostly coincident with a geological structure referred to as the Ocala Platform (Figure 7-8). This area in NW peninsular Florida has been previously referred to as the "Ocala Uplift," but this term is misleading. Although the bedrock in this area is higher than that surrounding it, this is apparently the result of the subsidence of this surrounding rock in relation to the Ocala Platform, which has remained more stable. The subsidence is thought to be related to the general Miocene subsidence of the North American Plate and the Gulf of Mexico Basin (T. Scott, pers. comm.). The Ocala Platform is one of the few areas in Florida where the aquifer is not confined by the Hawthorne Formation, which may result in better drainage in this area (Brinkmann et al. 2007). Although the Hawthorne Formation was deposited on the entire Florida Platform, these sediments were eroded from the Ocala Platform when this area was exposed above sea level during the Miocene (T. Scott, pers. comm.). Interestingly, the other area of distribution (Seminole County) is near another high, the Sanford High (Figure 7-8).

An area such as the Ocala Platform could be important for two reasons: First, *M. gaigei* could have originated on such a structure. Alternatively, the habitat for *M. gaigei* could have
formerly been more extensive (possibly because of a different climate or lower sea level) and a recent contraction in habitat could have resulted in remnant populations in this area.

The records in Seminole County are isolated from the rest of the distribution of *M. gaigei*, both geographically (being far away from the nearest records, which are in Marion County, Florida), and apparently ecologically as well, because the MaxEnt likelihood of occurrence map suggests that the favorable habitat that contains the Seminole County sites is more isolated and patchy (compared to the major area of distribution of *M. gaigei*). Geneva Hill, where the two Seminole County collecting sites that were used are situated, is an isolated, sandy hill. The lower, less well-drained nature of the surrounding area is evident when travelling north on Route 46 out of Geneva. There are sandy areas scattered to the southwest and west of Geneva Hill, toward Orlando, and there are records of *M. gaigei* in nearby Oviedo approximately 7 miles SW of the sites in Geneva (S. Fullerton, *in litt.*). The seemingly disjunct distribution of the Seminole County records from the rest of the known distribution begs for an explanation.

There are two simple (involving only one step) explanations:

1) (Dispersal) *M. gaigei* dispersed at some point from somewhere in the main body of distribution to the Seminole County area, or from the latter to the former area.

2. (Vicariance) There was, at a previous time, a relatively continuous distribution of *M. gaigei* from the main area to the Seminole County area, and some event (such as a rise in sea level) later split the two areas with no subsequent re-colonization of this intervening area.

The northern part of the distribution of *M. gaigei* is also coincident with an area of particularly dense records of the Southeastern Pocket Gopher, *Geomys pinetus* Rafinesque (Kovarik et al. 2000). *Geomys pinetus*, like *Mycotrupes*, digs deep burrows, and apparently
requires well-drained sandy soil. The gopher, however, has evidently been more successful at dispersal, as indicated by its wider geographic distribution.

**Conclusion**

Was MaxEnt successful in mapping the true distributions of *Mycotrupes* species, and was it successful in proposing additional areas where *Mycotrupes* may be found? The answer may depend on scale. Even with a species such as *M. gaigei*, where there are many occurrence localities, we will not have a complete idea of its distribution in space and time. Apart from extensive pitfall trapping across the mapped areas, there may not be an objective method to assess the success of the niche modeling. However, the high AUC values combined with (in some cases) the likelihood of occurrence maps showing presence localities nested within contiguous areas of apparently good habitat, suggests that the niche modeling was largely effective for *Mycotrupes*. Some large, contiguous areas were assigned a high likelihood of occurrence by MaxEnt for *Mycotrupes* species where they are not known. These areas may warrant surveying for *Mycotrupes*.

Two species that may have the least completely known distributions, *M. pedester* and *M. lethroides*, unfortunately have the fewest known presence localities. This means that the MaxEnt niche models for these species are likely to be inferior to the niche models of *M. gaigei*, a species for which many collecting localities were available. Of course, it is expected that a species with a smaller geographic distribution will be represented by a smaller number of occurrence points. In this case, questions of scale may have consequences for the ability of the niche of a given species to be modeled sufficiently. More collecting in the known distribution of a species, such as *M. pedester*, would be expected to yield additional occurrence localities, because the scale of this analysis is relatively fine (the raster cell size is approximately 111 meters on a side). Additional presence localities would give greater confidence in the resulting niche model.
A major part of my work on *Mycotrupes* is the biogeography of the genus. A central question to this issue is the link between the biogeography of *Mycotrupes* and the diversity of habitats occupied by the species. Are *Mycotrupes* found in different habitats because of biological adaptations to different habitats, or is the difference in habitat a product of geographic patterns in soil and vegetation and the insular nature of such habitat? The latter would imply that *Mycotrupes* has simple niche requirements, such as well-drained soil, that are satisfied by any of the habitats it is known to occupy. This would also be consistent with a vicariance-based hypothesis of speciation. For example: A species of *Mycotrupes* ("species X") may be distributed in a patch of land that has a unique geological origin (such as soils with a high clay content). Species X could just as easily live in soils with less clay in other areas, but because it is flightless and its dispersal is limited from those other areas by certain barriers (such as poorly-drained habitat or a river), it remains restricted in distribution to a relatively small area with a distinct soil type. The reality of the situation probably lies somewhere in between the two extremes.

Progress toward an answer to the questions of the degree of adaptation of *Mycotrupes* species to their present habitat, and their ability to live in other habitats where they are not presently known to occur (for instance, a well-drained habitat that happens to be cut off from a *Mycotrupes* population only because dispersal to it is not possible), could be made in several ways. One possible approach would be to study the biology of *Mycotrupes* in a controlled setting, varying certain environmental factors such as soil drainage. Comparing the fitness of *Mycotrupes* across a range of conditions might reveal important limiting factors. Such a study would be difficult, given the long lifecycle (probable at least one year), deep burrowing habits, and largely unknown biology of *Mycotrupes*. A simpler approach to study habitat specialization
would be to sample *Mycotrupes* along pitfall trap transects to find responses along gradients of habitat variables such as vegetation cover or soil drainage. Such a transect approach might also be used to quantify barriers to dispersal between habitat hosting a given species of *Mycotrupes* and the nearest unoccupied habitat of a type that is known to host other species of *Mycotrupes* in other areas.

Phillips et al. (2006) stated "If the realized niche and fundamental niche do not fully coincide, we cannot hope for any modeling algorithm to characterize the species' full fundamental niche; the necessary information is simply not present in the occurrence localities. This problem is likely exacerbated when occurrence records are drawn from too small a geographic area." They add "...the departure between the fundamental niche (a theoretical construct) and realized niche (which can be observed) of a species will remain unknown." Hence, when modeling the distribution of species, the results must be interpreted in light of the limitations of the data. Ideally, niche modeling should utilize biologically meaningful information rather than simply using factors that happen to be correlated with a species' distribution. This is difficult, however, when two things are considered: 1) That these techniques are used in order to discover biologically meaningful information, and 2) one has to consciously choose to include each layer of interest in the analysis. This is a philosophical problem that might be impossible to solve.
<table>
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<th>Species</th>
<th>State: County</th>
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<th>Longitude</th>
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<td>29.0230</td>
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<td>K. Beucke, specimen data.</td>
</tr>
<tr>
<td><em>M. gaigei</em></td>
<td>FL: Marion</td>
<td>Marion Oaks Manor and SW 56th Court</td>
<td>28.9962</td>
<td>-82.2129</td>
<td>K. Beucke, specimen data.</td>
</tr>
<tr>
<td><em>M. gaigei</em></td>
<td>FL: Marion</td>
<td>SW 59th Ave. Rd. and SW 158th Ln.</td>
<td>28.9920</td>
<td>-82.2197</td>
<td>K. Beucke, specimen data.</td>
</tr>
<tr>
<td><em>M. gaigei</em></td>
<td>FL: Marion</td>
<td>SR 474A and SE 1st Ave. Rd.</td>
<td>29.0093</td>
<td>-82.1350</td>
<td>K. Beucke, specimen data.</td>
</tr>
<tr>
<td><em>M. gaigei</em></td>
<td>FL: Suwannee</td>
<td>CR 137 N. of US Hwy. 27.</td>
<td>29.9938</td>
<td>-82.8106</td>
<td>P. Choate, specimen data.</td>
</tr>
<tr>
<td><em>M. gaigei</em></td>
<td>FL: Dixie</td>
<td>Old Town</td>
<td>29.5760</td>
<td>-82.9678</td>
<td>K. Beucke, specimen data.</td>
</tr>
<tr>
<td><em>M. gaigei</em></td>
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<td>CR 425, 0.6 mi N of US Hwy. 27.</td>
<td>29.9905</td>
<td>-82.9957</td>
<td>P. Choate, specimen data.</td>
</tr>
<tr>
<td><em>M. gaigei</em></td>
<td>FL: Lafayette</td>
<td>Mayo</td>
<td>30.0618</td>
<td>-83.1905</td>
<td>P. Choate, specimen data.</td>
</tr>
<tr>
<td><em>M. gaigei</em></td>
<td>FL: Lafayette</td>
<td>CR 354 N. of US Hwy. 27.</td>
<td>30.0716</td>
<td>-83.0961</td>
<td>P. Choate, specimen data.</td>
</tr>
<tr>
<td><em>M. gaigei</em></td>
<td>FL: Lafayette</td>
<td>Townsend</td>
<td>30.1494</td>
<td>-83.3287</td>
<td>P. Choate, specimen data.</td>
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<td><em>M. gaigei</em></td>
<td>FL: Madison</td>
<td>CR 53, N. of Madison Co. line.</td>
<td>30.2743</td>
<td>-83.2887</td>
<td>P. Choate, specimen data.</td>
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<tr>
<td><em>M. pedester</em></td>
<td>FL: Lee</td>
<td>US Hwy. 41, 0.1 mi S. of Constitution Blvd.</td>
<td>26.4745</td>
<td>-81.8361</td>
<td>K. Beucke, specimen data.</td>
</tr>
<tr>
<td><em>M. pedester</em></td>
<td>FL: Lee</td>
<td>Babcock Ranch</td>
<td>26.7611</td>
<td>-81.6808</td>
<td>K. Beucke, specimen data.</td>
</tr>
<tr>
<td>Database</td>
<td>Layer</td>
<td>Type</td>
<td></td>
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<td>-------------------------</td>
<td>--------------------------</td>
<td></td>
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</tr>
<tr>
<td><strong>Elevation</strong></td>
<td>Elevation</td>
<td>Continuous</td>
<td></td>
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<td><em>National Map Seamless Server</em></td>
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<tr>
<td><strong>Soil</strong></td>
<td>Drainage</td>
<td>Categorical, 9 classes</td>
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<td><em>U.S. General Soils Map - USDA</em></td>
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<td></td>
<td>Hydric Class</td>
<td>Categorical, 3 classes</td>
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<td></td>
<td>Bulk Density</td>
<td>Continuous</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Sand Percent (top layer)</td>
<td>Continuous</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Clay Percent (top layer)</td>
<td>Continuous</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Silt Percent (top layer)</td>
<td>Continuous</td>
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<td></td>
<td></td>
</tr>
<tr>
<td><strong>Bioclim</strong></td>
<td>1, Annual Mean Temperature</td>
<td>Continuous</td>
<td></td>
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</tr>
<tr>
<td><em>WorldClim</em></td>
<td></td>
<td></td>
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<td></td>
<td>2, Mean Diurnal Range</td>
<td>Continuous</td>
<td></td>
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<tr>
<td></td>
<td>3, Isothermality</td>
<td>Continuous</td>
<td></td>
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</tr>
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<td>4, Temperature Seasonality</td>
<td>Continuous</td>
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<tr>
<td></td>
<td>5, Max. Temperature Warmest Month</td>
<td>Continuous</td>
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<tr>
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<td>6, Min. Temperature Coldest Month</td>
<td>Continuous</td>
<td></td>
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<tr>
<td></td>
<td>7, Temperature Annual Range</td>
<td>Continuous</td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>8, Mean Temperature Wettest Quarter</td>
<td>Continuous</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>9, Mean Temperature Driest Quarter</td>
<td>Continuous</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>10, Mean Temperature Warmest Quarter</td>
<td>Continuous</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>11, Mean Temperature Coldest Quarter</td>
<td>Continuous</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>12, Annual Precipitation</td>
<td>Continuous</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>13, Precipitation Wettest Month</td>
<td>Continuous</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>14, Precipitation Driest Month</td>
<td>Continuous</td>
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<tr>
<td></td>
<td>15, Precipitation Seasonality</td>
<td>Continuous</td>
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<td></td>
<td>16, Precipitation Wettest Quarter</td>
<td>Continuous</td>
<td></td>
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<tr>
<td></td>
<td>17, Precipitation Driest Quarter</td>
<td>Continuous</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>18, Precipitation Warmest Quarter</td>
<td>Continuous</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>19, Precipitation Coldest Quarter</td>
<td>Continuous</td>
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<td></td>
</tr>
</tbody>
</table>
Table 7-3. Diagnostics and results from niche modeling.

<table>
<thead>
<tr>
<th>Species</th>
<th>With Bioclim AUC</th>
<th>P-value, threshold</th>
<th>Most Important Layers</th>
<th>Without Bioclim AUC</th>
<th>P-value, threshold</th>
<th>Most Important Layers</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. lethroides</td>
<td>.995</td>
<td>1.60E-6, .466</td>
<td>Bioclim 2, Bioclim 13, Clay percent, Drainage, Sand percent</td>
<td>.930</td>
<td>7.49E-4, .608</td>
<td>Clay percent, Drainage</td>
</tr>
<tr>
<td>M. retusus</td>
<td>.993</td>
<td>1.82E-4, .301</td>
<td>Bioclim 7, Silt percent, Bioclim 8</td>
<td>.974</td>
<td>2.46E-3, .121</td>
<td>Silt percent, Drainage, Bulk density, Elevation Drainage</td>
</tr>
<tr>
<td>M. cartwrighti</td>
<td>.845</td>
<td>1.267E-3, .091</td>
<td>Bioclim 19, Bioclim 8, Drainage, Bioclim 3, Bioclim 13 Drainage, Bioclim 8, Bioclim 2, Bioclim 14</td>
<td>.685</td>
<td>3.298E-2, .588</td>
<td>Silt percent, Drainage, Bulk density, Elevation, Sand percent Silt percent, Drainage, Sand percent</td>
</tr>
<tr>
<td>M. gaigei</td>
<td>.969</td>
<td>1.171E-11, .084</td>
<td>Bioclim 8, Bioclim 2, Bioclim 15, Silt percent, Bioclim 16</td>
<td>.872</td>
<td>2.551E-5, 0</td>
<td>Silt percent, Drainage, Sand percent</td>
</tr>
<tr>
<td>M. pedester</td>
<td>.999</td>
<td>1.1E-3, .691</td>
<td>Bioclim 15, Silt percent, Bioclim 16</td>
<td>.977</td>
<td>2.28E-2, .534</td>
<td>Silt percent, Elevation, Drainage</td>
</tr>
</tbody>
</table>
Table 7-4. Taxonomic classification of soil at each site used in niche modeling, according to the United States General Soils Map.

<table>
<thead>
<tr>
<th>Species</th>
<th>State: County</th>
<th>Location</th>
<th>Taxonomy</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. lethroides</td>
<td>GA: Burke</td>
<td>Yuchi Wildlife Management Area</td>
<td>Arenic kandiudults, loamy. siliceous, thermic</td>
</tr>
<tr>
<td>M. lethroides</td>
<td>GA: Burke</td>
<td>Yuchi Wildlife Management Area</td>
<td>Arenic kandiudults, loamy. siliceous, thermic</td>
</tr>
<tr>
<td>M. lethroides</td>
<td>GA: Richmond</td>
<td>US Hwy. 1, 0.5 mi E. of</td>
<td>Siliceous, thermic typic quartzipsamments</td>
</tr>
<tr>
<td>M. lethroides</td>
<td>GA: Richmond</td>
<td>Junction of I-520 and US Hwy. 1.</td>
<td>Typic kanhapldults, fine-loamy, siliceous, thermic</td>
</tr>
<tr>
<td>M. lethroides</td>
<td>GA: Richmond</td>
<td>Junction of US Hwy. 25 and SR 415.</td>
<td>Thermic, coated typic quartzipsamments</td>
</tr>
<tr>
<td>M. retusus</td>
<td>SC: Aiken</td>
<td>Junction of Oak Club Rd. and US</td>
<td>Typic quartzipsamments, thermic, coated</td>
</tr>
<tr>
<td>M. retusus</td>
<td>SC: Aiken</td>
<td>NE of White Pond on Webb Pond Rd.</td>
<td>Grossarenic kandiudults, loamy, siliceous, thermic</td>
</tr>
<tr>
<td>M. retusus</td>
<td>SC: Richland</td>
<td>US Hwy. 1, Near Sesquicentennial</td>
<td>Typic quartzipsamments, thermic, coated</td>
</tr>
<tr>
<td>M. cartwrighti</td>
<td>FL: Leon</td>
<td>Eleanor Klapp-Phipps Park</td>
<td>Fine-loamy, kaolinitic, thermic typic kandiudults</td>
</tr>
<tr>
<td>M. cartwrighti</td>
<td>FL: Leon</td>
<td>Tall Timbers Research Station</td>
<td>Fine-loamy, kaolinitic, thermic typic kandiudults</td>
</tr>
<tr>
<td>M. cartwrighti</td>
<td>FL: Jefferson</td>
<td>Avalon conservation easement.</td>
<td>Fine-loamy, kaolinitic, thermic typic kandiudults</td>
</tr>
<tr>
<td>M. cartwrighti</td>
<td>FL: Jefferson</td>
<td>Avalon conservation easement.</td>
<td>Fine-loamy, kaolinitic, thermic typic kandiudults</td>
</tr>
<tr>
<td>M. cartwrighti</td>
<td>GA: Thomas</td>
<td>Thomasville</td>
<td>Fine-loamy, kaolinitic, thermic plinthic kandiudults</td>
</tr>
<tr>
<td>M. cartwrighti</td>
<td>GA: Liberty</td>
<td>Hinesville</td>
<td>Ultic haplaquods, sandy, siliceous, thermic</td>
</tr>
<tr>
<td>M. cartwrighti</td>
<td>FL: Liberty</td>
<td>Torreya State Park</td>
<td>Loamy, kaolinitic, thermic grossarenic kandiudults</td>
</tr>
<tr>
<td>M. gaigei</td>
<td>FL: Alachua</td>
<td>Archer</td>
<td>loamy, siliceous, hyperthermic grossarenic paleudults</td>
</tr>
<tr>
<td>M. gaigei</td>
<td>FL: Columbia</td>
<td>OLeno State Park</td>
<td>Thermic, coated lamellic quartzipsamments</td>
</tr>
<tr>
<td>M. gaigei</td>
<td>FL: Columbia</td>
<td>SR 47, S. of Ft. White</td>
<td>Thermic, coated lamellic quartzipsamments</td>
</tr>
<tr>
<td>M. gaigei</td>
<td>FL: Sumter</td>
<td>Tillman Hammock</td>
<td>Hyperthermic, uncoated typic quartzipsamments</td>
</tr>
<tr>
<td>M. gaigei</td>
<td>FL: Seminole</td>
<td>Geneva, Cochran Rd.</td>
<td>Hyperthermic, uncoated typic quartzipsamments</td>
</tr>
<tr>
<td>M. gaigei</td>
<td>FL: Gilchrist</td>
<td>SR 26 W. of Newberry</td>
<td>Sandy, siliceous, hyperthermic aeric haplaquads</td>
</tr>
<tr>
<td>M. gaigei</td>
<td>FL: Levy</td>
<td>SR 24, near Alachua/Levy Co. line.</td>
<td>Hyperthermic, uncoated typic quartzipsamments</td>
</tr>
<tr>
<td>M. gaigei</td>
<td>FL: Marion</td>
<td>SR 484 and 76th Court.</td>
<td>Hyperthermic, uncoated typic quartzipsamments</td>
</tr>
<tr>
<td>M. gaigei</td>
<td>FL: Marion</td>
<td>Marion Oaks Manor and SW 56th</td>
<td>Hyperthermic, uncoated typic quartzipsamments</td>
</tr>
<tr>
<td>M. gaigei</td>
<td>FL: Marion</td>
<td>SW 59th Ave. Rd. and SW 158th Ln.</td>
<td>Hyperthermic, uncoated typic quartzipsamments</td>
</tr>
<tr>
<td>M. gaigei</td>
<td>FL: Marion</td>
<td>SR 474A and SE 1st Ave. Rd.</td>
<td>Loamy, siliceous, hyperthermic grossarenic paleudults</td>
</tr>
<tr>
<td>M. gaigei</td>
<td>FL: Suwannee</td>
<td>CR 137 N. of US Hwy. 27.</td>
<td>Thermic, coated lamellic quartzipsamments</td>
</tr>
<tr>
<td>M. gaigei</td>
<td>FL: Dixie</td>
<td>Old Town</td>
<td>Loamy, siliceous, subactive, thermic arenic paleudults</td>
</tr>
<tr>
<td>M. gaigei</td>
<td>FL: Lafayette</td>
<td>CR 425, 0.6 mi N of US Hwy. 27.</td>
<td>Fine-loamy, siliceous, semiactive, thermic aque</td>
</tr>
<tr>
<td>M. gaigei</td>
<td>FL: Lafayette</td>
<td>Mayo</td>
<td>Loamy, siliceous, subactive, thermic arenic paleudults</td>
</tr>
<tr>
<td>M. gaigei</td>
<td>FL: Lafayette</td>
<td>CR 354 N. of US Hwy. 27.</td>
<td>Loamy, siliceous, subactive, thermic arenic paleudults</td>
</tr>
<tr>
<td>M. gaigei</td>
<td>FL: Lafayette</td>
<td>Townsend</td>
<td>Loamy, siliceous, subactive, thermic arenic paleudults</td>
</tr>
<tr>
<td>M. gaigei</td>
<td>FL: Madison</td>
<td>CR 53, N. of Madison Co. line.</td>
<td>Loamy, siliceous, subactive, thermic arenic paleudults</td>
</tr>
<tr>
<td>M. pedester</td>
<td>FL: Lee</td>
<td>US Hwy. 41, 0.1 mi S. of Constitution</td>
<td>Sandy, siliceous, hyperthermic aeric haplaquads</td>
</tr>
<tr>
<td>M. pedester</td>
<td>FL: Lee</td>
<td>Babcock Ranch</td>
<td>Sandy, siliceous, hyperthermic ultic haplaquods</td>
</tr>
<tr>
<td>M. pedester</td>
<td>FL: Lee</td>
<td>Babcock Ranch</td>
<td>Sandy, siliceous, hyperthermic ultic haplaquods</td>
</tr>
<tr>
<td>Cryptic species?</td>
<td>FL: Taylor</td>
<td>Fenholloway</td>
<td>Loamy, siliceous, thermic aquic arenic haplaudals</td>
</tr>
</tbody>
</table>
Figure 7-1. Likelihood of occurrence map for *M. lethroides*, with Bioclim (left) and without (right).

Figure 7-2. Likelihood of occurrence map for *M. retusus*, with Bioclim (left) and without (right).
Figure 7-3. Likelihood of occurrence map for *M. cartwrighti*, with Bioclim (left) and without (right).

Figure 7-4. Likelihood of occurrence map for *M. gaigei*, with Bioclim (left) and without (right).
Figure 7-5. Likelihood of occurrence map for *M. pedester*, with Bioclim (left) and without (right).

Figure 7-6. Well-drained soil and *M. gaigei* collecting localities.
Figure 7-7. Marine terraces in Florida. The Wicomico terrace is in blue and the Penholloway is in green (Healy 1975).

Figure 7-8. The Ocala Platform area (labeled). The Sanford High is the smaller area, circled in red, in Eastern Florida (USGS 2002).
CHAPTER 8
CONCLUSION

My study is the result of almost four years of field and laboratory work on the genus *Mycotrupes*. This genus was selected for a variety of reasons, among them my interest in Geotrupidae, the poorly known biology of *Mycotrupes*, and the desire to test the biogeographical hypothesis of Hubbell (1954). *Mycotrupes* specimens were collected from much of the presently known distribution of the genus. This degree of sampling is a result of both knowledge of the distributions of the different species, gained from literature records, museum specimen data, and personal communication with experts, as well as persistence. Numerous sites that did not yield *Mycotrupes* were visited, thus the number of successful collections underrepresented the whole effort. Additional field work would have been preferable, but time and money were major limiting factors.

The phylogenetic hypothesis of *Mycotrupes* put forth in my study refutes that of Hubbell (1954), which he based on assumptions regarding the morphological evolution and biogeography of *Mycotrupes*. My study was based on newer techniques (not available to Hubbell), including nucleotide sequence data, and was conducted with modern phylogenetic methods. For these reasons I believe that the present phylogenetic hypothesis (see Chapter 5) is more accurate. There is little support for the hypotheses of Hubbell that *M. gaigei* is a "primitive" *Mycotrupes*, and that *M. cartwrighti* and *M. pedester* are sister taxa.

This phylogenetic study has also revealed a possible cryptic species from a location near what has been considered the distribution of *M. cartwrighti*. The possibility that this entity may be a separate species is further supported by the high mean pairwise distances between its sequence and *M. cartwrighti* from other locations. The collection of a larger series from this location (only four specimens, two of them sequenced, are currently known), increasing the
potential sample size for both morphological as well as molecular study, would enable a more robust test of the distinctness of this entity as a new species. It is therefore not formally named at this time.

Because the evolutionary hypothesis of Hubbell (1954) was rejected in this study, it is also necessary to reject his biogeographic hypothesis. Hubbell proposed that *M. gaigei* arose through a vicariance event in which an island of habitat in Central Florida was isolated during a rise in sea-level, from the ancestral habitat which receded to the Fall Line. In my phylogenetic study, I determined that *M. gaigei* is derived relative to other *Mycotrupes* species, so the existence of this Central Florida island habitat is not required. The allopatric distributions of *Mycotrupes* species, combined with the fact that the two most basal species (*M. lethroides* and *M. retusus*) are found on the highest, most inland habitat (Fall Line), strongly suggests that sea level played a major role in the evolution of *Mycotrupes*. The poorly constrained divergence date estimates produced by the BEAST analysis, combined with the lack of fossil data and a complex history of sea level change, makes it impossible to correlated divergence times in *Mycotrupes* with particular changes in sea level.

Many interesting biogeographic questions remain. Among them is the historical extent of *Mycotrupes* habitat, which was probably, at different times, more and less extensive than at present. This issue bears directly on the relative importance of vicariance versus dispersal in speciation within this group.

My niche modeling study indicated that a high degree of drainage of the soil is an important variable in determining the distribution of all *Mycotrupes* species. This may come as an obvious conclusion to entomologists who are familiar with *Mycotrupes* and its "typical" habitat, however, this had not been formally studied, and niche modeling provided evidence to
support it. Other environmental layers, such as climate and elevation, were found to be important for individual *Mycotrupes* species, but this may have been an artifact of the restricted geographic distributions of the species, as discussed in Chapter 7. There are many other possible data layers available now, and still others that could be constructed by biologists, that might be used in future niche modeling studies. One interesting possibility is integrating historical information into layers that could be used to study biogeography via niche modeling.

My phylogenetic study, central to this work, was based on a relatively small (by today's standards) nucleotide data set of 481 base pairs and a small number of specimens analyzed. It is suggested that future contributions of *Mycotrupes* character data will be in the form of additional nucleotide sequences and more specimens sampled per species. Although my search for a significant number of morphological characters in *Mycotrupes* was unsuccessful, it is not my intention to dissuade further morphological study of the genus. A comparative morphological study of larvae from different species of *Mycotrupes* might provide morphological character data for future phylogenetic study. At present, the larva has been described from only one species, *M. gaigei* (Hubbell 1954). A rearing experiment by P. Skelley (see Chapter 3) succeeded in yielding two larvae of *M. gaigei*. Captive rearing or burrow excavation of other species of *Mycotrupes* may yield larvae as well.

Observations made over the course of field work suggest that the depth of burrows in different species of *Mycotrupes* may be dependent on soil characteristics. For example, *M. cartwrighti* was seen to dig shallow burrows in clay-rich soil, while *M. gaigei* burrows were quite deep in sandy soil. With the limited data available, however, it is difficult to separate the importance of species versus regional conditions. The effect of species and soil type on burrow
depth and characteristics will require study and excavation of many burrows of different
\textit{Mycotrupes} species across a range of habitats within each species' distribution.

\textit{Mycotrupes} species have been listed as threatened (see Chapter 2). Because \textit{Mycotrupes} require well-drained habitat such as sandhill, they are uniquely threatened by development. Well-drained uplands are developed at a high rate in Florida for such uses as housing and agriculture (Enge et al. 1986). Any attempt to protect \textit{Mycotrupes} will be hampered by an incomplete knowledge of their biology and distribution. The distributions of certain species, such as \textit{M. pedester}, may be poorly known. Further collecting will be necessary in order to have a better idea of the complete distributions of \textit{Mycotrupes} species and the environmental conditions that this fascinating group requires for survival.

\textit{Mycotrupes} is one of many taxa, including other flightless insects, inhabiting the southeastern Atlantic Coastal Plain that offer an opportunity to unravel a biogeographical story. Theodore Hubbell, who was concerned with the biogeography of insects in the southeastern Atlantic Coastal Plain and coauthored the 1954 monograph on \textit{Mycotrupes} with Olson and Howden, recognized the potential in this region for the comparative study of biogeography across different insect taxa. In his words: "One may find concentrated here many kinds of evolutionary situations, the result of differences in vagility, ecological requirements, and original geographic location of the groups concerned." He also recognized the importance of considering all available evidence when framing a regional explanation of biogeography: "There was, of course, only one actual regional history, and a hypothesis that is set up to explain what happened in one group must be consistent with the evidence from others." (Hubbell 1956) It is my hope that my research on \textit{Mycotrupes} will provide an interesting comparison to future studies on the biogeography of life in the Atlantic Coastal Plain.
APPENDIX A
CYTOCHROME OXIDASE I SEQUENCE DATA

*Peltotrupes youngi*
TATTATTTGACAAGAAAGAAGAAAAAAAAGAAACATTGGGAACCTTTAGGTATAATTT
ATGCTATAATAGCAATTTGTTTTTATTAGGTTTTTATTGTATGAGCACTCATATATTTAC
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TGTCCTACAGGTTATTTGTGTTTTTATTGTATGAGCCTTTCATGGAACCTCAAAT
TAACACTCTCCCATCAATATTATGACCTTTAGGATTTGTATTTTTATTATACTACGTAGGA
GGACTAACCAGGGCGTTTATCTTTGGCAATTTGCAATATGATATTGTTTTTACATGATACG
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TAGGGGATTTAGTCATTTGACTTTTTATTTACTGGAACATAATATTAATAAGAAAAT
ATTTAAAAATTCAATTTTTATT

*Mycotrupes lethroides* A-1
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TTCTCAGAAATTTAAATTTTAGTATTAGCAGACCTTTACATGGAACAAATAA
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CTGTTTTCTCTTTGNCATTTGACTTTTTATTTACTGGAACATAATATTAATAAGAAAATTTTTA

*M. lethroides* A-2
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TTCTCAGAAATTTAAATTTTAGTATTAGCAGACCTTTACATGGAACAAATAA
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CTGTTTTCTCTTTGNCATTTGACTTTTTATTTACTGGAACATAATATTAATAAGAAAATTTTTA

*M. retusus* A-1
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**M. retusus A-2**
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**M. retusus B-1**
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**M. retusus C-1**
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**M. retusus D-1**
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M. retusus D-2
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GTTCCTAAGGAATAAATTTTCAGATGATTAGCAACCATTACATGGAAACACAAATT
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GCAGGATTTGTTGACTTCCCAACTATTTACAGGTAAAATATAAATATAATT
TAAAATAATCATTTTTATT

M. cartwrighti A-1
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M. cartwrighti B-1
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M. cartwrighti C-1
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M. cartwrighti C-2
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M. cartwrighti E-1
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M. cartwrighti F-1
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M. cartwrighti H-1
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**M. cartwrighti** H-2
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**M. cartwrighti** I-1
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**M. cartwrighti** I-2
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**M. cartwrighti** J-1
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**M. cartwrighti** J-2
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M. cartwrighti J-2
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M. cartwrighti L-1
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M. gaigei A-1
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M. gaigei A-2
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124
M. gaigei C-1
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ATTGGAAAAATTTCAATTTTTAATT

M. gaigei C-2
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M. gaigei D-1
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M. gaigei D-2
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M. gaigei E-1
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M. gaigei F-1
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M. gaigei M-1
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M. gaigei M-2
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M. gaigei N-1
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M. gaigei N-2
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M. gaigei O-1
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GGTTTAACAGGAGTTATTTTAGTTACATTAGTCTTTTCTATAGGAGCTGTATTGCTATTAT
AGCAGGATTGGTACATTGATTCCCTGTATTACAGGAGTAAATAATATAAAACAGAAATA
TTTTAAAAATCATTAAAAATTT

M. gaigei O-2
TATTATAGCCCAAGAAGAAAGAAAAAGGAAACATTGGAACCCTAGGAATAAT
TTATGCTATAATAGCTATTGGATTATTGTATGGGCCCATCATATATTTAC
TGTTTCTACAGGAATAAAAAATTTTTAGATGATTAGCAACTTTACAGGAAACAAAT
AAACTATCCCCCTCAATATGAGGTTTTTTTTTTTTTTATTTCTGTGGGGG
GGTTTAACAGGAGTTATTTTAGTTACATTAGTCTTTTCTATAGGAGCTGTATTGCTATTAT
AGCAGGATTGGTACATTGATTCCCTGTATTACAGGAGTAAATAATATAAAACAGAAATA
TTTTAAAAATCATTAAAAATTT

M. gaigei P-1
TATTATAGCCCAAGAAGAAAGAAAAAGGAAACATTGGAACCCTAGGAATAAT
TTATGCTATAATAGCTATTGGATTATTGTATGGGCCCATCATATATTTAC
TGTTTCTACAGGAATAAAAAATTTTTAGATGATTAGCAACTTTACAGGAAACAAAT
AAACTATCCCCCTCAATATGAGGTTTTTTTTTTTTTTATTTCTGTGGGGG
GGTTTAACAGGAGTTATTTTAGTTACATTAGTCTTTTCTATAGGAGCTGTATTGCTATTAT
AGCAGGATTGGTACATTGATTCCCTGTATTACAGGAGTAAATAATATAAAACAGAAATA
TTTTAAAAATCATTAAAAATTT

127
M. gaigei P-2
TATTATGCGCAAAGAAGAGAAAAAAAGAAAAACATTCCGGAACCCCTGGGTATAATTG
ATGCTATAATAGCTATTTGGAATTAGGATTTATAGTATGAGCACACATCATATATTAC
CGTAGAGAATAGTGGGATACCCGAGCTATTTCACTTTGCGAATACCTAATTGGCT
TATATGAGTGGACACATTTTACAGGATTAAATATAAATAGAAAATATTGAAAATTCAATTATTAAATT

M. pedester A-1
TATTATGTCAGAAAGAAGAGAAAAAAAGAAAAACCTTTTGGAACCTTGAAGAATATTCT
ATGCTATAATAGCTATTTGGAATTAGGATTTATAGTATGAGCACACATCATATATTAC
TGAGGATTAGAATGATGGCAGCTTTACTCCAGAATATATATATTGCT
TGCTTTCAGCGAATTAAATTATAGCTTTGCTTTTATGCAATTATTAGTATGAGCTTGT
TTTAACGGGAGTAATTTTAGCTAATTCAAGAATTGATATTGGTCTTCATGGGATACATAT
TATGAGTAGGCTACTTTCAATTTGCTTTTCCATAGGAGCTGTATTTGCAATTATAGCTGGATTTGTTCACTGATTTCCACTATTTACAGGATTAASATAAATAAGAAAATATTAAAATTCAATTAtTTTAATT

M. pedester A-2
TATTATGTCAGAAAGAAGAGAAAAAAAGAAAAACCTTTTGGAACCTTGAAGAATATTCT
ATGCTATAATAGCTATTTGGAATTAGGATTTATAGTATGAGCACACATCATATATTAC
TGAGGATTAGAATGATGGCAGCTTTACTCCAGAATATATATATTGCT
TGCTTTCAGCGAATTAAATTATAGCTTTGCTTTTATGCAATTATTAGTATGAGCTTGT
TTTAACGGGAGTAATTTTAGCTAATTCAAGAATTGATATTGGTCTTCATGGGATACATAT
TATGAGTAGGCTACTTTCAATTTGCTTTTCCATAGGAGCTGTATTTGCAATTATAGCTGGATTTGTTCACTGATTTCCACTATTTACAGGATTAASATAAATAAGAAAATATTAAAATTCAATTAtTTTAATT

M. pedester B-1
TATTATGTCAGAAAGAAGAGAAAAAAAGAAAAACCTTTTGGAACCTTGAAGAATATTCT
ATGCTATAATAGCTATTTGGAATTAGGATTTATAGTATGAGCACACATCATATATTAC
TGAGGATTAGAATGATGGCAGCTTTACTCCAGAATATATATATTGCT
TGCTTTCAGCGAATTAAATTATAGCTTTGCTTTTATGCAATTATTAGTATGAGCTTGT
TTTAACGGGAGTAATTTTAGCTAATTCAAGAATTGATATTGGTCTTCATGGGATACATAT
TATGAGTAGGCTACTTTCAATTTGCTTTTCCATAGGAGCTGTATTTGCAATTATAGCTGGATTTGTTCACTGATTTCCACTATTTACAGGATTAASATAAATAAGAAAATATTAAAATTCAATTAtTTTAATT

128
TTTAACAGGAGTAATTTTAGCTAATTCAAGAATTGACATTATTCTTCATGATACATATATAGTAGGATACCTCAGGATTTCCATATTATATTACAGGATTAAATATAATAAGAAAAATATTAAAAATTTAATT

*M. pedester C-1*

TATTATTAGTCAAGAAAGAAGAAAAAAGAAAAACCTTTGGAACTTTAGGAAATAATCTATGCTATAATAGCTATTGGATTATTAGGGTTTATTGTATGAGCAACATCATATATTTAC
TGTTAGGGTAAGGTTGATACCCCGAGCCTATTATTTTACTTCAGCAACACTATAATTTATTGCTTGTTCCTACAGGAATCAAAAATTTTAGATGGGCTAGCAACATTACATGGGAACACAAATAAATTATTCTCCTCCTCATTATTATGAGCCTTTAGGATTGTTTTTTATTTACTGTACGAGGTTTAACGGGAGTAATTTTAGCTAATTCAAGAATTGACATTATTCTTCATGATACATATATAGTAGGATACCTCAGGATTTCCATATTATATTACAGGATTAAATATAATAAGAAAAATATTAAAAATTTAATT
Approximately 1,500 specimens from the following institutions were studied:

AMNH  American Museum of Natural History
CNC  Canadian National Collection
CU  Clemson University
FSCA  Florida State Collection of Arthropods
HMOU  Hope Museum of Oxford University
KBPC  Kyle Beucke Private Collection
KPPC  Keith Philips Private Collection
KU  University of Kansas
NCSU  North Carolina State University
UMMZ  University of Michigan Museum of Zoology
TNHM  The Natural History Museum, London
USNM  United States National Museum

Note: All sequenced cryogenic material (indicated with a code, for example, A-1) is stored in the -80ºC freezer in the Branham Laboratory, University of Florida, Gainesville, Florida.

*M. lethroides*

**USA: GEORGIA:** No locality given other than Georgia, "Georgia-see Abbot's drawings," 5506, *retusus* McLeay," 1♂ (TNHM); no locality given other than Georgia, 1♂ (Holotype) (HMOU); no locality given except Georgia, "5506a," "Figured in Abbot's drawing of Georgian Insects," 1♂ (TNHM); 5 mi W of Augusta, 6-9-XII-1960, malt traps, R.E. Woodruff and E.W. Holder, 2♀ (FSCA); *Burke Co.*, Yuchi Wildlife Management Area, 3-6-XI-2007, pig dung and fermenting malt pitfall, K. Beucke, 2♂, 3♀ (KBPC); same except 6-XI-2007, K. Beucke, 1♂ (KBPC); sandy road off of Ebeneezer Church Road, turkey oak and pine with exposed sand, N33° 04.848', W81° 47.070', 3-6-XI-2007, pig dung and fermenting malt pitfalls, K. Beucke, 5♂, 8♀ (KBPC); same except 6-XI-2007, excavated from burrows, K. Beucke, 1♂, 1♀ (C-1) (KBPC); same except down sandy road from Ebeneezer Church Road, just past field, to right, in shaded oak forest, N33° 05.017', W81° 46.535', 3-XI-2007, excavated from burrows, K. Beucke, 4♂, 1♀ (KBPC); same except collected on surface of road feeding on acorn, K. Beucke, 1♂ (KBPC); same except
3-6-XI-2007, pig dung and fermenting malt pitfalls, K. Beucke, 2♂ (A-1, A-2), 1♀ (A-3) (KBPC); same except in oak-pine woods on side of sandy road, large field on other side of road, N33° 04.965', W 81° 46.786', 3-6-XI-2007, pig dung and fermenting malt pitfall, K. Beucke, 1♂ (B-1), 1♀ (B-2) (KBPC); same except 8.3 mi E of St. 80 on St. 23, 6-III-1992, collected from pushups, P. Skelley, 1♂, 1♀ (FSCA); same except 7-10-III-1992, P. Skelley, 10♂, 4♀ (FSCA); same except dung and malt pitfall, P. Skelley, 2♂, 4♀ (FSCA); same except 14-I-1995, Skelley and Kovarik, 1♀ (KPPC); same except 18-20-III-1996, Wappes and Turnbow, 3♂, 1♀ (FSCA);

Richmond Co., Augusta, date illegible, O.L. Cartwright, 1♂ (UMMZ); same except 27-IX-1930, T.H. Hubbell, 2♂ ("figured specimen"), 1♀ ("figured specimen") (UMMZ), 1♀ (FSCA); same except 2-III-1944, O.L. Cartwright, 1♂, 1♀ (USNM); same except Jct. I-520 and US-1, 4-14-1-1989, P. Skelley, 1♀ (FSCA); same except pig dung and malt pitfall in sand scrub, P. Skelley, 2♂ (FSCA); same except 13-31-XII-1989, P. Skelley, 3♂, 1♀ (FSCA); same except NW side of Hwy 25, 1.5 mi S. of Hwy 415, 18-XII-1992, T.K. and T.B. Phillips, 1♀ (KPPC).

M. pedester

no locality given, determined by H.F. Strohecker, 1♂ (FSCA); USA: FLORIDA: Charlotte Co., Punta Gorda, 6-IV-1940, H. Ramstadt, 1♀ (paratype) (UMMZ); same but 15-IV-1940, H. Ramstadt, 1♂ (paratype) (UMMZ); same but IV-1951, M. Casselberry, 1♂, 1♀ (topotype) (FSCA), 51♂, 90♀ (AMNH), 1♂, 2♀ (CNC), 1♂, 1♀ (USNM); same but no collector given, 1♂ (paratype) (UMMZ), 3♂, 1♀ (all paratypes) (USNM); same but V-1953, H. Ramstadt, "fr. type series," 1♀ (CNC); DeSoto Co., Arcadia, X-1930, T.H. Hubbell, 1♂ (paratype) (UMMZ); Lee Co., Babcock Ranch, near herp. array. in open pine woods with saw palmetto, N26° 45.665' W81° 40.848', 30-I-6-II-2008, pig dung and fermenting malt pitfall, K. Beucke, 1♀ (B-1) (KBPC); same except N26° 45.578' W81° 40.869', K. Beucke, 2♂ (C-1) (KBPC); Estero, 29-III-1962, malt traps, R.E. Woodruff, 2♂ (FSCA); same but 6-IV-1962, R.E. Woodruff, 2♂ (FSCA); same but 14-IV-1964, B.K. Dozier, 1♂ (USNM); same but 13-I-1965, B.K. Dozier, 1♂ (NCSU); same but malt trap 10AM-4PM, B.K. Dozier, 3♂ (USNM), 1♂, 2♀ (FSCA); same but 10-III-1965, malt trap 9AM-11AM, B.K. Dozier, 3♂, 2♀ (FSCA), 3♂, 1♀ (USNM); same but malt trap 11AM-1:30PM, B.K. Dozier, 2♂, 2♀ (FSCA); same but malt trap 11AM-? (time illegible), B.K. Dozier, 1♂ (USNM); same but 4-IV-1965, malt trap, B.K. Dozier, 1♂, 2♀ (FSCA); same but Rt. 41 just S of Constitution Blvd., at border of dense sandhill woods and parking lot for furniture store, N26° 28.467' W81° 50.168', 30-I-6-II-2008, pig dung and fermenting malt pitfall, K. Beucke, 1♂, 1♀ (KBPC); N of Estero, 0.1 mi S of Constitution Blvd. on US-41, N26° 28.472' W81° 50.165', 17-19-III-2007, pitfall trap, P. Skelley and B. Warner, 2♂ (FSCA), 3♀ (A-1, A-2, A-3) (KBPC); Ft. Myers, VI-1967, 1♂ (CNC); San Carlos (San Carlos Park?) on Hwy. 41, 26-31-I-1992, fert. malt/dung trap, R. Morris, 1♀ (FSCA); 3.4 mi N of Koreshan State Historical Site, 6-9-IV-1991, M. Thomas and R. Turnbow, 11♂, 6♀ (FSCA); Tice, 1.3 mi S of the Caloosahatchee River, on Ortiz Road, 28-30-III-1962, malt trap, R.E. Woodruff, 1♂ (FSCA, in alcohol).

M. retusus

USA: SOUTH CAROLINA: no locality given, with "93:" "67.45," 1♀ (TNHM); no locality given, from Hope Westwood collection, 3♂, 2♀ (HMOU); no locality given, with "5/5"/"73," 1♂
M. cartwrighti

USA: FLORIDA: No location given other than Florida, "From collection of Chas. Schaeffer," 1♀ (paratype) (USNM), 1♀ (AMNH); same but Hubbard and Schwarz, 1♀ (CNC); Dade Co., Miami, 6-IV-1919, H. Klages, 1♂ (paratype) (UMMZ); Duval Co., Atlantic Beach, A.T. Slosson, Ac. 26226, 1♂ (AMNH); Jefferson Co., Avalon conservation easement, oak/hickory forest with pine and Quercus falcata on slope, excavated from burrows approximately 18cm deep, N30° 23.660’ W83° 53.763’, 7-III-2007, K. Beucke, 2♂ (C-1, C-2) (KBPC); same but 25-X-1932, O.L. Cartwright, 2♂ (USNM), 1♂, 2♀ (CU); same but 29-X-1933, O.L. Cartwright, 2♂ (UMMZ), 1♂ (CNC); same but 23-IV-1934, O.L. Cartwright, 1♂ (USNM); near entrance to Sesquicentennial State Park in pine-oak woods, N34.1020° W80.9112°, 4-6 XI-2007, pig dung and fermenting malt pitfall, K. Beucke, 1♀ (D-1), 1♂ (D-2) (KBPC); Kershaw Co., Blaney, 13-X-1936, O.L. Cartwright, 2♂, 1♀ (USNM); Lexington Co., near Hwy. 342, W of Gaston, N33° 49.406’ W81° 11.853’, 26-XII-2006, T.K. Phillips, 1♂, 1♀ (FSCA), 4♂ (A-3, A-4, A-5, A-6), 2♀ (A-1, A-2) (KBPC); 5 mi NE of Pelion, 29-XII-1988, 1♂ (KPPC); Murray, 13-X-1936, O.L. Cartwright, 3♂ (USNM); "Hury 210"?, 1-V-1945, O.L. Cartwright, 1♀ (USNM); Richland Co., Columbia, 3-III-1932, O.L. Cartwright, 1♂ (UMMZ), 1♀ (USNM), 1♀ (TNHM); same but 21-III-1932, O.L. Cartwright, 1♂ (UMMZ), 1♀ (CNC), 1♀ (USNM), 1♀ (TNHM); same but 24-III-1932, O.L. Cartwright, 1♀ ("Figured specimen Olson + Hubbell ’54; Figure 60.") (UMMZ); same but 25-IV-1932, O.L. Cartwright, 2♀ (USNM), 1♂, 2♀ (CU); same but 29-X-1933, O.L. Cartwright, 1♂ (TNHM), 1♂ (CNC); same but 23-IV-1934, O.L. Cartwright, 1♀ (USNM); same but 25-III-1937, O.L. Cartwright, 1♀ (USNM); near entrance to Sesquicentennial State Park in pine-oak woods, N34.1020° W80.9112°, 4-6 XI-2007, pig dung and fermenting malt pitfall, K. Beucke, 1♀ (B-1) (KBPC).
VI-1969, pitfall, Whitcomb, 1♂, 2♀ (FSCA); same but trail one pitfall, 26-V-1969, Whitcomb, 1♀ (FSCA); same but trail two pitfall, 26-V-1969, Whitcomb, 1♀ (FSCA); same but trail three pitfall, beech magnolia hammock, 28-IV-1969, Whitcomb, 1♀ (FSCA); Leon Co., no location given, 10-VI-1922, J.S. Alexander, 1♂ (paratype) (UMMZ); same but 18-VI-1924, C.O. Handley, 1♂ (USNM); Bradfordville, 2-V-1985, pig dung trap, S. Roman, 4♀ (FSCA); Elinor Klapp-Phipps Park, in field near woods, 27-II-2007, D. Almquist, 1♂ (G-1), 3♀ (G-2, G-3) (KBPC); same but recently burned upland forest, N30° 32.310' W84° 17.363', 7-III-2007, excavated from 24cm deep burrow, K. Beucke, 1♂ (B-1) (KBPC); same but N30° 32.294' W84° 17.340', 7-III-2007, excavated from 20cm deep burrow, K. Beucke, 1♂ (A-1) (KBPC); Miccosukee Canopy Greenway, NW of intersection of Miccosukee Road (CR-146) and Crump Road in disturbed former red oak woods (pine oak hickory forest) with shortleaf and loblolly pines in vicinity. (2 specimens with following information; cannot attribute collecting data to either specimen: 30.519153° W 84.133495°, 13-XII-2007, excavated from 20cm deep burrow under old horse dung on sandy trail; N 30.51817° W 84.134624°, found walking on surface of sandy trail at 2:00PM), D. Almquist and A. Johnson, 1♂, 1♀ (KBPC); Tallahassee, 10-12-IX-1929, T.H. Hubbell, 1♂ (paratype) (FSCA), 1♀ (paratype) (UMMZ), 1♀ (paratype) (CNC); same but 10-13-IX-1929, T.H. Hubbell, 1♀ (paratype) (TNHM); same but 5-X-1960, crawling on ground, R.E. Woodruff, 1♂ (FSCA); same but 5-X-1960, dug from 1" burrow, R.E. Woodruff, 1♂, 3♀ (FSCA); same but 5-7-X-1960, malt trap, R.E. Woodruff, 8♂, 4♀ (FSCA); same but 15-24-IV-1963, malt trap, R.E. Woodruff, 8♂, 4♀ (FSCA); same but 15-24-IV-1967, R.E. Woodruff, 3♀ (USNM); 6 mi E of Tallahassee, 5-7-X-1960, R.E. Woodruff, 1♀ (NCSU); Tall Timbers Research Station, a large series of over 10,000 specimens collected on a weekly basis for 2.5 years by D.L. Harris, W.H. Whitcomb, and W.W. Baker (a small number were examined) (FSCA); same but 2-X-1972, Ross Arnett Jr., 1♂, 1♀ (FSCA); same but 16-23-XI-1970, pitfall, W. Rosenberg, 4♂, 7♀ (USNM); same but 27-XI-7-XII-1970, Harris, 2♂ (FSCA); same but 29-30-IX-1989, P. Skelley, 2♀ (FSCA); same but 11-VIII-1969, D. Harris, 12♂, 6♀ (FSCA); same but 15-IX-1969, pitfall, D. Harris, 6♂, 5♀ (FSCA); same but 19-IX-1970, D. Harris, 2♂, 3♀ (FSCA); same but 27-XI-7-XII-1970, D. Harris, 3♂, 1♀ (FSCA); same but disturbed pine upland, N30.66683°, W84.24455°, 18-XII-2007, excavated from 12cm deep burrow in very hard packed clay soil in road, appeared to be burying dense, tan, puffball-like fungi, D. Almquist and M. Paulsen, 1♀ (M-1) (KBPC); Tallahassee, excavated from 1" burrow with tiny pushup) in packed clay soil, K. Beucke, 1♂ (F-1) (KBPC); same but "rep. old corn field," 9-15-XI-1971, D. Harris, 9♂, 28♀ (FSCA); same but 13-20-XII-1971, D. Harris, 6♀ (CNC); same but 3-10-I-1972, D. Harris, 4♂, 21♀ (FSCA), 3♀ (FSCA), 1♀ (CNC); same but 20-27-XII-1972, D. Harris, 4♂, 14♀ (FSCA); same but "by F5A," "NB66," 7-III-1968, Wilson Baker, 1♀ (FSCA); same but rep. 1-A, 2-9-XI-1970, pitfall, D. Harris, 6♂, 6♀ (FSCA); same but rep. 2-E, 21-29-V-1973, pitfall, D. Harris, 1♂ (FSCA); same but small mammal trap B3A, 5-III-1970, Wilson Baker, 1♀ (FSCA); same but small mammal trap C-2, 9-III-1969, Wilson Baker, 1♀ (FSCA); same but in C5, Wilson Baker, 1♀ (FSCA); same but small mammal trap C5X, 9-III-1969, Wilson Baker, 1♀ (FSCA); same but small mammal trap D4X, 9-III-1969, Wilson Baker, 1♀ (FSCA); same but "by G4," 10-III-1968, Wilson Baker, 1♀ (FSCA); same but in G4X, 10-III-1968, Wilson Baker, 1♀ (USNM); Liberty Co., Torreya State Park, on dung, 26-XI-2000, Gino Nearns, 2♂ (FSCA); same but just inside entrance, in upland pine habitat, N30.55893° W84.95027°, 12-16-IV-2007, D. Almquist, 1♂ (L-1) (KBPC); GEORGIA: Location illegible (Pelham?), 22-IV-1995, Department of Agriculture,
"injuring (illegible) vines at Pelham, Ga"?, 1♂ (USNM); Baker Co., Newton, 30-III-1956, walking on dirt road 5:30PM, H. Howden, 1♀ (CNC); 6 mi N.E. Newton, 30-III-1956, walking on dirt road, 5PM, H. Howden, 1♂ (FSCA); Decatur Co., 2.7 mi WSW Faceville, Dist. 21, Lot 352, 22-25-III-1954, T.H. Hubbell, 1♂, 2♀ (UMMZ); Dooley Co., Vienna, 24-VII-1930, "Leng No. 13300," H. Spieth, 1♂ (UMMZ); Grady Co., no location given, 1935 (rest of date illegible), H.S. Peters, 1♂ (USNM); Liberty Co., Ft. Stewart, Old Sundbury Road, N31° 52.479' W81° 34.452', 15-24-V-2007, pig dung and fermenting malt pitfall, K. Beucke, 1♂ (J-1) (KBPC); same but N31° 52.485' W81° 34.440', 29-XI-3-XII-2007, K. Beucke, 1♀ (J-3) (KBPC); same but N31° 52.571' W81° 34.279', 15-24-V-2007, K. Beucke, 1♀ (K-1) (KBPC); same but N31° 52.580' W81° 34.259', 29-XI-3-XII-2007, K. Beucke, 1♀ (J-2) (KBPC); Hinesville, Camp Stewart, 9-VII-1941, J.G. Watts, 1♂ (USNM); Peach Co., Fort Valley, 14-VI-1928, M.C. Swingle, 1♂, 1♀ (USNM); Sumter Co., Americus, 1-V-1950, O.L. Cartwright, 1♂, 1♀ (USNM); Thomas Co., 1.8 mi S of Metcalf, swine feces baited pitfall, 8-15-I-2006, R. Turnbow, 5♂, 7♀ (FSCA); 2 mi S of Metcalf on Metcalf Highway, 13-I-1995, Skelley and Kovarik, 1♀ (KPPC); Thomasville, no date or collector given, 1♂ (AMNH); same but Chas. Schaeffer, 1♂, 1♀ (AMNH); same but 28-III-(year illegible), R.C. Casselberry, 1♂ (AMNH); same but 24-VIII-1938, P.W. Fattig, 1♂, 1♀ (CNC), 1♂ (paratype (FSCA), 2♂ (all paratypes), 4♀ (all paratypes) (USNM), 1♂ (paratype), 1♀ (paratype) (UMMZ); same but 28-VIII-1938, P.W. Fattig, 1♀ (paratype #8997) (CNC), 1♂ (paratype) (UMMZ); same but 3-III-1939, W.H. Thames, Jr., 2♂ (FSCA); Pinetree Blvd. and Lower Cairo Road, 0.3 mi W of W Thomasville Bypass, on north side of road in ditch and in adjacent pine plantation (burned recently, with scant undergrowth), N30° 49.693' W84° 00.690', 15-III-2007, excavated from burrows, K. Beucke, 6♀ (H-1, H-2, H-3, H-4, H-5, H-6), 3♀ (H-7, H-8, H-9) (KBPC); same but pitfall trap, P. Choate, 1♂ (KBPC).

**M. gaigei**

No locality given, 1♂ (FSCA); USA: FLORIDA: "near Inverness," 28-III-1932, F.M.V and A.L.N., 1♀ (USNM); Alachua Co., no location given, 26-III-1959, H.V. Weems, Jr., 1♂ (AMNH); Archer, 21-23-III-1959, R.E. Woodruff, 1♂, 1♀ (FSCA), 1♀ (NCSU); same but 28-III-1960, R.E. Woodruff, 2♀ (AMNH); same but 30-III-1960, malt, Howden, 1♂ (FSCA), 1♂, 1♀ (NCSU), 8♂, 12♀ (CNC), 4♂, 2♀ (USNM); same but in malt trap, 8-IV-1960, R.E. Woodruff, 1♂ (FSCA); same but 22-III-1967, J. Mellott, USNM, 4♂, 2♀ (USNM); same (Levy Co. on label) but 5-III-1977, unknown collector, "NX 651," 1♂ (FSCA); W of Archer, N29° 31.553' W82° 32.100', 19-22-II-2007, pig dung and fermenting malt pitfall, K. Beucke, 1♀ (G-1) (KBPC); 0.5 mi SW of Archer, 21-II-1989, P. Skelley, 1♀ (FSCA); 2 mi W of Archer, 24-III-1953, Howden and Dozier, 1♂ (paratype #6526) (CNC); 2.2 mi SW of Archer, 25-III-1949, F.N. Young, 1♀ (paratype) (AMNH); Gainesville, 3-X-1996, P. Harpootlian, 1♀ (CU); High Springs, 31-III-1938, no collector given, 1♂ (paratype) (NCSU); same but 1-4-II-1960, R.E. Woodruff, 1♂ (FSCA); same but in malt trap, 4♂, 5♀ (FSCA); CR-178, 0.4 mi S of CR-38, 27-IV-1985, blacklight trap, K.W. Vick, 1♂ (FSCA); Columbia Co., no location given, 26-30-X-1929, T.H. Hubbell, 1♂ (paratype) (TNHM); Ft. White, Paisley Drive and Rt. 27 (just off 27), N29° 54.816' W82° 42.229', 23-24-I-2007, P. Choate, 5♂ (Q-4, Q-5), 14♀ (Q-1, Q-2, Q-3) (KBPC); Rt. 47, S of Ft. White, N29° 52.886' W82° 44.036', III-2007, pig dung and fermenting malt pitfall, P. Choate, 3♂ (L-1, L-2, L-3), 2♀ (L-4, L-5) (KBPC); High Springs, 26-X-1929, T.H. Hubbell, 1♂, 2♀ (paratypes) (FSCA), 2♂, 1♀ (paratypes) (USNM); same but 26-30-X-1929, T. Hubbell, 1♂, 1♀ (all paratypes) (FSCA), 2♀ (paratypes) (USNM), 1♀ (paratype)
(TNHM); 4 mi N of High Springs, 25-III-1953, Howden and Dozier, 2♂ (paratype #6526) (CNC); same but 19-III-1953, Howden and Dozier, 2♂, 1♀ (paratypes) (FSCA), 3♂ (paratype) (USNM), 1♂ (paratype) (HMOU), 1♂ (paratype) (NCSU); same but 22-III-1953, malt trap, Howden and Dozier, 3♂, 2♀ (all paratypes) (FSCA), 1♂ (paratype) (USNM), 1♂ (paratype #6526) (CNC), 1♂, 1♀ (paratypes) (TNHM); same but 25-III-1953, Howden and Dozier, 3♂ (all paratypes) (FSCA), 4♂ (all paratypes) (USNM), 1♂ (paratype) (NCSU), 1♀ (paratype #6526) (CNC); Ichetucknee River, yeast and dung pitfall trap, date illegible, L.R. Davis, Jr., 1♂ (FSCA); Ichetucknee River (?) (illegible) on Rt. 27, 15-IX-1974, Lloyd R. Davis, Jr., 1♀ (FSCA); O'Leno State Park, on sandy road in sandhill vegetation, N29° 55.216' W82° 35.060', 20-22-X-2006, pig dung and fermenting malt pitfall, K. Beucke, 1♂ (S-1) (KBPC); same but N29° 55.065 W82° 35.058, 18-21-I-2007, pig dung and fermenting malt pitfall, K. Beucke, 8♂, 13♀ (KBPC); same but N29° 55.222' W82° 35.057', 18-21-I-2007, pig dung and fermenting malt pitfall, K. Beucke, 1♂, 1♀ (KBPC); same but N29° 55.001' W82° 35.057', 18-21-I-2007, pig dung and fermenting malt pitfall, K. Beucke, 2♂ (KBPC); Note: The following M. gaigei sequences are from the combined 18-21-I-2007 catch from the O'Leno sites: ♀ (P-7, P-8, P-9, P-10), ♀ (P-1, P-2, P-3, P-4, P-5, P-6); Dixie Co., Old Town, edge of sandy road, N29° 34.559' W82° 58.069', 6-8-III-2007, pig dung and fermenting malt pitfall, K. Beucke and P. Skelley, 2♂ (K-1, K-2) (KBPC); same but 8-14-III-2007, 5♂ (K-3, K-4, K-5, K-6), 4♀ (K-7, K-8, K-9) (KBPC);
Gilchrist Co., 6.5 mi W of High Springs at US-27 in Rt. 340 (jct. Sarvis and Billy Brown Ave.), 10-15-XII-1998, Geomys burrow, P.S. Skelley, 1♂ (FSCA); on edge of SR-26, W of Newberry, sandhill vegetation with much exposed sand, N29° 37.879' W82° 42.313', 28-X-2-XI-2006, fermenting malt pitfall, K. Beucke (KBPC); same but 18-21-I-2007, pig dung and fermenting malt pitfall, K. Beucke, 1♂ (KBPC); same but 10-I-1984, K.W. Vick, 2♂ (FSCA); same but 16-20-I-1984, K.W. Vick, 2♂, 1♀ (FSCA); 6 mi W of Jct. US-27 on Rt. 27, W of Newberry, N29° 37.878' W82° 42.281', 22-24-III-2007, P. Skelley, 8♂, 1♀, 1 larva (first instar?) obtained in rearing experiment (see Chapter 3) (FSCA); R 16E T 10S S 10, 30-III-1949, F.N. Young, 9♂ (all paratypes) (UMMZ); Lafayette Co., Mayo, Rt. 27 1.2 mi W of Rt. 51, N30° 03.706' W83° 11.427', 1-I-2007, pig dung pitfall, P. Choate, 1♂ (A-1) (KBPC); same but 27-28-I-2007, P. Choate, 2♂ (A-2), 6♀ (A-3, A-4) (KBPC); 4.5 mi E of Mayo, road to Convict Springs and CR-354, 1.2 mi N of Rt. 27, under live oak tree at corner of NE Rowan Rd and Convict Springs road, N30° 04.295' W83° 04.281', 19-22-II-2007, pig dung pitfall, P. Choate, 2♂ (I-1), 9♀ (I-2, I-3, I-4, I-5) (KBPC); 5 mi E of Mayo on US-27, 13-18-II-1960, R.E. Woodruff and H.V. Weems, Jr., 9♂, 6♀ (FSCA); same but 16-20-V-1960, R.E. Woodruff, 19♂, 17♀ (FSCA); same but in pure pine stand, 16-20-V-1960, malt trap, R.E Woodruff, 4♀ (FSCA); Townsend, Rt. 348 off Rt. 27, N30° 08.964' W83° 19.719', 27-III-2007, pig dung and fermenting malt pitfall, P. Choate, 2♀ (M-1, M-2) (KBPC); CR-425, 0.6 mi N of Rt. 27, N29° 59.431' W82° 59.744', 23-24-I-2007, P. Choate, 4♂ (R-4, R-5), 8♀ (R-1, R-2, R-3) (KBPC); same but 25-I-2007, pig dung pitfall, P. Choate, 3♂, 7♀ (KBPC); same but 27-28-I-2007, P. Choate, 11♂ (B-6, B-7, B-8, B-9, B-10), 6♀ (B-1, B-2, B-3, B-4, B-5) (KBPC); Levy Co., no locality given, 31-V-1956, R.A. Morse, 1♂ (NCSU), 1♂, 4♀ (FSCA); same but 19-21-II-1959, R.E. Woodruff, 1♀ (FSCA); same but in 21-23-II-1959, malt trap, R.E. Woodruff, 1♂, 5♀ (FSCA), 2♀ (USNM); Alachua/Leyve County line, 23-25-II-1959, R.E. Woodruff, 2♀ (FSCA); Alachua/Leyve County line, 1 mi N of SR-24, 19-26-II-1983, yeast and dung traps, M.C. Thomas and T. Zoebisch, 1♂, 1♀ (FSCA); "Area 3" (believed to be on the Alachua/Leyve County line, R.E. Woodruff, pers. comm.), 23-25-II-1959, R.E. Woodruff, 4♂, 6♀ (CNC); 0.2 mi W of Alachua/Leyve County line
on SR-24, N29° 30.605' W82° 33.598', 7-9-X-2007, pig dung and fermenting malt pitfall, K. Beucke, 1♀ (KBPC); close to previous site, N29° 30.530' W80° 33.735', 24-II-4-III-2007, pig dung and fermenting malt pitfall, K. Beucke, 2♀ (J-1, J-2) (KBPC); Rt. 24 at Alachua/Levy County line, 24-26-III-1978, J.D. Glaser, 1♀ (FSCA); W of Archer, 1-III-1987, malt trap, P. Skelley, 1♂ (FSCA); 2 mi W of Archer, 23-III-1953, Howden and Dozier, 1♀ (paratype) (FSCA), 1♂, 2♀ (all paratypes) (USNM); same but 24-III-1953, malt and propionic acid trap, 3♂, 4♀ (all paratypes) (FSCA), 2♂, 2♀ (AMNH) (all paratypes), 2♂ (paratypes) (TNHM), 1♂ (HMOU), 4♂, 3♀ (paratypes) (USNM); 3 mi W of Archer, Shirley Ct., 10-VI-1986, Cicero, 1♀ (AMNH), 2♀ (FSCA), 1♂ (paratype #6526) (CNC), 1♀ (paratype) (USNM); 4 mi W of Archer on Rt. 24, 29-III-1991, dung traps, P.E. Skelley and R.E. Woodruff, 123♂, 101♀ (FSCA); Bronson, 19-III-1959, malt trap, R.E. Woodruff, 5♂, 3♀ (USNM); same but 21-23-III-1959, R.E. Woodruff, 3♂, 4♀ (FSCA); same but 23-25-III-1959, R.E. Woodruff, 1♂, 2♀ (USNM); 8 mi E of Bronson on Rt. 27, 24-26-III-1978, J.D. Glaser, 1♂ (FSCA); Bronson, 19-II-1959, malt trap, R.E. Woodruff, 5♂, 3♀ (USNM); same but 21-23-III-1959, R.E. Woodruff, 3♂, 4♀ (FSCA); same but 23-25-III-1959, R.E. Woodruff, 1♂, 2♀ (USNM); 8 mi E of Bronson on Rt. 27, 24-26-III-1978, J.D. Glaser, 1♂ (FSCA); Meredith, 23-II-1959, malt traps, R.E. Woodruff, 3♂, 12♀ (FSCA), 6♂, 5♀ (AMNH), 2♂, 2♀ (USNM); Oak Ridge Estates, 4-10-XII-1990, malt trap, R. Morris, 7♂, 7♀ (FSCA); T11S R17E sec 24/25, 25-III-1976, pitfall traps, L.R. Davis, Jr., 17♂, 13♀ (USNM); Madison Co., on CR-53, N of county line, N30° 16.459' W83° 17.320', 9-II-2007, pig dung pitfall, P. Choate, 1♀ (D-1) (KBPC); same but 10-II-2007, P. Choate, 5♂ (D-2, D-3, D-4) (KBPC); same but III-2007, P. Choate, 1♂ (D-5) (KBPC); Marion Co., Barge Canal Surv. (?) T17S R21E 4 (east central), 4-IV-1975, herp. pitfall (H-7). Christman, 1♂, 1♀ (FSCA, in alcohol); Marion Oaks Manor and SW 56th Court, N28.9962° W82.2197°, 9-VII-2007, pig dung and fermenting malt pitfall, K. Beucke, 1♂ (KBPC); Village of Rainbow Springs, 5-V-1982, M.C. Thomas, 4♂, 7♀ (FSCA); same but 26-III-1989, M.C. Thomas, 1♀ (FSCA); Summerfield, La Casta Estates, 14641 SE 1st Ave. Road, N29° 00.618' W82° 21.229°, 9-VII-2007, pig dung and fermenting malt pitfall, K. Beucke, 1♂ (KBPC); SW 59th Avenue Road, in power line cut near SW 158th Lane, N28.9920° W82.2197°, 9-VII-2007, pig dung and fermenting malt pitfall, K. Beucke, 1♂ (KBPC); 76th Court, off of 484 East, N29° 01.382' W82° 14.770', 19-22-II-2007, pig dung and fermenting malt pitfall, K. Beucke, 2♂ (F-1, F-2), 2♀ (F-3, F-4) (KBPC); Seminole Co., Geneva, 20-IV-1960, R.E. Woodruff, 19♂, 13♀ (FSCA); same but 20-21-IV-1960, in malt trap, R.E. Woodruff, 9♂, 8♀ (FSCA); same but VI-1976, M. Thomas, 2♂ (CNC), 10♂, 8♀ (USNM); same but in turkey oak, 13-20-III-1976, yeast trap, M.C. Thomas, 28♂, 13♀ (FSCA); Geneva, sand pit on Cochran Road, W of SR-46, NW edge of pit in pine woods, N28° 44.754 W81° 07.801, 6-9-VIII-2006, fermenting malt pitfall, K. Beucke, 1♂ (KBPC); same but 27-III-3-IV-2007, pig dung and fermenting malt pitfall, K. Beucke, 3♂, 4♀ (N-1) (KBPC); same but 25-29-V-2007, K. Beucke, 1♀ (N-2) (KBPC); Geneva, on side of Ridge Road near SR-46, N28° 44.817' W81° 07.772', 29-VIII-1-IX-2006, fermenting malt pitfall, K. Beucke, 1♂ (KBPC); Sumter Co., Tillman Hammock on CR-425, N28° 57.218' W82° 08.001', 19-22-II-2007, pig dung and fermenting malt pitfall, K. Beucke, 1♂ (H-1) (KBPC); Suwannee Co., Hildreth, 2.9 mi N of Rt. 27 on road to Ichetucknee Baptist Church, N29° 59.626' W82° 48.633', 27-28-I-2007, pig dung pitfall, P. Choate, 19♂ (C-6, C-7, C-8, C-9, C-10), 22♀ (C-1, C-2, C-3, C-4, C-5) (KBPC); 3.6 mi N of
Obrien, 17-III-1956, malt, Howden, 1♂ (FSCA); on Rt. 349, 6.9 mi S. of Jct. with Rt. 252, T5S R13E section 6, 15-XII-1980, on carrion opossum, L. Davis, Jr., 1♀ (FSCA, in alcohol).

Cryptic *Mycotrupes* species?

APPENDIX C:
MYCOTRUPES LOCALITY RECORDS FROM WHICH NO SPECIMENS WERE STUDIED

M. lethroides

USA: GEORGIA: Burke Co., 7 miles northwest of Girard on SR-23 (Harpootlian 1995); Jefferson Co., near Wrens on US Hwy. 1 (Harpootlian pers. comm.); Richmond Co., 0.5 mi east of the Richmond/Jefferson County line (Brier Creek) on US Hwy. 1 (Harpootlian 1995).

M. retusus

USA: SOUTH CAROLINA: Calhoun/Orangeburg County line on Interstate 26, (Harpootlian 2001); Aiken Co., Gopher Tortoise Heritage Preserve (Harpootlian 2006); Aiken, Hitchcock Woods (Harpootlian 2006); picnic area 17.5 miles north of Aiken (Olson and Hubbell 1954).

M. cartwrighti

USA: FLORIDA: Duval Co., Jacksonville (Olson and Hubbell 1954); Leon Co., 6.5 mi east of Tallahassee, north of U.S. Highway 90 (Olson and Hubbell 1954); GEORGIA: Dooley Co., U.S. Highway 41 at Pennahatchee Creek, 2 miles north of Vienna (probably same as specimen on loan from UMMZ) (Olson and Hubbell 1954).

M. gaigei

USA: FLORIDA: Alachua Co., Warren's Cave, about 8 miles NW of Gainesville, in pineland near entrance (Olson and Hubbell 1954); Citrus Co. (Olson and Hubbell 1954); Levy Co., Andrews Wildlife Management Area, near "Zone C," xeric hammock dominated by Quercus geminata (D. Almquist pers. comm.); 5 miles SW of Archer on State Highway 13 (Olson and Hubbell 1954); Marion Co., Dunnellon (Olson and Hubbell 1954); Seminole Co., Oviedo, SR-434 6.5 mi N of University of Central Florida (S. Fullerton, pers. comm.).
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BIOGRAPHICAL SKETCH

Kyle August Beucke was born in Annaheim, CA, in 1979. He began his undergraduate studies as a freshman at the University of Arizona, where he was hopelessly infected with a love of the desert Southwest. He transferred to Cornell University his sophomore year and earned a B.S. in entomology. After graduating, Beucke worked at the American Museum of Natural History in New York City for three years. In the fall of 2009, he received his Ph.D. from the University of Florida.